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Proteomic Analysis, Immune Dysregulation and Pathway Interconnections with Obesity

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Abstract

Proteomic studies can offer information on hundreds to thousands of proteins and potentially provide researchers with a comprehensive understanding of signaling response during stress and disease. Large datasets, such as those obtained in high-dimensional proteomic studies, can be leveraged for pathway analysis to discover or describe the biological implications of clinical disease states. Obesity is a worldwide epidemic that is considered a risk factor for numerous other diseases. We performed analysis on plasma proteomic data from 3 separate sample sets of post-menopausal women to identify the pathways that are altered in subjects with a high body mass index (BMI) compared to normal BMI. We found many pathways consistently and significantly associated with inflammation dysregulated in plasma from obese/overweight subjects compared to plasma from normal BMI subjects. These pathways indicate alterations of soluble inflammatory regulators, cellular stress, and metabolic dysregulation. Our results highlight the importance of high-dimensional pathway analysis in complex diseases as well as provide information on the interconnections between pathways that are dysregulated with obesity. Specifically, overlap of obesity related pathways with those activated during cancer and infection could help describe why obesity is a risk factor for disease and help devise treatment options that mitigate its effect.

Graphical Abstract

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ASSOCIATED CONTENT

Supporting information:

Supplemental Table 1: Table of significant proteins in each study, Supplemental Table 2: Table of significant KEGG pathways, Supplemental Table 3: Table of significant WikiPathways pathways, Supplemental Table 4: Table of significant GO Biological Process pathways, Appendix: List of WHI investigators

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Author Contributions

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Keywords

Proteomics; Pathways; Antibody Array; Obesity; Inflammation; Immune System

Introduction

High-dimensional -omics studies, such as transcriptomics and proteomics have transformed biomedical research by enabling comprehensive real-time monitoring of a biological system. Proteomic studies are generally performed in one of two ways, with mass spectrometry (MS) or immunoassays. New developments have allowed both methods to provide information on thousands of proteins at a given point in time. Thus, these new levels of proteomic coverage allow a more comprehensive reporting of disease etiology than was previously only accessible via mRNA expression array analysis. Furthermore, since the transcriptome and proteome can vary significantly and proteins can be regulated and modified post-translationally, proteomic analysis can yield a more comprehensive picture of actual cellular status. Additionally, by identifying pathways that differ between two conditions, one can have more explanatory power of the difference between the two states than with individual proteins alone. By examining the sum effect of the different pathways, a comprehensive overview of disease can be obtained. Validation of the up or down regulated pathways in multiple studies to identify the most important biological pathways can yield conclusions about even a complicated disease process like obesity.

Obesity is currently a worldwide epidemic, more prevalent in developed countries, that shows little evidence for declining or plateauing^{1,2}. In the United States, more than one-third of adults (78.6 million) and 17% of children (12.7 million) are obese³. Worldwide there are more than 1.9 billion overweight and over 600 million obese adults⁴. Obesity is included in the global non-communicable diseases that are being targeted for change by the World Health Organization, with the intention of halting the rise of obesity to its 2010 level by 2025². Body-mass index (BMI) is clinically used to identify individuals who may have high body fat. BMI can help to screen patients for certain weight categories, such as overweight or obese, but is not a singular diagnostic tool for the health of an individual⁵. A high BMI is considered a risk factor for cardiovascular disease, kidney disease, diabetes, musculoskeletal disorders, and some cancers⁶⁻¹². Males and females differ in how and where they store body fat and post-menopausal women are more likely to be obese than pre-menopausal women¹³.

Adipose tissue is an important part of the endocrine system that helps to maintain a balance of energy homeostasis and immune system reactivity by regulating lipid storage and controlling the production and secretion of a wide range of adipokines and cytokines¹⁴.

Additionally, in post-menopausal women, adipose tissue is the major source of steroid hormone production with estrogen regulating body adiposity and fat distribution¹⁵ and potentially modifying risk for disease. Several proteomic studies have been employed in multiple tissue types such as adipose tissue, isolated adipocytes¹⁶, and plasma¹⁷ to analyze gene expression changes in obese patients. These studies have identified extensive upregulation of inflammatory pathways. It is thought that these alterations induce chronic inflammation which contributes to the development of the many obesity-related illnesses including type-2 diabetes (T2D), cardiovascular disease and cancer¹⁸. To further examine the biology behind the adverse inflammatory response in obesity, we utilized a high-density antibody microarray platform to examine plasma from post-menopausal women with a wide range of BMI in one autoantibody and three proteomic studies.

Experimental Section

Clinical Samples

Plasma samples from the Women's Health Initiative (WHI) Observational Study, a prospective cohort of 93,676 post-menopausal women enrolled from 1993 to 1998 in the United States were used for these studies^{19,20}. Plasma from women with no prior history of any type of cancer and no cancer diagnosis two years after collection were used in four separate studies and profiled on customized antibody arrays^{21–23}. The four studies were comprised of 208, 308, 322, and 86 samples and were well matched in age across studies (between 50 and 79 years of age for all studies) (Table 1). The use of human samples and all patient information provided was done so in accordance with the Institutional Review Board at the Fred Hutchinson Cancer Research Center.

Antibody Arrays

Arrays were populated with more than 3000 distinct full-length antibodies primarily from SDIX (now sold by Novus), Aviva Biosciences, R&D Systems, Abnova, Sigma-Aldrich, and a few other manufacturers to more than 2100 different proteins involved in a diverse array of signaling pathways. Although there was significant overlap in content, each study had some variation in the specific antibodies used. A detailed list of the antibodies used in study 3 has previously been published²⁴. Details on array fabrication have been previously reported^{25,26}.

Proteomics

Relative protein levels were detected as previously described^{23,25,27,28}. Briefly, albumin and IgG were depleted from plasma. The depleted plasma was concentrated to its original volume, measured for total protein concentration, labeled with the amine reactive dyes Cy3- and Cy5-maleimide and unincorporated dye was removed. Individual Cy5-labeled subject samples were incubated with an equal amount of Cy3-labeled reference (a common pool of plasma comprised of samples collected from 7 women aged 45–72 years was used as a reference for all samples). Labeled lysates were incubated on arrays for 90 minutes, washed serially to remove excess dye, and slides were scanned in an Axon GenePix 4000B microarray scanner and data extracted using GenePix Pro 6.0 software (Molecular Devices,

Sunnyvale, CA). All proteomic data is publically available in the National Center for Biotechnology Information's (NCBI) Gene Expression Omnibus (GEO) repository.

Autoantibody-antigen

Autoantibody-antigen complexes were detected as previously described²⁴. Briefly, undepleted human plasma was diluted 1:80, pipetted onto the array at the microarray/coverslip junction and incubated for 60 min at room temperature. Autoantigen-antibody complexes were detected after incubation with Alexa Fluor 546-goat anti-human IgG and Alexa Fluor 647-goat anti-human IgM for 60 min at room temperature. As a control to determine background levels of signal, the arrays were incubated with just secondary antibody (no plasma added) and the resulting signals were used for background subtraction. Finally, the slides were scanned on an Axon GenePix 4000B microarray scanner and data was extracted using GenePix Pro 6.0 software. Autoantibody-antigen data (Study 4) is publically available in the GEO repository.

Statistical Analysis

For each protein study, fold change of signal (red channel) compared to reference (green channel), the M value, was calculated as $\log_2(Rc/Gc)$; where Rc is red corrected and Gc is green corrected for each antibody spot^{27,29} (using the normexp background correction method developed by Smyth)³⁰. Technical sources of variation were normalized using loess procedures developed for microarrays, including within-array print-tip loess and between-arrays quartile normalization. Following normalization, triplicate features were summarized using their median. M values were further normalized using linear regression to remove the systematic bias due to factors such as incubation day, hormone replacement therapy, age and ethnicity. For autoantibody-antigen complex analysis, cases and controls red and green spot intensities are analyzed separately for IgG and IgM specific autoantibody content²⁴.

Pathway Analysis

To perform pathway analysis, only proteins that were significantly altered ($p < 0.05$) in overweight/obese compared to normal BMI were considered. Each study was analyzed independently using Enrichr³¹ a gene set enrichment analysis web server that is a comprehensive resource for curated gene sets. Specifically, Kyoto Encyclopedia of Genes and Genomes (KEGG), WikiPathways and Gene Ontology (GO) Biological Process results were analyzed^{32–34}. For each study, input of the significantly altered proteins into Enrichr resulted in an adjusted p-value, which is correcting for the total number of pathways queried in each individual pathway dataset using the Benjamini-Hochberg method. The adjusted p-value is based on the assumption that all the pathway members are represented on our array so it would underestimate significance (since all are not covered). Pathways that were deemed significant in all three proteomic studies via this method were then further analyzed as a conglomerate to measure the significance of the pathway, taking into account the fact that not all pathway proteins are measured on the arrays. All three studies array platforms were combined to determine the spectrum of proteins that were analyzed and the significant proteins from all three studies were also combined. This information was then used to

calculate the hypergeometric probability of each pathway being modulated given the total number of proteins analyzed, the total number of significant proteins, the number of proteins in the pathway that are represented in our arrays, and the number of those proteins in the pathway that are significant.

Pathway Network Maps

Pathway maps were created to visualize the interaction between distinct pathways. These maps were created by the authors based on the KEGG and WikiPathways databases, which show upstream and downstream pathway interactors in their pathway maps.

Immunoblotting

Plasma lysates were prepared in Laemmli sample buffer, standardized for equal protein levels, separated by 4–12% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were first stained with ponceau S for equal loading and then probed with a Stat3 primary antibody (2438.00.02) from SDIX.

Results and Discussion

Study Design

We examined plasma from women participating in the WHI Observational Study with no prior history of any type of cancer as a sub-analysis of 4 previous studies examining breast and pancreatic cancer^{21–23}. The demographics of subjects and their classification as normal (BMI<24.9kg/m²) or overweight/obese (BMI>25.0kg/m²) in each of the studies is presented in Table 1. The arrays used for each of the studies had significant overlap in antibody content but were not identical. Additionally, all of the plasma samples used in each proteomic study were distinct.

Because the antibodies used in the immunoassay arrays were not entirely consistent across all four datasets, with some proteins being targeted by different antibodies depending on the array and other proteins absent in one or more of the arrays, we chose to perform pathway analysis of each individual dataset and then compare the pathways that were deemed significantly changed across the studies. By doing this, we allow for study-to-study content variability while still probing the biological implications of obesity in our datasets. Using proteins that were statistically different between overweight/obese and normal BMI (all significantly changed proteins from the 4 studies are presented in Supplemental Table 1), we specifically probed three pathway databases. Using KEGG, 62, 87, 44, and 89 pathways met our threshold for statistical significance in studies 1, 2, 3 and 4, respectfully (Supplemental Table 2, corrected for multiple hypothesis testing). Of those, 31 pathways were significant in all three proteomic studies (Supplemental Table 2). Using WikiPathways, 75, 86, 35, and 81 pathways met the threshold for statistical significance in studies 1, 2, 3 and 4, respectfully with 26 of those pathways significant across all three proteomic studies (Supplemental Table 3). Using GO Biological Process, 648, 621, 343, and 604 pathways met our threshold for statistical significance in studies 1, 2, 3 and 4, respectfully, and 138 pathways were significant across all three proteomic studies (Supplemental Table 4). We identified 25.8% of

significant KEGG pathways and 69.2% of significant pathways from WikiPathways as involved in inflammation. As GO Biological Process terms describe a series of events accomplished by organized assemblies of molecular functions and are not equivalent to the broad pathways identified with KEGG and WikiPathways, we did not explore the number of inflammation terms identified. Since the majority of pathways found in KEGG and WikiPathways were involved inflammation we focused our study on biological processes in inflammation and the pathways we identified with sufficient coverage in our arrays with implications of inflammation in obesity.

Soluble Inflammatory Mediators

Adipokines are soluble proteins released from adipocytes and are believed to play an important role in obesity development³⁵. They exert their effects in an autocrine, paracrine and endocrine manner and the balance between pro- and anti-inflammatory adipokines influences disease development. There have been many adipokines identified to date including leptin, adiponectin, resistin, retinol binding protein-4, TNF- α , IL-1, IL-6, IL-10, CCL2, and IL-8³⁶. Many of these adipokines and their pathways were altered in all three of our proteomic datasets, including leptin, oncostatin M, and IL-6 (Table 2). We also see consistent dysregulation of a number of other cytokine and adipokine pathways in our data such as the IL-4, IL-11, IL-3, IL-2, IL-5, TGF-beta, and Kit receptor signaling pathways as well as EPO receptor signaling (Table 2).

Leptin was the first adipokine discovered and remains the most well described inflammatory adipokine. The level of leptin in human blood closely correlates to adipose tissue mass³⁷. Leptin can alter immune function and the long form of the leptin receptor is found on almost all immune cells. Engagement of leptin with its receptor can activate multiple intracellular signaling pathways through JAK2 including STAT3, MAPK-ERK, and PI3K³⁸ (Figure 1). In our data we see dysregulation of PI3K-Akt signaling as well as dysregulation of prolactin signaling which activates pathways such as PI3K and JAK-STAT (Table 2, Figure 1). These signaling pathways result in the production of a host of pro-inflammatory molecules including IL-6, IL-12, IL-18, TNF- α , IL-8, CCL2, CXCL5, PGE2, ROS, IFN- γ , and leukotrienes (Figure 1). In particular, IL-6, IL-1 and TNF- α also stabilize the expression of leptin mRNA creating a feed-forward loop of chronic inflammation³⁹ (Figure 1). In post-menopausal women, IL-6, IL-1 and TNF- α cytokines are known to be increased⁴⁰. While we saw significant dysregulation of the PI3K pathway in all three of our proteomic studies, the JAK-STAT pathway was only significant in two. Regardless, the JAK-STAT pathway plays a critical intermediary role in inflammation in obesity (Figure 1). In a study of peripheral blood mononuclear cells analyzed by shot-gun MS proteomic profiling approaches from lean and obese human males, 47 proteins were found to be differentially expressed. As a consequence, obese subjects had overexpression of NF- κ B and TNF- α which were decreased after exercise¹⁷. Leptin expression is also diametrically opposed to the expression of another adipokine, adiponectin. Adiponectin is the most abundant adipokine in human serum and has potent anti-inflammatory properties. Adiponectin plasma concentration negatively correlates with amassing body fat and low adiponectin levels are associated with many obesity-related disorders. Binding to either adiponectin receptors AdipoR1 or AdipoR2 on immune cells or tissues activates AMPK and blocks NF- κ B

activation. This leads to decreased production of the pro-inflammatory cytokines TNF- α , IL-6 and IL-18. In our dataset of overweight/obese subjects, we see changes in the IL-6 pathway that are consistent with reduced adiponectin confirming that these two pathways are diametrically opposed (Figure 1). It is clear leptin plays an important role in obese patients. However, many proteomic studies do not identify leptin in obese patients compared to controls^{41–43}. As an individual protein, leptin was significantly upregulated in one of our data sets, but the leptin signaling pathway was identified as significant in all 3 studies, indicating the importance of using pathway analyses to understand complex disease states.

We also wanted to validate one of our top candidates by using an additional assay. We chose Stat3 because it is significantly upregulated in our proteomic studies in the plasma of overweight/obese women compared to women with normal BMI (Figure 2). Additionally, Stat3 has previously shown to be an important player in obesity. For example, Leptin and IL-6 can increase Stat3 signaling and chronic Stat3 expression induces obesity in mouse models^{112,113}. Stat3 is upregulated in plasma from overweight/obese subjects compared to normal by immunoblotting with one of the same antibodies used on our arrays.

While identifying dysregulated pathways is an important first step in understanding the changes associated with obesity, examining individual players in signaling pathways to determine overall signaling consequences will also be important. For instance, as shown in supplementary table 1 there are 4 antibodies for Stat3 expression in study 2 and study 3 that show significant dysregulation. At first, the data seem conflicting as two antibodies significantly increase and two antibodies significantly decrease Stat3 expression in overweight/obese subjects compared to normal BMI. Further investigation demonstrated that the antibodies associated with a decrease in Stat3 expression actually recognize phosphorylation sites on Stat3, while the other two antibodies recognize whole protein Stat3. This type of pattern is seen with several top proteins in our analysis including AKT and STAT1 (Supplemental Table 1). Careful examination of the up and down regulation of important pathways identified here will be a critical next step to understanding a complex disease like obesity.

Another key inflammatory signaling pathway we observe is the advanced glycation end-products/receptor for advanced glycation end-products (AGE/RAGE) pathway. This pathway is consistently ranked as highly significant in our datasets and appears in both KEGG and WikiPathways results (Table 2). AGEs are heterogeneous glycosylated proteins, lipids and nucleotides⁴⁴. The RAGE receptor is another danger signal receptor and is expressed on immune cells and adipocytes⁴⁵. Downstream signals of RAGE include NF- κ B, ERK, and JNK all of which increase inflammatory cytokine production including the precursor of IL-1, IL18, IL6 and TNF- α ⁴⁶ (Figure 1). The importance of IL-1 β in obesity-related inflammation has been demonstrated by the clinical efficacy of a human IL-1 receptor antagonist in reducing systemic inflammation and improving T2D symptoms in several clinical trials⁴⁷.

A consequence of the pro-inflammatory signaling pathways we observe is that cytokines and other immune cells in adipose tissue drive macrophage and T cell phenotypes⁴⁸. These different cellular subsets are critical to the inflammatory-driven metabolic diseases of

obesity. Macrophages can be driven into M1 or M2 cells and are characterized by the cytokines they produce. M2 macrophages participate in wound healing, secrete the anti-inflammatory cytokine IL-10 and are the predominant macrophage found in normal adipose tissue. Adiponectin also stimulates production of M2 macrophage related genes like arginase-1 which stimulates production of anti-inflammatory mediators like IL-10 and IL-1Ra. Conversely, M1 macrophages are the principle macrophage in obese adipose tissue, and produce a multitude of inflammatory cytokines including IL-6, IL-1 and TNF- α which skew both innate and adaptive immune cells towards an activated immune response (Figure 1). The change in the balance of immune effectors in adipose tissue increases inflammatory cells like M1 macrophages, neutrophils, eosinophils, CD8⁺ and Th1 CD4⁺ cells while decreasing Th2 CD4⁺ T cells, M2 macrophages and T regulatory cells. Mantonvni et al. used MS to compare the plasma membrane proteomes of polarized macrophages⁴⁹. M1 macrophages had proteomic signatures of leukocyte activation, IFN- γ induced GTPase activity and defense responses while M2 macrophages had signatures of phagocytosis and angiogenesis. T cells also play a critical role in obesity development and both CD4⁺ and CD8⁺ T cells are increased alongside macrophages in obese adipose tissue^{50,51}. We consistently see a change in T cell receptor signaling in our datasets (Table 2). CD4⁺ T cells also differentiate into Th1 cells instead of the Th2 cells found in lean adipose tissue. Th1 cells also promote the differentiation of M1 macrophages.

Integrating our significantly altered pathways into a biological map (Figure 1) identified some surprising intermediaries including regulation of actin cytoskeleton and calcium signaling as important mediators of the biology of soluble inflammatory mediators in obesity. Across all four of our studies, we only probe for 29 of the 180 proteins involved in calcium signaling (KEGG) and 82 of 214 proteins involved in the regulation of actin cytoskeleton (KEGG), limiting our power to see dysregulation of these pathways. By creating our biological map we were able to use the pathways we did have power to observe to uncover other potential pathways involved in obesity. Currently, the knowledge of the role of the calcium signaling pathway in obesity is limited, but parts of the pathway are implicated in selective insulin resistance⁵². Calcium signaling is more extensively studied in relation to various types of cancer^{53–55}. The actin cytoskeleton has been studied in relation to obesity^{56,57} and is known to be highly involved in cancer development and progression^{58–61}. We see some evidence of actin cytoskeleton modulation in our GO Biological Process data, specifically in pathways involving cell migration, cell motility, and cellular component movement (Supplemental Table 4). Future studies using targeted immunoassay arrays could provide critical information regarding the effect of obesity on diseases such as cancer, in which there is much more information regarding the roles of these pathways. The fact that the populations we studied were limited to post-menopausal women can be viewed both as a limitation and strength. Examining the literature, many of the inflammatory markers and inflammatory mediator pathways that we identified are found in most overweight/obese populations. By limiting to the studies to older women, we may find associations that would be masked by sex or age differences. However, it is possible that some of the associations we find, particularly the novel ones might be only associated with our particular demography.

Cellular Stress

In all three proteomic datasets analyzed, we found a significant number of pathways that contribute to or are a product of cellular stress dysregulated in our overweight/obese samples compared to normal. These include the HIF-1, FoxO, Phospholipase D, TNF, AGE/RAGE, IL-6 and, apoptotic signaling pathways as well as the regulation of cellular response to stress (Table 3, Figure 3). With weight gain, existing adipose tissue expands through hypertrophy of present adipocytes and differentiation of pre-adipocytes to accommodate the increasing energy storage demands⁶². The rapid expansion of adipose tissue causes cellular stress via biological conditions such as hypoxia and oxidative stress, which persist in the obese state, and we see these dysregulations in pathways such as HIF-1, FoxO, AGE/RAGE, and angiogenesis (Table 3). Cellular stress drives an immune imbalance, stemming from adipocyte secretion of inflammatory cytokines, infiltration of inflammatory cells like CD8⁺ T, B, and M1-polarized macrophages, and the simultaneous reduction in the presence and function of anti-inflammatory cells like CD4⁺ T regulatory cells and M2-polarized macrophages⁶³. Cellular stress can also cause dysfunction of the endoplasmic reticulum, eventually triggering a regulatory mechanism termed the unfolded protein response (UPR)⁶⁴. The process of UPR is associated with the increase production of inflammatory mediators like ROS, IL-8, and TNF- α . In our data, we specifically see a dysregulation in the TNF signaling pathway and DNA damage response (Table 3, Figure 3). Cumulatively these changes result in functional maladaptation and tissue remodeling of adipose tissue to sustain a low level state of chronic inflammation^{65–69}. Pathways such as Nanoparticle-mediated activation of receptor signaling (Table 3) are important in the creation of a sustained inflammatory response. Nanoparticles are shown to interact with cellular receptors such as EGFR, activating the MAPK signaling cascade and inflammation⁷⁰ (Figure 3). The inflammation discussed can be reversible as changes in diet, high intensity exercise or nutritional supplementation significantly decrease multiple inflammatory pathways^{71–74}.

Previous proteomic analyses have been done in human adipose tissue of lean, healthy subjects, overweight subjects and morbidly obese subjects. Studies of the proteome, specifically in abdominal adipocytes of lean versus obese humans, demonstrate an increase in inflammatory and metabolic pathways⁷⁵. A study of fat biopsies from lean or insulin-resistant obese patients analyzed by 2D gels followed by MALDI-TOF MS, identified an enrichment of proteins involved in inflammation and the UPR⁷⁶. Specifically, IRE1 and XBP1, which are ER stress sensors and activated JNK (which is involved both in inflammation and can be activated by ER stress) were identified. In our datasets we see dysregulation of the TNF signaling pathway and the AGE/RAGE pathway (Table 3), both of which can regulate JNK activation to mediate inflammation via the MAPK signaling pathway (Figure 3). For as important as the MAPK signaling pathway is in cellular stress, it's intriguing that we don't see the specific pathway significantly dysregulated in all of our proteomic datasets. Looking at the WikiPathways and KEGG pathway results, only study 3 is not significantly dysregulated.

In addition to the pathways discussed in relation to overall cellular stress, we see significant dysregulation of pathways such as Phospholipase D, Notch, and the Alpha 6 Beta 4 ($\alpha 6\beta 4$ -integrin) signaling pathway (Table 3), which are all important for cell survival. Obesity is

associated with a certain degree of apoptosis in adipose tissue, a common response to cellular stress. In our plasma proteomic studies we see an alteration in the regulation of apoptotic signaling pathway (Table 3) as well pathways that contribute to apoptosis such as Oncostatin M (Table 3, Figure 3). Previously performed transcriptome analysis of whole blood demonstrated an enrichment for genes in apoptotic pathways in obese compared to lean subjects⁷⁷. Additionally, macrophages that accumulate in white adipose tissue of both mice and humans promote adipose and systemic inflammation^{78,79}. The prevalence of these white adipose tissue macrophages is localized to individual dead adipocytes⁸⁰. These macrophages remain activated and fuse to form multinucleate giant cells that acutely produce proinflammatory cytokines such as IL-1 α and TNF- α ⁸¹. The frequency of adipocyte death is increased >30 fold in a mouse model of obesity and in obese humans with BMI>30, suggesting that adipocyte death promotes macrophage recruitment, accumulation and persistence in the white adipose tissue of obese individuals⁷⁹, consistent with our findings related to apoptosis in a high BMI population.

With cellular stress, we see ubiquitin mediated proteolysis as an end result of many of our significantly altered pathways (Figure 3). Ubiquitin mediated proteolysis is an important part of the cellular process that primarily functions by signaling for protein degradation via the addition of ubiquitin to the protein of interest. There have been numerous gene expression studies that identify ubiquitin mediated proteolysis as significantly altered with obesity⁸²⁻⁸⁴. In our studies, the pathway itself isn't significantly dysregulated, which is unsurprising considering that only 33 of the 137 proteins in the pathway (KEGG) were represented across all four of our arrays, resulting in reduced power to make a significant observation. Thus, further research is needed to determine the specific role of ubiquitin mediated proteolysis in obesity especially as it relates to the risk of cancer. Ubiquitin mediated proteolysis has been extensively studied in cancer⁸⁵ and could potentially provide insight into the link between obesity and cancer. Post-menopausal women are more likely to develop certain types of cancer, such as breast cancer, than pre-menopausal women⁸⁶. Future studies looking into ubiquitin mediated proteolysis in post-menopausal women of varying BMI could help explain the biology behind the dysregulation of the pathway in high BMI women and describe its role as a link between cancer and obesity.

Metabolic Dysregulation

Notably, we see pathways involving metabolic dysregulation in our datasets (Table 4). A large percentage of obese individuals are known to be in a state of metabolic dysregulation (typically characterized by an increased waist circumference, hyperglycemia, hypertension, hypertriglyceridemia, and insulin resistance)⁸⁷. With metabolic dysregulation, increased levels of circulating insulin, insulin-like growth factor 1, leptin, inflammatory factors, and vascular integrity-related factors are typically observed⁸⁸. We see the alteration of pathways such as response to insulin and the insulin receptor signaling pathway in our studies (Table 4). While the insulin signaling pathway (KEGG) is not significantly dysregulated in all three proteomic analysis, it is significantly altered in two of them. Additionally, in obese individuals there tends to be an induction in the release of free-fatty acids, glucose levels, ROS, inflammatory chemokines, and cytokines in a feed-forward loop of inflammation and metabolic dysregulation. In our studies, we see the AGE-RAGE signaling pathway in

diabetic complications, a pathway that can be caused by hyperglycemia, as well as the PI3K-Akt and FoxO signaling pathways (Table 4). The FoxO signaling pathway is phosphorylated by PI3K in response to insulin (Figure 4). Furthermore, Foxo1 has been shown to directly upregulate the pro-inflammatory cytokine IL-1 β via NF- κ B signaling in cultured macrophages⁸⁹. We also observe dysregulation of cellular response to hormone stimulus and response to peptide hormone, both indicators of cytokine activity in our samples.

Mann et al. conducted a comprehensive study of the mouse preadipocyte cell line 3T3-L1⁹⁰, identifying insulin signaling as a key regulatory pathway. They completed a follow up study in adipose tissue by using high resolution quantitative MS-based proteomic tools to identify and quantify SILAC and anti-pY immunoprecipitation of 40 proteins involved in the insulin signaling pathway⁹¹. We see a number of insulin related pathways altered in our data (Table 4, Figure 4). Another study used several approaches to compare the normal human adipose tissue proteome to expression data from obese subjects⁹². RNA-Seq data from the adipose tissue of three healthy females was followed-up with immunochemistry analysis of identified transcripts to determine the localization and expression levels of a number of different candidate proteins. Identified proteins were compared to 26 other tissues throughout the body. Using a genome-scale metabolic model they compared the adipose tissue data to gene expression data obtained from subjects in the Swedish Obese Subjects Sib Pair study. In their studies they demonstrated major mitochondrial metabolic differences between normal and obese subjects. Others have shown that nutritional imbalance leads to mitochondrial dysfunction and susceptibility to apoptosis with consequences such as insulin resistance⁹³.

Obesity and Disease

With the copious number of pathways in our data showing inflammatory mediators, cellular stress, metabolic dysregulation, it is not surprising that obesity is closely linked to a myriad of diseases⁹⁴⁻⁹⁷. Of the 31 KEGG pathways identified as significant in all three proteomic studies (Supplemental Table 2), close to half are cancer specific pathways and five are pathways of other diseases (Table 5). Cancer is the most represented disease in our studies with broad coverage such as pathways in cancer, proteoglycans in cancer and choline metabolism in cancer, as well as more specific cancer pathways such as pancreatic cancer, chronic myeloid leukemia, prostate cancer, glioma, endometrial cancer, colorectal cancer, renal cell carcinoma, non-small lung cancer, acute myeloid leukemia, integrated breast cancer pathway, integrated pancreatic cancer pathway, and the TCA cycle nutrient utilization and invasiveness of ovarian cancer (Table 5). From the data we have collected on our cohorts, we know the post-menopausal women in these studies were cancer free at the time of collection, indicating that the pathways that are dysregulated in the overweight/obese population are potentially drivers of future cancers. Several studies using the expansive WHI dataset have demonstrated an increased risk of breast and pancreatic cancer in postmenopausal women with high BMI^{98,99}. Additionally, the duration of time women are overweight or obese correlates with increased risk for breast and ovarian cancer¹⁰⁰.

While many non-sex organ cancers, such as renal cell, colorectal, pancreatic, and acute myeloid leukemia, attribute obesity as a risk factor in either gender, gliomas appear to be

only linked to obesity in women¹⁰¹. It's interesting that we see a significant dysregulation of glioma related genes in our studies, as our cohorts are exclusively post-menopausal women. While outside the scope of this paper, it would be intriguing to further analyze the WHI cohort as a whole (93,676 women) in an epidemiological study to see if obesity correlates to the incidence of glioma. Additionally, we see dysregulation of breast cancer, endometrial cancer, and ovarian pathways, all female dominant cancers that are prevalent in post-menopausal women (Table 5). Because our studies were done in post-menopausal women, these results may be driven by our subject population and may not be relevant in obese children, pre-menopausal women, and men.

Interestingly, diseases outside the scope of cancer were also significant in our datasets. We see diseases that occur due to internal dysregulation such as non-alcoholic fatty liver disease and a number of diseases that are a result of infection, including hepatitis B, hepatitis C, tuberculosis, and HTLV-1 infection (Table 5). While it's doubtful a significant number of our subjects had these infections, these results are interesting in that they indicate in our overweight/obese population there is a response to "infection", highlighting the prevalence of the immune system in obesity. Unfortunately, this response and chronic inflammation in obesity also interferes with the ability of the human body to combat a true infection¹⁰².

Obesity Related Autoantibody-Antigen Complexes

Recent research is making it increasingly clear that B cells play a central role in obesity-induced inflammation. B cells accumulate in adipose tissue during obesity in large numbers¹⁰³. Compared to healthy controls, B cells isolated from obese T2D patients secrete more pro-inflammatory cytokines and less anti-inflammatory IL-10 cytokines¹⁰⁴. Treating diet-induced obese mice with a B cell depleting CD20 antibody attenuates disease, while transfer of IgG antibodies from diet-induced obese mice rapidly induces insulin resistance in obese but otherwise healthy B cell null mice¹⁰⁵. B cells can promote and sustain inflammation by presenting antigens, secreting cytokines and producing pathogenic antibodies¹⁰⁶.

Autoantibodies are a type of antibody produced by B cells in response to self-antigens. These self-molecules trigger B cell activation usually because of aberrant expression, mislocalization, sequencing errors^{107,108}, and/or altered post-translational modifications¹⁰⁹. Thus, autoantibodies can lend interesting insights into disease processes. With the understanding that B cells play a prominent role in obesity-related inflammation, we decided to examine autoantibody-antigen complexes via immunoassay microarray. Pathway analysis of both IgM and IgG significant autoantibody-antigen complexes revealed many of the same pathways observed in the proteomics analysis including AGE/RAGE, Leptin signaling, and TNF signaling (Table 6). This lends support to the proposal that these pathways are key players in obesity. Additionally, several of the inflammatory pathways previously discussed had both IgG and IgM autoantibody-antigen complexes, including macrophage activation (IL-34, CSF3R) and cytokine signaling (STAT1, MAPKAPK3). Interestingly, Type 2 diabetes was significant in Study 4 but not in any of the proteomic studies. In earlier studies, we have observed that proteins complexed to autoantibody appear to decrease during proteomic analysis. This is likely due to the fact that we deplete plasma of IgG before

proteomic analysis. Thus type 2 diabetes related proteins present in a complex with IgG along with any other proteins that interacted would be depleted prior to proteomic analysis preventing their identification as upregulated or dysregulated. This emphasizes the importance of combining data from multiple -omics methods to gain a more comprehensive insight into complex diseases and can potentially explain why some of the very important intermediary pathways identified in our pathway maps (Figure 1, Figure 3, Figure 4), such as NF- κ B, MAPK, JAK-STAT, and insulin signaling are not significant in all three proteomic studies but are significant in the autoantibody-autoantigen complex analysis (Supplemental Table 2).

Obese humans are known to have a unique profile of IgG autoantibodies compared to controls. This is evident in studies such as one in which serum from 32 overweight/obese subjects and matched normal weight male human serum was analyzed on ProtoArrays¹⁰⁵. Another study performed functional protein microarrays, created by spotting individually purified proteins onto a solid surface¹¹⁰, and found 122 IgG targets to be associated with obesity. Additionally, a different study compared the serum of individuals categorized as metabolically healthy obese or metabolically unhealthy obese. Using MS combined with Ingenuity pathway analysis, they demonstrated an enrichment of proteins involved in the humoral inflammatory processes in metabolically unhealthy obese¹¹¹. These studies and our own data lend further strength to using multiple proteomic approaches to understanding a complex disease like obesity.

Conclusions

Our pathway analysis of three separate proteomic studies identified inflammation related pathways as significantly altered in the plasma of overweight/obese post-menopausal women compared to normal BMI. By visualizing the pathways that were significantly dysregulated in all three proteomic studies in a map format, we were able to identify pathways that, while not significant in all three studies, are important regulators of obesity, such as the JAK-STAT and NF- κ B pathways. A fourth dataset analyzing the autoantibody-antigen complexes in plasma of overweight/obese compared to normal post-menopausal women corroborated with proteomic findings by identifying the same inflammatory pathways significant in our proteomic studies. A major strength of our study is the three relatively large independent sample sets we used for our proteomic analysis. These numerous datasets allowed us to validate significantly dysregulated pathways, regardless of the coverage of each individual dataset. Even though the same immunoassay array technology was used for each of our studies, the antibodies printed and the proteins targeted varied, yet we were able to identify many of the same pathways in each dataset. In creating a map of the altered pathways, we were additionally able to identify whole biological systems (inflammatory mediators, cellular stress, and metabolic dysregulation) altered due to obesity as well as identify pathways that may have been missed in looking at only one study (NF- κ B, MAPK, and JAK-STAT). Due to the population we used in our studies, post-menopausal women, it is possible that some of our results are not applicable to other obese populations such as children, pre-menopausal women, and men. Additional studies will need to be conducted to identify common and unique pathway alterations among the different populations. While proteomic research in obesity has focused on inflammation in the past

and the immune system more recently, our data highlights the importance of pathway analysis for discovering new potential disease connections. Specifically, we found ubiquitin mediated proteolysis, regulation of actin cytoskeleton, and calcium signaling pathways to be intermediary and downstream of dysregulated pathways, even though these pathways themselves were not found to be statistically significant in our studies. This could be due to a number of reasons, most notably that we did not have enough coverage of the pathways on the immunoassay arrays that were used in the studies. A closer examination of these pathways is needed to better understand their importance in obesity and the methods by which they work to link obesity with disease. Other future analyses could include additional obesity qualifiers, such as waist circumference, to refine normal body fat and high body fat groupings. In summary, the studies presented provide evidence for potential novel mechanisms linking obesity, inflammation, and disease and highlight the importance of pathway analysis as we develop a more comprehensive clinical understanding of the key factors in disease etiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

AdipoR1	adiponectin receptor 1
AdipoR2	adiponectin receptor 2
AGE	advanced glycation endproducts
Akt	protein kinase B
AMPK	AMP-activated protein kinase
BMI	body mass index
CCL2	chemokine (C-C motif) ligand 2
CSF3R	colony stimulating factor 3 receptor
CXCL5	C-X-C motif chemokine 5

EGFR	epidermal growth factor receptor
EPO	erythropoietin
ERK	extracellular signal-related kinase
FoxO	forkhead box protein O
GO	gene ontology
HIF-1	hypoxia-inducible factor 1
HTLV-1	human T-cell leukemia virus type 1
IFN-γ	interferon gamma
IL-1	interleukin 1
IL-10	interleukin 10
IL-11	interleukin 11
IL-12	interleukin 12
IL-18	interleukin 18
IL-1Ra	interleukin 1 receptor antagonist
IL-1β	interleukin 1 beta
IL-3	interleukin 3
IL-34	interleukin 34
IL-4	interleukin 4
IL-5	interleukin 5
IL-6	interleukin 6
IL-8	interleukin 8
IRE1	endoplasmic reticulum to nucleus signaling 1
JAK2	janus kinase 2
JNK	c-Jun N-terminal kinase
KEGG	Kyoto encyclopedia of genes and genomes
MALDI	matrix-assisted laser desorption/ionization
MAPK	mitogen-activated protein kinase
MAPKAPK3	mitogen-activated protein kinase-activated protein kinase 3
MS	mass spectrometry

NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
PGE2	prostaglandin E2
PI3K	phosphatidylinositol-4,5-bisphosphate 3-kinase
RAGE	receptor for advanced glycation endproducts
ROS	reactive oxygen species
SILAC	stable isotope labeling with amino acids in cell culture
STAT1	signal transducer and activator of transcription 1
STAT3	signal transducer and activator of transcription 3
T2D	type-2 diabetes
TCA	tricarboxylic acid
TGF-β	transforming growth factor beta
Th1	T helper 1
Th2	T helper 2
TNF	tumor necrosis factor
TNF-α	tumor necrosis factor alpha
TOF	time of flight
UPR	unfolded protein response
WHI	Women's Health Initiative
XBP1	X-box binding protein 1

REFERENCES

1. Goodwin PJ, Stambolic V. Annu. Rev. Med. 2015; 66(1):281–296. [PubMed: 25423596]
2. Global action plan for the prevention and control of noncommunicable diseases 2013–2020. Geneva: WHO Media Centre: World Health Organization; 2013.
3. Ogden CL, Carroll MD, Kit BK, Flegal KM. JAMA. 2014; 311(8):806–814. [PubMed: 24570244]
4. WHO | Obesity and overweight. [accessed Aug 24, 2016] <http://www.who.int/mediacentre/factsheets/fs311/en/>
5. Assessing Your Weight and Health Risk. [accessed Jun 28, 2016] http://www.nhlbi.nih.gov/health/educational/lose_wt/risk.htm
6. Singh GM, Danaei G, Farzadfar F, Stevens GA, Woodward M, Wormser D, Kaptoge S, Whitlock G, Qiao Q, Lewington S, Angelantonio ED, Hoorn S, vander Lawes CMM, Ali MK, Mozaffarian D, Ezzati M. Group G. B. of M. R. F. of C. D. C.; (apcsc), A.-P. C. S. C., (decode), D. E. C. analysis of D. criteria in E, (erfc), E. R. F. C., (psc), P. S. C. PLOS ONE. 2013; 8(7):e65174. [PubMed: 23935815]
7. Ni Mhurchu C, Rodgers A, Pan WH, Gu DF, Woodward M. Asia Pacific Cohort Studies Collaboration. Int. J. Epidemiol. 2004; 33(4):751–758. [PubMed: 15105409]

8. Berrington de Gonzalez A, Hartge P, Cerhan JR, Flint AJ, Hannan L, MacInnis RJ, Moore SC, Tobias GS, Anton-Culver H, Freeman LB, Beeson WL, Clipp SL, English DR, Folsom AR, Freedman DM, Giles G, Hakansson N, Henderson KD, Hoffman-Bolton J, Hoppin JA, Koenig KL, Lee I-M, Linet MS, Park Y, Pocobelli G, Schatzkin A, Sesso HD, Weiderpass E, Willcox BJ, Wolk A, Zeleniuch-Jacquotte A, Willett WC, Thun MJ. *N. Engl. J. Med.* 2010; 363(23):2211–2219. [PubMed: 21121834]
9. Zheng W, McLerran DF, Rolland B, Zhang X, Inoue M, Matsuo K, He J, Gupta PC, Ramadas K, Tsugane S, Irie F, Tamakoshi A, Gao Y-T, Wang R, Shu X-O, Tsuji I, Kuriyama S, Tanaka H, Satoh H, Chen C-J, Yuan J-M, Yoo K-Y, Ahsan H, Pan W-H, Gu D, Pednekar MS, Sauvaget C, Sasazuki S, Sairenchi T, Yang G, Xiang Y-B, Nagai M, Suzuki T, Nishino Y, You S-L, Koh W-P, Park SK, Chen Y, Shen C-Y, Thornquist M, Feng Z, Kang D, Boffetta P, Potter JD. *N. Engl. J. Med.* 2011; 364(8):719–729. [PubMed: 21345101]
10. Emerging Risk Factors Collaboration Wormser D, Kaptoge S, Di Angelantonio E, Wood AM, Pennells L, Thompson A, Sarwar N, Kizer JR, Lawlor DA, Nordestgaard BG, Ridker P, Salomaa V, Stevens J, Woodward M, Sattar N, Collins R, Thompson SG, Whitlock G, Danesh J. *Lancet Lond. Engl.* 2011; 377(9771):1085–1095.
11. Prospective Studies Collaboration Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, Qizilbash N, Collins R, Peto R. *Lancet Lond. Engl.* 2009; 373(9669):1083–1096.
12. Lu Y, Hajifathalian K, Ezzati M, Woodward M, Rimm EB, Danaei G. Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration (BMI Mediated Effects). *Lancet Lond. Engl.* 2014; 383(9921):970–983.
13. Brown LM, Gent L, Davis K, Clegg DJ. *Brain Res.* 2010; 1350:77–85. [PubMed: 20441773]
14. Balistreri CR, Caruso C, Candore G. *Mediators Inflamm.* 2010; 802078:1–19.
15. Brown L, Clegg D. *J. Steroid Biochem. Mol. Biol.* 2010; 122(1–3):65–73. [PubMed: 20035866]
16. Xie X, Yi Z, Bowen B, Wolf C, Flynn CR, Sinha S, Mandarino LJ, Meyer C. *J. Proteome Res.* 2010; 9(9):4521–4534. [PubMed: 20812759]
17. Abu-Farha M, Tiss A, Abubaker J, Khadir A, Al-Ghimlas F, Al-Khairi I, Baturcam E, Cherian P, Elkum N, Hammad M, John J, Kavalakatt S, Warsame S, Behbehani K, Dermime S, Dehbi M. *PLoS ONE.* 2013; 8(9):e75342. [PubMed: 24086512]
18. O'Rourke RW. *Surgery.* 2009; 145(3):255–259. [PubMed: 19231576]
19. Hays J, Hunt JR, Hubbell FA, Anderson GL, Limacher M, Allen C, Rossouw JE. *Ann. Epidemiol.* 2003; 13(9 Suppl):S18–S77. [PubMed: 14575939]
20. *Control. Clin. Trials.* 1998; 19(1):61–109. [PubMed: 9492970]
21. Mirus JE, Zhang Y, Li CI, Lokshin AE, Prentice RL, Hingorani SR, Lampe PD. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 2015; 21(7):1764–1771.
22. Li CI, Mirus JE, Zhang Y, Ramirez AB, Ladd JJ, Prentice RL, McIntosh MW, Hanash SM, Lampe PD. *Breast Cancer Res. Treat.* 2012; 135(2):611–618. [PubMed: 22903690]
23. Buas MF, Rho J, Chai X, Zhang Y, Lampe PD, Li CI. *Breast Cancer Res. Treat.* 2015; 153(2):445–454. [PubMed: 26319120]
24. Rho J, Lampe PD. *J. Proteome Res.* 2013; 12(5):2311–2320. [PubMed: 23541305]
25. Loch CM, Ramirez AB, Liu Y, Sather CL, Delrow JJ, Scholler N, Garvik BM, Urban ND, McIntosh MW, Lampe PD. *Mol. Oncol.* 2007; 1(3):313–320. [PubMed: 19383305]
26. Ramirez AB, Loch CM, Zhang Y, Liu Y, Wang X, Wayner EA, Sargent JE, Sibani S, Hainsworth E, Mendoza EA, Eugene R, LaBaer J, Urban ND, McIntosh MW, Lampe PD. *Mol. Cell. Proteomics MCP.* 2010; 9(7):1449–1460. [PubMed: 20467042]
27. Mirus JE, Zhang Y, Li CI, Lokshin AE, Prentice RL, Hingorani SR, Lampe PD. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 2015; 21(7):1764–1771.
28. Ramirez AB, Lampe PD. *Cancer Biomark. Sect. Dis. Markers.* 2010; 8(4–5):293–307.
29. Mirus JE, Zhang Y, Hollingsworth MA, Solan JL, Lampe PD, Hingorani SR. *Mol. Cell. Proteomics MCP.* 2014; 13(12):3484–3496. [PubMed: 25225358]
30. Smyth GK, Speed T. *Methods San Diego Calif.* 2003; 31(4):265–273.
31. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, Clark NR, Ma'ayan A. *BMC Bioinformatics.* 2013; 14:128. [PubMed: 23586463]

32. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M. Nucleic Acids Res. 2016; 44(D1):D457–D462. [PubMed: 26476454]
33. Kutmon M, Riutta A, Nunes N, Hanspers K, Willighagen EL, Bohler A, Mélius J, Waagmeester A, Sinha SR, Miller R, Coort SL, Cirillo E, Smeets B, Evelo CT, Pico AR. Nucleic Acids Res. 2016; 44(D1):D488–D494. [PubMed: 26481357]
34. Consortium TGO. Nucleic Acids Res. 2015; 43(D1):D1049–D1056. [PubMed: 25428369]
35. Howe LR, Subbaramaiah K, Hudis CA, Dannenberg AJ. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 2013; 19(22):6074–6083.
36. Mancuso P. ImmunoTargets Ther. 2016;47. [PubMed: 27529061]
37. Friedman JM, Halaas JL. Nature. 1998; 395(6704):763–770. [PubMed: 9796811]
38. Tilg H, Moschen AR. Nat Rev Immunol. 2006; 6(10):772–783. [PubMed: 16998510]
39. Paz-Filho G, Mastronardi C, Franco CB, Wang KB, Wong M-L, Licinio J. Arq. Bras. Endocrinol. Metabol. 2012; 56:597–607. [PubMed: 23329181]
40. Pfeilschifter J, Köditz R, Pfohl M, Schatz H. Endocr. Rev. 2002; 23(1):90–119. [PubMed: 11844745]
41. Szalowska E, Van Hijum SAFT, Roelofsens H, Hoek A, Vonk RJ, te Meerman GJ. Biotechnol. Prog. 2007; 23(1):217–224. [PubMed: 17269691]
42. Wang P, Mariman E, Keijer J, Bouwman F, Noben J-P, Robben J, Renes J. Cell. Mol. Life Sci. CMLS. 2004; 61(18):2405–2417. [PubMed: 15378209]
43. Roca-Rivada A, Alonso J, Al-Massadi O, Castelao C, Peinado JR, Seoane LM, Casanueva FF, Pardo M. Mass Spectrom. - One Pillars Proteomics. 2011; 74(7):1068–1079.
44. Soman S, Raju R, Sandhya VK, Advani J, Khan AA, Harsha HC, Prasad TSK, Sudhakaran PR, Pandey A, Adishesha PK. J. Cell Commun. Signal. 2013; 7(1):19–23. [PubMed: 23161412]
45. Yamamoto Y, Yamamoto H. Front. Endocrinol. 2013;4.
46. Soman S, Raju R, Sandhya VK, Advani J, Khan AA, Harsha HC, Prasad TSK, Sudhakaran PR, Pandey A, Adishesha PK. J. Cell Commun. Signal. 2013; 7(1):19–23. [PubMed: 23161412]
47. Donath MY. Nat Rev Drug Discov. 2014; 13(6):465–476. [PubMed: 24854413]
48. Mantovani Alberto Sica Antonio Locati M. Immunity. 23(4):344–346.
49. Becker L, Liu N-C, Averill MM, Yuan W, Pamir N, Peng Y, Irwin AD, Fu X, Bornfeldt KE, Heinecke JW. PLoS ONE. 2012; 7(3):e33297. [PubMed: 22428014]
50. Pacifico L, Di Renzo L, Anania C, Osborn JF, Ippoliti F, Schiavo E, Chiesa C. Eur. J. Endocrinol. Eur. Fed. Endocr. Soc. 2006; 154(5):691–697.
51. Gerriets VA, MacIver NJ. Front. Immunol. 2014;5. [PubMed: 24478774]
52. Ozcan L, Cristina de Souza J, Harari AA, Backs J, Olson EN, Tabas I. Cell Metab. 2013; 18(6): 803–815. [PubMed: 24268736]
53. Raynal NJ-M, Lee JT, Wang Y, Beaudry A, Madireddi P, Garriga J, Malouf GG, Dumont S, Dettman EJ, Gharibyan V, Ahmed S, Chung W, Childers WE, Abou-Gharbia M, Henry RA, Andrews AJ, Jelinek J, Cui Y, Baylin SB, Gill DL, Issa J-PJ. Cancer Res. 2016; 76(6):1494–1505. [PubMed: 26719529]
54. Greenberg EF, Lavik AR, Distelhorst CW. Biochim. Biophys. Acta BBA - Mol. Cell Res. 2014; 1843(10):2205–2210.
55. Schaal C, Padmanabhan J, Chellappan S. Cancers. 2015; 7(3):1447–1471. [PubMed: 26264026]
56. Palanivel R, Ganguly R, Turdi S, Xu A, Sweeney G. Metabolism. 2014; 63(10):1363–1373. [PubMed: 25108566]
57. Yang W, Thein S, Wang X, Bi X, Ericksen RE, Xu F, Han W. Hum. Mol. Genet. 2013; ddt444.
58. Yamaguchi H, Condeelis J. Biochim. Biophys. Acta BBA - Mol. Cell Res. 2007; 1773(5):642–652.
59. Hall A. Cancer Metastasis Rev. 2009; 28(1–2):5–14. [PubMed: 19153674]
60. Fife CM, McCarroll JA, Kavallaris M. Br. J. Pharmacol. 2014; 171(24):5507–5523. [PubMed: 24665826]
61. Shankar J, Nabi IR. PLoS ONE. 2015; (3):10.
62. Berry DC, Stenesen D, Zeve D, Graff JM. Development. 2013; 140(19):3939–3949. [PubMed: 24046315]

63. Exley MA, Hand L, O'Shea D, Lynch L. J. Endocrinol. 2014; 223(2):R41–R48. [PubMed: 25228503]
64. Gregor MF, Hotamisligil GS. J. Lipid Res. 2007; 48(9):1905–1914. [PubMed: 17699733]
65. Schenk S, Saberi M, Olefsky JM. J. Clin. Invest. 2008; 118(9):2992–3002. [PubMed: 18769626]
66. Hotamisligil GS. Nature. 2006; 444(7121):860–867. [PubMed: 17167474]
67. Medzhitov R. Nature. 2008; 454(7203):428–435. [PubMed: 18650913]
68. Rocha VZ, Libby P. Nat. Rev. Cardiol. 2009; 6(6):399–409. [PubMed: 19399028]
69. Berg AH, Scherer PE. Circ. Res. 2005; 96(9):939–949. [PubMed: 15890981]
70. Rauch J, Kolch W, Laurent S, Mahmoudi M. Chem. Rev. 2013; 113(5):3391–3406. [PubMed: 23428231]
71. Navarro SL, White E, Kantor ED, Zhang Y, Rho J, Song X, Milne GL, Lampe PD, Lampe JW. PLoS ONE. 2015; 10(2):e0117534. [PubMed: 25719429]
72. Gu Y, Zhao A, Huang F, Zhang Y, Liu J, Wang C, Jia W, Xie G, Jia W. J. Proteome Res. 2013; 12(12):5801–5811. [PubMed: 24224694]
73. CLÉMENT K, VIGUERIE N, POITOU C, CARETTE C, PELLOUX V, CURAT CA, SICARD A, ROME S, BENIS A, ZUCKER J-D, VIDAL H, LAVILLE M, BARSH GS, BASDEVANT A, STICH V, CANCELLO R, LANGIN D. FASEB J. 2004; 18(14):1657–1669. [PubMed: 15522911]
74. Leggate M, Carter WG, Evans MJC, Vennard RA, Sribala-Sundaram S, Nimmo MA. J. Appl. Physiol. 2012; 112(8):1353–1360. [PubMed: 22267387]
75. Benabdelkamel H, Masood A, Almidani GM, Alsadhan AA, Bassas AF, Duncan MW, Alfadda AA. Mol. Cell. Endocrinol. 2015; 401:142–154. [PubMed: 25498962]
76. Boden G, Duan X, Homko C, Molina EJ, Song W, Perez O, Cheung P, Merali S. Diabetes. 2008; 57(9):2438–2444. [PubMed: 18567819]
77. Ghosh S, Dent R, Harper M-E, Gorman SA, Stuart JS, McPherson R. BMC Med. Genomics. 2010; 3:56–56. [PubMed: 21122113]
78. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. J. Clin. Invest. 2003; 112(12):1796–1808. [PubMed: 14679176]
79. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. J. Clin. Invest. 2003; 112(12):1821–1830. [PubMed: 14679177]
80. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, Wang S, Fortier M, Greenberg AS, Obin MS. J. Lipid Res. 2005; 46(11):2347–2355. [PubMed: 16150820]
81. Hernandez-Pando R, Bornstein QL, Aguilar Leon D, Orozco EH, Madrigal VK, Martinez Cordero E. Immunology. 2000; 100(3):352–358. [PubMed: 10929057]
82. Waller-Evans H, Hue C, Fearnside J, Rothwell AR, Lockstone HE, Caldérari S, Wilder SP, Cazier J-B, Scott J, Gauguier D. PLoS ONE. 2013; 8(12):e82825. [PubMed: 24324835]
83. Ghosh S, Dent R, Harper ME, Stuart J, McPherson R. Obesity. 2011; 19(2):457–463. [PubMed: 20885385]
84. Das UN, Rao AA. Lipids Health Dis. 2007; 6:35. [PubMed: 18078524]
85. Mani A, Gelmann EP. J. Clin. Oncol. 2005; 23(21):4776–4789. [PubMed: 16034054]
86. Cheraghi Z, Poorolajal J, Hashem T, Esmailnasab N, Doosti Irani A. PloS One. 2012; 7(12):e51446. [PubMed: 23236502]
87. Ervin RB. Natl. Health Stat. Rep. 2009; (13):1–7.
88. Hursting SD, Berger NA. J. Clin. Oncol. 2010; 28(26):4058–4065. [PubMed: 20697088]
89. Su D, Coudriet GM, Hyun Kim D, Lu Y, Perdomo G, Qu S, Slusher S, Tse HM, Piganelli J, Giannoukakis N, Zhang J, Henry Dong H. Diabetes. 2009; 58(11):2624–2633. [PubMed: 19651810]
90. Adachi J, Kumar C, Zhang Y, Mann M. Mol. Cell. Proteomics. 2007; 6(7):1257–1273. [PubMed: 17409382]
91. Krüger M, Kratchmarova I, Blagoev B, Tseng Y-H, Kahn CR, Mann M. Proc. Natl. Acad. Sci. 2008; 105(7):2451–2456. [PubMed: 18268350]
92. Mardinoglu A, Kampf C, Asplund A, Fagerberg L, Hallström BM, Edlund K, Blüher M, Pontén F, Uhlen M, Nielsen J. J. Proteome Res. 2014; 13(11):5106–5119. [PubMed: 25219818]

93. Pintus F, Floris G, Rufini A. Aging. 2012; 4(11):734–741. [PubMed: 23211444]
94. Weight Control and Physical Activity - IARC Handbook of Cancer Prevention Volume 6. [accessed Jun 20, 2016] <http://www.iarc.fr/en/publications/pdfs-online/prev/handbook6/>
95. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington DC: AICR; 2007.
96. Reeves GK, Pirie K, Beral V, Green J, Spencer E, Bull D. Million Women Study Collaboration. BMJ. 2007; 335(7630):1134. [PubMed: 17986716]
97. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Lancet Lond. Engl. 2008; 371(9612): 569–578.
98. Neuhouwer ML, Aragaki AK, Prentice RL, et al. JAMA Oncol. 2015; 1(5):611–621. [PubMed: 26182172]
99. Luo J, Margolis KL, Adami H-O, LaCroix A, Ye W. Br. J. Cancer. 2008; 99(3):527–531. [PubMed: 18628761]
100. Arnold M, Jiang L, Stefanick ML, Johnson KC, Lane DS, LeBlanc ES, Prentice R, Rohan TE, Snively BM, Vitolins M, Zaslavsky O, Soerjomataram I, Anton-Culver H. PLOS Med. 2016; 13(8):e1002081. [PubMed: 27529652]
101. Sergentanis TN, Tsivgoulis G, Perlepe C, Ntanas-Stathopoulos I, Tzanninis I-G, Sergentanis IN, Psaltopoulou T. PLOS ONE. 2015; 10(9):e0136974. [PubMed: 26332834]
102. Milner JJ, Beck MA. Proc. Nutr. Soc. 2012; 71(2):298–306. [PubMed: 22414338]
103. Winer DA, Winer S, Shen L, Chng MHY, Engleman EG. Int J Obes Supp. 2012; 2(S1):S4–S7.
104. DeFuria J, Belkina AC, Jagannathan-Bogdan M, Snyder-Cappione J, Carr JD, Nersesova YR, Markham D, Strissel KJ, Watkins AA, Zhu M, Allen J, Bouchard J, Toraldo G, Jasuja R, Obin MS, McDonnell ME, Apovian C, Denis GV, Nikolajczyk BS. Proc. Natl. Acad. Sci. 2013; 110(13):5133–5138. [PubMed: 23479618]
105. Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, Tsui H, Wu P, Davidson MG, Alonso MN, Leong HX, Glassford A, Caimol M, Kenkel JA, Tedder TF, McLaughlin T, Miklos DB, Dosch H-M, Engleman EG. Nat Med. 2011; 17(5):610–617. [PubMed: 21499269]
106. Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, Tsui H, Wu P, Davidson MG, Alonso MN, Leong H, Glassford A, Caimol M, Kenkel JA, Tedder TF, McLaughlin T, Miklos DB, Dosch H-M, Engleman EG. Nat. Med. 2011; 17(5):610–617. [PubMed: 21499269]
107. Soussi T. Cancer Res. 2000; 60(7):1777–1788. [PubMed: 10766157]
108. Ulanet DB, Torbenson M, Dang CV, Casciola-Rosen L, Rosen A. Proc. Natl. Acad. Sci. U. S. A. 2003; 100(21):12361–12366. [PubMed: 14519847]
109. von Mensdorff-Pouilly S, Petrakou E, Kenemans P, van Uffelen K, Verstraeten AA, Snijdwint FGM, van Kamp GJ, Schol DJ, Reis CA, Price MR, Livingston PO, Hilgers J. Int. J. Cancer. 2000; 86(5):702–712. [PubMed: 10797294]
110. Phizicky E, Bastiaens PIH, Zhu H, Snyder M, Fields S. Nature. 2003; 422(6928):208–215. [PubMed: 12634794]
111. Doumatey AP, Zhou J, Zhou M, Prieto D, Rotimi CN, Adeyemo A. Obesity. 2016; 24(6):1257–1265. [PubMed: 27106679]
112. Wunderlich CM, Hövelmeyer N, Wunderlich FT. JAK-STAT. 2013; 2(2)
113. Chang C-C, Wu M-J, Yang J-Y, Camarillo IG, Chang C-J. Cancer Res. 2015; 75(11):2375–2386. [PubMed: 25840984]

Figure 1.

Basic overview of soluble inflammatory mediator pathways. Arrows indicate a relationship between pathways with the direction of the arrow indicating the pathway that is upstream of the other. Pathways in red are significantly dysregulated ($p < 0.05$) in all three of our proteomic studies.

Figure 2.

Top protein associated with obesity. Immunoblotting to confirm higher expression of Stat3 protein in overweight/obese plasma compared to plasma from women with normal BMI. The M values are the expression coefficients of Stat3 expression via array. N, OW and OB stand for normal, overweight and obese BMI, respectively.

Figure 3.

Basic overview of cellular stress pathways. Arrows indicate a relationship between pathways with the direction of the arrow indicating the pathway that is upstream of the other.

Pathways in red are significantly dysregulated ($p < 0.05$) in all three of our proteomic studies.

Figure 4.

Basic overview of metabolic dysregulation pathways. Arrows indicate a relationship between pathways with the direction of the arrow indicating the pathway that is upstream of the other. Pathways in red are significantly dysregulated ($p < 0.05$) in all three of our proteomic studies.

Table 1

Study demographics. Subsets of the data from studies 1²², 2²¹, and 3²³ have been previously published as controls in comparisons to cancer cases. Study 4 is unpublished work.

Characteristic		Normal (BMI<24.9kg/m ²)	Overweight/Obese (BMI>25.0kg/m ²)
Study 1	Sample Numbers	103	105
	Age	64.8±7.8	62.8±7.4
	HRT	52 Yes 51 No	53 Yes 52 No
Study 2	Sample Numbers	135	173
	Age	67.5±7.7	66.7±6.6
	HRT	64 Yes 71 No	64 Yes 109 No
	Race/Ethnicity	115 White (not of Hispanic origin) 3 Black or African-American 12 Asian or Pacific Islander 5 Other	149 White (not of Hispanic origin) 16 Black or African-American 2 Asian or Pacific Islander 6 Other
Study 3	Sample Numbers	129	193
	Age	63.8±7.5	64.2±6.7
	HRT	89 Yes 38 No	104 Yes 82 No
	Race/Ethnicity	123 White 5 Non-white	164 White 29 Non-white
Study 4	Sample Numbers	38	48
	Age	69.2±7.8	66.5±7.3
	HRT	16 Yes 22 No	17 Yes 31 No
	Race/Ethnicity	31 White (not of Hispanic origin) 4 Black or African-American 1 Asian or Pacific Islander 2 Other	44 White (not of Hispanic origin) 1 Black or African-American 2 Asian or Pacific Islander 1 Other

Table 2

Soluble inflammatory mediator pathways. Study P-value's are adjusted using the Benjamini-Hochberg method for correction for multiple hypotheses testing. Overlap shows the number of unique proteins in our combined datasets that are significantly dysregulated compared to the number of proteins in the pathway that are present within the three arrays over the total number of proteins in that pathway (significant/on arrays/total in pathway). Overlap P-value is calculated using hypergeometric probability and reflects the overall significance of each pathway based on the number of proteins in each pathway that we test on the three arrays.

Tool	Term	Study 1 P-Value	Study 2 P-Value	Study 3 P-Value	Overlap	Overlap P-Value
KEGG 2016	T cell receptor signaling pathway	1.08E-02	1.15E-04	3.77E-02	16/72/104	4.00E-02
	PI3K-Akt signaling pathway	5.04E-06	1.13E-08	1.91E-02	44/178/341	4.32E-04
	Prolactin signaling pathway	1.04E-04	1.10E-05	1.45E-02	17/47/72	3.28E-04
	AGE-RAGE signaling pathway in diabetic complications	1.61E-06	1.30E-13	1.18E-02	28/69/101	3.36E-07
	Leptin signaling pathway	9.71E-06	8.32E-09	1.24E-03	23/60/75	1.13E-05
WikiPathways 2016	Oncostatin M Signaling Pathway	1.33E-05	9.93E-08	2.44E-03	24/58/65	1.55E-06
	IL-4 Signaling Pathway	2.42E-05	5.16E-05	4.05E-02	16/46/54	7.69E-04
	Interleukin-11 Signaling Pathway	3.44E-05	1.75E-05	3.00E-02	15/33/44	3.88E-05
	IL-3 Signaling Pathway	4.56E-05	1.08E-03	3.45E-02	15/40/49	4.67E-04
	IL-2 Signaling Pathway	1.18E-04	5.70E-04	2.86E-02	12/36/42	4.62E-03
	IL-6 signaling pathway	1.20E-04	9.43E-05	2.86E-02	13/33/43	6.43E-04
	EPO Receptor Signaling	7.59E-04	4.54E-03	3.82E-02	8/23/26	1.41E-02
	TGF-beta Signaling Pathway	1.85E-03	1.87E-03	1.16E-02	21/80/132	5.35E-03
	IL-5 Signaling Pathway	2.42E-05	7.00E-05	2.77E-02	14/36/40	4.71E-04
	Kit receptor signaling pathway	3.00E-05	4.74E-04	2.04E-02	16/48/59	1.25E-03
GO Biological Process 2015	Interferon type I signaling pathways	8.13E-03	5.16E-05	4.05E-02	13/38/54	2.62E-03
	AGE/RAGE pathway	4.52E-08	8.13E-07	8.58E-03	23/53/66	9.54E-07
	Transforming growth factor beta receptor signaling pathway	2.63E-03	8.54E-04	3.81E-02	18/62/134	3.28E-03
	Response to transforming	6.35E-03	6.55E-04	2.72E-02	21/74/166	2.19E-03

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growth factor beta					
Tool	Term	Study 1 P-Value	Study 2 P-Value	Study 3 P-Value	Overlap P-Value

Cellular stress pathways. Study P-value's are adjusted using the Benjamini-Hochberg method for correction for multiple hypotheses testing. Overlap shows the number of unique proteins in our combined datasets that are significantly dysregulated compared to the number of proteins in the pathway that are present within the three arrays over the total number of proteins in that pathway (significant/on arrays/total in pathway). Overlap P-value is calculated using hypergeometric probability and reflects the overall significance of each pathway based on the number of proteins in each pathway that we test on the three arrays.

Table 3

Tool	Term	Study 1 P-Value	Study 2 P-Value	Study 3 P-Value	Overlap	Overlap P-Value
KEGG 2016	HIF-1 signaling pathway	1.61E-06	1.12E-04	5.68E-03	19/68/103	3.97E-03
	TNF signaling pathway	2.05E-04	3.51E-03	1.63E-02	18/69/110	9.42E-03
	FoxO signaling pathway	8.25E-03	5.14E-10	2.86E-02	23/83/133	1.95E-03
	Phospholipase D signaling pathway	1.16E-02	3.53E-03	3.64E-02	16/51/144	2.41E-03
WikiPathways 2016	AGE/RAGE pathway	4.52E-08	8.12E-07	8.58E-03	23/53/66	9.54E-07
	Oncostatin M Signaling Pathway	1.33E-05	9.92E-08	2.44E-03	24/58/65	1.55E-06
	Angiogenesis	3.82E-03	4.74E-04	3.45E-02	9/22/24	3.12E-03
	Nanoparticle-mediated activation of receptor signaling	5.65E-03	1.37E-05	4.23E-02	11/25/28	5.71E-04
GO Biological Process 2015	DNA Damage Response (only ATM dependent)	8.75E-03	4.03E-05	3.32E-02	20/83/111	1.43E-02
	Alpha 6 Beta 4 signaling pathway	9.12E-03	8.33E-03	1.98E-02	10/29/33	7.11E-03
	Notch Signaling Pathway	4.81E-02	1.24E-02	6.85E-03	11/39/62	2.02E-02
	Regulation of apoptotic signaling pathway	1.08E-04	6.69E-05	5.35E-03	34/140/356	2.23E-03
	Regulation of cellular response to stress	7.23E-03	6.11E-04	1.48E-03	30/147/404	2.61E-02

Metabolic dysregulation pathways. Study P-value's are adjusted using the Benjamini-Hochberg method for correction for multiple hypotheses testing. Overlap shows the number of unique proteins in our combined datasets that are significantly dysregulated compared to the number of proteins in the pathway that are present within the three arrays over the total number of proteins in that pathway (significant/on arrays/total in pathway). Overlap P-value is calculated using hypergeometric probability and reflects the overall significance of each pathway based on the number of proteins in each pathway that we test on the three arrays.

Table 4

Tool	Term	Study 1 P-Value	Study 2 P-Value	Study 3 P-Value	Overlap	Overlap P Value
KEGG 2016	AGE-RAGE signaling pathway in diabetic complications	1.61E-06	1.30E-13	1.18E-02	28/69/101	3.36E-07
	PI3K-Akt signaling pathway	5.04E-06	1.13E-08	1.91E-02	44/178/341	4.32E-04
	FoxO signaling pathway	8.25E-03	5.14E-10	2.86E-02	23/83/133	1.95E-03
	Response to insulin	4.76E-06	5.32E-04	3.38E-02	25/98/246	3.91E-03
GO Biological Process 2015	Insulin receptor signaling pathway	9.65E-06	3.31E-04	4.66E-02	20/61/148	4.33E-04
	Regulation of cellular response to insulin stimulus	1.47E-04	1.88E-04	4.48E-02	12/22/45	2.51E-05
	Cellular response to hormone stimulus	2.40E-07	1.59E-06	1.47E-03	37/164/462	4.90E-03
	Response to peptide hormone	1.01E-06	5.56E-06	2.87E-02	37/151/364	1.31E-03

Table 5

Disease pathways. Study P-value's are adjusted using the Benjamini-Hochberg method for correction for multiple hypotheses testing. Overlap shows the number of unique proteins in our combined datasets that are significantly dysregulated compared to the number of proteins in the pathway that are present within the three arrays over the total number of proteins in that pathway (significant/on arrays/total in pathway). Overlap P-value is calculated using hypergeometric probability and reflects the overall significance of each pathway based on the number of proteins in each pathway that we test on the three arrays.

Tool	Term	Study 1 P-Value	Study 2 P-Value	Study 3 P-Value	Overlap	Overlap P-Value
KEGG 2016	Pathways in cancer	2.88E-05	1.16E-11	3.66E-08	58/249/397	3.09E-04
	Hepatitis B	8.89E-05	2.61E-10	6.23E-03	28/96/146	3.12E-04
	Non-alcoholic fatty liver disease (NAFLD)	9.22E-05	1.56E-02	4.15E-02	19/53/151	1.73E-04
	Proteoglycans in cancer	1.98E-04	3.63E-15	1.39E-08	44/133/203	1.80E-07
	Pancreatic cancer	3.19E-04	1.13E-08	1.18E-02	15/54/66	9.55E-03
	Chronic myeloid leukemia	5.27E-04	7.95E-05	5.68E-03	16/59/73	9.59E-03
	Tuberculosis	9.03E-04	3.38E-03	2.92E-02	20/76/178	6.17E-03
	Prostate cancer	1.37E-03	2.12E-07	2.46E-02	19/69/89	4.63E-03
	Glioma	1.66E-03	9.09E-07	3.64E-02	14/45/65	4.57E-03
	Choline metabolism in cancer	2.39E-03	1.01E-04	1.18E-02	15/45/101	1.73E-03
	HTLV-1 infection	2.83E-03	7.43E-06	1.16E-02	31/145/258	1.57E-02
	Endometrial cancer	3.57E-03	1.10E-05	6.61E-03	13/43/52	7.63E-03
	Colorectal cancer	7.26E-03	4.45E-06	1.14E-02	14/49/62	9.42E-03
	Hepatitis C	8.25E-03	2.73E-05	5.68E-03	19/66/133	2.87E-03
	Renal cell carcinoma	8.34E-03	9.27E-07	1.24E-03	16/50/66	1.95E-03
	Non-small cell lung cancer	2.36E-02	2.92E-07	8.44E-03	13/44/56	9.15E-03
	Acute myeloid leukemia	2.46E-02	1.21E-04	8.53E-03	11/45/57	4.43E-02
	Integrated Breast Cancer Pathway	4.33E-05	3.04E-09	2.44E-03	34/85/155	2.78E-08
	Integrated Pancreatic Cancer Pathway	1.55E-04	3.43E-10	2.44E-03	37/137/192	2.07E-04
	TCA Cycle Nutrient Utilization and Invasiveness of Ovarian Cancer	1.57E-02	1.35E-02	2.86E-02	3/5/5	2.78E-02

Table 6

Autoantibody-antigen complex pathways. Study P-value is adjusted using the Benjamini-Hochberg method for correction for multiple hypotheses testing. Overlap shows the number of proteins complexed with autoantibody in our datasets that are significantly dysregulated compared to the number of proteins in the pathway that are present on our arrays over the total number of proteins in that pathway (significant/on array/total in pathway). Overlap P-value is calculated using hypergeometric probability and reflects the overall significance of each pathway based on the number of proteins in each pathway that we tested on the array.

Tool	Term	Study 4 P-Value	Overlap	Overlap P-Value
KEGG 2016	AGE-RAGE signaling pathway in diabetic complications	3.67E-08	15/60/101	2.58E-04
	TNF signaling pathway	1.44E-05	12/56/110	3.76E-03
	T cell receptor signaling pathway	1.60E-04	10/60/104	3.20E-02
WikiPathways 2016	Type II diabetes mellitus	7.13E-03	5/16/48	1.16E-02
	TNF alpha Signaling Pathway	6.88E-05	11/53/92	6.69E-03
	AGE/RAGE pathway	7.04E-06	11/45/66	1.95E-03
	Leptin signaling pathway	4.95E-06	12/50/75	1.47E-03
	IL-4 Signaling Pathway	2.56E-04	8/39/54	1.92E-02
Go Biological Processes 2015	Interleukin-11 Signaling Pathway	4.42E-04	7/26/44	7.12E-03
	Response to insulin	1.00E-04	14/83/246	1.35E-02
	Insulin receptor signaling pathway	1.11E-04	11/52/148	5.85E-03
	Leukocyte differentiation	4.02E-06	17/95/251	4.38E-03
	T cell activation	6.72E-06	15/72/198	1.76E-03
	Lymphocyte activation	8.21E-06	18/103/304	4.41E-03
	Leukocyte migration	1.99E-05	15/88/226	1.02E-2