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BRAIN RNA EXPRESSION IN OBESE VS LEAN MICE AFTER LPS-INDUCED SYSTEMIC INFLAMMATION

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1. ABSTRACT

Mortality of obese patients with severe sepsis is higher than non-obese patients. Thus far, a pathophysiologic mechanism has not been identified that explains this higher mortality. The central nervous system is now becoming increasingly recognized as a target organ in sepsis and the systemic inflammatory response syndrome and may hold clues to the deleterious affects of obesity in patients with sepsis syndrome. In this study, obese and non-obese mice were given LPS IP and the brains were harvested 2 hours after injection. The brains were processed and mRNA isolated and hybridized to a microarray chip and processed. Analysis of gene expression demonstrated distinct expression difference between the lean and obese animals. Ontology data supports clear differences between the lean and obese groups in the coagulation system, neuro-endocrine system, lipid transport and insulin receptors. Approximately eighty genes were identified to show 10-fold differential expression between the obese and lean mice.

2. INTRODUCTION

Mortality of obese patients with sepsis is higher than in non-obese patients, and reported to be 23% as compared to 6% in the non-obese (1, 2). Although the mechanism of worse outcomes in obese patients with sepsis is unknown, it has been suggested that obesity is associated with an altered immune response to a septic or inflammatory insult (3-5). Since obesity has been an exclusion criterion in many sepsis trials, little is known about how obesity affects outcome or if the obese respond differently to therapy. In particular, little is known about how obesity affects cerebral function in sepsis. Because of the complex interactions associated with obesity and sepsis, standard investigational strategies do not allow examination of numerous tissues and biologic pathways simultaneously, and may not uncover the mechanisms by which obesity alters the immune response to a septic or inflammatory insult.

The deleterious effects of sepsis on organs such as the brain, liver, kidney and heart are well-recognized. The central nervous system is now becoming increasingly recognized as a target organ in sepsis and the systemic inflammatory response syndrome. Cerebral dysfunction manifests with a spectrum ranging from mildly altered sensorium to coma. Historically, the encephalopathy of sepsis was attributed to functional effects of false neurotransmitters (6, 7) or alterations in cerebral blood flow (8). More recent investigations in animal models have revealed morphological changes, including perimicrovascular edema and neuronal apoptosis, suggesting loss of blood-brain barrier function and/or the involvement of cytokine-mediated injury (9).

A technique of examining numerous biologic pathways and tissues simultaneously is to identify cellular RNA expression through microarray technology. This technology allows large-scale identification of the RNA expressed within a tissue, and may allow identification of pathways not previously associated with obesity, inflammation or the cellular response to an infectious or inflammatory insult.

In this study we compared RNA expression in brain tissue between obese and non-obese mice after LPS

injection in an attempt to identify expression patterns that may provide clues to the cerebral dysfunction in sepsis, and those that may differentially expressed in obese subjects following the inflammatory insult.

3. METHODS

3.1. Animal Model

Obese transgenic mice (-actin promoter driving agouti) and non-transgenic lean littermates were used as the model for the presence and absence of obesity, respectively (10-13). This mouse strain appears to provide a model of obesity having less immunologic interference than leptin deficient mice (14). All mice were 6 weeks of age. The obese group mean weight was 51 grams and the lean was 29 grams.

3.2. Experimental Design

The study was designed as a two-way comparison between obese and non-obese mice (n=12), with half of each group receiving saline and the other receiving lipopolysaccharide (LPS).

Group 1 consisted of obese transgenic mice subjected to LPS challenge. Mice received 12 mg/kg of *E. coli* LPS (Sigma-Aldrich, St Louis, Mo) by intraperitoneal injection. Group 2 consisted of obese transgenic mice subjected to saline challenge. Mice in this group were treated identically to group 1 above except they received an equal volume sterile saline rather than LPS intraperitoneally. Group 3 consisted of non-transgenic littermates (non-obese) mice subjected to LPS challenge. Mice in this group were treated identically to group 1 above. Group 4 consisted of non-transgenic littermates (non-obese) mice subjected to saline challenge. Mice in this group were treated identically to group 2 above.

3.3. Animal procedures

All mice received either 12 mg/kg of E. coli LPS or equal volume of saline by intraperitoneal injection. The mice were observed and sacrificed two hours after receiving the injection. After the mice were euthanized, the brain was recovered by dissection of the skull and removal of the cortex. A coronal section was cut out of the mid cortex, 0.5 cm thick and included both hemispheres. This tissue was subsequently placed in a RNA preservative (RNALater, Quiagen, Valencia, CA, http://www.qiagen.com) and frozen to -70°C. for later RNA extraction and analysis. Animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee.

3.4. RNA isolation and microarray hybridization

The tissue was removed from the RNA preservative and homogenized in a buffered solution. The isolation was performed utilizing the RNeasy® Mini Kit (Qiagen). A highly denaturing cell-lysis buffer containing guanidine isothiocyanate (GITC) immediately generates an RNase-free environment, stabilizes the RNA, and simultaneously releases the DNA. The cellular extract is prepared, the conditions adjusted to allow separation of RNA and DNA, and the extract loaded onto the QIAGEN-tip. Total RNA and a portion of the genomic DNA present in the sample bind to QIAGEN Resin while the remaining DNA passes through in the first flow-through fraction. Residual proteins, metabolites and low-molecular-weight impurities are removed by washing the QIAGEN-tip with a medium-salt buffer. Pure RNA is eluted in a high-salt buffer while DNA remains bound to the resin. The RNA is then concentrated and desalted by isopropanol precipitation.

For simultaneous isolation of genomic DNA and RNA from the same sample, the first flow-through is reapplied to the QIAGEN-tip after elution of the RNA in order to bind the rest of the genomic DNA. The column is washed again, and the genomic DNA is eluted. Concentration and desalting of the RNA and DNA by isopropanol precipitation can then be performed in parallel. RNA samples were test for quantity and quality utilizing photospectrometry.

An initial test hybridization was performed, once these samples satisfied this quality control measure, they were hybridized to the MU 74 Av2® oligonucleotide chip (Affymetrix, Santa Clara, CA, http://www.affymetrix.com), hybridized and labeled. The chips were scanned with an Agilent Technologies (Palo Alto, CA, http://www.agilent.com) GeneArray® scanner.

3.5. Data analysis

Initial data review and preparation was conducted with Microarray Suite 5.0 (Affymetrix, Santa Clara, CA, http://www.affymetrix.com). The acquired image files were visually inspected for aberrations. The mean intensity of the four quadrants of each image file was determined to ascertain that data acquisition on each chip was uniform (< 5% deviation). Grid alignment was manually verified on each sample before calculation of probe intensities. Cell expression values were obtained using the standard Affymetrix calculations, which include (1) background subtraction with a smoothing adjustment, (2) noise correction to avoid negative values, (3) subtraction of ideal mismatch values, (4) application of Tukey's biweight algorithm on log-transformed values to obtain a robust mean estimate, and (5) scaling using the mean trimmed of the upper and lower 2% of observations (15). The resulting expression datasets were filtered to remove the Affymetrix control probe sets, and all probe sets in which the detection calls were absent across all four groups, leaving 7341 expression levels for further analysis.

Clustering analysis was performed with Hierarchical Clustering Explorer 2.0 (University of Maryland) (16). Prior to clustering, the dataset was log transformed then normalized with the z-transform across the overall mean expression value. The distance metric used was the Pearson correlation distance, and the clustering was performed with the UPGMA algorithm. Differential expression analysis was performed on the dataset prepared by normalizing the mean values to that of the baseline (lean saline, LS) group.

4. RESULTS

The heat map derived from hierarchical clustering of the filtered, normalized dataset, along with greatest differential expression sub-clusters and their general annotation, is given in Figure 1. After evaluating the

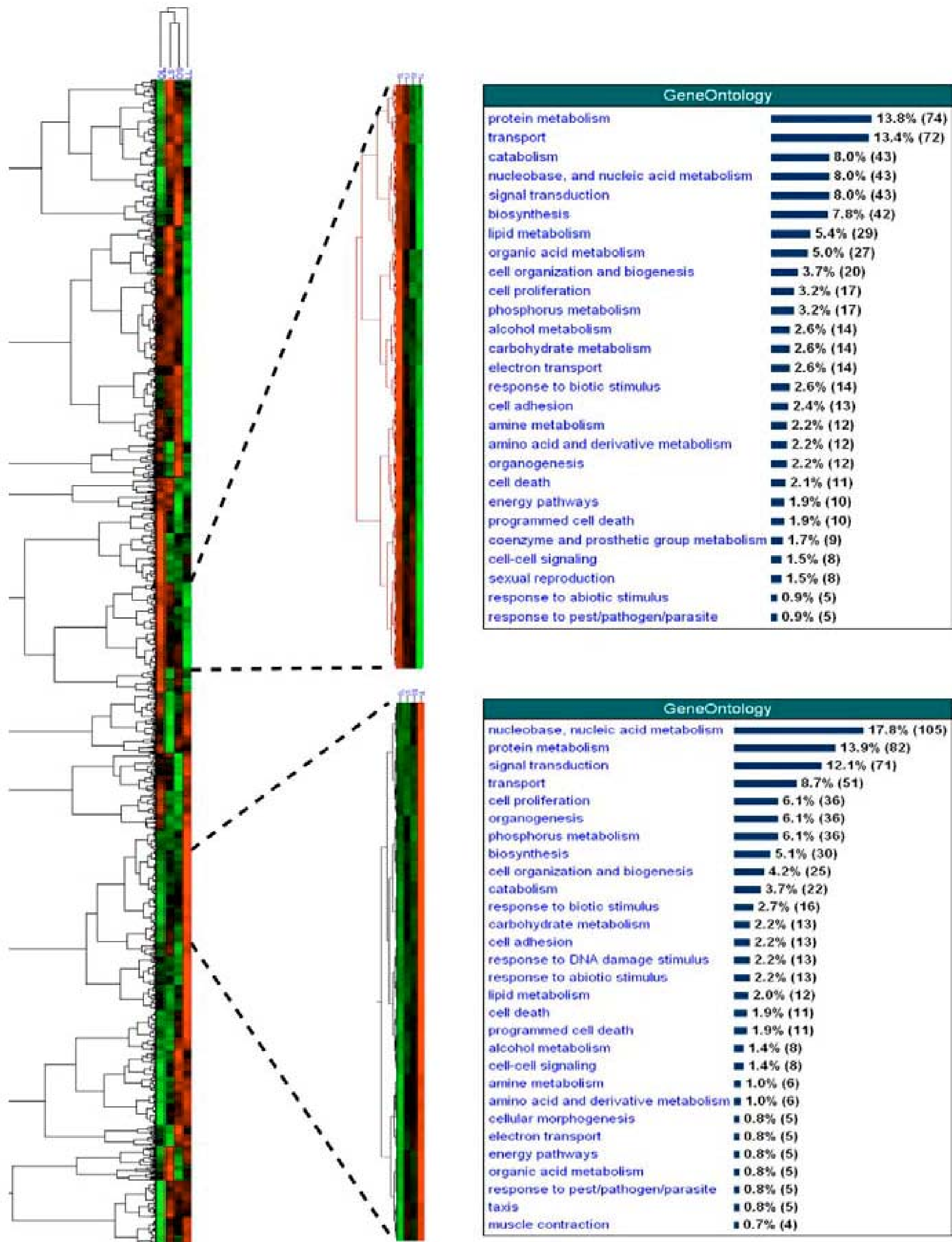

Figure 1. Heat map of differentially expressed genes (left). Two subclusters were identified and are shown expanded (middle). The upper subcluster shows genes that are up-regulated in the obese LPS group, down-regulated in the lean LPS group, and unchanged in the lean and obese saline controls. The lower subcluster shows the converse. Gene ontology classifications for each of the sub-clusters are shown on the right.

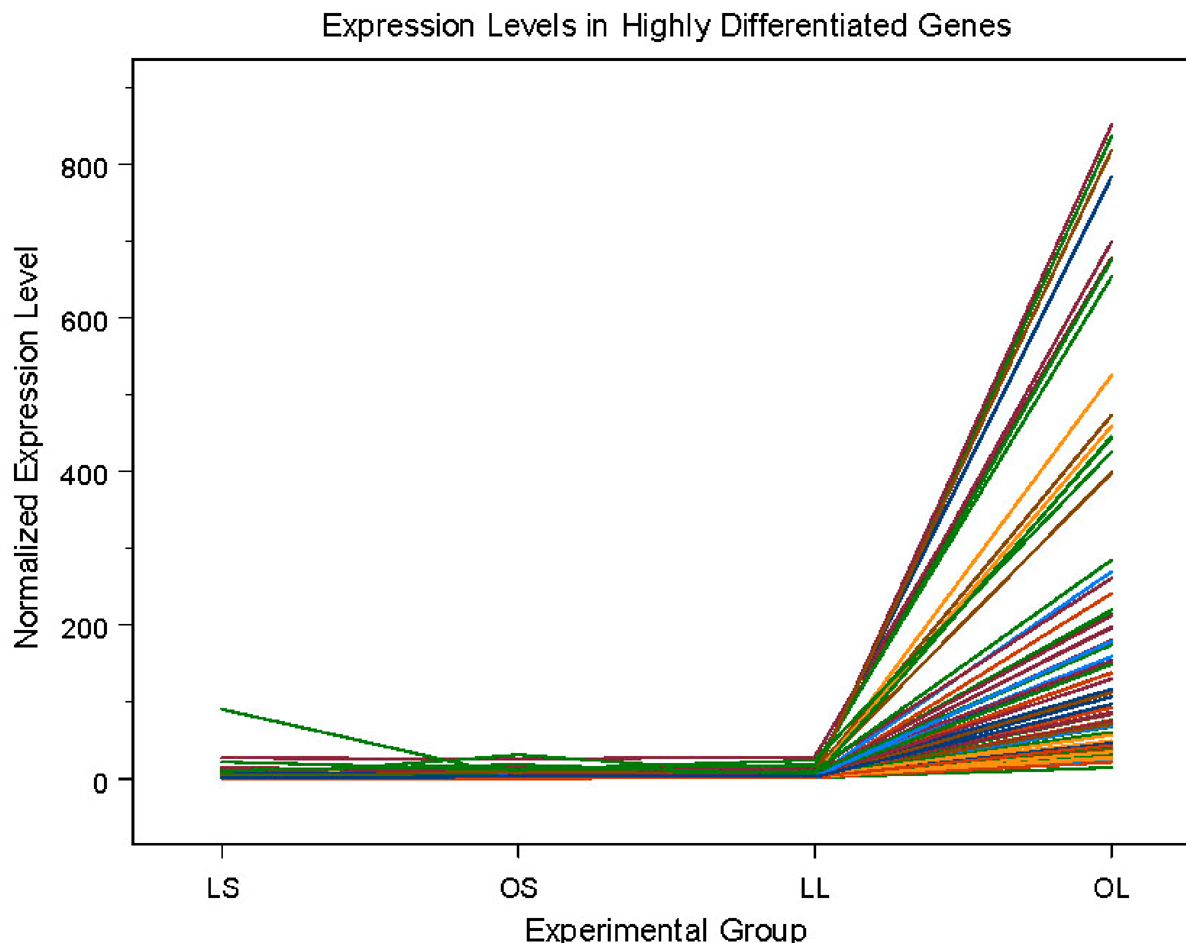

Figure 2. Cross-sectional plot of the 78 genes with 10-fold or greater expression in the obese LPS-stimulated group (OL) as compared with the lean LPS-stimulated group (LL). The expression levels of genes in the lean and obese saline groups (LS, OS) are similar to those in the LL group. This represents the effect of the difference in expression in inflammation due to obesity as the major differentiating factor. The list of gene annotations for this group are given in Table 1.

data of the most highly expressed genes, several systems that are known to be relevant in sepsis and inflammation deserve special mention.

4.1. Coagulation System

The microarray data in this study show that the protein C receptor expression is less in the obese LPS group compared to the lean LPS group although it did not reach 10 fold differences. The opposite is true for plasminogen activator inhibitor 1 (PAI-1) which shows an 8.5 fold higher expression level in the obese LPS group compared to the lean LPS group. Plasminogen expression is also higher in the obese LPS group demonstrating a 5 fold increase. No difference was noted in either expression of these genes in the saline groups. Coagulation factor II gene (Locus Link 14061) also demonstrated increased expression in the obese LPS group compared to the lean along with coagulation factor V (Locus Link 14067). These fold changes are shown in Figure 3.

4.2. Neuro-endocrine System

Analysis of RNA expressed in neuro-endorine function (Figure 4) demonstrates increased expression of insulin-like growth hormones, serine protease inhibitors and vasopressin. Further, there is a 16-fold increase oxytocin-neurophysin expression in obese mice administered LPS compared to what lean mice injected with LPS. Of note is the down regulation of the insulin receptor after LPS stimulation in lean mice challenged with LPS. These expression patterns are specific for the obese LPS mice and not seen in the lean mice given LPS or the obese mice subjected to saline administration.

4.3. Lipid Transport

A significant increase in RNA expression is each of the majority of the apolipoproteins (A-I, A-II, A-IV, A-V, C-II, C-IV, F and H) was demonstrated in only the obese LPS-treated mice as compared with the lean LPS-treated mice (Figure 5). The remaining apolipoproteins had no change in expression level between these two groups.

4.4. Insulin

Comparing the lean and obese mice that received LPS, one finding stands out. The insulin receptor in the obese group is significantly down-regulated compared to the lean group. This difference is shown in Figure 6. With

Table 1. List of genes that are highly differentiated in the obese LPS group as compared with the other three groups (see Figure 2 for differential expression graph)

Affyid	Locus Link	Gene Name	Gene Ontology
100329_at	20700	serine (or cysteine) proteinase inhibitor, clade A, member 1a	acute-phase response; serine-type endopeptidase inhibitor activity
100333_at	20209	serum amyloid A 2	acute-phase response; acute-phase response protein activity; extracellular; lipid transporter activity; protein binding
100436_at	18405	orosomucoid 1	acute-phase response; extracellular space; transport; transporter activity
100437_g_at	18405	orosomucoid 1	acute-phase response; extracellular space; transport; transporter activity
100634_at	54150	retinol dehydrogenase 7	catalytic activity; extracellular space; metabolism; oxidoreductase activity
100967_at	26458	solute carrier family 27 (fatty acid transporter), member 2	catalytic activity; extracellular space; fatty acid metabolism; integral to membrane; ligase activity; metabolism; peroxisome
101287_s_at	13101	cytochrome P450, family 2, subfamily d, polypeptide 10	electron transport; endoplasmic reticulum; integral to membrane; membrane; microsome; monooxygenase activity; oxidoreductase activity; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen
101531_at	230163	aldolase 2, B isoform	fructose-bisphosphate aldolase activity; glycolysis; lyase activity
101553_at	14161	fibrinogen, alpha polypeptide	extracellular space
101565_f_at	20700	serine (or cysteine) proteinase inhibitor, clade A, member 1a	acute-phase response; serine-type endopeptidase inhibitor activity
101566_f_at	17840	major urinary protein 1	immediate hypersensitivity response; pheromone binding; transport; transporter activity
101572_f_at	20700	serine (or cysteine) proteinase inhibitor, clade A, member 1a	acute-phase response; serine-type endopeptidase inhibitor activity
101574_f_at	20704	serine (or cysteine) proteinase inhibitor, clade A, member 1e	acute-phase response; peptidase activity; serine-type endopeptidase inhibitor activity
101576_f_at	20701	serine (or cysteine) proteinase inhibitor, clade A, member 1b	acute-phase response; endopeptidase inhibitor activity; peptidase activity; serine-type endopeptidase inhibitor activity
101635_f_at	17844	major urinary protein 5	extracellular space; pheromone binding; transport; transporter activity
101638_s_at	13114	cytochrome P450, family 3, subfamily a, polypeptide 16	electron transport; endoplasmic reticulum; membrane; microsome; monooxygenase activity; oxidoreductase activity; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen
101682_f_at	17843	major urinary protein 4	extracellular space; membrane; pheromone binding; transport; transporter activity
101899_at	14061	coagulation factor II	acute-phase response; blood coagulation; blood coagulation factor activity; calcium ion binding; chymotrypsin activity; extracellular; extracellular space; hydrolase activity; proteolysis and peptidolysis; serine-type endopeptidase activity; thrombin activity; trypsin activity
101909_f_at	17842	major urinary protein 3	pheromone binding; transport; transporter activity
101910_f_at	17840	major urinary protein 1	immediate hypersensitivity response; pheromone binding; transport; transporter activity
101912_at	66107	RIKEN cDNA 1100001G20 gene	
102096_f_at	17841	major urinary protein 2	pheromone binding; transport; transporter activity
102712_at	20210	serum amyloid A 3	acute-phase response; acute-phase response protein activity; extracellular; extracellular space; lipid transporter activity
102748_at	14067	coagulation factor V	blood coagulation; blood coagulation factor activity; cell adhesion; copper ion binding; extracellular space
102799_at	12269	complement component 4 binding protein	complement activation; complement activation, classical pathway; complement activity
103407_at	71775	RIKEN cDNA 1300017J02 gene	extracellular; extracellular space; ferric iron binding; iron ion homeostasis; iron ion transport
103465_f_at	20209	serum amyloid A 2	acute-phase response; acute-phase response protein activity; extracellular; lipid transporter activity; protein binding
103896_f_at	16006	insulin-like growth factor binding protein 1	extracellular; extracellular space; growth factor binding; insulin-like growth factor binding; regulation of cell growth
104424_at	12279	complement component 9	complement activation; complement activation, alternative

			pathway; complement activation, classical pathway; complement activity; cytolysis; extracellular space; integral to membrane; membrane attack complex
104588_at	66438	RIKEN cDNA 1810073K19 gene	
104726_at	69379	RIKEN cDNA 1700013L23 gene	extracellular space; transport; transporter activity
160375_at	12350	carbonic anhydrase 3	carbonate dehydratase activity; lyase activity; one-carbon compound metabolism; zinc ion binding
160481_at	18534	phosphoenolpyruvate carboxykinase 1, cytosolic	GTP binding; carboxy-lyase activity; gluconeogenesis; glycerol biosynthesis from pyruvate; kinase activity; lipid metabolism; lyase activity; phosphoenolpyruvate carboxykinase (GTP) activity; phosphoenolpyruvate carboxykinase activity
161626_f_at	18815	plasminogen	apoptosis; apoptosis activator activity; blood coagulation; calcium ion binding; chymotrypsin activity; extracellular; extracellular space; hormone activity; hydrolase activity; negative regulation of angiogenesis; negative regulation of blood coagulation; plasmin activity; proteolysis and peptidolysis; serine-type endopeptidase activity; thrombin activity; trypsin activity
161815_f_at	17841	major urinary protein 2	pheromone binding; transport; transporter activity
161827_f_at	15458	hemopexin	acute-phase response; extracellular space; transport
161924_f_at	11807	apolipoprotein A-II	extracellular space; lipid transport; lipid transporter activity; regulation of cholesterol absorption; transport
92606_at	22262	urate oxidase	oxidoreductase activity; peroxisome; purine base metabolism; urate oxidase activity
92837_f_at	17836	murinoglobulin 1	endopeptidase inhibitor activity; extracellular space; membrane; receptor activity; serine-type endopeptidase inhibitor activity; transport; transporter activity; wide-spectrum protease inhibitor activity
93096_at	99571	fibrinogen, gamma polypeptide	blood coagulation; extracellular space
93097_at	11846	arginase 1, liver	arginase activity; arginine catabolism; arginine metabolism; catalytic activity; hydrolase activity; manganese ion binding; urea cycle
93109_f_at	20703	serine (or cysteine) proteinase inhibitor, clade A, member 1d	acute-phase response; endopeptidase inhibitor activity; peptidase activity; serine-type endopeptidase inhibitor activity
93354_at	11812	apolipoprotein C-I	extracellular; extracellular space; lipid transport; lipid transporter activity; lipoprotein metabolism; transport
93381_at	11998	arginine vasopressin	extracellular; extracellular space; hormone activity; neurohypophyseal hormone activity; regulation of blood pressure
93433_s_at	11889	asialoglycoprotein receptor 1	endocytosis; heterophilic cell adhesion; integral to membrane; membrane; receptor activity; sugar binding
93497_at	12266	complement component 3	complement activation; complement activation, alternative pathway; complement activation, classical pathway; complement activity; endopeptidase inhibitor activity; extracellular; extracellular space; inflammatory response
93766_at	19733	regucalcin	calcium ion binding; cytoplasm; enzyme regulator activity; nucleus
93770_at	13112	cytochrome P450, family 3, subfamily a, polypeptide 11	electron transport; endoplasmic reticulum; membrane; microsome; monooxygenase activity; oxidoreductase activity; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen
93824_at	227231	hypothetical protein 4732433M03	
93837_at	16644	kininogen	blood coagulation; cysteine protease inhibitor activity; extracellular space; inflammatory response; regulation of blood pressure
94045_at	11699	alpha 1 microglobulin/bikunin	endopeptidase inhibitor activity; extracellular space; serine-type endopeptidase inhibitor activity; transport; transporter activity
94049_at	12116	betaine-homocysteine methyltransferase	homocysteine S-methyltransferase activity; methionine biosynthesis; methyltransferase activity; transferase activity
94075_at	14080	fatty acid binding protein 1, liver	binding; fatty acid binding; lipid binding; transport; transporter activity

94318_at	11818	apolipoprotein H	extracellular space; heparin binding
94440_at	104910	expressed sequence AI876593	
94540_at	76279	cytochrome P450, family 2, subfamily d, polypeptide 26	electron transport; endoplasmic reticulum; integral to membrane; membrane; microsome; monooxygenase activity; oxidoreductase activity
94777_at	11657	albumin 1	carrier activity; extracellular space; lipid binding; transport
95043_at	226105	cytochrome P450, family 2, subfamily c, polypeptide 70	monooxygenase activity; oxidoreductase activity
95407_at	18478	phenylalanine hydroxylase	amino acid binding; aromatic amino acid family metabolism; catalytic activity; iron ion binding; metabolism; monooxygenase activity; oxidoreductase activity; phenylalanine 4-monooxygenase activity; phenylalanine catabolism
95727_at	66113	apolipoprotein A-V	extracellular space lipid binding lipid transport; triglyceride binding
96092_at	15439	haptoglobin	acute-phase response; chymotrypsin activity; extracellular space; hemoglobin binding; proteolysis and peptidolysis; trypsin activity
96094_at	11806	apolipoprotein A-I	cholesterol metabolism; extracellular space; lipid binding; lipid transport; lipid transporter activity; protein binding; regulation of cholesterol absorption
96326_at	234724	tyrosine aminotransferase	amino acid metabolism; biosynthesis; phenylalanine catabolism; transaminase activity; transferase activity; tyrosine catabolism; tyrosine transaminase activity
96334_f_at	13099	cytochrome P450, family 2, subfamily c, polypeptide 40	electron transport; endoplasmic reticulum; extracellular space; membrane; microsome; monooxygenase activity; oxidoreductase activity
96792_at	104779	expressed sequence AI315052	
96796_f_at	22238	UDP-glucuronosyltransferase 2 family, member 5	glucuronosyltransferase activity; integral to membrane; metabolism; microsome; transferase activity; transferase activity, transferring glycosyl groups; transferase activity, transferring hexosyl groups
96828_at	14711	glycine N-methyltransferase	S-adenosylmethionine-dependent methyltransferase activity; catalytic activity; folic acid binding; glycine N-methyltransferase activity; methyltransferase activity; transferase activity
96846_at	11905	serine (or cysteine) proteinase inhibitor, clade C (antithrombin), member 1	blood coagulation; extracellular space; heparin binding; serine-type endopeptidase inhibitor activity
96868_at	110135	fibrinogen, B beta polypeptide	blood coagulation; extracellular space
96918_at	14121	fructose bisphosphatase 1	carbohydrate metabolism; catalytic activity; fructose-bisphosphatase activity; gluconeogenesis; hydrolase activity; phosphoric ester hydrolase activity
97216_at	11287	pregnancy zone protein	endopeptidase inhibitor activity; extracellular; extracellular space; serine-type endopeptidase inhibitor activity; wide-spectrum protease inhibitor activity
98116_at	15458	hemopexin	acute-phase response; extracellular space; transport
98467_at	16427	inter alpha-trypsin inhibitor, heavy chain 4	
98589_at	11520	adipose differentiation related protein	biological_process unknown; membrane; molecular_function unknown
98612_at	13101	cytochrome P450, family 2, subfamily d, polypeptide 10	electron transport; endoplasmic reticulum; integral to membrane; membrane; microsome; monooxygenase activity; oxidoreductase activity; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen
99197_at	14473	group specific component	actin binding; carrier activity; extracellular space; transport
99862_at	11625	alpha-2-HS-glycoprotein	cysteine protease inhibitor activity; extracellular space; ossification
99927_at	12630	complement component factor i	chymotrypsin activity; complement activation; complement activation, classical pathway; complement activity; extracellular space; hydrolase activity; membrane; proteolysis and peptidolysis; scavenger receptor activity; serine-type endopeptidase activity; trypsin activity
99941_at	13909	esterase 31-like	

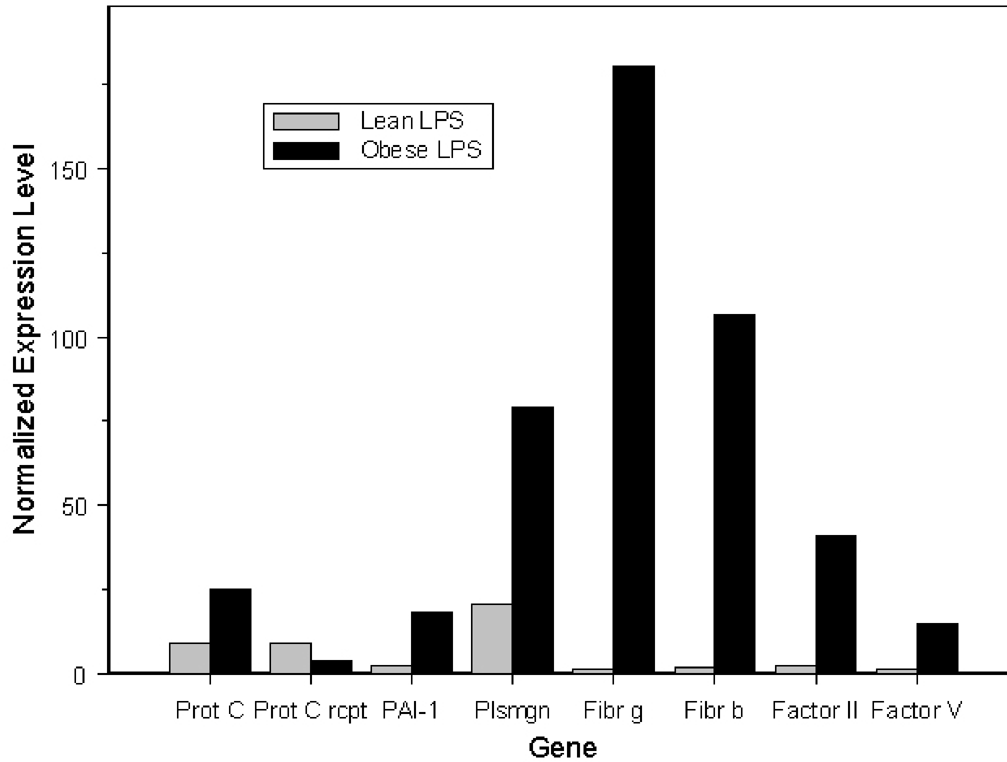

Figure 3. Differential expression of major coagulation system genes in the obese LPS (OL) group and the lean LPS (LL) group. This graph demonstrates an increased expression of Protein C, but a decreased expression of the protein C receptor gene in the obese LPS group. Also noted are significant increased expression of PAI-1, plasminogen, fibrinogen, coagulation factor II and V compared to the lean LPS group.

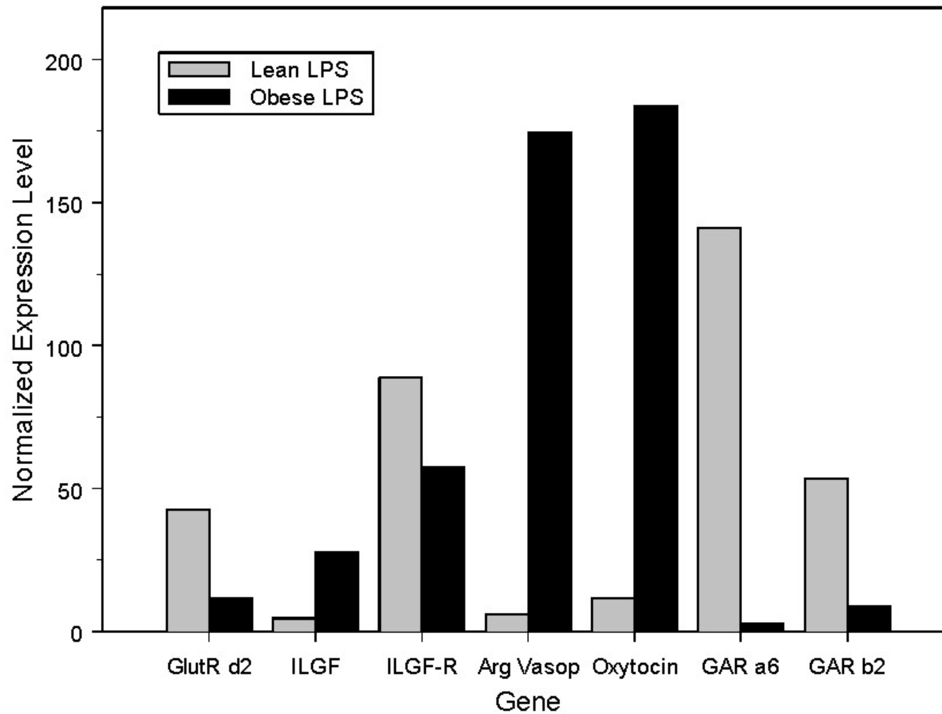

Figure 4. Brain RNA expression of neuro-endocrine receptors and hormones in obese and lean mice challenged with LPS. Considerable up-regulation is noted in GABA A (GA-R) and GABA β-2 (GB-R) receptors, glutamate receptor (GR) and insulin-like growth hormone. Marked down-regulation is noted in oxytocin-neurophysin (O-N) and vasopressin II (B-II).

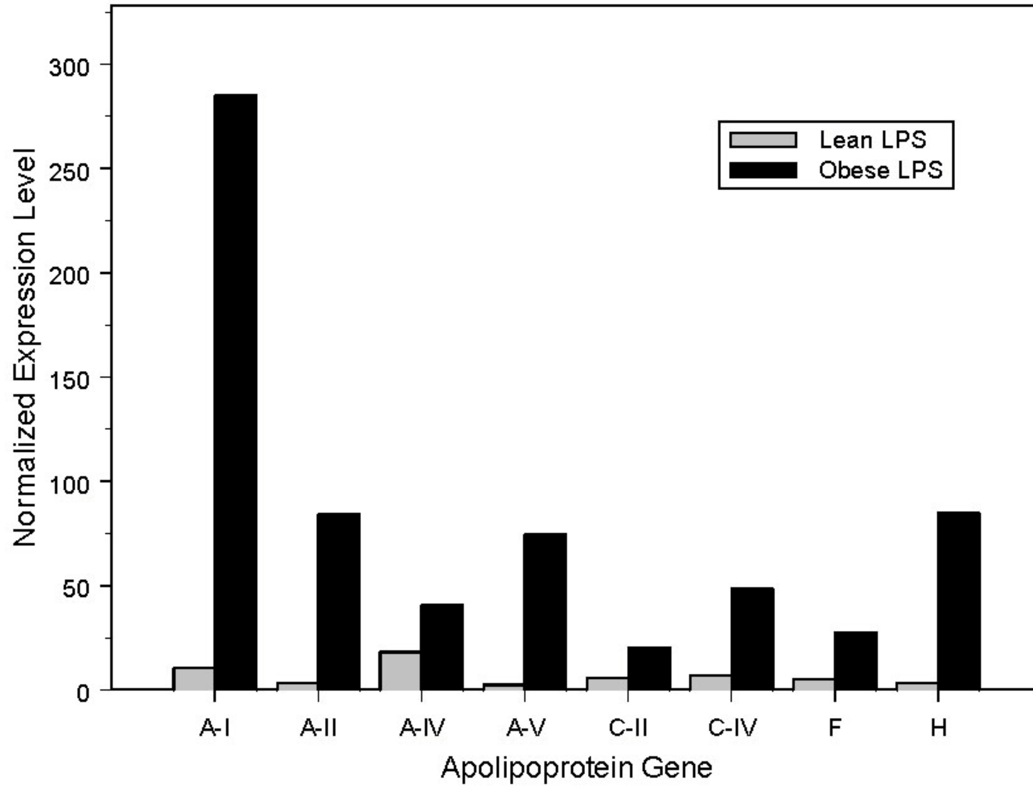

Figure 5. Brain RNA expression of apolipoprotein in obese and lean mice following LPS stimulation.

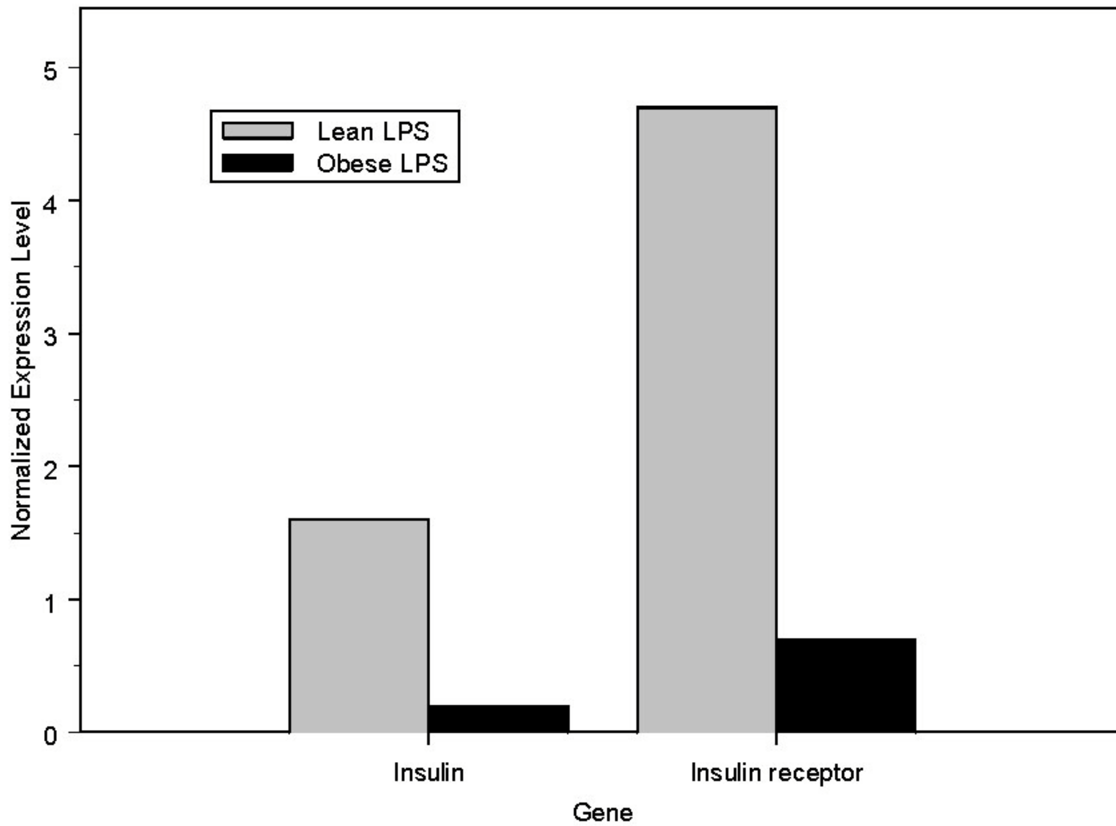

Figure 6. Differences in brain RNA expression in insulin and the insulin receptor in obese mice subjected to LPS or saline administration.

our recent understanding of insulin's intricate involvement in the inflammatory process, this decreased expression may offer some insight into the chronic inflammatory state of obesity and the response of the obese to an inflammatory insult (17).

5. DISCUSSION

The use of microarray in profiling biologic functions or as a discovery tool has proven itself as a valuable tool in many disciplines including neuroscience. The ability to investigate numerous biologic functions and pathways has added an additional tool in neuroscience research. Studying complex issues like sepsis, in a complex model such as obesity, would be daunting if not impossible, without this technology. However, there is a paradigm shift away from isolated molecular investigation to multi-system approach. This has placed more burdens on the bioinformatist or biostatistician and has positioned computational methods and technologies central in the research plan and design.

The recent use of microarray technology has allowed the examination of large-scale gene expressions in a variety of diseases including inflammatory states (18-21). Examining large-scale gene expression provides an opportunity to develop an expression pattern or profile that is unique to a stimulus or the host. This unique profile can provide define the biologic pathways that contribute to or are activated by the host's response to an insult or injury.

Current microarray chips allow whole genome evaluation in both the human and mouse. Using this whole genome approach, a large-scale analysis of the RNA signature of multiple organs is possible. Specific gene chips that identify major cytokines require pre-determination of areas to investigate and prevent observation of the genomic influences of unrecognized pathways that may be operational. Narrowing the genes investigated also reduces the chance of identifying pathways or genes that may be involved in obesity and immune response that have not previous been identified.

This study demonstrates the power of microarray technology by identifying several pathways that may help better understand the neuro-endocrine response to an inflammatory insult and identities pathways that may help in the understanding of the influence obesity has on the brains response to sepsis or inflammation. The most obvious standout is the changes noted in the coagulation systems. Two genes deserve special mention due to their relation to sepsis. In the past several years the interplay between sepsis, organ failure and the coagulation system has been extensively investigated (22). Patients with severe sepsis demonstrate decreased concentration of protein C and elevated PAI-1 resulting in decreased fibrinolysis and increased thrombogenesis (23). This tendency promotes microvascular clotting, tissue hypoxia and subsequent organ dysfunction. As shown in Figure 3, the expression pattern would appear to promote intravascular coagulation and poor fibrinolysis. This requires validation and further investigation noting the heightened interest in the sepsis/coagulation axis.

The neuro-endocrine and lipid transport systems also require further investigation and validation. Noting the changes in GABA receptor expression may expand our understanding of the brain response to severe infection and inflammation and may lead to further insights in understanding sepsis associated encephalopathies.

The differential expression of the obese and lean in the insulin gene and receptor is intriguing. Obesity is known to be a "low grade" inflammatory process with well documented increases in TNF and known exaggerated cytokines responses to inflammatory stimulus (3, 5). Insulin is known to inhibit NF kappa B activity which is the nuclear trigger of numerous inflammatory cytokines. The reduced expression of the insulin gene and, more importantly the insulin receptor may provide some insight into this inflammatory state documented in the obese.

6. CONCLUSIONS

This study has demonstrated that substantial differences the genomic expression profiles of obese and lean mice after LPS injection exist. Seventy eight genes were statistically isolated to show a greater than 10-fold expression difference between the obese and lean mice following induction of inflammation. These expression profiles suggest unfavorable coagulation expressions in the obese, along with alterations in GABA receptors, lipid transport and down regulation in insulin and insulin receptor.

7. ACKNOWLEDGEMENTS

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