Diabetologia Springer

This is a post-print of an article published in Diabetologia. The final authenticated version is available online at: https://doi.org/10.1007/s00125-014-3205-0

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Journal:	*Diabetologia*
Manuscript ID:	Diab-13-1690.R1
Manuscript Type:	Article
Keywords:	1.01 Basic science, 2.02 Animal - rat, 3.02.01 Islets, 4.02 Microarray, 4.08.Proteomics, 4.09 Metabonomics

INTEGRATIVE ANALYSIS REVEALS NOVEL PATHWAYS MEDIATING THE INTERACTION BETWEEN ADIPOSE TISSUE AND PANCREATIC ISLETS IN OBESITY

Rita Malpique[1,2], Hugo Figueiredo[1,2], Yaiza Esteban[1,2], Sandra A. Rebuffat[1,2], Felicia A. Hanzu[1,6], Maria Vinaixa[2,3], Oscar Yanes[2,3], Xavier Correig[2,3], Sílvia Barceló-Batllori[4], Rosa Gasa[1,2], Susana G. Kalko[5], Ramon Gomis[1,2,6,7]

[1] Diabetes and Obesity Laboratory, *Institut d'Investigations Biomediques August Pi i Sunyer* (IDIBAPS), Carrer Rosselló 153, 08036, Barcelona, Spain; [2] *Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas* (CIBERDEM), Carrer de Bonanova 69, 08017, Barcelona, Spain; [3] Center for Omics Sciences (COS) - *Universitat Rovira i Virgili*, Avinguda Universitat 1, 43204 Reus, Spain; [4] Proteomics Unit, *Fundació Institut d'Investigació Biomèdica de Bellvitge* (IDIBELL), Avinguda de la Granvia de l'Hospitalet 199, 08908 Barcelona, Spain; [5] Bioinformatics Core Facility, IDIBAPS – CEK, Carrer Rosselló 153, 08036 Barcelona, Spain; [6] Endocrinology and Diabetes Unit, *Hospital Clínic*, 08036 Barcelona, Spain; [7] Barcelona University, Barcelona, 08036 Spain

Corresponding author: Ramon Gomis, M.D., Ph.D.,

IDIBAPS - *Hospital Clínic*/*Universitat de Barcelona*

Carrer de Rosselló 153, 08036 Barcelona, Spain

Phone: +34 932275400; Fax: +34 933129409; rgomis@clinic.ub.es

Word Count Abstract: 245; Word Count Main Text: 4034

ABSTRACT

Aims/hypothesis: Comprehensive characterization of the interrelation between the peripancreatic adipose tissue and the pancreatic islets promises novel insights into the mechanisms that regulate beta-cell adaptation to obesity. Here, we sought to determine the main pathways and key molecules mediating the cross-talk between these two tissues during adaptation to obesity by the way of an integrated inter-tissue, multi-platform analysis.

Methods: Wistar rats were fed a standard or cafeteria diet for 30 days. Transcriptomic variations by diet in islets and peripancreatic adipose tissue were examined through microarray analysis. Secretome from peripancreatic adipose tissue was subjected to a non-targeted metabolomics and proteomics analysis. Gene expression variations in islets were integrated with changes in peripancreatic adipose tissue gene expression and protein and metabolite secretion using an integrated inter-tissue pathway and network analysis.

Results: The highest level of data integration, linking genes differentially expressed in both tissues with secretome variations, allowed the identification of significantly enriched canonical pathways, such as the Activation of Liver/Retinoid X Receptors, Triacylglycerol Degradation and Regulation of Inflammatory and Immune Responses, and underscored interaction network hubs, such as cholesterol and the fatty acid binding protein 4, which were unpredicted through single-tissue analysis and have not been previously implied in the peripancreatic adipose tissue crosstalk with beta-cells.

Conclusions/interpretation: The integrated analysis reported hereby allowed the identification of novel mechanisms and key molecules involved in the peripancreatic adipose tissue interrelation with beta-cells during the development of obesity, which might help developing novel strategies to prevent type 2 diabetes.

Key words: Peripancreatic Adipose Tissue, Secretome, Pancreatic Islets, Obesity, Transcriptomics, Proteomics, Metabolomics, Data Integration

Abbreviations: PM-WAT – peripancreatic white adipose tissue, E-WAT – epididymal white adipose tissue; OB – Wistar rats fed a high caloric cafeteria diet for 30 days; STD – Wistar rats fed a standard chow diet for 30 days.

INTRODUCTION

Obesity is known to be associated with an increased risk of developing insulin resistance and type 2 diabetes (T2D) [1]. During evolution of obesity, the adaptation of the pancreatic beta-cell mass is crucial to compensate the rise in insulin demand and maintain glucose homeostasis (2,3).

Inter-organ communication plays an essential role in maintaining systemic metabolic regulation. It is now widely accepted that adipose tissue has a central role in regulation of other organs metabolism via secretion of a large number of endocrine and autocrine/paracrine factors (adipokines) and free fatty acids (FFAs) [2, 3], but few studies have analyzed the cross-talk between the adipose tissue and the pancreas [4, 5]. Recently, in a model of diet-induced obesity, we have shown that changes in the secretome from the peripancreatic white adipose tissue (PM-WAT) depot, but not the secretome from the epididymal (E-WAT) or subcutaneous depots, are able to increase the rate of beta-cell replication [4], which might affect beta-cell mass, suggesting a paracrine action of the PM-WAT in beta-cell physiology.

The main objective of this work was to assess the interrelation between changes in the PM-WAT and the beta-cells during adaptation to obesity. We hypothesized that an integrated analysis of the molecular variations in PM-WAT and pancreatic islets caused by diet-induced obesity would give a more complete molecular picture of this interrelation when compared to focusing on each tissue separately or a single 'omic'. Thus, here we have used a combined multi-platform inter-tissue analysis to investigate transcriptomic changes in islets and in PM-WAT together with proteomics- and metabolomics-level PM-WAT secretome perturbations in response to diet-induced obesity (Fig. 1).

RESEARCH DESIGN AND METHODS

Adult male Wistar rats were fed a high caloric cafeteria diet (OB group) or a standard chow diet (STD group) for 30 days as described [4, 6]. All animal procedures were approved by the Animal Research Committee of the University of Barcelona. Adipose tissue from the E-WAT and PM-WAT depots was collected and either used for the preparation of conditioned medium (secretome) as described [4], or frozen. Islets were obtained by collagenase digestion as described [7]. Secretomes and islets were stored at -80ºC until analysis.

Transcriptomic Analysis. Total RNA extraction, isolation, and preparation were conducted as described [4]. Labelled cRNA (10 μg) was fragmented and hybridized to oligonucleotide GeneChip® Rat Genome 230 2.0 (Affymetrix, Santa Clara, CA) whole genome arrays. Fifteen microarrays were hybridized, three (four) with independent samples coming from STD rats and three (five) with independent samples coming from OB rats in islets (PM-WAT) tissue. Expression values were summarized after background correction and normalization using *RMA* methodology [8]. Differential statistical analysis was performed using the non-parametric approach *Rank Prod* (*q*-value < 0.05) [9]. The microarray datasets were submitted to the GEO (NCBI) repository (GSE44372, GSE44373, and GSE44374).

Gene expression. mRNA levels were quantitated by real time PCR (qRT-PCR) as previously described [4]. All primers used are listed in Supplementary Table 3.

Proteomic Analysis. Secretomes from OB and STD rats were analysed by 2D differentially gel electrophoresis (DIGE) as previously described [10]. Image and statistical analysis (Two-way ANOVA or the two-tailed Student's T-test, p-value<0.05) were performed on the DeCyder 2D software v6.5 using published recommendations [11, 12]. Statistically different proteins were excised from a preparative Flamingo-

stained gel containing 300 μg protein from PM-WAT secretome from OB rats, and analyzed by mass spectrometry (MS) (LTQ-VELOS-Orbitrap, Thermo Fisher Scientific, Waltham, MA). Results were submitted to the Proteomic Discover 1.3 software using UniProt-Swissprot Release 2012. Validation studies were performed, according to the manufacturer's recommendations, through the following quantitative ELISA immunoassays: Rat Complement C3 (Innovative Research, Novi, MI), Rat Vitamin D Binding Protein (Innovative Research), Rat Adipocyte Fatty Acid Binding Protein (Aviscera Bioscience, Santa Clara, CA).

Secretome prediction. Significant transcriptomic and proteomic data from PM-WAT were submitted to the SecretomeP 2.0 server [13] for classification in terms of secretion pathways. Proteins were considered to be prone to be secreted if containing a predicted signal peptide or their NN score exceeded the value of 0.5.

Metabolomic Analysis. PM-WAT and E-WAT secretomes from OB and STD rats were analysed through non-targeted metabolomics using gas chromatography-mass spectrometry (GC-MS), according to Agilent's specifications [14], [15], and nuclear magnetic resonance (NMR) as previously described [16]. GC-MS analysis was performed. For both techniques, OB vs STD differences for each secretome were evaluated using the nonparametric Mann Whitney U test (q-value<0.05).

Integrative Pathways Enrichment and Network Derivation Analysis. The *Ingenuity Pathways Analysis* tool (IPA; Ingenuity Systems, Redwood City, CA) was used for the canonical pathways enrichment analysis and the derivation of mechanistic networks. The best scored networks were selected. Highly interconnected networks are likely to represent significant biological functions, where features added by the tool ("non-focus") represent interconnections that were not perceived in the experiment.

Culture of islets with PM-WAT secretome. Pancreatic islets were obtained by collagenase digestion from 8-wk-old rats as described [7] and cultured for 48 hrs in culture medium alone or containing 1/3 volume of PM-WAT secretome from OB or STD rats (4).

RESULTS

To address the relationship between changes in the PM-WAT and the beta-cells during adaptation to obesity, we used the cafeteria diet-induced obesity rat model, which has been recognized as one of the most appropriate experimental models to study obesity [4, 10, 17]. We were interested in the early-phase of diet-induced obesity and performed a 30 days cafeteria-diet, which has been shown to be sufficient to promote beta-cell proliferation and a rise in beta-cell mass [4].

Expression of inflammatory and immune response signaling genes is downregulated in PM-WAT and islets

The transcriptomic analysis identified 141 annotated genes (81 upregulated and 60 downregulated) in PM-WAT and 111 annotated genes (59 upregulated and 52 downregulated) in islets that were differentially expressed between OB and STD rats (Supplementary Tables 1 and 2). Fig. 2a supplies a summary of the top 20 up- and down-regulated annotated genes between OB and STD groups. Microarray results were validated by quantitative qRT-PCR analysis for several of these genes (Fig. 2b).

Differentially expressed genes were classified according to function (Supplementary Tables 1 and 2). In PM-WAT, the expression levels of genes related to transport, hormonal signalling and transcriptional regulation were increased in OB vs. STD rats, whereas genes encoding for proteins responsible for the regulation of immune and inflammatory responses were found to be downregulated in PM-WAT of OB in

comparison to STD rats. Only 2 genes related to the positive regulation of the immune response, *RT1-A3* and *RT1-EC2*, were upregulated due to diet-induced obesity. Genes involved in apoptosis, cell proliferation, cytoskeleton organization and lipid and cholesterol metabolism were differentially changed (up- and down-regulated) in OB in relation to STD rats. Remarkably, 67% of significantly differentially expressed genes encode for proteins able to be secreted and thus to interact with the surrounding tissues.

In islets, the expression levels of genes related to transcriptional regulation and transport were increased in OB as compared to STD rats. Moreover, genes involved in proteolysis, transport and fatty acid metabolism, which, as far as we are aware, had not been previously shown to be differentially expressed in islets during the adaptation stage of obesity progression, were found to be up-regulated in OB vs. STD rats. Similarly to the adipose tissue, the expression of genes encoding for proteins that regulate immune and inflammatory responses was decreased in islets of OB in comparison to STD rats.

Secretion of acute-phase response proteins by PM-WAT is increased

Adipose tissue secretomes from OB and STD rats were subjected to a non-targeted 2D-DIGE proteomic analysis. From a total of 529 protein spots annotated, only 36 spots showed significant differences between OB and STD groups (Supplementary Fig. 1). MS analysis of these spots identified 21 unique secreted proteins (following the selection criteria presented in Fig.3a) that included 11 proteins differentially secreted by the E-WAT and 19 proteins differentially secreted by the PM-WAT in response to obesity (Table 1). Nine differentially secreted proteins were common for E-WAT and PM-WAT. These are included in transport, acute-phase response, protease inhibition, guanine metabolism and xenobiotics detoxification functional groups (Table 1). The 10 proteins specifically changed in the PM-WAT secretome are involved in positive

regulation of the acute-phase response, protease inhibition or lipid transport. Only 2 proteins were selectively modified in the E-WAT secretome of OB rats compared to controls. Changes have been validated through quantitative ELISA assays for some of these proteins (Fig. 3b-d).

PM-WAT lipolysis and cholesterol secretion is increased

Secretomes from PM-WAT and E-WAT depots were analysed through a non-targeted metabolic approach. Since the culture media *per se* contain several different metabolites, the non-supplemented culture medium (CM) was also analysed to determine whether the significantly changed metabolites were either consumed or secreted to the medium (Fig. 4). Lactate, α-ketoglutarate, citrate, glycerol, cholesterol, acetoacetate and the amino acids glutamate and glycine were considered to be a product of the fat pads as they were detected in secretome samples exclusively, regardless of obesity. On the other hand, glucose was considered as an uptaken metabolite as lower levels were detected in the secretomes from both fat pads as compared to the CM. In addition, obesity was found to induce the secretion of lauric and oleic acids, nicotinamide, aspartate and myo-inositol by both depots as their levels were significantly increased in the secretomes from OB rats in comparison to CM. This suggests a dramatic change on the adipose tissue metabolism of nicotinamide and aspartate as their levels in the secretomes from STD animals were consistently lower than in CM.

In Table 2, metabolites are classified by their key biological pathway. Fourteen metabolites were significantly changed in the secretomes from both depots, which are involved mainly in glycolysis and TCA cycle, fatty acid biosynthesis, amino acids metabolism and NAD biosynthesis. Four additional metabolites were found to be changed uniquely in the secretome from the PM-WAT: α-ketoglutarate, cholesterol,

oleic acid and threonine, which are involved in the TCA cycle, fatty acid and steroid biosynthesis and amino acids metabolism, respectively.

Metabolic nuclear receptors and immune and inflammatory response signalling pathways are involved in the crosstalk PM-WT-islets

In order to explore the relevancy of each individual dataset and the added value of combining datasets to construct integrated models of how the PM-WAT and islets communicate we considered initially the single-tissue, single-platform analysis of significantly changed features; and then the inter-tissue, multi-platform analysis of islet transcriptomics data combined with different datasets of PM-WAT.

Overall, analysis of the different datasets revealed top over-represented canonical pathways that were distinct for each type of -omic approach and tissue analyzed (Supplementary Table 4). Nevertheless, enrichment examination of differentially expressed genes in PM-WAT and islets identified common top over-represented pathways related to the regulation of the immune system. Analysis of over-represented pathways related to proteomic changes in the WAT secretomes revealed the disruption of canonical pathways mainly related to activation of the LXR/RXR nuclear receptors, whereas significant metabolite changes were mainly related to the synthesis of amino acids and nucleotides.

To relate islet transcriptomic changes with alterations in PM-WAT, we used the islet transcriptome dataset as the basis for network generation and sequentially added the different datasets of PM-WAT changes. Progressive combination of different types of information should progressively increase our understanding of the complex relationship between both tissues. As the last step, to search for main upstream effectors and better evaluate how PM-WAT alterations might affect beta-cell gene expression, we integrated the four different datasets (Fig. 5).

When compared with the single-platform analyses, increasing the level of data integration significantly enriched the related canonical pathways in the number of involved molecules and the significance. “LXR/RXR Activation” and “Triacylglycerol Degradation” pathways were found to be the top identified pathways for the three levels of data integration analysed (Supplementary Table 5). When all data was combined (Supplementary Table 6), cholesterol and the acute-phase response proteins were shown to be the strongest secretome contributors to the top enriched pathway (“LXR/RXR Activation”), while significant tissue contributors include genes involved mainly in lipid and cholesterol metabolism (*Acaca*, *ApoB* and *Hadh*), activation of the innate immune system (*Il1rl1* and *C4b*) and regulation of growth and apoptosis (*Clu*). Other pathways related to the regulation of the immune and inflammatory response signalling, were also identified.

Concerning the generated networks, the connectivity and functional clustering degree increased and the number of “non-focus” features decreased with increased data integration (Supplementary Fig. 3 and Fig. 5). Top scored networks generated by the combination of islet transcriptomics with PM-WAT metabolomics data clearly show potential interactions between metabolite markers and several genes implicated in cholesterol transport and metabolism, triacylglycerol degradation and immune response signalling. Embedding the proteomic dataset into the previous one allowed the identification of sets of interconnected genes and secretome proteins and metabolites, such as the ones involved in cholesterol transport and metabolism, and revealed acute phase response proteins as central network hubs.

Finally, the complete inter-tissue network analysis clearly revealed interactions between the two tissues (Fig. 5) and allowed to correlate specific PM-WAT perturbations with islet transcriptomic changes through discovering of “cause-effect”

modules, including the effect of disrupted cholesterol metabolism on increased acute-phase response secretome proteins and islet inflammation. Analysis of functional modules identified major integrative hubs connecting secretome perturbations with PM-WAT and islet gene expression changes, including cholesterol, secretome molecules involved in lipid transport (FAPB4) or acute-phase response (PLG, C3), and genes involved in transcriptional regulation (*histone h3*) or control of proliferation and apoptosis (*Igfbp3*).

Validation of selected identified pathways in islets from OB rats and islets exposed to PM-WAT secretome *in vitro*

Our integrative analysis suggested a prominent role for cholesterol as an effector of the PM-WAT secretome from OB rats and the pathway enrichment analysis identified LXR/RXR signalling as one of the top enriched pathways. Hence, we postulated that one of the mechanisms implicated in the PM-WAT-beta-cell crosstalk might entail the direct effect of PM-WAT-derived cholesterol on the LXR/RXR pathway in islets. In order to validate this model, we first verified differential expression of genes related to the LXR/RXR pathway in islets from OB and STD rats by qRT-PCR (Fig. 6). mRNAs for both *Lxr* isoforms and *Rxrα* were found upregulated in islets of OB vs. STD rats. Likewise, we were able to confirm the upregulation of genes involved in cholesterol influx (*Lrp5* and *Ldlr*) as well as key LXR targets, including genes involved in lipogenesis (*Srebp1c* and *Fasn*) and cholesterol absorption and efflux (*Idol*, *Abcg1*) (Fig. 6a-d). As our integrated analysis highlighted also immune and inflammatory response as key signalling pathways involved in the PM-WAT-islets crosstalk and LXRs are known to modulate inflammatory responses through transrepression of NF-κB (Joseph SB 2003), we also quantified changes in the expression of genes of the NF-

κB signalling pathway (*Il-6*, *Il-1β*, *Cox-2* and *Vcam*). We found all these genes to be downregulated in islets of OB as compared to STD rats (Fig. 6e).

Finally, we asked if alterations in these pathways could be reproduced *in vitro*. To this end, we incubated islets isolated from control rats with PM-WAT secretome from OB or STD rats. We confirmed the activation of *Lxrβ* and *RXRα* as well as the upregulation of LXR/RXR target genes involved in lipogenesis and cholesterol transport (Fig. 6g-h). Concerning the NF-κB signalling pathway, only *Il-6* was found to be downregulated in islets incubated with secretome from OB rats as compared to secretome from STD rats (Fig. 6i).

DISCUSSION

In the current study, we hypothesize and proved that an integrated, inter-tissue analysis provides an enriched view of the interaction between PM-WAT and beta-cells as compared to focusing on each tissue separately or on a specific PM-WAT secreted factor.

Both PM-WAT and pancreatic islets were found to share common response mechanisms, such as lipid and cholesterol metabolism and apoptosis and proliferation which are known to be activated during the development of obesity and insulin resistance, [18-21]. Another shared pathway is the immune and inflammatory response that, surprisingly, stands as the most represented group among down-regulated genes in both tissues. In PM-WAT, this group includes CC chemokines and its receptors, such as *Ccl5* and *Ccr5*, immunoglobulin receptors, such as *Fcer1a* and *Fcgrb2,* and components of the MHC class I and II, such as *H2-t24* and *Hla-dqa1*. These results were unexpected as previous studies on adipose tissue transcriptomic changes due to obesity have shown a significant increase in genes encoding for proteins responsible for the regulation of

immune and inflammatory responses [22-25]. Indeed, visceral obesity is coupled to a general low-grade chronic inflammatory state characterized by macrophage activation and inflammatory cytokine production [26, 27]. In islets, our transcriptomic analysis revealed the down-regulation of genes involved in chemokine and cytokine signaling, such as *Cxcr4* and *Il2rg,* lymphocyte function, such as *Cd69* and *Tcrb,* and antigen processing, such as *Cd74* and *RT1-DB1*. Again, these results were unexpected as increased cytokine expression and macrophage infiltration has been described in mouse islets during the time course of a high-fat diet, concomitant with the onset of glucose intolerance [28, 29]. Increased islet-associated immune cells have also been observed in genetic animal models of obesity and T2D [28-32] but, to our knowledge, no previous studies were published indicating an islet immune and inflammatory response at the early stage of diet-induced obesity. Different models of obesity as well as different experimental parameters, namely diet type and duration, may explain the discordance with previous findings in islets as well as in PM-WAT [17]. Since our study focused on the early-phase of diet-induced obesity, the observed gene expression changes may reflect the early beginning of the inflammatory response, characterized by the simultaneous infiltration of innate and adaptive immune cells and the decrease in the number or functionality of beneficial immune cells. In this line, we have found changes in PM-WAT secretome proteins involved in the positive regulation of the acute-phase response, which is viewed as the primary defense mechanism of the body. Notably, immune and inflammatory response genes were found to be up-regulated in islets from rats fed a cafeteria diet for 6 months (Supplementary Fig. 5).

Consistent with previous studies, our transcriptomic analysis revealed the differential expression of several PM-WAT genes linked to enhanced lipolysis and FFA release and imbalanced cholesterol metabolism, characteristic of obesity progression and insulin

resistance [20, 21, 24, 33-35]. Our metabolomic analysis revealed increased lipid and cholesterol content in the PM-WAT secretome of OB rats, thus supporting our transcriptomic findings [35]. In addition, decreased glucose consumption and lactate secretion and increased glutamine consumption and α-ketoglutarate, malate and oleic acid secretion in PM-WAT suggest decreased glycolysis and enhanced TCA anaplerosis in diet-induced OB rats. Again, these data are in agreement with metabolomic changes characteristic of obesity progression and insulin resistance [34, 35]. Finally, significant differences in the metabolism of essential and non-essential amino acids were observed, revealing that obesity broadly blunt amino acid metabolism in adipose tissue. These results are in line with previous reports on human adipose tissue secretomes [15] and human serum metabolome [36, 37].

Enrichment analyses highlighted the activation of LXR/RXR nuclear receptors, triacylglycerol degradation and regulation of immune and inflammatory response signalling as the best scored pathways. LXRs form functional heterodimers with RXRs and play crucial roles in cholesterol homeostasis by acting as cholesterol sensors and regulating its transport out of the cells [38]. The important role of cholesterol in the impact of PM-WAT on the islets is suggested in the combined analysis of islet transcriptomics with secretome metabolomic datasets and further evidenced in the inter-tissue multi-platform network analysis. Moreover, we were able to confirm upregulation of genes involved in cholesterol influx as well as genes related to activation of the LXR/RXR pathway in islets of OB as compared to STD rats. Remarkably, similar gene expression changes were found in islets incubated with PM-WAT secretome from OB rats, thus suggesting the direct implication of the adipose tissue in the deregulation of these pathways in islets. It is noteworthy that, in addition to their role in cholesterol homeostasis, LXRs exert anti-inflammatory actions in response to metabolic signalling

[39]. LXRs mediate inhibition of the acute phase response in the liver [39] and negatively regulate macrophage inflammatory gene expression [40]. Here we have confirmed the downregulation of inflammatory mediators of the NF-κB signalling pathway in islets from OB as compared to STD rats and the repression of *Il-6* in control islets incubated with PM-WAT secretome from OB rats as compared to islets incubated with PM-WAT from STD rats. Therefore, our results support the role of cholesterol and the involvement of LXRs/RXRs signalling in this inter-tissue communication, thus validating our model and evidencing once more the utility of this integrated approach. Whether changes in the activation state of islets LXRs and RXRs are due to increased secretion of cholesterol and FFAs by PM-WAT as suggested in our study, and whether these changes mediate inflammatory response signalling in islets through NF-κB transrepression by LXRs remain to be further elucidated. In fact, cholesterol uptake by beta-cells leads to reduced beta-cell function and increased apoptosis [41], but further studies are required to dissect its exact role in beta-cells as well as to determine its involvement in *in vivo* situations.

In conclusion, we have successfully integrated and explored distinct high-throughput datasets of different origin and size on the same model. This has allowed the identification of novel molecular perturbations, such as activation of metabolic nuclear receptors, disruption of lipid and cholesterol metabolism, and dysregulation of inflammatory and immune response signalling pathways, which may all, contribute to the effect of PM-WAT on beta-cells in obesity. Although these pathways had already been linked to obesity, they had not previously been correlated with the specific impact of PM-WAT on pancreatic beta-cells.

ACKNOWLEDGMENTS

This work was developed at the Centro Esther Koplowitz, Barcelona, Spain. We acknowledge the Unit of Proteomic from the CCiT-UB (Barcelona, Spain), a member of ProteoRed network, for technical help. We acknowledge the Genomics Unit of the IDIBAPS (Barcelona, Spain), where part of the transcriptomic analysis was carried out, for technical help. We acknowledge the Bioinformatics Unit of the IDIBAPS (Barcelona, Spain), where the statistical transcriptomics and IPA analyses were carried out, for bioinformatics resources support.

FUNDING

This works was supported by grants from the *Ministerio de Ciencia y Innovación* (Spain), SAF2010-19527, and from *Generalitat de Catalunya* (Spain), 2009SGR1426. *Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas* is an initiative of *Instituto de Salud Carlos III (Madrid, Spain)*. RM was a recipient of a grant from European Commission for financial support (Marie Curie Grant - FP7 PEOPLE-2007-3-1-IAPP-218130).

DUALITY OF INTEREST

The authors declare that there is no duality of interest associated with this manuscript.

CONTRIBUTION STATEMENT

RM designed the study, carried out the tissue and secretome collections, proteomic and transcriptomic analysis and results validation, supervised the work, analyzed the data, prepared the figures and wrote the manuscript. HF contributed to the results

validation, analyzed the generated data and discussed results. YE contributed to the proteomic analysis. SR contributed to the study design, carried out the tissue and secretome collections, discussed results and revised the manuscript. FAH revised the manuscript. MV, OY and XC carried out the metabolomic analysis. SB contributed to the analysis of proteomics data and preparation of figures. RGasa discussed results and revised the manuscript. SK contributed to the transcriptomic analyses and preparation of figures, performed the data integration, discussed results and revised the manuscript. RGomis designed the study, supervised the work, discussed results and revised the manuscript.

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TABLES

Table 1. Significantly changed proteins in the secretome from PM-WAT and E-WAT depots in response to diet-induced obesity (Two-way ANOVA and/or Student's T-test, *p*-value < 0.05). Fold-change values used as IPA input values.

UNIPROT NO.	GENE NAME	IDENTIFIED PROTEIN	FOLD-CHANGE OB vs STD		BIOLOGICAL PROCESS	SECRETOME P
			PM-WAT	E-WAT		
P02770	**Alb**	**Serum albumin**	3.07	1.79	Fatty acid binding; antioxidant defence; Acute-phase response	SignalP
Q6IE52	**Mug2**	**Murinoglobulin-2**	2.98	1.79	Protease inhibition	SignalP
F1LM84	**Nid1**	**Nidogen-1**	- 2.96	-1.80 1.64	Cell-matrix adhesion	0.5
Q63041	**A1m**	**Alpha-1-macroglobulin**	1.71	-	Protease inhibition; Acute-phase response	SignalP
P14046	**A1i3**	**Alpha-1-inhibitor 3**	- 2.96	-1.73 1.64	Protease inhibition	SignalP
Q01177	**Plg**	**Plasminogen**	1.71	-	Proteolysis	SignalP
P09811	**Pygl**	**Glycogen phosphorylase**	1.77	-	Carbohydrate metabolism; Glycogen metabolism	0.678
P12346	**Tf**	**Serotransferrin**	1.85 1.54	1.21 -	Iron transport; Acute-phase response	SignalP
P16303	**Ces3**	**Carboxylesterase 3**	- -1.73	1.80 -	Detoxification of xenobiotics	0.668
P17475	**Serpina1**	**Alpha-1-antiproteinase**	-1.73 2.36	- -	Serine protease inhibition; Acute-phase response	SignalP
P04785	**P4hb**	**Protein disulfide-isomerase**	-1.73 1.36	- -	Intracellular estrogen-binding; protein folding	SignalP
P24090	**Ahsg**	**Alpha-2-HS-glycoprotein**	1.36	-	Acute-phase response	SignalP
Q68FY4 P04276	**Dbp**	**Vitamin D-binding protein**	- 3.30	-1.75 -	Vitamin D transport and metabolic process; acute-phase response	SignalP
Q9JKB7	**Gda**	**Guanine deaminase**	2.19 -1.73	- -1.49	Guanine catabolic process; purine nucleobase metabolic process	SignalP
D4AEP0	**Adss**	**Adenylsuccinate synthase**	2.19	-	Purine biosynthesis	SignalP
Q4V8N0	**Tinagl1**	**Lipocalin 7**	2.19	-	Transport	SignalP
P01026	**C3**	**Complement C3**	2.49	-	Activation of the complement system; Acute-phase response	SignalP
P02651	**Apoa4**	**Apolipoprotein A-IV**	-	1.79	Lipid / cholesterol transport and metabolism	SignalP
Q6AYJ9	**Art3**	**ADP-ribosyltransferase 3**	-	1.79	Protein ADP-ribosylation	SignalP
P07632	**Sod1**	**Superoxide dismutase**	-1.47	-	Antioxidant defence; Regulation of apoptotic process	0.728
P70623	**Fabp4**	**Fatty-acid-binding protein**	2.86	-	Fatty acids binding and transport	0.787

Table 2. Metabolites significantly changed in the secretome from PM-WAT and E-WAT adipose tissue in response to diet-induced obesity (non-parametric Mann-Witney *U* test, *p*-value < 0.05). Fold-change values used as IPA input values.

CAS No.	METABOLITE	Fold Change OB *vs* STD		PATHWAY
		PM-WAT	E-WAT	
50-99-7	**Glucose**	2.18	1.48	Glycolysis
72-17-3	**Lactate**	-2.23	-7.27	
50-41-9	**Citrate**	-4.67	-4.41	TCA cycle
328-50-7	**α-Ketoglutarate**	3.59	-	
57-88-5	**Cholesterol**	2.38	-	Steroid biosynthesis and degradation
112-80-1	**Oleic acid**	2.22	-	Fatty acid biosynthesis
143-07-7	**Lauric acid**	1.62	1.47	Fatty acid biosynthesis
56-81-5	**Glycerol**	-1.81	-1.97	Glycerolipid metabolism, galactose metabolism
87-89-8	**Myo-inositol**	1.47	1.52	Galactose metabolism
142-47-2	**Glutamate**	1.94	2.35	Alanine, aspartate and glutamate metabolism
56-85-9	**Glutamine**	-1.60	-1.42	
617-45-8	**Aspartate**	4.86	6.34	
80-68-2	**Threonine**	-8.13	-	Glycine, serine and threonine metabolism
56-40-6	**Glycine**	-1.87	-2.48	
56-87-1	**Lysine**	-1.35	-1.26	Lysine metabolism
51-35-4	**4-hydroxyproline**	-1.99	-2.00	Arginine and proline metabolism
105-45-3	**Acetoacetate**	-4.39	-3.30	Valine, leucine and isoleucine degradation, tyrosine metabolisme
98-92-0	**Nicotinamide**	2.44	2.70	Nicotinamide metabolism, NAD biosynthesis

FIGURE LEGENDS

Figure 1. Overview of the study design, generated datasets and main findings.

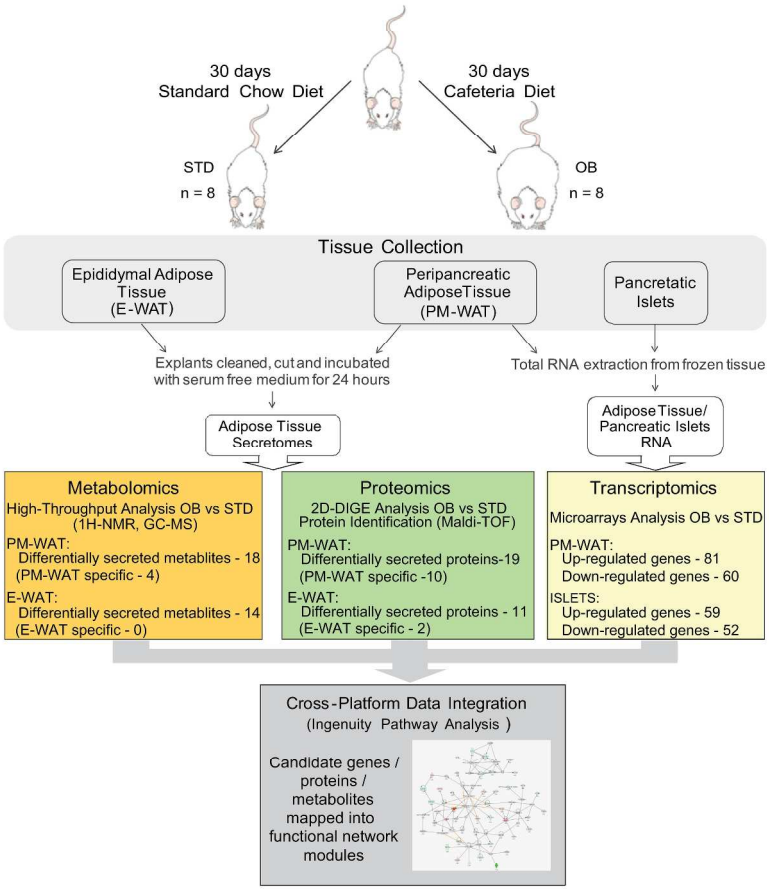
Figure 2. Gene expression changes in response to diet-induced obesity. (a, b) List of the top 20 up- and down-regulated genes differentially expressed between OB and STD animals in PM-WAT and islets. Gene products that can be potentially secreted are indicated as (+). (c, d) qRT-PCR results for statistically significant genes in PM-WAT and islets. The expression of each gene was normalized against expression of the gene *Tbp*. All values are presented as mean ± SEM from 4 to 12 animals per group. **P* < 0.05, ***P* < 0.01 and ****P* < 0.005. Black bars = STD group; white bars = OB group.

Figure 3. Proteome composition changes in PM-WAT and E-WAT secretomes in response to diet-induced obesity. (a) Selection criteria for protein identification. (b, c, d) Quantitative ELISA results for the statistically significant proteins complement protein C3, vitamin D binding protein (DBP) and fatty acid binding protein 4 (FABP4): Black bars = STD group; white bars = OB group. All values are presented as mean ± SEM from 4 to 12 animals per group. ***P* < 0.01 and ****P* < 0.005.

Figure 4. Metabolite changes in PM-WAT and E-WAT secretomes in response to diet-induced obesity and examples of metabolic pathways in which they are involved. Black bars = STD group; White bars = OB group. All values are presented as mean ± SEM from 6 to 8 animals per group. **P* < 0.05, ***P* < 0.01 and ****P* < 0.005.

Figure 5. Inter-tissue integrative highest score network: PM-WAT secretome metabolome-centric view network with islets transcriptomics and PM-WAT transcriptomics and secretome proteomics results overlaid. Bold Line: islet transcriptomic changes; Blue Letters: PM-WAT secretome proteomic changes; Bold Lilac Lines: transcriptomic changes shared by PM-WAT and islets.

Figure 6. Gene expression changes of the LXR/RXR signalling pathway in islets in response to diet-induced obesity. (a) Schematic representation of the LXR/RKX pathway indicating those genes whose expression was found to be changed between islets from OB and STD rats: green – dowregulated genes; red – upregulated genes (b, c, d, e) qRT-PCR results for the indicated genes in islets from OB and STD rats after a 30-day cafeteria diet. Black bars = STD group; white bars = OB group. (f, g, h, i) Isolated islets were cultured with PM-WAT secretome from OB or STD rats for 48 h. qRT-PCR for the indicated genes. Black bars = islets incubated with STD secretome; white bars = islets incubated with OB secretome. The expression of each gene was normalized against expression of the gene *Gadph*. All values are presented as mean ± SEM from 6 to 10 animals per group. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.005$.



Overview of the study design, generated datasets and main findings
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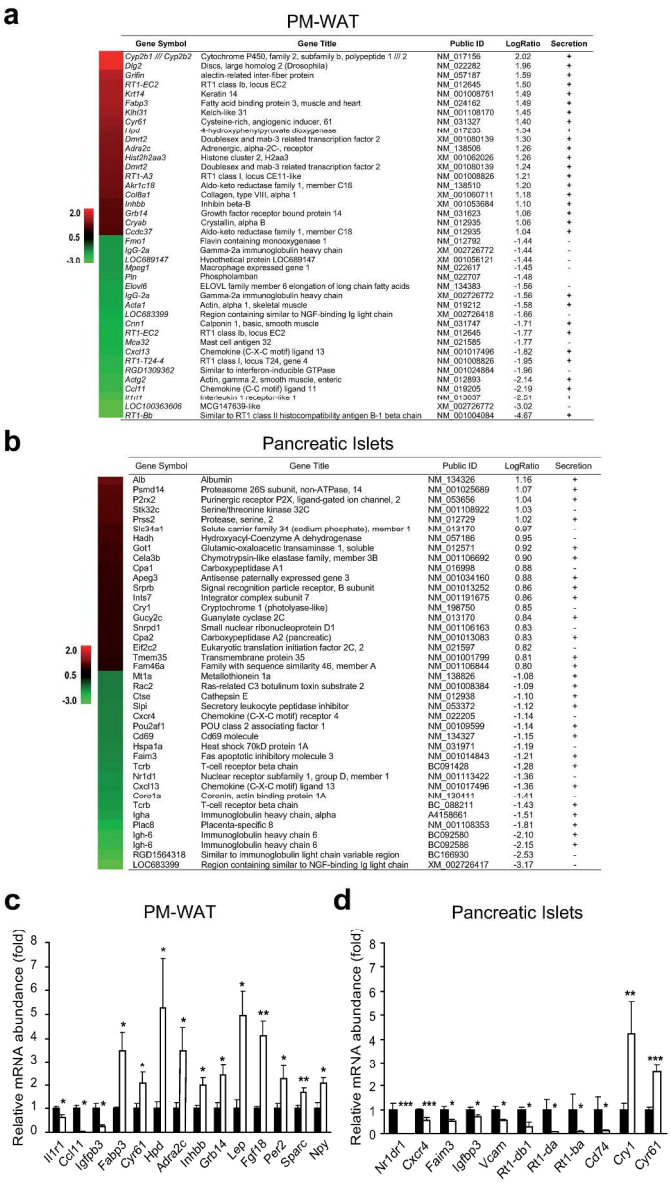

Gene expression changes in response to diet-induced obesity. (a, b) List of the top 20 up- and down-regulated genes differentially expressed between OB and STD animals in PM-WAT and islets. Gene products that can be potentially secreted are indicated as (+). (c, d) qRT-PCR results for statistically significant genes in PM-WAT and islets. The expression of each gene was normalized against expression of the gene *Tbp*. All values are presented as mean ± SEM from 4 to 12 animals per group. **P* < 0.05, ***P* < 0.01 and ****P* < 0.005. Black bars = STD group; white bars = OB group.
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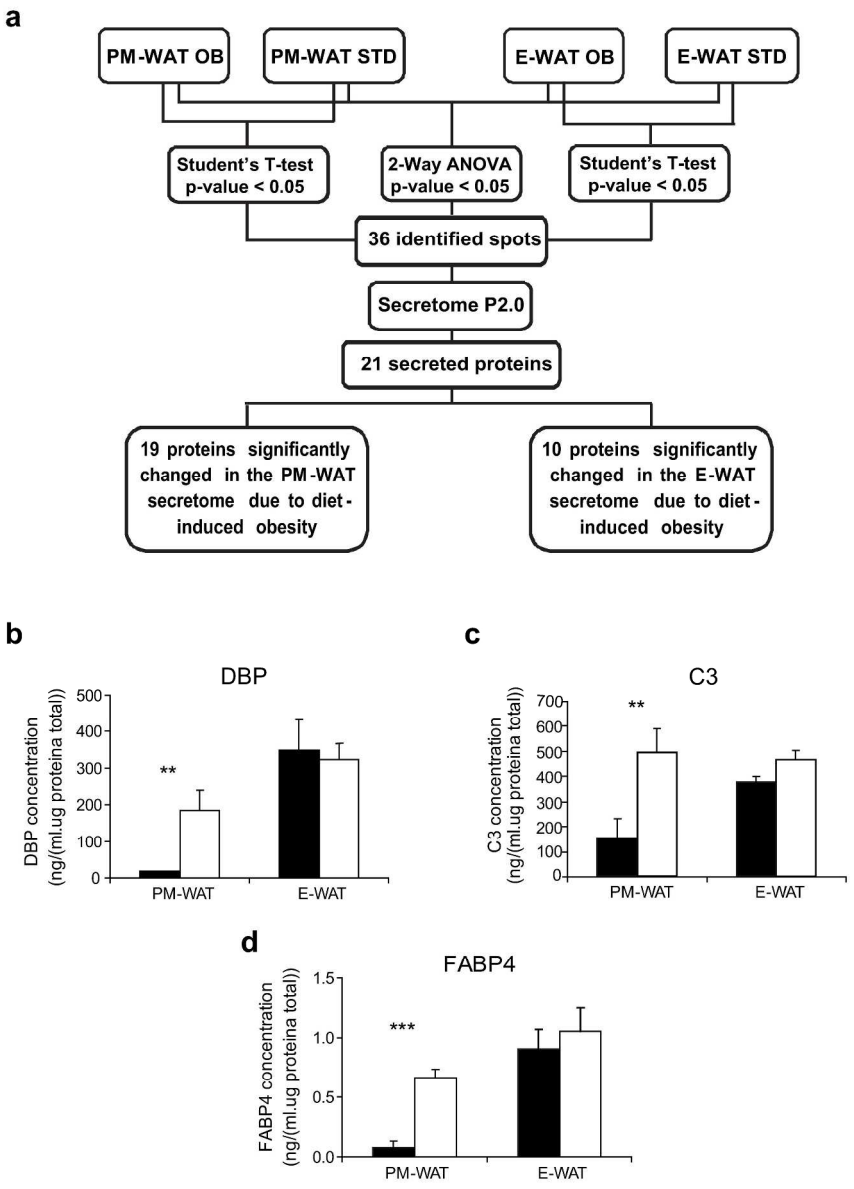
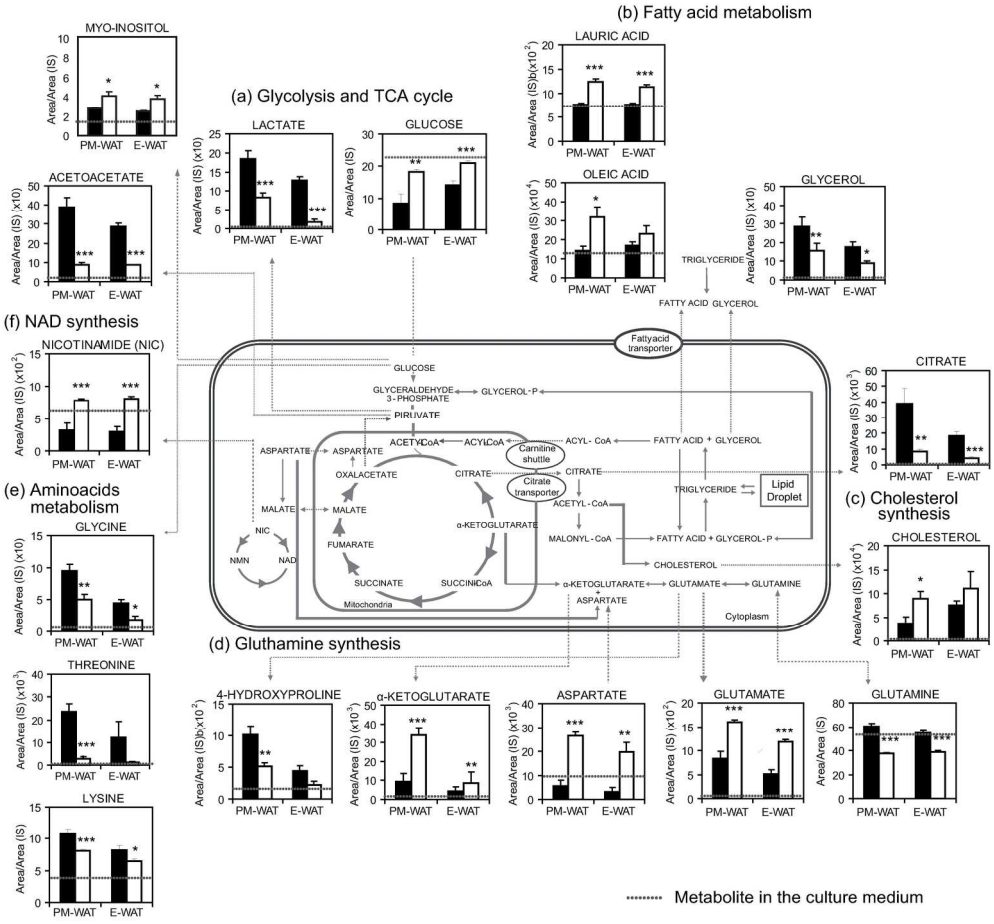


Proteome composition changes in PM-WAT and E-WAT secretomes in response to diet-induced obesity. (a) Selection criteria for protein identification. (b, c, d) Quantitative ELISA results for the statistically significant proteins complement protein C3, vitamin D binding protein (DBP) and fatty acid binding protein 4 (FABP4). Black bars = STD group; white bars = OB group. All values are presented as mean ± SEM from 4 to 12 animals per group. $^{**}P < 0.01$ and $^{***}P < 0.005$.
257x357mm (300 x 300 DPI)



Metabolite changes in PM-WAT and E-WAT secretomes in response to diet-induced obesity and examples of metabolic pathways in which they are involved. Black bars = STD group; White bars = OB group. All values are presented as mean ± SEM from 6 to 8 animals per group. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.005$.
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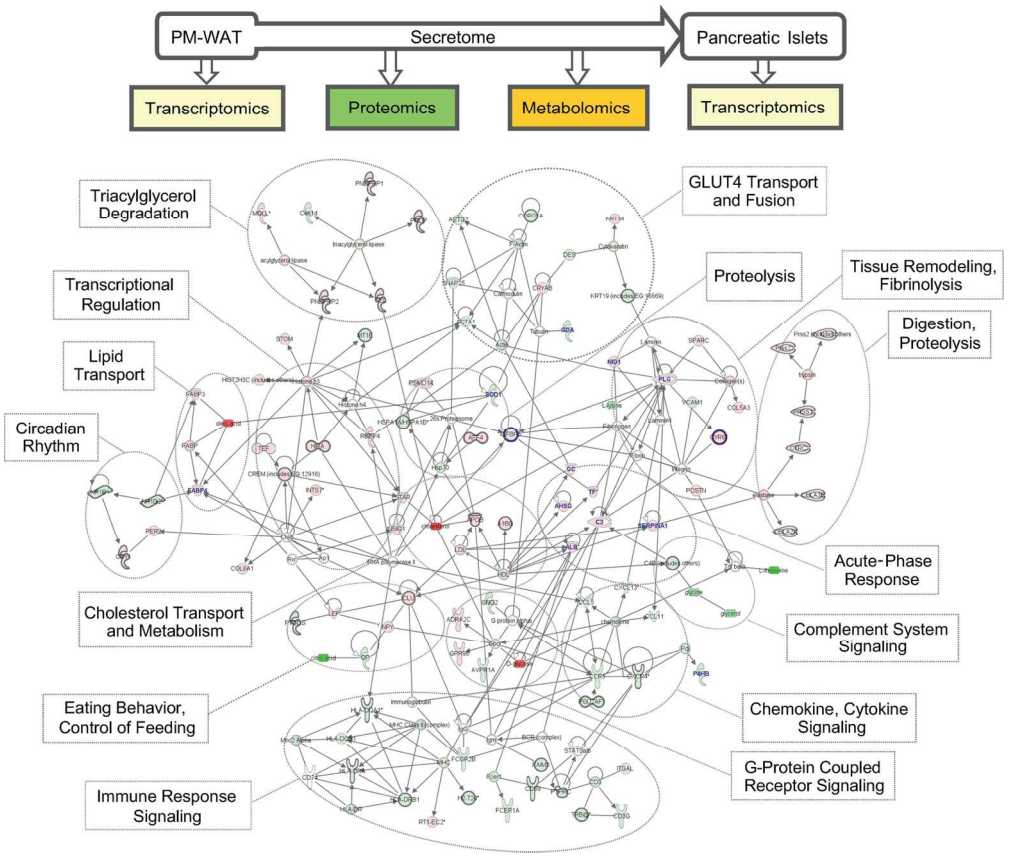

Inter-tissue integrative highest score network: PM-WAT secretome metabolome-centric view network with islets transcriptomics and PM-WAT transcriptomics and secretome proteomics results overlaid. Bold Line: islet transcriptomic changes; Blue Letters: PM-WAT secretome proteomic changes; Bold Lilac Lines: transcriptomic changes shared by PM-WAT and islets.
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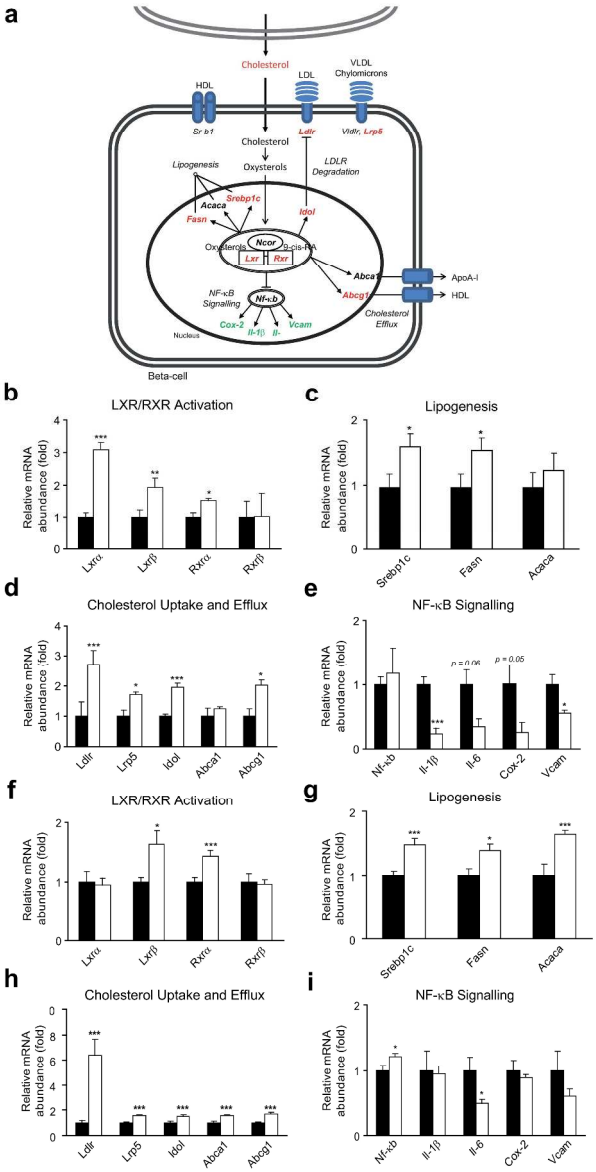

Gene expression changes of the LXR/RXR signalling pathway in islets in response to diet-induced obesity. (a) Schematic representation of the LXR/RKX pathway indicating those genes whose expression was found to be changed between islets from OB and STD rats: green – dowregulated genes; red – upregulated genes (b, c, d, e) qRT-PCR results for the indicated genes in islets from OB and STD rats after a 30-day cafeteria diet. Black bars = STD group; white bars = OB group. (f, g, h, i) Isolated islets were cultured with PM-WAT secretome from OB or STD rats for 48 h. qRT-PCR for the indicated genes. Black bars = islets incubated with STD secretome; white bars = islets incubated with OB secretome. The expression of each gene was normalized against expression of the gene with the constitutive expression of the gene *Gadph*. All values are presented as mean ± SEM from 6 to 10 animals per group. **P* < 0.05, ***P* < 0.01 and ****P* < 0.005.
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SUPPLEMENTAL MATERIALS

SUPPLEMENTARY TABLES

Supplementary Table 1A. Genes down-regulated in PM-WAT in response to diet-induced obesity (q-value < 0.05).

Gene Symbol	Public ID	Log Ratio	SecretomeP
Inflammatory / Immune response			
RT1-Bb	NM_022531	-4.67	SignalP
LOC100363606	NM_012588	-3.02	0.620
Il1rl1	NM_031747	-2.51	0.791
Ccl11	NM_080767	-2.19	SignalP
RGD1309362	NM_134389	-1.96	0.544
RT1-T24-4	NM_031598	-1.95	SignalP
Cxcl13	NM_012532	-1.82	SignalP
Mca32	NM_024375	-1.77	---
RT1-EC2	NM_133416	-1.77	SignalP
IgG-2a	NM_053960	-1.56	SignalP
Mpeg1	NM_019212	-1.45	SignalP
IgG-2a	NM_053559	-1.44	0.678
Itgal	NM_001106314	-1.33	SignalP
Ccr5	---	-1.33	0.919
RT1-Ba	NM_031828	-1.32	0.554
Igha	NM_053861	-1.31	0.672
Lilrb3l	NM_001170606	-1.27	SignalP
Fcer1a	NM_001024884	-1.27	SignalP
Tcrb	---	-1.25	SignalP
Spink5	---	-1.22	SignalP
Fcgr2a	---	-1.22	0.297
Cd3g	---	-1.19	SignalP
Ccl5	NM_134393	-1.15	SignalP
Psmb8	NM_022193	-1.12	0.484
Ifitm1	---	-1.06	0.934
Spink5	---	-1.01	SignalP
Igtp	---	-1.00	0.305
MGC108823	NM_001170606	-0.99	0.370
Fatty acid and cholesterol metabolism			
Elovl6	NM_022617	-1.56	0.239
Acaca	NM_001037357	-1.41	0.335
Elovl6	NM_001008831	-1.40	0.239
Acsbg1	XM_001056121	-1.20	0.527
Ch25h	NM_012724	-1.10	0.393
Pla2g2a	NM_134383	-1.05	SignalP
Apoptosis / Proliferation			
Grem1	NM_053843	-1.35	SignalP
Slfn3	NM_001025415	-1.23	0.717
Igfbp3	NM_012893	-1.18	SignalP
Gdf10	XM_002726772	-1.05	SignalP
Bcl2a1d	---	-1.04	0.499
Adhesion / Cytoskeleton organization / Cell motility			
Actg2	NM_019370	-2.14	0.572
Cnn1	NM_013198	-1.71	0.594
Acta1	NM_019282	-1.58	0.504
Des	NM_001127682	-1.33	---
Tnc	NM_001012353	-1.30	SignalP
Transport			
Pln	NM_053019	-1.48	0.496

Kcnma1	1370924_at	-1.39	SignalP
Cp	---	-1.29	SignalP
Other biological processes			
LOC683399	NM_021585	-1.66	SignalP
LOC689147	NM_031116	-1.44	---
Fmo1	NM_022707	-1.44	0.499
Vit	---	-1.39	0.455
Sh3bgr	---	-1.26	0.106
Avpr1a	---	-1.23	0.345
Snap25	---	-1.23	0.754
Trpc6	NM_001077646	-1.20	0.389
LOC681290	XM_001064219	-1.20	---
Pmfbp1	NM_019205	-1.13	0.145
Maob	NM_001033998	-1.00	0.325

Supplementary Table 1B. Genes up-regulated in PM-WAT adipose tissue in response to diet-induced obesity (q-value < 0.05).

Gene Symbol	Public ID	Log Ratio	SecretomeP
Transport			
Fabp3	NM_024162	1.49	0.566
Slc7a10	NM_053726	0.93	0.648
Rab3b	NM_031091	0.86	0.287
Sfxn5	NM_153298	0.89	0.176
Slc2a13	NM_133611	0.84	0.334
Lfng	NM_133393	0.83	SignalP
Abcd2	NM_033352	0.76	0.591
P2rx5	NM_080780	0.74	0.592
Hormonal response and signalling			
Adra2c	NM_138506	1.26	0.925
Akr1c18	NM_138510	1.20	0.557
Inhbb	NM_080771	1.10	SignalP
Lep	NM_013076	0.98	SignalP
Pmepa1	NM_001107807	0.96	SignalP
Npr3	NM_012868	0.79	SignalP
Npy	NM_012614	0.75	SignalP
Apoptosis / Proliferation			
Cyr61	NM_031327	1.40	SignalP
Cryab	NM_012935	1.06	0.886
Fgf18	NM_019199	0.96	SignalP
Crlf1	NM_001106074	0.87	0.305
Aplp1	NM_001100802	0.81	SignalP
Sparc	NM_012656	0.78	SignalP
Hspa1a	NM_031971	0.76	0.259
Transcriptional regulation			
Klhl31	NM_001108170	1.45	0.669
Dmrt2	NM_001107597	1.30	0.419
Hist2h2aa3	XM_001062079	1.26	0.494
Dmrt2	NM_001107597	1.24	0.419
Obp3	NM_001033958	0.96	SignalP
Per2	NM_031678	0.96	0.456
Cytoskeleton organization / ECM remodelling			
Krt14	NM_001008751	1.49	0.379
Col8a1	NM_001107100	1.18	SignalP
Postn	NM_001108550	0.92	SignalP
Col5a3	NM_021760	0.91	SignalP
Fbln2	XM_001062865	0.85	SignalP
Dact2	NM_001107464	0.85	0.682

Fatty acid and cholesterol metabolism			
Mgll	NM_138502	0.91	0.590
Star	NM_031558	0.89	0.734
Lpin2	NM_001108236	0.88	0.338
Insig1	NM_022392	0.84	0.660
Mgll	NM_138502	0.75	0.590
Mgll	NM_138502	0.91	0.590
Immune Response			
RT1-A3	NM_001109495	1.84	0.132
RT1-EC2	NM_012645	1.50	0.434
Insulin signalling pathway			
Grb14	NM_031623	1.06	0.508
Irs3	NM_032074	0.78	0.471
L-phenylalanine catabolic process			
Hpd	NM_017233	1.34	0.450
Pcbd1	NM_001007601	0.74	0.461
Other biological processes			
Cyp2b1	NM_001134844	2.02	SignalP
Dlg2	NM_022282	1.96	0.489
Grifin	NM_057187	1.59	0.559
Ccdc37	XM_001076288	1.04	0.072
RGD1565033	NM_001109050	1.00	0.853
Ccdc3	XM_001071191	0.99	SignalP
Nnat	NM_053601	0.98	SignalP
RGD1306739	NM_001134576	0.97	0.657
Trim16	NM_001135033	0.96	0.562
Uba5	NM_001009669	0.95	0.363
Fcgbp	NM_001164656	0.94	0.587
Eif1a	NM_001008773	0.93	0.337
LOC367597	---	0.93	0.791
Obfc2a	NM_001014216	0.92	0.560
Acyp2	NM_001169145	0.90	0.664
St3gal5	NM_031337	0.89	0.560
Mmp23	NM_053606	0.88	SignalP
LOC363331	XM_002727158	0.86	---
Cdr2	NM_001025682	0.86	0.606
Fam19a5	NM_001191991	0.84	0.604
RGD1563516	XR_006811	0.84	0.681
Gcsh	NM_133598	0.81	SignalP
RGD1561849	NM_001109260	0.80	0.452
Gpr98	XM_001063571	0.78	0.471
Eepd1	NM_001014088	0.77	0.435
Fam25a	NM_001134849	0.76	0.828
Tmem120a	NM_001010945	0.76	0.339
RGD1359108	NM_001007702	0.75	0.475
Fbxo23	NM_001013138	0.75	0.570
Stom	NM_001011965	0.75	0.228
Ak3	NM_013218	0.74	0.580
Fbxo23	NM_001013138	0.74	0.570
Akr1c12	NM_001170342	0.73	0.385
Cpa1	NM_016998	0.54	SignalP

Supplementary Table 2A. Genes down-regulated in pancreatic islets in response to diet-induced obesity (q-value < 0.05).

Gene Symbol	Public ID	Log Ratio	SecretomeP
Inflammatory / Immune response			
Ig lambda-1	---	-2.53	SignalP
Igha	---	-1.51	0.672
Igh-6	---	-2.10	0.62
Igh-6	---	-2.15	0.62
Tcrb	---	-1.43	SignalP
Cxcl13	NM_001017496	-1.36	SignalP
Cxcr4	NM_022205	-1.14	0.369
Tcrb	---	-1.28	---
Cd69	NM_134327	-1.15	---
Cd74	NM_013069	-1.01	0.38
Faim3	NM_001014843	-1.21	---
IgG-2a	XM_002726794	-0.83	SignalP
Ltb	NM_212507	-0.92	---
Cxcr4	NM_022205	-0.91	0.369
Ctse	NM_012938	-1.10	SignalP
RT1-T24-4	NM_001008826	-0.72	SignalP
Cd24	NM_012752	-0.87	SignalP
Ptprc	NM_001109887	-1.06	---
RT1-Db1	NM_001008884	-0.86	SignalP
C4-2	NM_001002805	-0.83	SignalP
RT1-Da	NM_001008847	-0.81	0.735
Vcam1	NM_012889	-0.86	SignalP
Cxcr4	NM_022205	-0.77	0.369
RT1-Ba	NM_001008831	-0.74	0.554
Dmbt1	NM_022849	-0.93	0.756
Il2rg	NM_080889	-0.81	0.866
Transport / Fatty acid metabolism			
Ptgds	NM_013015	-0.79	SignalP
Kcnk3	NM_033376	-0.71	SignalP
Dbp	NM_012543	-0.68	0.550
Stress response			
Hspa1a	NM_031971	-1.19	0.252
Hspa1a	NM_031971	-0.95	0.259
Apoptosis / Proliferation			
Igfbp3	NM_012588	-0.86	SignalP
Dnase1l3	NM_053907	-0.87	SignalP
Cytoskeleton organization			
Coro1a	NM_130411	-1.41	0.781
Rac2	NM_001008384	-1.09	0.693
Transcriptional regulation			
Nr1d1	NM_001113422	-1.36	0.378
Nr1d2	NM_147210	-0.69	0.318
Nr1d2	NM_147210	-0.68	0.318
Pou2af1	NM_001109599	-1.14	0.856
Zfp36	NM_133290	-0.80	0.192
Other biological processes			
Plac8	NM_001108353	-1.81	0.687
Slpi	NM_053372	-1.12	SignalP
Mt1a	NM_138826	-1.08	SignalP
Krt19	NM_199498	-0.98	0.705
Ints7	NM_001191675	-0.90	0.521
Gstm1	NM_017014	-0.89	0.362
Prr12	---	-0.72	---
Prr12	---	-0.62	---

Supplementary Table 2B. Genes up-regulated in pancreatic islets in response to diet-induced obesity (q-value < 0.05).

Gene Symbol	Public ID	Log Ratio	SecretomeP
Proteolysis			
Psmd14	NM_001025689	1.07	0.594
Prss2	NM_012729	1.02	SignalP
Cela3b	NM_001106692	0.90	SignalP
Cpa1	NM_001108922	0.88	SignalP
Cpa2	NM_001013083	0.83	SignalP
Ctrc	NM_001077649	0.77	SignalP
Ctrb1	NM_012536	0.74	SignalP
Cpb1	NM_012533	0.58	SignalP
Cela2a	NM_012553	0.56	SignalP
Prss3	NM_173127	0.43	SignalP
Prss1	NM_012635	0.47	0.892
Transcriptional regulation			
Cry1	NM_198750	0.85	0.415
Snrpd1	NM_001106163	0.83	0.391
Eif2c2	NM_021597	0.82	0.352
Rpl7	NM_001100534	0.80	0.293
Nlk	---	0.78	---
Aff4	NM_001107001	0.75	0.323
Hira	NM_001135760	0.70	---
Mllt10	NM_001012162	0.69	0.366
Crem	NM_001110860	0.62	0.917
Transport			
Alb	NM_134326	1.16	SignalP
P2rx2	NM_053656	1.04	0.825
Slc34a1	NM_013030	0.97	0.351
Trim72	NM_001077675	0.71	0.46
Alb	NM_134326	0.67	SignalP
ApoB	NM_019287	0.69	SignalP
P2rx2	NM_053656	0.63	---
Fatty acid metabolism			
Hadh	NM_057186	0.95	0.432
Pnlip	NM_013161	0.72	SignalP
Pnliprp2	NM_057206	0.72	SignalP
Pnliprp1	NM_032081	0.65	SignalP
Cel	NM_016997	0.49	SignalP
Apoptosis / Proliferation			
Gucy2c	NM_013170	0.84	SignalP
Cyr61	NM_031327	0.74	SignalP
Dapl1	NM_001108582	0.67	0.653
Microtubule stability			
Stmn2	NM_053440	0.78	0.665
Kif5c	NM_001107730	0.78	0.181
Stmn2	NM_053440	0.73	0.665
Translational regulation			
Srprb	NM_001013252	0.86	0.910
Ate1	NM_001106300	0.66	0.483
Cell cycle			
Spc25	NM_001009654	0.68	0.638
Kif20a	NM_001108426	0.62	0.233
Other biological processes			
Stk32c	NM_001108922	1.03	0.458
Got1	NM_012571	0.92	0.446
Apeg3	NM_001034160	0.88	0.908
Ints7	NM_001191675	0.86	SignalP
Tmem35	NM_001001799	0.81	SignalP

Fam46a	NM_001106844	0.80	---
Clu	NM_053021	0.77	0.799
Fam46a	NM_001106844	0.74	0.564
Ssb	NM_031119	0.72	0.497
Fam46a	NM_001106844	0.65	0.564
Rrm2	NM_001025740	0.64	0.46
A1bg	NM_022258	0.62	SignalP
Rnase1	NM_001029904	0.51	SignalP

Supplementary Table 3. List of primers used in quantitative real-time PCR.

Gene Name	Representative Public ID	Forward	Reverse
Il1r1	NM_013123.3	Cggcggtatgcgcggtctt	tttcacccctgccgggtaggt
Ccl11	NM_019205.1	Tgccacaaagcacctggaccaa	ccttgggggatgggtgccgat
Igfbp3	NM_012588.2	Tgctgggagtgtggaaagc	gagtggatggaacttggaatcag
Fabp3	NM_024162.1	Cactcggtgtgggctttgcca	ccgtccagtgtcacgaccgac
Cyr61	NM_031327.2	Agcgccctagagaagcgcct	tgcggtcagatgagggccga
Hpd	NM_017233.1	Gttggcaacgccaagcaggc	cagcccttgtaggccagcg
Adra2c	NM_138506.1	Ctggcagccgtggtgggttt	gcagcgctcggcttgtcaac
Inhbb	NM_080771.1	Gcatgcgtggcctgaaccct	tcatgttgggcacgtcccgc
Grb14	NM_031623.1	Cgcctcgcggtcgatgactc	gggtcctgggcgtctccctt
Lep	NM_013076.3	Atttcacacacgcagtcg	agcccaggaatgaagtccaa
Fgf18	NM_019199.1	Aactacacggccctgatgtca	gccgccccttcttggt
Per2	NM_031678.1	Acccctcctgacatcctctc	ctcattccaccaggggtcac
Sparc	NM_012656.1	Ctgcgcagcgtgactggc	agcctccaggcgcttctcgt
Npy	NM_012614.2	Ccgcccgccatgatgctagg	ggccatgtcctctgctggcg
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Cxcr4	NM_022205.3	Cccaccaacagccagaggcc	ggctgtcggggtaaaggcgg
Faim3	NM_001014843.1	Gcgtagccgaatggcgagg	gcatcccggcgctgtgtagg
Vcam	NM_012889.1	Gccagcgagggtctaccagc	aggtgagggtggcatttcccga
Rt1-Db1	NM_001008884.2	Tcctgaactgaggtgaggtga	gtctggccaggggttaaaca
Rt1-Da	NM_001008847.2	Gtcctgtgtgttctcgggtt	aatgagctctcacggaagcc
Rt1-Ba	NM_001008831.3	Ccccgagtttggacaactga	gacttggaaaacacggtcgc
Cd74	NM_013069.2	Agcgcccgtgaagaatgtta	ctgtgggagttcacgggtc
Cry1	NM_198750.2	Gctctagcccgcgctatcc	gggaggctcgtcacggaaa
Tbp	NM_001004198.1	Gacatcaccctgcagca	gcagtgccgcccaagta
Gadph	NM_017008.4	Gaagggctcatgaccacagt	Ggagcaggatgatgttct
Lxrα	NM_031627.2	Agggctgcaagggattcttc	Gacacactcctccctcatgc
Lxrβ	NM_031626.1	Ccaacagttagttgccgtgc	Ggctagctcagtgaagtggg
Rxrα	NM_012805.2	Ctgccgctcgacttctctac	Cccatgccattgatgggaga
Rxrβ	NM_206849.3	Ttcctccctacctctggacg	Ctctgtcagcacccgatcaa
Srebp1c	NM_001276707.1	Cccggtttcccaggaactttt	Agcatgtcttcgatgcggt
Fasn	NM_017332.1	Gtggaagacactggctcgaa	Tggtacactttcccgctcac
Acaca	NM_022193.1	Aaggctatgtgaaggatgtgg	Gaggttagggaagtcatctgg
Ldlr	NM_175762.2	Aggagtgcaagaccaacgag	Catggatccagtctaccgcc
Lrp5	NM_001106321.2	Gctgatgatctgccctaccc	Gcccagttcaatgctatgcag
Idol	NM_001107344.2	Ccacccacaccagtcttctc	Atactgcagcttgtggggac
Abca1	NM_178095.2	Aaccctggatgtataacgagc	Aaggagtgtttgggattggg
Abcg1	NM_053502.1	Ccagttctgcatcctcttcaag	Ttcgttcccaatcccaagg
Nf-κB	NM_001276711.1	Aacgcgtccaacctgaagat	Tgtctgtgaacatccgtggg
Il-1β	NM_031512.2	Tcctctgtgactcgtgggat	Tcagacagcacgaggcattt
Il-6	NM_012589.2	Agagacttccagccagttgc	Agtctcctccggacttgt

Supplementary Table 4. Top 3 most enriched functional categories among up- and down-regulated genes in PM-WAT and islets and up- and down-regulated proteins and metabolites in PM-WAT secretome due to diet-induced obesity. Count is the number of contributors and *P*-value is the significance of the pathway.

Canonical Pathway	Count	*P*-value
PM-WAT Transcriptomics		
Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells	6	3.6E-05
OX40 Signalling Pathway	6	5.8E-05
CCR5 Signalling in Macrophages	5	1.2E-04
Pancreatic Islets Transcriptomics		
Altered T Cell and B Cell Signalling	6	8.9E-06
B Cell Development	4	2.5E-05
iCOS-iCOSL Signalling in T Helper Cells	6	2.8E-05
PM-WAT Secretome Proteomics		
LXR/RXR Activation	6	3.4E-09
Acute Phase Response Signalling	6	2.3E-08
Coagulation System	2	7.4E-04
PM-WAT Secretome Metabolomics		
tRNA Charging	5	1.0E-08
Purine Nucleotides De Novo Biosynthesis II	3	8.7E-06
Asparagine Biosynthesis I	2	2.4E-05

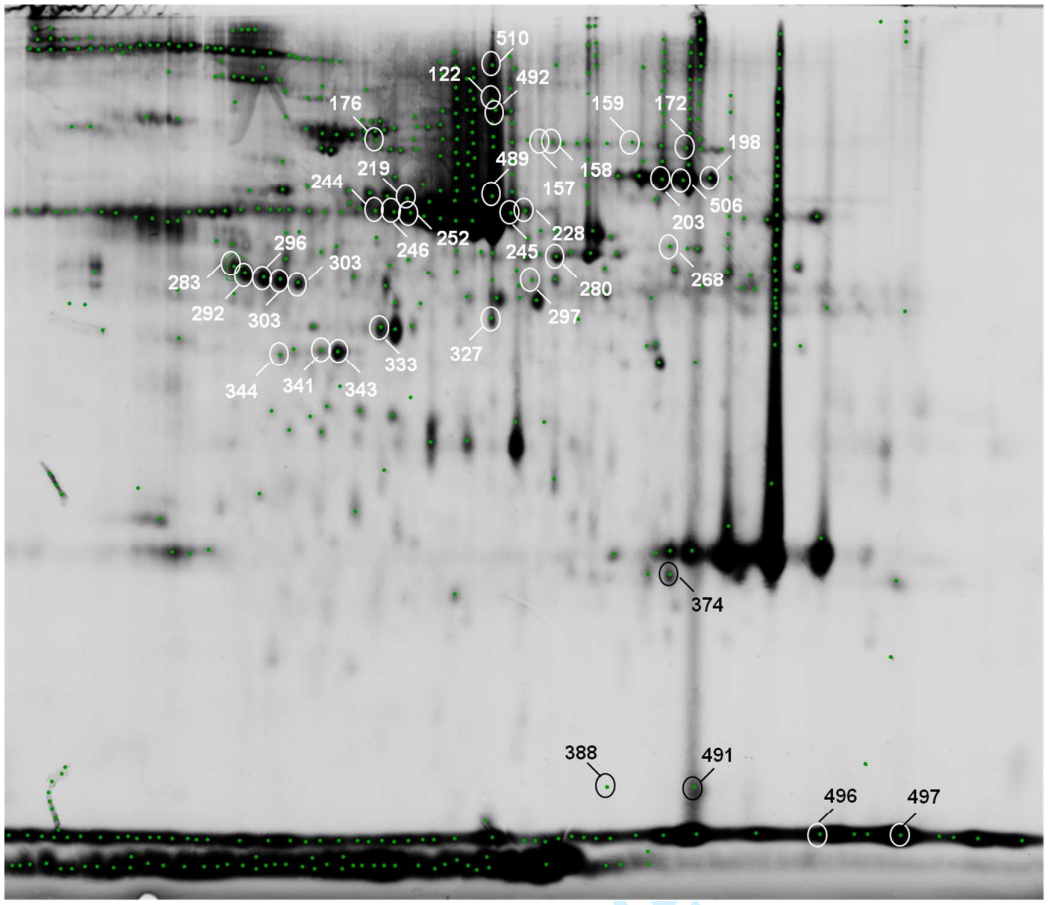
Supplementary Table 5. Top 5 most enriched functional categories among islet transcriptomic changes and PM-WAT secretome metabolic and proteomic perturbations due to diet-induced obesity. Count is the number of contributed contributors and *P*-value is the significance of the pathway.

Canonical Pathway	Count	*P*-Value
Islets Transcriptomics + PM-WAT Secretome Metabolomics		
Triacylglycerol Degradation	5	1.2E-06
LXR/RXR Activation	7	1.3E-05
Altered T Cell and B Cell Signalling	6	1.5E-05
B Cell Development	4	4.4E-05
iCOS-iCOSL Signalling in T Helper Cells	6	6.9E-05
Islets Transcriptomics + PM-WAT Secretome Proteomics and Metabolomics	**Count**	***P*-Value**
LXR/RXR Activation	12	1.1E-10
Triacylglycerol Degradation	6	7.8E-08
Altered T Cell and B Cell Signalling	6	3.6E-05
Retinol Biosynthesis	5	7.1E-05
B Cell Development	4	7.8E-05

Supplementary Table 6. Top 5 most enriched functional categories among PM-WAT and islet transcriptomic changes in and proteomic and metabolomic perturbations in PM-WAT secretome due to diet-induced obesity. Count is the number of contributed contributors and *P*-value is the significance of the pathway.

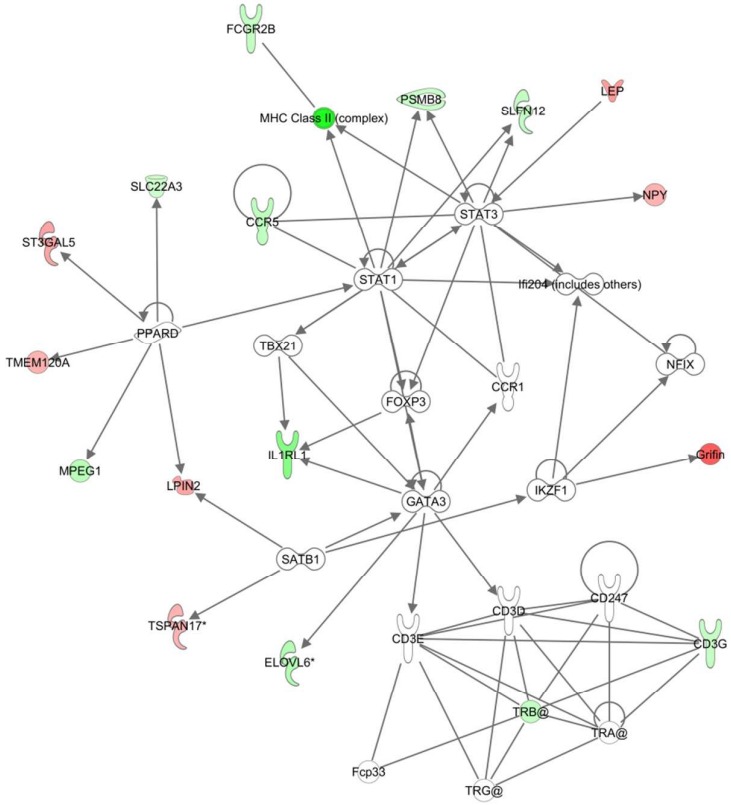
Canonical Pathway	Count	Molecules	*P*-value
LXR/RXR Activation	14	Apob, C3, Il1rl1 AHSG, Clu, A1BG, Cholesterol, Alb, Tf, C4B, Acaca, Serpina1, Gc, Hadh	1.1E-09
Triacylglycerol Degradation	7	Pnilprp2, Cel, Pnliprp1, Mgll, Ces1d, Glycerol, Pnlip	1.2E-07
Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells	8	Cd3g, H2-T24,Trb@, Rt1-Ec2, Hla-Dqa1, Hla-Drb1, Hla-Dqb1	9.8E-06
OX40 Signalling Pathway	8	Cd3g, H2-T24,Trb@, Rt1-Ec2, Hla-Dqa1, Hla-Drb1, Hla-Dqb1	1.5E-05
Crosstalk between Dendritic Cells and Natural Killer Cells	8	Il2rg, Cd69, Hla-Dra, Hl-Drb1, Ltb, Actg2, Itgal, Acta1	2.3E-05

SUPPLEMENTARY FIGURES



Supplementary Figure 1. 2D-DIGE differential analysis of adipose tissue secretomes from OB *vs* STD rats. Representative image gel evidencing the 36 spot locations selected for excision after applying the statistical analysis (Two-way ANOVA and the two-tailed Student's T-test for paired samples, p-value<0.05).

a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STAT1
Ifi204 (includes others)
PPARD
TBX21
NFIX
TMEM120A
FOXP3
CCR1
IL1RL1
Grifin
MPEG1
LPIN2
GATA3
IKZF1
SATB1
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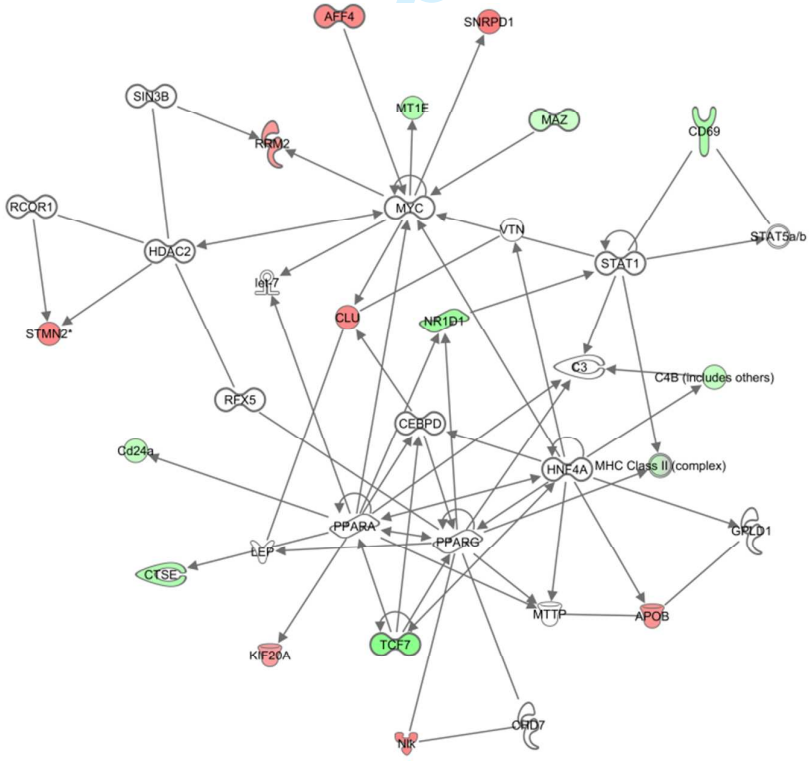
TRB@
TRA@
Fcp33
TRG@

b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MAZ
CD69
RRM2
RCOR1
MYC
VTN
STAT5a/b
HDAC2
STAT1
IgH7
CLU
NR1D1
STMN2*
C3
C4B (includes others)
REX5
CEBPD
Cd24a
HNF4A
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PPARG
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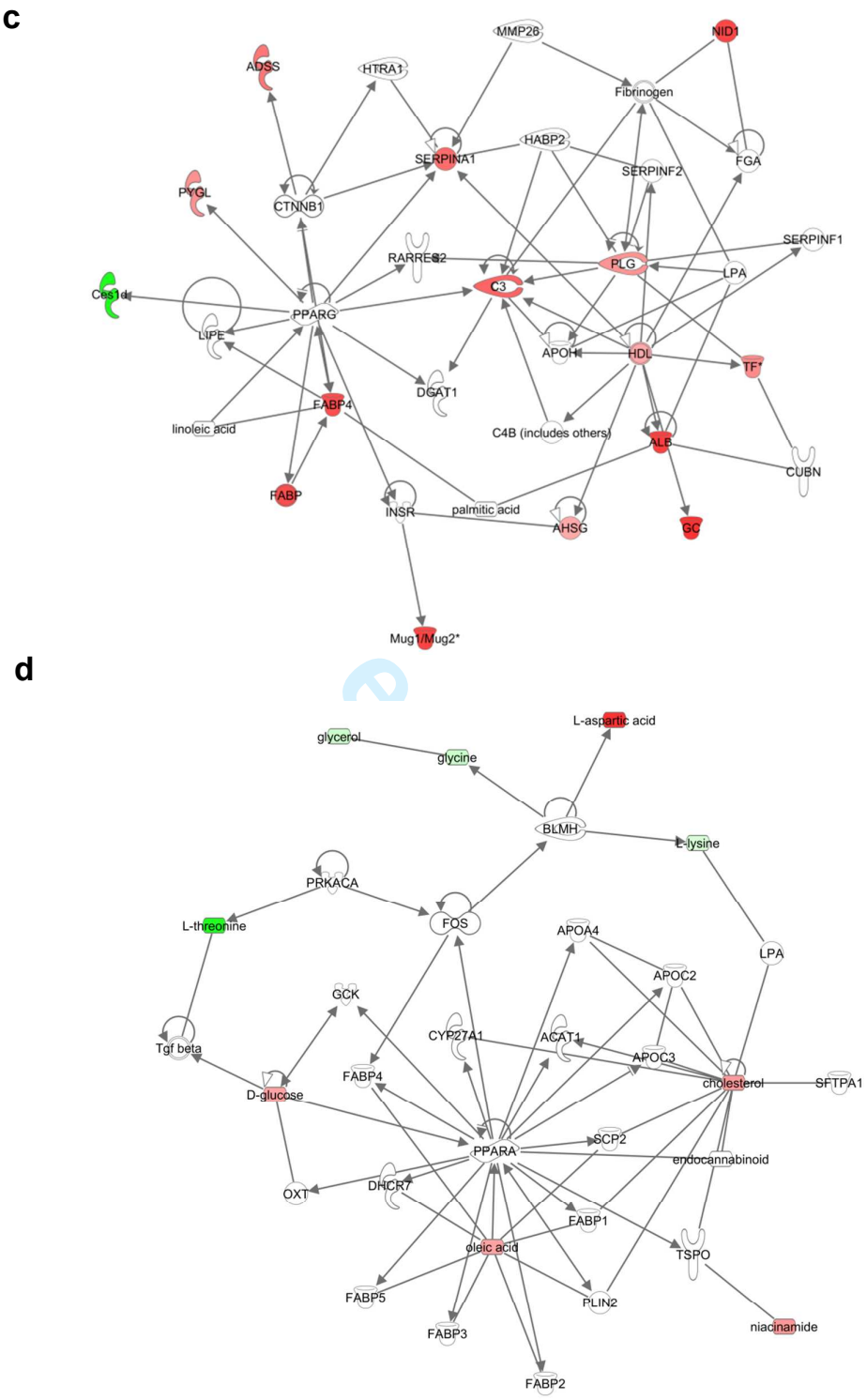
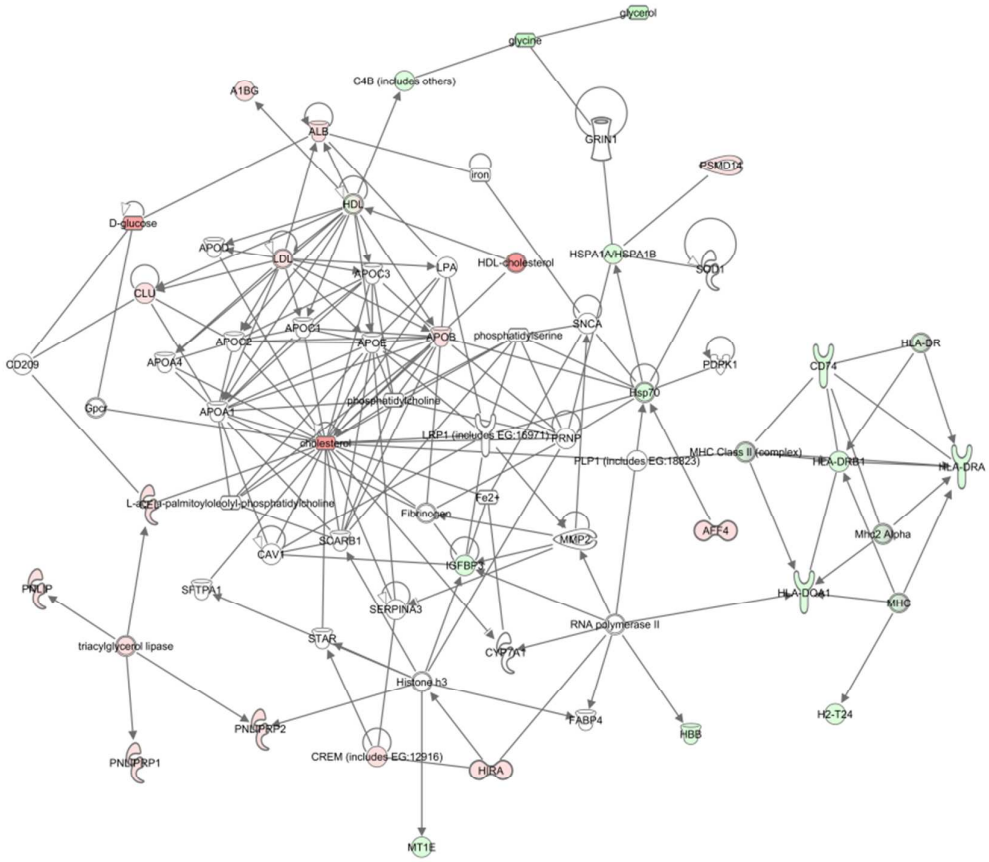
LEP
CTSE
MTTP
APOB
TCF7
KIF20A
CRD7
NIK



Supplementary Figure 2. Single-tissue, single-platform pathway mapping. PM-WAT (a) and pancreatic islets (b) transcriptomics integrative pathway mapping and PM-WAT secretome proteomics (c) and metabolomics (d) integrative pathway mapping (highest score network), considering only direct interactions**.**

a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HDL
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LDL
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LPA
HDL-cholesterol
HSPA1A/HSPA1B
SOD1
CLU
APOC1
APOC2
APOE
APOB
phosphatidylserine
SNCA
CD209
APOA4
PDPK1
HLA-DR
CD74
Hsp70
Gpc
APOA1
phosphatidylcholine
cholesterol
LRP1 (includes EG:16971)
PRNP
PLP1 (includes EG:18823)
MHC Class II (complex)
HLA-DRB1
HLA-DRA
L-alpha-palmitoyloleoyl-phosphatidylcholine
Fe2+
Fibrinogen
AEF4
MMP2
SCARB1
CAV1
Mhc2 Aloha
IGFBP3
PNLIP
SFTPA1
SERPINA3
HLA-DQA1
MHC
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triacylglycerol lipase
STAR
CYP7A1
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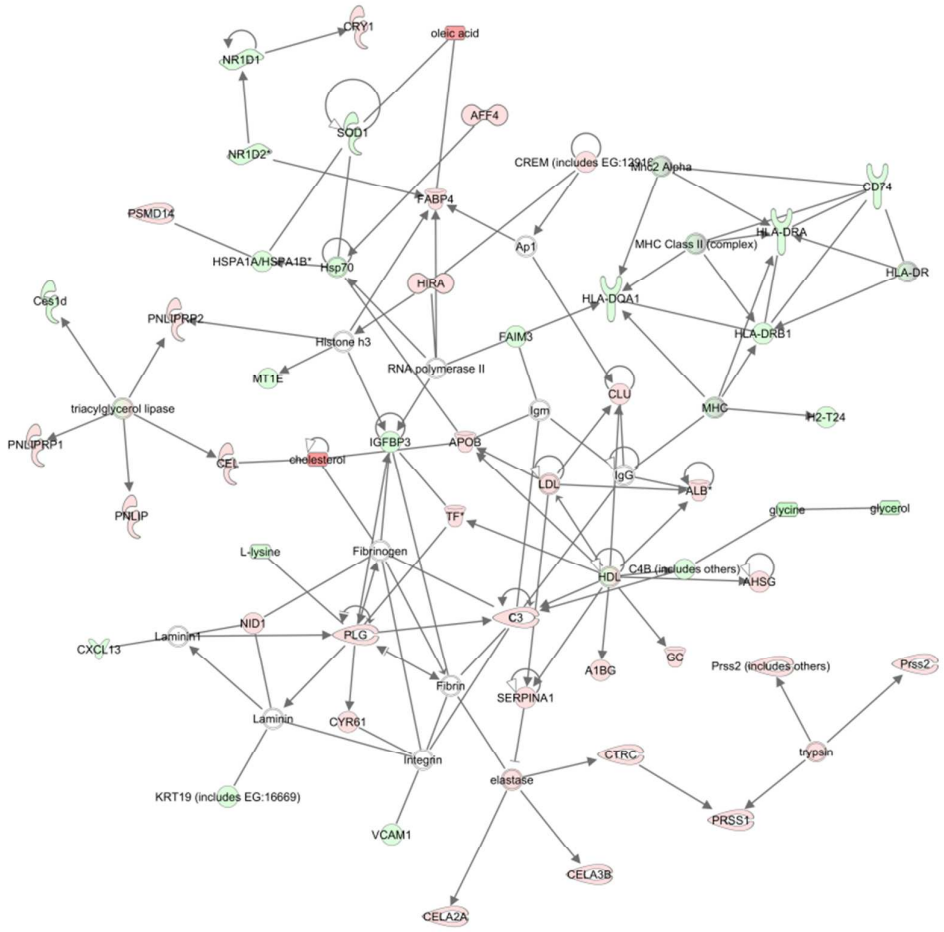
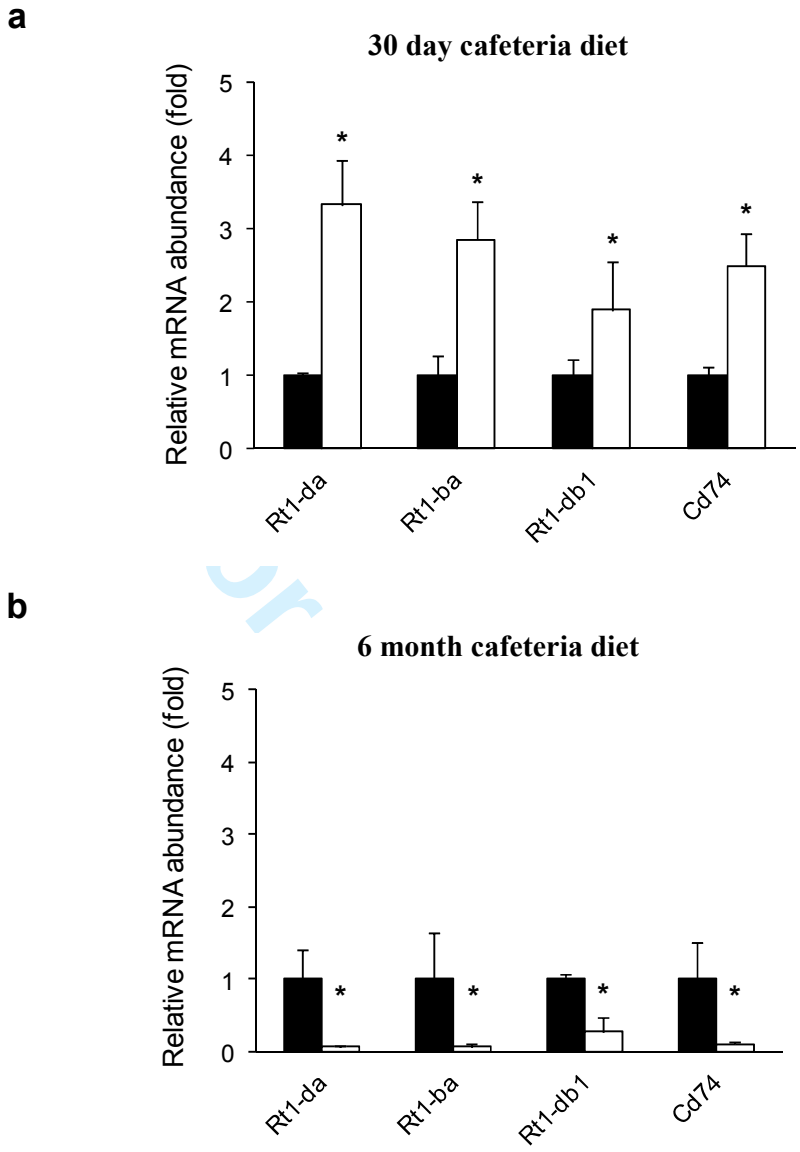
MT1E

b



Supplementary Figure 3. Inter-tissue, multi-platform integrative pathway mapping, considering only direct interactions: (a) Pancreatic transcriptomics data superimposed on the secretome metabolite network (b) Pancreatic islets transcriptomics data and PM-WAT secretome proteomics data superimposed on the PM-WAT secretome metabolite network.



Supplementary Figure 4. Gene expression changes in islet immune and inflammatory response signalling due to 30 day (a) and 6 month (b) cafeteria diet-induced obesity. Real-time PCR results for statistically significant genes: black bars = STD group; white bars = OB group. The expression of each gene was normalized with the constitutive expression of the gene *Gadph*. All values are presented as mean ± SEM from 6 to 10 animals per group. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.005$.

Editor's comments:

The referees raised a few major concerns. One of the key issues is that although the work is informative, it is largely descriptive in nature and lacking experimental validation.
As commented one of the referees, the authors should make an attempt to provide experimental validation rather than concluding the manuscript with mere speculation/without testing their hypothesis.

Reviewer comments:

Referee: 1

In this work, the goal of Rita Malpique and coauthors was to determine the main pathways and key molecules mediating the cross-talk between peripancreatic adipose tissue and the pancreatic islets during adaptation to obesity by the way of an integrated inter-tissue, multi-platform analysis. These two tissues were extracted from Wistar rats which were fed a standard or cafeteria diet for 30 days, and transcriptomic analysis were done on peripancreatic adipose tissue through microarray analysis. Also secretome from peripancreatic adipose tissue was subjected to a non-targeted metabolomics and proteomics analysis. The results of these analyses led to the identification of the activation of Liver/Retinoid X Receptors, degradation of triacylglycerol and up and down regulation of mediators of Inflammatory and immune responses. The study is well done and well designed, however, some conclusions made by the authors could be a little speculative.

- The authors explain that the main objective of this study was to assess the interrelation between changes in the PM-WAT and the beta-cells during adaptation to obesity. The inter-tissue network analysis may help here in hypothesizing the potential implication of the identified mediators in controlling pancreatic beta-cells functions, however this study did not show the specific impact of the studied parameters in PM-WAT on beta cells. The study was limited to analyse separately the two tissues. To conclude on specific impact of PM-WAT on beta cells function, mechanistic experiments of cause-effect relationship, involving both tissues, should be done.

We agree with the reviewer that mechanistic experiments of cause-effect relationship, involving both tissues, are crucial to validate our multi-platform approach. To this aim, we have performed additional experiments in which we have cultured islets from control rats with PM-WAT secretome from OB or STD rats for 48 hrs and studied gene expression of relevant pathways identified in our –omic analysis. Namely, we have confirmed changes in LXRs and RXRs and in some of their key target genes involved in lipogenesis and cholesterol transport as well as the down-regulation of *Il-6*. These data have now been included as new Figure 6. These results confirm the direct effect of the PM-WAT secretome from OB rats on islets LXR/RXR activation and signalling, thus validating our model.

- The text should be shortened, there many repetitions throughout the text. The authors have discussed the results in both sections (results and discussion). Moreover, the hypothesis of the study is repeated throughout the text many times (introduction and discussion sections).

Following the reviewer's request, we have shortened the text, removed repetitions throughout the text and discussed the results in the discussion section.

Referee: 2

Diet induced obesity is characterized by peripheral insulin resistance and can lead to β-cell dysfunction and loss, causing hyperglycemia and a higher risk of type II diabetes. In the early stages of obesity, β-cell mass expansion by replication and/or hyperplasia compensates for the rise of insulin demand. This β-Cell plasticity governs the adaptation of β-cell mass and function to ensure euglycemia. The molecular understanding on how β-cell mass is controlled during the onset of obesity and the identification of signaling molecules are active fields of research.
Malpique et al. in the manuscript have demonstrated the interelations between adipose tissues and pancreatic islets crosstalks as well as signaling pathways activated by the early stage of high fat diet induced obesity. The authors used high level data integration from transcriptomics analysis, metabonomics and proteomics secretome of the pancreatic islets, epididymal (E-WAT) and peripancreatic (PM-WAT) white adipose tissues in rats subjected to normal or cafeteria diet for 30 days. The authors elegantly identified and described significant changes in signaling molecules involved in apoptosis, fatty acid and cholesterol transport and metabolism, inflammatory and immune response between the PM-WAT and the pancreatic islets. However, the authors should address the following concerns:

- This paper sheds light on the adaptive capacity of the beta cells without going to the depth of the signaling pathways and on the crosstalk between PM-WAT and islets. Particularly, the observed decrease of immune and inflammatory response at the early stage of diet induce obesity is unexpected might key to the process of beta cell adaptation to obesity. For that reason, it should be followed up and compared to a longer term treatment (60days) as previously shown by the authors (Palau et al 2012). It would be insightful to determine how long this decreased persist as it might be important for the promotion of beta cell function and proliferation in the early stage and dysfuncton in the later stages of diet induced obesity models.

We agree with the point raised by the reviewer regarding the possible key role of the decreased immune and inflammatory response at early stages of diet on the beta cell adaptation to obesity. As suggested by the reviewer, we have compared gene expression changes in islets after a 30 day-diet with changes after a 6-month diet. As expected, immune and inflammatory response genes that we had found to be downregulated at 30 days were significantly upregulated at 6 months in islets from OB rats relative to STD rats. We have included these new data as a Supplementary Figure in the revised manuscript (Supplementary Fig. 4). Unfortunately, we could not include additional time points between 30 days and 6 months as these tissue samples from a 6-month diet were the only ones available to us at present. Given the time given to us to resubmit the manuscript it was not possible to perform a diet for longer than 30 days.

- The author should also determine the impact of the increase in cholesterol secretion by PM-WAT, LXR/RXR signaling and acute phase response secreted protein in combination with the decrease inflammatory response on islets function and maintenance in ex vivo setting for instance by looking at the expression ABCa1, LRP and of proliferation and survival markers.

As suggested by the reviewer, we have assessed the expression of genes involved in cholesterol uptake and efflux such as *Ldlr*, *Lrp5* and *Abcg1* as well as target genes of the LXR/RXR signalling pathway, such as *Srebp1c* and *Idol*. We have determined these changes not only in islets of OB as compared to STD rats but also in islets of control rats incubated with PM-WAT secretome. These data have been included as Figure 6.
We agree with the reviewer on the importance of determining the effect of the induced obesity on islet function and survival. However, the main objective of our present work was to to identify key molecules and signalling pathways involved in the crosstalk between PM-WAT and islets during evolution of obesity using a novel multi-platform approach. Concerning the effect of PM-WAT on

islet proliferation, this was already reported in a previous study by our group (Palau et al. Endocrinology, 153(1): 177–187. 2012), in which we showed the effect of the PM-WAT secretome on the proliferation of islets isolated from control rats and INS1 cells. We plan to evaluate additional parameters on our upcoming investigations.

- The authors have previously addressed the effect of diet induced PM-WAT secretome on beta cell proliferation with the implication of IGFBP3 a factor secreted by the PM-WAT and islet IGF1 receptor signaling in the proliferation of beta cell in response to diet induced obesity. The authors should show insulin secretion and the glucose tolerance test in their model of early phase of diet induced obesity.

The effects of a 30 day cafeteria diet on rats glycemia and insulinemia have already been demonstrated in a previous study published by our group (Palau et al. Endocrinology, 153(1):177–187. 2012), in which we show that diet-induced obese rats present increased levels of glycemia and insulinemia during an intravenous glucose tolerance test (IGTT) and higher fasted plasma levels of insulin (1).

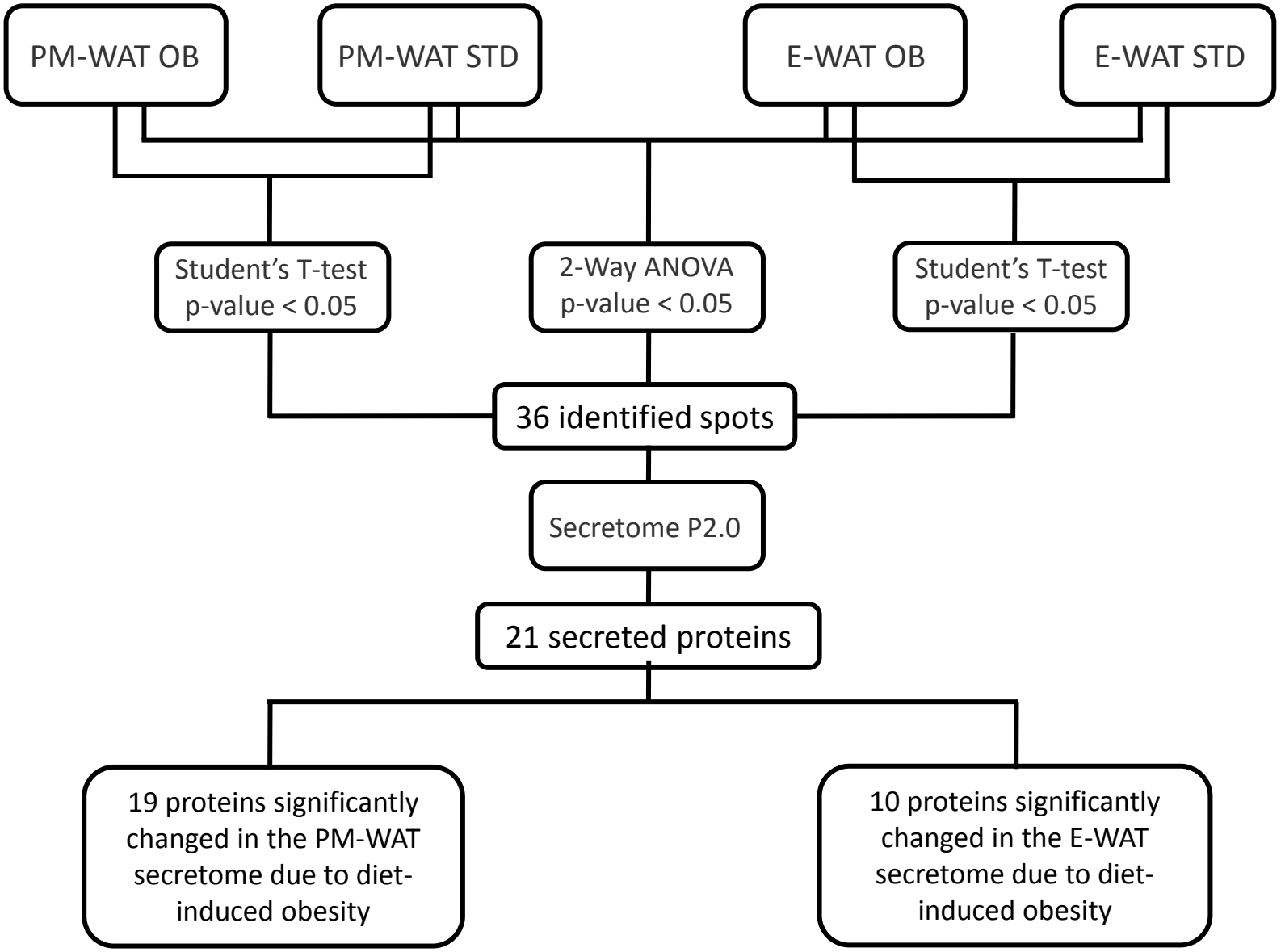
Referee: 3

Malpique and coworkers aimed to characterize the interrelation between peripancreatic adipose tissue and the pancreatic islets with idea to obtain novel insights about the mechanisms regulating beta-cell function/adaptation in/to obesity. The authors applied a microarray-based integrated inter-tissue, multiplatform analysis on the results of a 30 day feeding study of Wistar rats. In addition to these microarray analyses, islets and peripancreatic adipose tissue secretomes were examined with a non-targeted metabolomics and proteomics analysis. Although such an approach is not completely novel, the model system used has not investigated using such systematic und unbiased approach. The authors identified critical pathways including activation of Liver/Retinoid X Receptors, TG degradation and regulation of inflammatory/immune responses. Criticists may argue that these are the expected pathways know from similar approaches comparing liver (or muscle) and adipose tissue expression and function. However, I think data are sufficiently novel and may further stimulate research into the more in depths characterization of the major players in these pathway hits.

The manuscript is highly descriptive, but very nicely written and contains many novel hypothesis-generating data. The manuscript could benefit by demonstrating a direct (could be in an in vitro model) effect of one of the secreted adipose tissue markers C3, FABP, PLG on beta cell function ex vivo. One example should be sufficient to convince the reader that this multiplex approach leads to physiological meaningful candidates. The advantage of some of the molecules is that data on AT beta-cell function are already published. So, it may be also possible to (more extensively than in this version) discuss individual molecules in this context.

As suggested by the reviewer, we have used an *in vitro* model to demonstrate the direct effect of the PM-WAT secretome on pancreatic islets. However, rather than focusing on a single tissue marker, we have centred our attention on the top pathway identified in our inter-tissue network analysis as we hypothesize it being critical in the cross-talk between these two tissues. We have confirmed the activation of this pathway not only on islets from rats subjected to the 30 days cafeteria diet but also in islets directly incubated with secretome from OB rats, as opposed to islets incubated with secretome from STD rats (Figure 6). We also demonstrate, in both OB islets and control islets incubated with PM-WA secretome from OB rats, the downregulation of genes involved in the inflammatory response through the NF-κB signalling pathway,

which has been shown to be a target of LXR trans repression in macrophages (Joseph SB et al. Nature Medicine 2(9):213-219. 2003). Our results validate our model and evidence cholesterol as a potential key molecule involved in the crosstalk between these two tissues. Whether activation of the LXR/RXR pathway in pancreatic islets of OB rats is due to increased cholesterol uptake by the islets as a result of its increased secretion by the PM-WAT, as suggested in our study, and whether activation of these nuclear receptors results in the trans repression of NF-κB remain to be elucidated and are the subject of studies already initiated in our group.


PM-WAT OB
PM-WAT STD
E-WAT OB
E-WAT STD
Student's T-test p-value < 0.05
2-Way ANOVA p-value < 0.05
Student's T-test p-value < 0.05
36 identified spots
Secretome P2.0
21 secreted proteins
19 proteins significantly changed in the PM-WAT secretome due to diet-induced obesity
10 proteins significantly changed in the E-WAT secretome due to diet-induced obesity