Aging Cell (2013) **12**, pp194–206 Doi: 10.1111/acel.12040

Gene expression profile of mouse white adipose tissue during inflammatory stress: age-dependent upregulation of major procoagulant factors

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Summary

Tolerance to physiological stress resulting from inflammatory disease decreases significantly with age. High mortality rates, increased cytokine production, and pronounced thrombosis are characteristic complications of aged mice with acute systemic inflammation induced by injection with lipopolysaccharide (LPS). As adipose tissue is now recognized as an important source of cytokines, we determined the effects of aging on visceral white adipose tissue gene expression during LPS-induced inflammation in male C57BL/6 mice. Microarray analysis revealed that the expression of 6025 genes was significantly changed by LPS; of those, the expression of 667 showed an age-associated difference. Age-associated differences were found in many genes belonging to the inflammatory response and blood clotting pathways. Genes for several procoagulant factors were upregulated by LPS; among these, tissue factor, thrombospondin-1, and plasminogen activator inhibitors-1 and -2, exhibited age-associated increases in expression which could potentially contribute to augmented thrombosis. Further analysis by qRT–PCR, histological examination, and cell fraction separation revealed that most inflammatory and coagulant-related gene expression changes occur in resident stromal cells rather than adipocytes or infiltrating cells. In addition, basal expression levels of 303 genes were altered by aging, including increased expression of component of Sp100-rs *(Csprs)*. This study indicates that adipose tissue is a major organ expressing genes for multiple inflammatory and coagulant factors and that the expression of many of these is significantly altered by aging during acute inflammation. Data presented here provide a framework for future studies aimed at elucidating the impact of adipose tissue on age-associated complications during sepsis and systemic inflammation.

Key words: adipose tissue; aging; coagulation; lipopolysaccharide; microarray; systemic inflammation.

Introduction

The rate of hospitalization and mortality from critical illnesses associated with the systemic inflammatory response syndrome (SIRS) is known to increase with age (Angus *et al.*, 2001; Marik, 2006; Martin *et al.*, 2006; Xin *et al.*, 2008; Ellis *et al.*, 2009; Pisani, 2009); however, the exact mechanisms for this age-associated vulnerability remain largely unknown. SIRS results in widespread inflammation and disseminated intravascular coagulation (DIC) which are major contributors to mortality in critical illnesses. While many attribute a decrease in physiological capacity and reduced ability to respond to stress as primary factors involved in age-associated susceptibility, augmented inflammation and coagulation in the aged are likely the driving factors.

Previous studies have demonstrated that aged mice, as compared with young-adult mice, are significantly more susceptible to death in models of SIRS induced by injection with lipopolysaccharide (LPS) (Chorinchath *et al.*, 1996; Tateda *et al.*, 1996; Saito *et al.*, 2003; Gomez *et al.*, 2006; Starr *et al.*, 2010). During LPS-induced inflammatory stress, visceral white adipose tissue, among other major organs, produces the highest levels of inflammatory cytokine interleukin-6 (IL-6), and the levels of adipose-derived IL-6 are significantly greater in aged compared with young mice (Starr *et al.*, 2009).

While adipose tissue dysfunction in obesity is a heavily studied area of research, age-associated differences in adipose tissue regulation remain largely understudied. Recent evidence suggests that the mechanisms of adipose tissue dysfunction in obesity differ from that of aging (Tchkonia *et al.*, 2010; Lumeng *et al.*, 2011). In obesity, macrophage infiltration due to adipocyte death is well documented to promote adipose tissue inflammation, however, adipocyte death and macrophage infiltration are not commonly observed in adipose tissue due to aging. Furthermore, aged IL-6 knockout mice, known to develop adult-onset obesity (Wallenius *et al.*, 2002), are more resistant to LPS than normal weight wild-type mice (Gomez *et al.*, 2006; Starr *et al.*, 2009), which suggests that the quality of the adipose tissue rather than the size of the organ plays a major role in regulating inflammation.

Based on our previous studies we hypothesized that adipose tissue likely produces a number of factors which may be significantly augmented in an age-dependent fashion and negatively affect tolerance to SIRS in the aged. The purpose of this study was to identify such factors by profiling adipose tissue for age-associated changes in gene expression during acute inflammatory stress induced by LPS. Results from our gene array analysis identify a role for adipose tissue in the regulation of inflammatory and coagulant processes which may contribute to age-associated vulnerability to inflammatory disorders such as SIRS and sepsis.

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Accepted for publication *15 December 2012*

Results

Hypothermia and plasma cytokine parameters confirm an age-associated increase in severity of systemic inflammation

Young and aged mice were injected intraperitoneally with bacterial endotoxin lipopolysaccharide (LPS) to elicit systemic inflammation. The average body weight of young mice (n = 12) and aged mice (n = 12) was 28.3 ± 1.45 and 32.8 ± 2.15, respectively; aged mice were significantly heavier than young ($P < 0.001$). These mice were sacrificed 3, 6, and 12 h after LPS injection along with control mice (0 h) which did not receive injection (n = 3 per time point in each age group). No mortality occurred during this 12-h period. For the purpose of understanding physiological mechanisms for age-associated vulnerability to SIRS, the dose of LPS (2.5 mg kg^{-1}) was chosen based on our previous data which show no mortality in young mice and 80% mortality in aged mice which usually occurs more than 12 h after LPS injection (Starr *et al.*, 2010). Hypothermia was evident after LPS injection in both age groups with a more pronounced decline in body temperature observed in the aged (31.9 ± 1.50°C vs. 28.6 ± 1.87°C, 12 h after LPS in young and aged, respectively, $P < 0.01$), confirming that the aged suffer more severe systemic inflammation (Saito *et al.*, 2003).

In addition, plasma levels of inflammatory cytokines tumor necrosis factor alpha (TNFα), interleukin 1 beta (IL-1β), and IL-6 were measured (Fig. 1A–C). Induced plasma levels of the two early cytokines, IL-1β and TNFα, tended to be higher in aged mice compared with young mice, although statistical significance was achieved only for TNFα 6 h after LPS injection. Plasma IL-6 levels were elevated in both young and aged mice 3 h after LPS injection with more pronounced and prolonged elevation in the aged. The augmented plasma cytokine levels and profound hypothermia in aged mice after LPS injection reflect an age-associated increase in the severity of systemic inflammation, providing evidence that the response of these mice, although small in number, is representative of previously published findings (Saito *et al.*, 2003; Starr *et al.*, 2009) and that adipose tissue obtained from them for microarray would produce results reasonably representative of a larger population.

LPS-induced plasma levels of adiponectin decreased approximately twofold; there was no age-associated difference in the extent or rate of the decrease (Fig. 1D). Plasma levels of leptin were similar in young and aged mice before LPS injection and exhibited a bimodal curve after LPS injection, peaking first at 3 h and then again at 12 h in both young and aged mice with the difference in induced leptin level at 3 h significantly higher in aged compared with young (Fig. 1E).

Total RNA was isolated from the epididymal fat pads of these young and aged mice that were sacrificed 0, 3, 6, and 12 h after LPS injection; the RNA samples were used for the following gene expression analyses.

Global analysis of adipose tissue gene expression changes during systemic inflammation

Gene expression in the epididymal adipose tissues of young and aged mice 0, 6, and 12 h after LPS injection was compared by Affymetrix Mouse Genome 430 2.0 Arrays. We previously showed that LPS-induced IL-6 expression was significantly stronger in epididymal adipose tissue compared with other types of adipose tissue (Starr *et al.*, 2009), thus we focused this gene expression analysis on the epididymal adipose tissue depot. Principal components analysis (PCA), a multivariate method of partitioning sources of variability, was used to explore the overall effects of aging on LPS-induced gene expression changes from adipose tissue. In short, PCA is a means of identifying how similar or dissimilar groups of samples are in relation to one another. PCA demonstrated that systemic

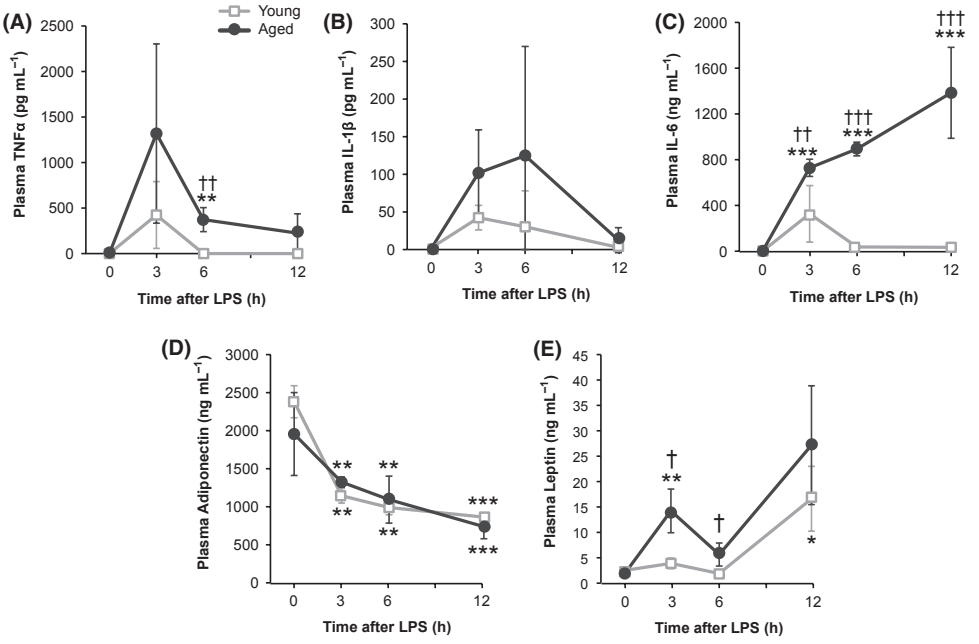

Fig. 1 Plasma cytokine/hormone levels. Systemic inflammation was induced in young (4 months) and aged (24 months) mice by LPS (2.5 mg kg^{-1}, i.p.). Plasma (A) TNFα, (B) IL-1β, (C) IL-6, (D) adiponectin, and (E) leptin levels were measure by ELISA. Open squares: young; Closed circles: aged. Data are expressed as the mean ± standard deviation, n = 3 for each time point of each age group. * indicates a statistically significant change as compared with the 0-h time point of the same age group. † indicates a statistically significant difference between young and aged of the same time point. One, two, or three symbols signify $P < 0.05$, 0.01, or 0.001, respectively.

inflammation, induced by injection with LPS, results in substantial gene expression changes within the adipose tissue. Furthermore, within the LPS-treated groups, gene expression varies significantly by age and time point, as depicted by the well-defined clustering of treatment groups (Fig. S1).

To refine the data into manageable numbers, the gene probes were classified into specific categories of interest based on type of gene expression change. A thorough explanation of this classification system can be found in the Supporting Information (Fig. S2). Briefly, of all the probes expressed in adipose tissue (30 043), 97.8% were constitutively expressed (i.e. expressed at the 0-h time point) and the remaining 2.2% were expressed only after LPS treatment. The expression level of 26.4% (7938 probes) was significantly changed ($P < 0.01$) by LPS treatment and had a fold change ≥ 2; among these, 10.5% (831 probes) exhibited age-dependent changes with a fold change ≥ 1.5. Genes with the highest LPS-induced fold change, regardless of age, are listed in Table 1; this list is limited to the ten most upregulated and ten most downregulated genes. Filtered gene lists (Tables S1–S3, Supporting Information) were generated after statistical analysis and genes were separated into groups based on significantly changed gene expression after LPS injection (Table S1), where the effect of LPS was different by aging (Table S2), and changes by aging *per se* (Table S3).

The time points of 6 and 12 h were used in our microarray study to avoid missing a significant portion of gene expression changes. Our experimental design increases the likelihood that most gene expression changes were observed; if only the 6-h time point had been used, approximately 10–15% of gene expression changes would have been missed, and if only the 12-h time point had been used, approximately 55% of gene expression changes would have been missed.

Pathway analysis: LPS-induced changes

To further evaluate the data on a biological level, pathway analysis was performed using Gene Map Annotator and Pathway Profiler (GenMAPP) software package, a computer program designed for analyzing genome-scale experiments (Dahlquist *et al.*, 2002; Doniger *et al.*, 2003; Salomonis *et al.*, 2007). Table 2 lists biological pathways which are overrepresented upon LPS injection (pathways in which the expression level of significantly more genes were changed than would be expected by chance). A significant change is defined by an LPS-induced fold change greater than 2.0. The upper half of the table lists pathways which were significantly upregulated after LPS injection; among the highest ranking were *Cytokines and Inflammatory Response, Apoptosis, Inflammatory Response Pathway,* and *Blood Clotting Cascade*. The lower half of the table lists significantly downregulated pathways; *Cholesterol Biosynthesis, Fatty Acid Synthesis, Fatty Acid Oxidation,* and *Adipogenesis* were among the highest ranking. In addition to evaluating pathways for LPS-mediated change, statistical measures were employed to

Table 1 List of genes in the adipose tissue with the highest fold change after lipopolysaccharide (LPS) injection

Probeset ID	Gene title	Gene symbol	Fold change†	*P* value
Upregulated by LPS				
1427381at	Immunoresponsive gene 1	Irg1	1164.9 (A6 h)	<0.001
1419427at	Colony stimulating factor 3 (granulocyte)	Csf3	947.8 (A6 h)	<0.001
1450788at	Serum amyloid A 1	Saa1	702.2 (Y12 h)	0.005
1420330at	C-type lectin domain family 4, member e	Clec4e	596.0 (Y12 h)	<0.001
1450297at	Interleukin 6	Il6	554.6 (A6 h)	<0.001
1419728at	chemokine (C-X-C motif) ligand 5	Cxcl5	522.6 (A12 h)	<0.001
1438148at	Chemokine (C-X-C motif) ligand 3	Cxcl3	483.4 (A12 h)	0.001
1450322sat	Schlafen 3	Slfn3	448.9 (Y6 h)	<0.001
1417256at	Matrix metallopeptidase 13	Mmp13	309.9 (A6 h)	0.004
1450808at	Formyl peptide receptor 1	Fpr1	307.1 (Y12 h)	<0.001
Downregulated by LPS				
1453588at	Carbonic anhydrase 3	Car3	−241.2 (Y12 h)	<0.001
1421555at	Adrenergic receptor, beta 3	Adrb3	−207.3 (Y12 h)	<0.001
1437672at	Insulin receptor substrate 3	Irs3	−147.0 (Y12 h)	<0.001
1423439at	Phosphoenolpyruvate carboxykinase 1, cytosolic	Pck1	−135.6 (Y12 h)	<0.001
1449918at	CD209 g antigen	Cd209 g	−87.0 (A12 h)	<0.001
1438309at	Activin A receptor, type IC	Acvr1c	−84.7 (Y12 h)	<0.001
1429679at	Leucine rich repeat containing 17	Lrrc17	−72.2 (A12 h)	<0.001
1428954at	Solute carrier family 9, member 3 regulator 2	Slc9a3r2	−58.0 (A6 h)	<0.001
1449860at	HIG1 domain family, member 1B	Higd1b	−49.9 (Y6 h)	<0.001
1419564at	Zinc finger protein 467	Zfp467	−45.1 (A12 h)	<0.001

Gene Lists were compiled from the top ten genes which had the largest fold change after LPS injection, regardless of age.
†Indicates fold change of gene expression as compared with 0-h control of the same age group; age and time point in parenthesis refer to which sample the fold change was observed in. Positive values indicate upregulation by LPS and negative values indicate downregulation by LPS. Rikens, predicted genes, and nameless probesets were excluded.

Table 2 List of biological pathways that are significantly up- or downregulated in the adipose tissue after lipopolysaccharide (LPS) injection

Pathway name in GenMAPP	LPS-induced changes in young			LPS-induced changes in aged			Age-associated changes			Age-associated[§] significance
	%[†] changed	*z* score	*P* value	%[†] changed	*z* score	*P* value	%[‡] changed	*z* score	*P* value	
Upregulated by LPS										
Cytokines and inflammatory response	76.5	**5.52**	**<0.001**	82.4	**6.03**	**<0.001**	70.6	**6.05**	**<0.001**	***
Apoptosis	41.5	**4.44**	**0.001**	46.3	**5.52**	**<0.001**	28.0	**2.89**	**0.004**	**
Inflammatory response pathway	51.4	**4.32**	**<0.001**	51.4	**4.23**	**<0.001**	51.4	**5.63**	**<0.001**	***
Blood clotting cascade	61.5	**3.51**	**0.002**	46.2	**2.11**	**0.033**	38.5	**2.15**	**0.055**	*
Hypertrophy model	50.0	**2.94**	**0.012**	61.1	**4.02**	**<0.001**	50.0	**3.86**	**<0.001**	***
IL-1 NetPath 13	40.5	**2.82**	**0.009**	48.6	**3.94**	**<0.001**	24.3	1.31	0.250	
IL-2 NetPath 14	34.2	**2.66**	**0.011**	45.2	**4.85**	**<0.001**	31.5	**3.53**	**<0.001**	***
TGF beta signaling pathway	35.4	**2.35**	**0.022**	37.5	**2.62**	**0.008**	35.4	**3.59**	**<0.001**	***
TNF-alpha-NF-kB NetPath 9	28.5	**2.27**	**0.025**	30.2	**2.69**	**0.006**	18.6	0.80	0.458	
MAPK signaling pathway KEGG	29.4	**2.17**	**0.038**	28.6	1.83	0.075	20.6	1.31	0.191	
IL-7 NetPath 19	30.0	1.30	0.239	47.5	**3.92**	**<0.001**	27.5	1.91	0.091	
Striated muscle contraction	29.4	1.12	0.294	47.1	**3.55**	**0.001**	29.4	2.06	0.059	
Adipogenesis	27.1	1.49	0.140	32.2	**2.74**	**0.009**	22.9	1.94	0.064	
IL-4 NetPath 16	31.6	1.85	0.080	36.8	**2.74**	**0.009**	26.3	**2.04**	**0.044**	*
IL-6 NetPath 18	26.3	1.14	0.315	32.6	**2.55**	**0.009**	16.8	0.12	1.000	
IL-5 NetPath 17	31.7	1.98	0.072	34.9	**2.51**	**0.019**	23.4	1.95	0.069	
IL-9 NetPath 20	38.1	1.85	0.105	42.9	**2.32**	**0.035**	14.3	−0.26	1.000	
Kit receptor NetPath 6	25.0	0.67	0.56	32.8	**2.12**	**0.048**	20.3	0.85	0.398	
Downregulated by LPS										
Cholesterol biosynthesis	80.0	**6.15**	**<0.001**	80.0	**4.77**	**<0.001**	20.0	−0.37	0.786	
Fatty acid synthesis BiGCaT	61.9	**5.14**	**<0.001**	66.7	**4.26**	**<0.001**	23.8	−0.03	1.000	
Adipogenesis	33.9	**4.40**	**<0.001**	34.7	**2.20**	**0.029**	17.8	−1.64	0.114	
Fatty acid beta Oxidation meta	46.7	**4.00**	**0.001**	46.7	**2.59**	**0.008**	23.3	−0.10	1.000	
Mitochondrial fatty acid betaoxidation	56.3	**3.90**	**0.001**	75.0	**4.47**	**<0.001**	18.8	−0.50	0.787	
Glycogen metabolism	45.5	**3.27**	**0.004**	50.0	**2.57**	**0.009**	23.9	**3.18**	**0.004**	##
Id (Inhibitor of DNA) NetPath 5	36.6	**3.01**	**0.005**	34.1	1.19	0.308	26.8	0.41	0.721	
Heme biosynthesis	55.6	**2.87**	**0.013**	88.9	**4.30**	**<0.001**	66.7	**2.99**	**0.008**	**
Nuclear receptors	37.5	**2.79**	**0.008**	50.0	**3.10**	**0.003**	21.9	−0.3	0.836	
Triacylglyceride synthesis BiGCaT	40.9	**2.72**	**0.015**	36.4	1.11	0.325	27.3	**2.49**	**0.032**	#
Eicosanoid synthesis	40.0	**2.49**	**0.029**	50.0	**2.45**	**0.013**	25.0	0.09	1.000	
Focal adhesion KEGG	24.4	**2.07**	**0.060**	30.2	1.29	0.225	22.1	−0.64	0.577	
Amino acid metabolism	27.2	**2.04**	**0.046**	28.4	0.49	0.709	21.0	−0.67	0.540	
Hedgehog Netpath 10	33.3	1.76	0.086	52.4	**2.76**	**0.010**	42.9	2.01	0.074	
Glycolysis and gluconeogenesis	25.6	1.16	0.289	41.0	**2.15**	**0.049**	30.8	0.98	0.347	

Pathway analysis was performed using the Mm_Contributed_20080619 pathway set in GenMAPP. Pathways listed include those whose differentially expressed genes were significantly overrepresented in either young or aged mice after LPS injection. Bolded values indicate statistical significance where criteria were set at $z > 2.0$ and $P < 0.05$.

[†]% Changed for "LPS-induced changes in young or aged" indicates the percent of genes within a respective pathway exhibiting a greater than twofold change after LPS injection.

[‡]% Changed for "Age-associated Changes" indicates the percent of genes within a respective pathway where the change in aged was 1.5-fold different than the change in young.

[§]* indicates >twofold up- or downregulation by LPS which is further enhanced 1.5-fold by aging; # indicates >twofold downregulation by LPS in young which is either not downregulated in aged or the degree of downregulation differed by <1.5-fold; * or # indicates $P < 0.05$, ## or ** indicates $P < 0.01$, and *** indicates $P < 0.001$.

discover pathways which showed significant age-associated differences after LPS (young 6 h compared with aged 6 h OR young 12 h compared with aged 12 h). The significantly upregulated pathways showing age-associated differences were *Cytokines and Inflammatory Response; Apoptosis; Inflammatory Response Pathway; Blood Clotting Cascade; Hypertrophy Model;* and *IL-2, IL-4, and TGFβ signaling pathways*. Individual gene expression changes within the top four pathways are listed in Table S5. Only three significantly downregulated pathways showed an age-dependent difference: *Glycogen metabolism, Heme Biosynthesis*, and *Triacylglyceride Synthesis*.

Real-time qRT–PCR validation of microarray results: LPS-induced changes

To confirm our microarray data, we selected several inflammatory and coagulant genes for validation by real-time qRT–PCR. For these validation experiments we included an additional time point of 3 h after LPS injection to better characterize the expression kinetics of these LPS-inducible genes in the adipose tissue. Figure 2(A–D) shows comparisons between qRT–PCR data and microarray results for the selected inflammatory genes; for all genes validated, the results are in strong agreement with the original microarray data.

TNFα was induced by LPS approximately 20- to 30-fold, although not in an age-dependent manner (Fig. 2A). IL-1α was present in the adipose tissue of both young and aged mice and a statistically significant induction was observed 3, 6, and 12 h later in aged animals only (Fig. 2B). Both IL-1β and IL-6 mRNA show a strong age-dependent induction in the adipose tissue after LPS injection. At the peak time point of 3 h, IL-1β and IL-6 were increased more than 400- and 3000-fold, compared with 0 h controls, respectively (Fig. 2C and D). The overall expression kinetics of TNFα, IL-1β, and IL-6 in adipose tissue tend to correlate with plasma levels of these proteins (shown in Figure 1); however, mRNA expression of these factors in the aged was sustained at significantly higher than basal levels for a longer period of time than circulating protein levels were elevated.

Figure 2(E–J) shows results of both qRT–PCR and microarray analyses for the selected coagulant genes; for all genes, the results from the PCR validation are similar to the original microarray data. Thrombospondin-1 (Thbs-1) expression in the adipose tissue from aged mice increased significantly (more than 15-fold) after LPS injection, whereas no significant rise in Thbs-1 levels was observed in young adipose tissue (Fig. 2E). PAI-1 also showed a strong age-dependent induction (70-fold) in the adipose tissue 3 h after LPS injection (Fig. 2F). PAI-2 showed an even more dramatic age-dependent induction than PAI-1; PAI-2 mRNA levels in the adipose tissue were significantly elevated (200- to 300-fold, $P < 0.001$) in aged but not young mice after LPS injection (Fig. 2G). Tissue factor (TF) was also induced only in aged adipose tissue after LPS injection (Fig 2H). Fibrinogen-like protein 2 (Fgl-2) was induced gradually after LPS injection in both young and aged adipose tissue for 6 h; after 6 h the level of Fgl-2 mRNA in aged adipose tissue continued to rise, whereas the level of Fgl-2 mRNA in young adipose tissue remained constant (Fig. 2I). Interestingly, the mRNA levels of coagulation factor XIIIa, a protein responsible for stabilizing fibrin clots, declined in both age groups after LPS injection (Fig. 2J), suggesting that factor XIIIa is not causally involved in the previously reported age-associated increase in blood clotting during systemic inflammatory stress (Starr *et al.*, 2010).

To evaluate the contribution of adipose-derived inflammatory cytokine and coagulant factor gene expression relative to other major organs, the expression of selected genes was compared between adipose tissue, liver, and kidney by qRT–PCR. As shown in Figure 3, many of the above-mentioned inflammatory and coagulant factors are more abundantly expressed in the adipose tissue compared with the kidney and liver which is more commonly known to highly express coagulation factors. Six hours after LPS injection, IL-6, Thbs-1, PAI-1, and PAI-2 are more strongly expressed in the adipose tissue compared with liver and kidney; in addition, these factors have significant age-associated differences in expression (Fig. 3A–D). Tissue factor (TF) mRNA expression was significantly higher in aged adipose tissue, liver, and kidney compared with young, with the kidney being the major source of TF among the three tissues (Fig. 3E). Fgl-2 was more strongly expressed in the adipose tissue compared with liver and kidney, but no significant age-associated difference was observed in the adipose tissue at this time point (Fig. 3F). In addition, mRNA expression of classical coagulation factors X, VIII, and V was compared among adipose tissue, liver, and kidney (Fig. 3G–I), whereas the liver is the predominant source of these coagulation factors, FVIII also showed strong expression in the adipose tissue and kidney.

LPS-induced gene expression changes in adipose tissue are not due to infiltrating cells

Histological analysis of H&E stained adipose tissue sections from young and aged mice 0, 6, or 12 h after LPS injection did not reveal any significant signs of acute inflammation or inflammatory cell infiltration (Fig. 4A). Sporadic areas of dense lymphocyte populations were observed in some sections, usually occurring around the perimeter of the tissue; however, these areas were not specific to any particular treatment or age group. In addition, analysis of gene expression of macrophage markers in the adipose tissue did not indicate significant age-associated increases by LPS injection. The expression of F4/80 (Fig. 4B) was 1.4-fold higher in adipose tissue from aged mice compared with young at the 0-h time point; however, F4/80 expression decreased in both age groups in response to LPS injection, without any significant age-related difference. Another general macrophage marker, CD11b (also known as integrin alpha M, a component of Mac-1, Fig. 4C), showed a 2.3-fold increase in gene expression 12 h after LPS injection; this increase was significant ($P < 0.01$), however, not altered by aging. CD11b is not specific to macrophages and can also be expressed by monocytes, granulocytes, and lymphocytes. Gene expression of the more specific macrophage markers, CD11c (specific for infiltrating M1 type macrophages, Fig. 4D) and CD206 (specific for resident M2 type macrophages, Fig. 4E) were both significantly downregulated by LPS in adipose tissue from young and aged mice, without age-associated significance. Taken together, these data do not give any indication that there was significant inflammatory cell infiltration into the adipose tissue upon LPS injection which could account for the large age-associated differences in cytokine and coagulation factor gene expression.

LPS-induced gene expression changes are localized primarily in the stromal vascular fraction (SVF)

To determine which fraction or cell type within the adipose tissue was more sensitive to LPS-induced changes in gene expression of cytokines and coagulant factors, we harvested epididymal adipose tissue from young and aged mice 6 h after injection with LPS. Noninjected mice were used as controls. After adipocytes and stromal vascular fraction (SVF) cells were separated by collagenase digestion and centrifugation, total RNA was isolated from each fraction for analysis by qRT–PCR. Due to low RNA yield, samples were pooled ($n = 5$ for each age and treatment group). The minimum level of adiponectin expression (specific to adipocytes) in the SVF demonstrates that the SVF was relatively pure and largely free of contamination with adipocytes (Fig. 5A). Likewise, the expression of F4/80 was limited to the SVF fraction and largely absent in the adipocyte fraction (Fig. 5B); a slight elevation in F4/80 expression in the aged adipocyte fraction with LPS could indicate a small amount of contaminating SVF cells. Gene expression of IL-6,

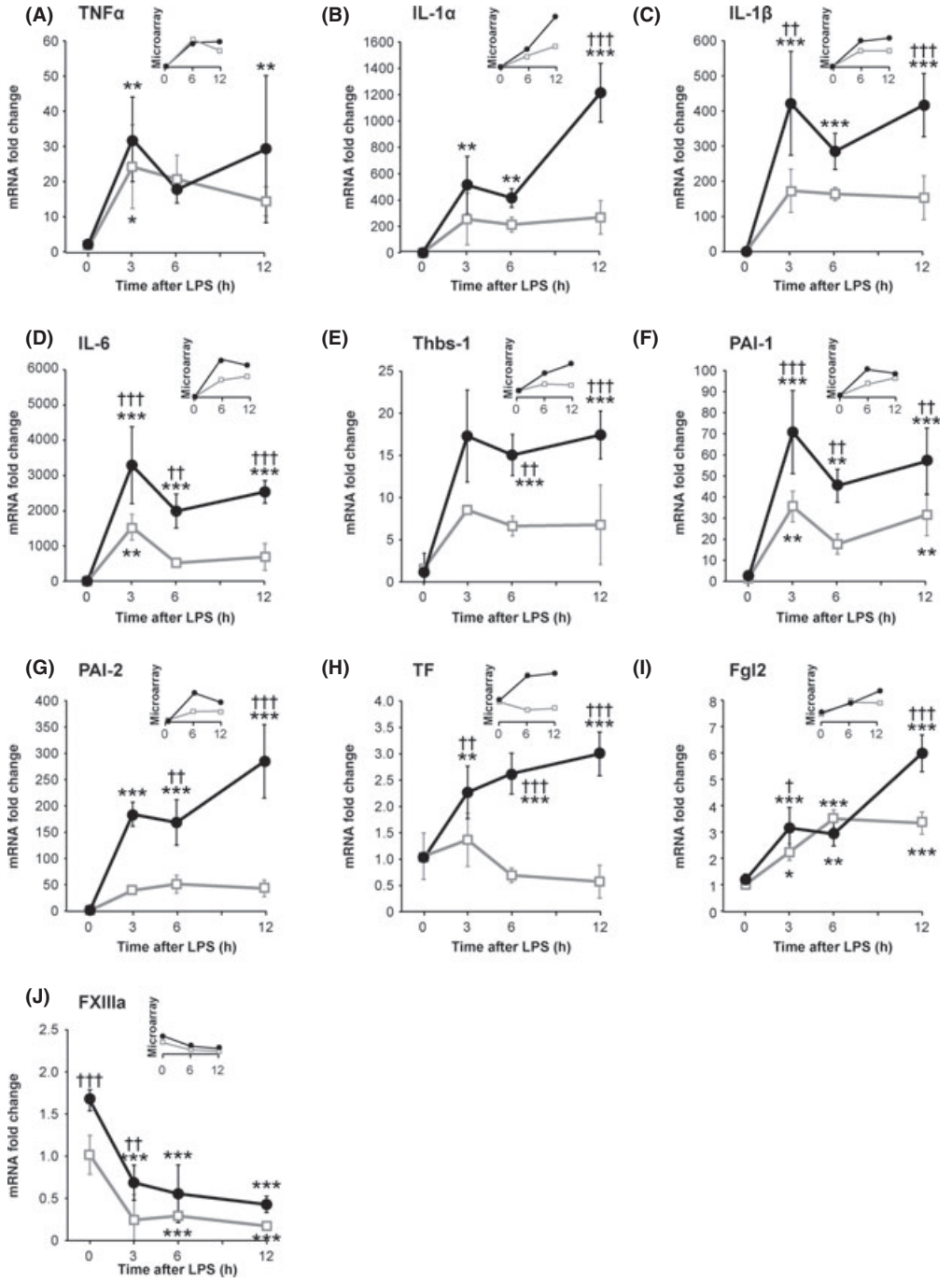

Fig. 2 Real-time quantitative RT–PCR validation of selected inflammatory and procoagulant genes. Time course of LPS-mediated induction of (A) TNFα, (B) IL-1α, (C) IL-1β, (D) IL-6, (E) thrombospondin-1 (Thbs-1), (F) plasminogen activator inhibitor 1 (PAI-1), (G) plasminogen activator inhibitor 2 (PAI-2), (H) tissue factor (TF), (I) fibrinogen-like protein 2 (Fgl-2), and (J) coagulation factor XIIIa (F13a) in adipose tissue from young and aged mice. Open squares: young; Closed circles: aged. Data are expressed as the mean ± standard deviation, $n = 3$ for each time point of each age group. * indicates a statistically significant change as compared with the 0-h time point of the same age group. † indicates a statistically significant difference between young and aged of the same time point. One, two, or three symbols signify $P < 0.05$, 0.01, or 0.001, respectively. Statistical significance between young and aged adipose tissue mRNA of PAI-2 and Thbs-1 at the 3-h time point could not be calculated due to deficient number of young samples.

TNFα, and IL-1β was largely localized to the SVF of the adipose tissue (Fig. 5C–E). Similar results were obtained by analysis of procoagulant factors PAI-1, PAI-2, Thbs-1, and TF (Fig. 5F–I); an increase in expression after LPS injection was observed in the SVF component of the adipose tissue. Upregulation of PAI-1, Thbs-1, and TF in adipocytes, particularly from aged mice, was more sensitive to LPS than for other genes. This data indicate that whereas adipocytes, particularly from the aged, express cytokines and

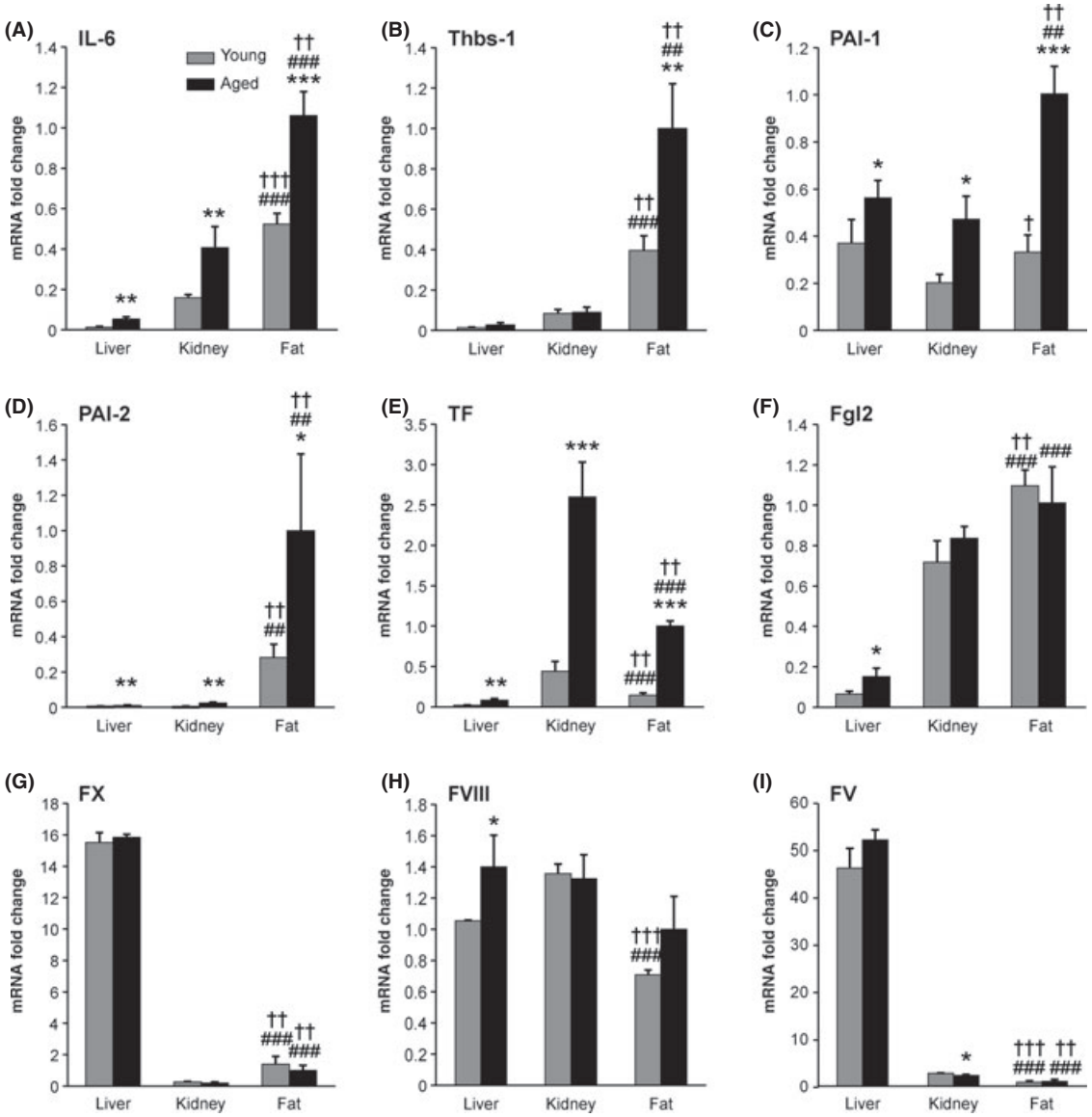

Fig. 3 Comparison of selected inflammatory genes and coagulation factors among adipose tissue, liver, and kidney. The expression of (A) IL-6, (B) Thbs-1, (C) PAI-1, (D) PAI-2, (E) TF, (F) Fgl-2, (G) coagulation factor X (FX), (H) coagulation factor VIII (FVIII), and (I) coagulation factor V (FV) was compared among liver, kidney, and adipose tissue (Fat) of young and aged mice 6 h after LPS injection. Gray bars: young; Black bars: aged. Data are expressed as the mean ± standard deviation, $n = 3$ for each age group. * indicates a statistically significant change between young and aged of the same tissue. # indicates a statistically significant change between Fat and Liver of the same age group. † indicates a statistically significant change between Fat and Kidney of the same age group. One, two, or three symbols signify $P < 0.05$, 0.01, or 0.001, respectively.

coagulation factors at modest levels, cells of the SVF are more responsive to LPS and overall express more of these factors when accounting for total RNA yield from the epididymal adipose depot.

Analysis of adipose tissue gene expression changes by aging *per se*

Because of the abundant interest in 'normal aging' or chronic age-associated changes, we devoted part of our analysis to evaluating age-associated changes in the adipose tissue from control animals only. Of the 29,382 probes in the microarray that were normally expressed, the expression of 811 or 2.8% was significantly changed by aging ($P < 0.01$). Of those 811, the expression of 399 or 1.3% was significantly changed with a fold change greater than 1.5; approximately 72% of these were upregulated, whereas the remaining 28% were downregulated. The ten most upregulated and ten most downregulated genes are listed in Table S6. Interestingly, the gene with the highest fold change (approximately 36-fold higher in aged adipose tissue compared with young) was component of Sp100-rs (Csprs). The function of this gene is unknown although it likely encodes a G-protein coupled receptor with homology to a thrombin receptor precursor (Weichenhan *et al.*, 2001). The age-associated induction of Csprs expression was confirmed by qRT–PCR (Fig. 6A). Both microarray and qRT–PCR confirm a significant increase in Csprs expression with aging at the 0-h time point (basal level); however, the original microarray data did not reveal a statistically significant

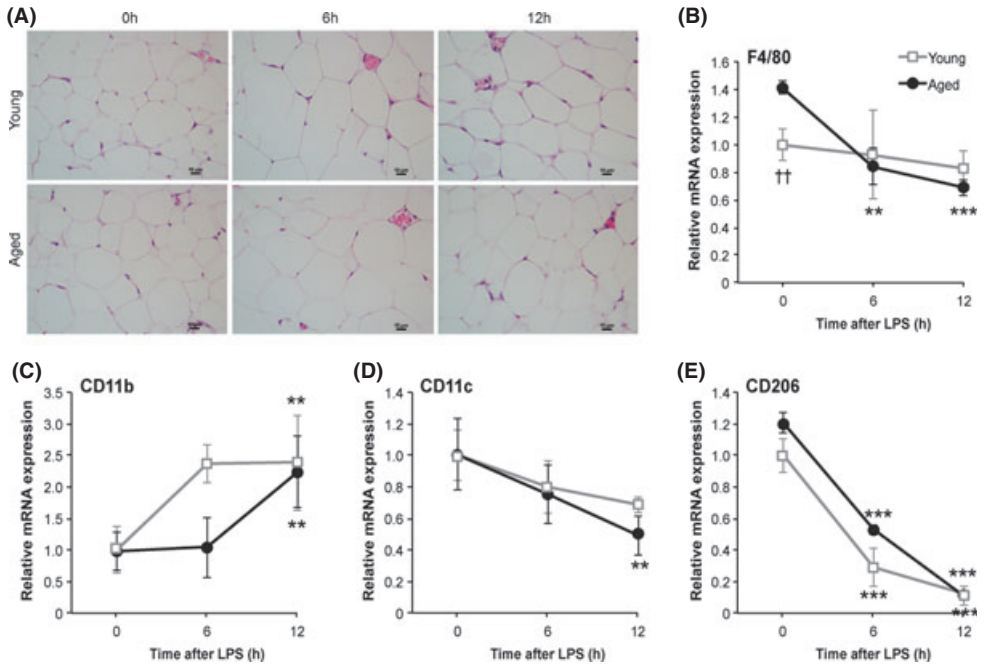

Fig. 4 LPS-induced gene expression changes are not due to infiltrating cells. (A) Histological analysis demonstrating a lack of inflammatory cell infiltration in adipose tissue after LPS injection. Sections from formalin-fixed, paraffin-embedded epididymal adipose tissue samples were stained with H&E. (B–E) Gene expression of general macrophage markers (B) F4/80 and (C) CD11b, infiltrating M1 type macrophages (D) CD11c, and resident M2 type macrophages (E) CD206 were evaluated for signs of infiltration. Open squares: young; Closed circles: aged. Data are expressed as the mean ± standard deviation, $n = 3$ for each age group. * indicates a statistically significant change as compared with the 0-h time point of the same age group. † indicates a statistically significant difference between young and aged of the same time point. Two or three symbols signify $P < 0.01$ or 0.001, respectively.

induction after LPS injection in young or aged mice, whereas qRT–PCR confirmation showed both a significant age-related induction (80-fold) and a significant, although modest, LPS-induced induction (1.8-fold, only in aged at 6 h and 12 h). In addition, Csprs expression was compared among liver, kidney, and adipose tissue (Fig. 6B), and aged adipose tissue Csprs expression was more than fivefold higher than that of liver or kidney.

GenMAPP pathway analysis was performed to determine which biological pathways were significantly changed by aging at basal levels (Y0 h vs. A0 h, only). The cutoff for significantly changed pathways was set at $z > 2.0$ and $P < 0.05$. Biological pathways which were overrepresented by aging *per se* are listed in Table S7. Only three pathways met the criteria for significant upregulation by aging: *Peptide G-Protein Coupled Receptors (GPRCs)*, *Inflammatory Response Pathway*, and *T-cell Receptor signaling pathway*. No pathways were significantly downregulated by aging. The genes constituting these pathways with significant gene expression changes are listed in Table S8.

Discussion

This study is the first of its kind to compare gene expression changes in visceral white adipose tissue between young and aged animals with acute inflammatory stress at several different time points. Based on our previous studies demonstrating age-dependent augmentation of adipose-derived IL-6 (Starr *et al.*, 2009) and increased mortality in the aged (Starr *et al.*, 2010) during LPS-induced systemic inflammation, we hypothesized that adipose tissue likely produces a number of other factors which would be expressed in an age-dependent fashion and negatively affect the survival outcome of aged animals during acute inflammatory stress. Importantly, in this study we showed that the gene expression of many procoagulant factors is upregulated in adipose tissue in an age-dependent manner upon stimulation with LPS and this likely contributes to age-associated exaggerated thrombosis and vulnerability to inflammatory stress. Furthermore, we showed that the adipose tissue, as compared with liver (a predominant site of coagulation factor synthesis), is a major site of expression of numerous inflammatory and procoagulant genes. We also demonstrated that the resident stromal vascular cells, rather than adipocytes or infiltrating inflammatory cells, are the component of adipose tissue which contributes most to the high expression of cytokines and coagulation factors which are upregulated in an age-associated manner upon acute inflammatory stress.

Several pathways of interest were upregulated upon LPS injection and many of the genes within these pathways showed significant age-associated upregulation. The majority of the upregulated pathways were in some way tied to immune or inflammatory responses. This, no doubt, plays a major role in influencing the proinflammatory nature of the adipose tissue, particularly during inflammatory stress. The *Inflammatory Response Pathway* was also significantly upregulated in the adipose tissue by aging *per se*, which supports the well-established idea that aged adipose tissue has an inflammatory nature, even in healthy individuals.

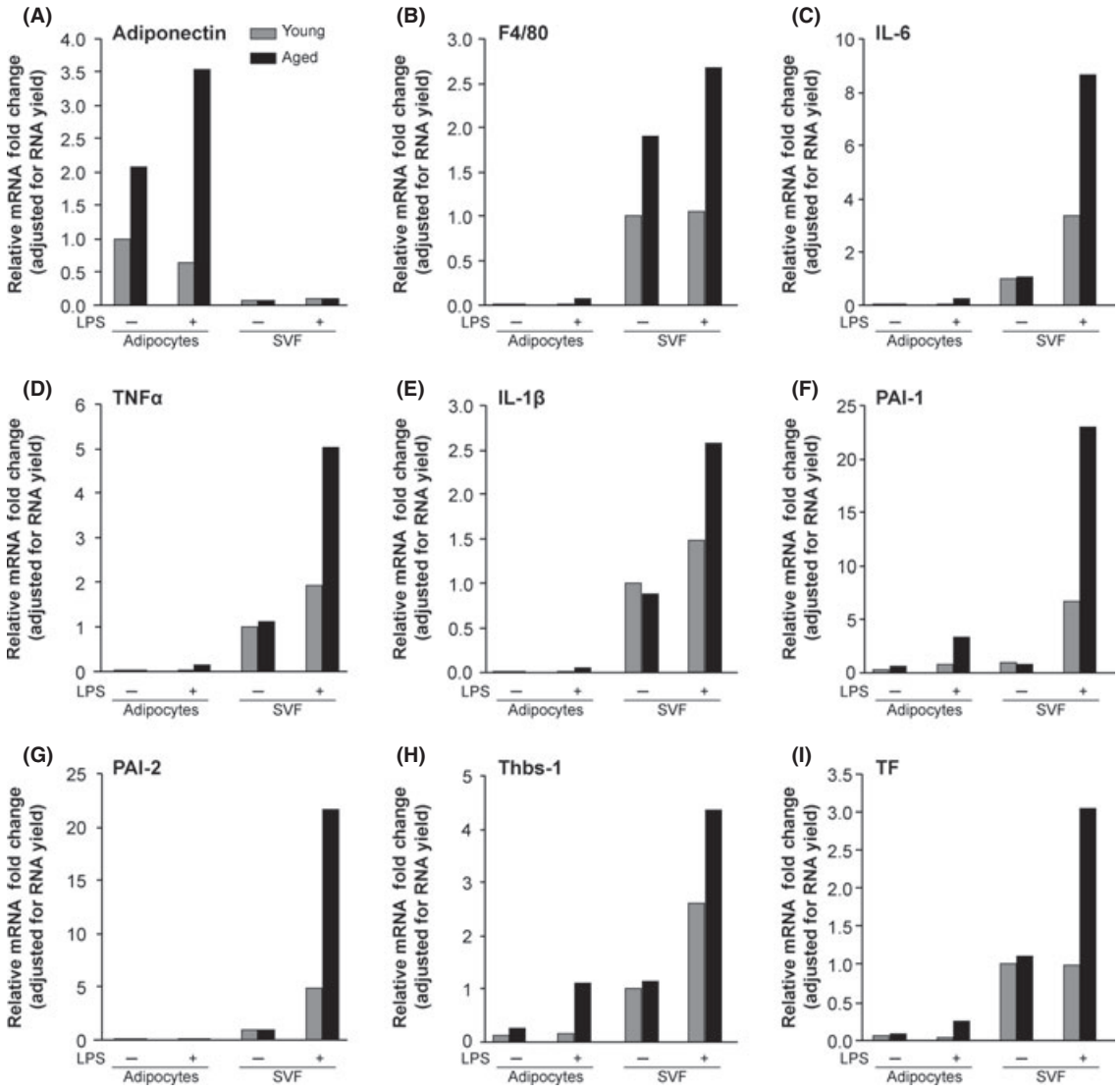

Fig. 5 LPS-induced gene expression changes are localized primarily in the stromal vascular fraction (SVF). The expression of (A) adiponectin, (B) F4/80, (C) IL-6, (D) TNFα, (E) IL-1β, (F) PAI-1, (G) PAI-2, (H) Thbs-1, (I) TF was compared between adipocytes and SVF cells from adipose tissue of young and aged mice 6 h after LPS injection. Adipocytes and SVF cells from noninjected mice were used as controls. Gray bars: young; Black bars: aged.

In addition to inflammation, the blood clotting pathway was significantly activated upon LPS injection with further augmentation by aging. Our data indicate that many procoagulant genes are synthesized much more abundantly by the adipose tissue than was previously thought and in many cases stress-induced adipose gene expression exceeds that of the liver as shown for PAI-1, PAI-2, Thbs-1, TF, and Fgl-2. We recently demonstrated that a low dose of LPS (2.5 mg kg^{-1}) causes 0% mortality in young mice, but 80% mortality in aged mice with dramatic age-dependent thrombosis (as assessed by fibrin formation) in multiple organs including the lung, liver, and kidney (Starr *et al.*, 2010). When a higher dose of LPS (20 mg kg^{-1}) was administered to young mice, mortality was equivalent to that of aged mice with the low dose; however, the high dose did not cause significant thrombosis in the young, suggesting that coagulation is a major age-related cause of death in mice under acute systemic inflammatory stress. This study also revealed that age-dependent thrombosis is partly due to a decrease in activation of the anticoagulant, protein C (Starr *et al.*, 2010). Yamamoto *et al.* (Yamamoto *et al.*, 2002, 2005) previously described that age-dependent thrombosis is partly due to increases in PAI-1 expression. Our current study suggests that, in addition to these mechanisms, there are additional procoagulant factors which are overexpressed in an age-dependent manner during systemic inflammation and by varying mechanisms likely also contribute to accelerated thrombus formation and DIC in the aged.

Although the apoptosis pathway ranked as second most upregulated pathway, we did not see any significant signs of apoptosis in the adipose tissue by TUNEL assay (data not shown) or histological analysis. The upregulation of this pathway may be artifactual as gene expression of caspase 3 and Bax (prominent markers of apoptosis) was not significantly changed by LPS injection and also because many of the genes included in the apoptosis pathway are

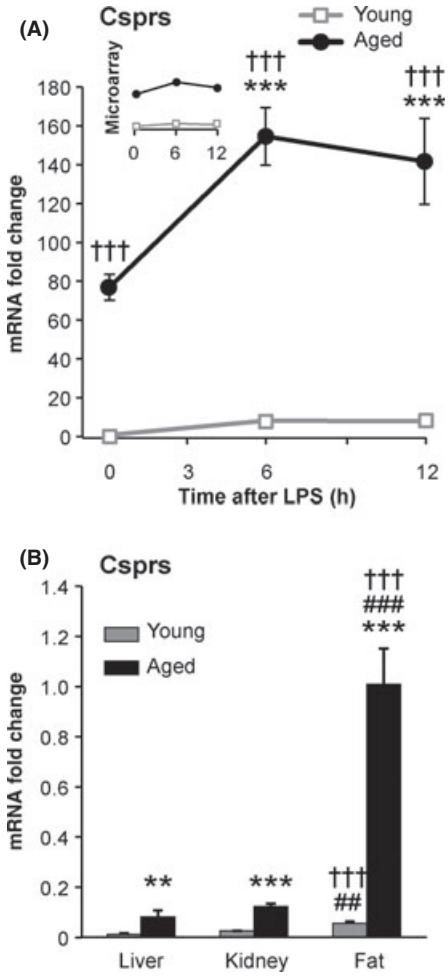

Fig. 6 Real-time quantitative RT–PCR validation of component of Sp100-rs (Csprs). (A) The time course of Csprs induction after LPS was validated by qRT–PCR. Open squares: young; Closed circles: aged. * indicates a statistically significant change as compared with the 0-h time point of the same age group. # indicates a statistically significant difference between young and aged of the same time point. Three symbols signify $P < 0.001$. (B) The expression of Csprs was compared among liver, kidney, and adipose tissue (fat) of young and aged mice 6 h after LPS injection. Gray bars: young; Black bars: aged. Data are expressed as the mean ± standard deviation, $n = 3$ for each group. * indicates a statistically significant change between young and aged of the same tissue. # indicates a statistically significant change between Fat and Liver of the same age group. † indicates a statistically significant change between Fat and Kidney of the same age group. One, two, or three symbols signify $P < 0.05$, 0.01, or 0.001, respectively.

overlapping with such inflammation-related pathways as TNF and NFκB signaling. It is also possible that apoptosis of adipose tissue cells indeed occurs at a later time point than evaluated in these analyses and the upregulation of some apoptosis-related genes in our analysis is a preliminary event.

The majority of the downregulated pathways, such as *Adipogenesis, Glycogen Metabolism,* and *Triacylglyceride Synthesis*, seemed to coincide with the 'classical' role of adipose tissue as an energy storage/metabolic site. The downregulation of these pathways is expected as SIRS is known to be a hypermetabolic state. The *Adipogenesis* pathway was both up- and downregulated; this is most likely related to the way we analyzed the data. We combined both the 6-h and 12-h time points in this analysis, thus some genes may have been initially upregulated, but then later downregulated, or vice versa. In addition, several cytokines including IL-6, LIF, and TNFα are included in the adipogenesis pathway and could have contributed to it being categorized as upregulated only in the aged as these factors tend to be higher in the aged with enhanced inflammation by LPS. Overall, our analysis indicates that the adipogenesis pathway is more significantly downregulated than upregulated.

We showed a decrease in circulating levels of adiponectin after LPS injection which was not altered by aging. Previously, Tsuchihashi *et al.* (Tsuchihashi *et al.*, 2006) showed a similar downregulation of plasma adiponectin levels in a polymicrobial rat model of sepsis and indicated that decreased plasma adiponectin might be due to its LPS-binding activity. Interestingly, the mRNA level of adiponectin increased after LPS injection ($P < 0.01$) in an age-associated manner, although the fold increase was below our 1.8-fold cutoff (Table S4). Although the stability or immunoreactivity of LPS-bound adiponectin remains uncertain, our data indicate that the drop in plasma adiponectin levels is not transcriptionally regulated. An increase in mRNA levels of adiponectin may serve as a positive feedback mechanism to replenish diminished circulating levels.

The largest fold change when comparing adipose tissue of young and aged mice at basal levels was observed for Csprs. Recently, Park *et al.* (Park *et al.*, 2009) reported that Csprs had the largest fold change by aging in the heart in multiple strains of mice, and indicated its potential use as a transcriptional biomarker of aging. Here, we report that not only is Csprs strongly expressed in an age-associated fashion in the adipose tissue, it is highly expressed in aged adipose tissue compared with liver, kidney, and young adipose tissue. Analysis in cardiac tissue was not performed in the current study. Collectively, our present study and the work by Park *et al.* indicate an age-associated induction of Csprs in the heart, liver, kidney ,and adipose tissue. Park *et al.* also reported that middle age-onset caloric restriction significantly attenuated the age-related upregulation of Csprs. Despite the lack of functional status, Csprs has obvious potential for use as a biomarker of aging and perhaps adipose tissue health.

The adipose tissue, like other organs, is highly heterogeneous, consisting of a variety of cell types including mature adipocytes, preadipocytes, monocytes, macrophages, lymphocytes, endothelial cells, and fibroblasts. Our cell fraction separation experiments demonstrate that the stromal vascular fraction (SVF) cells, rather than the adipocytes, are more responsive to LPS-mediated inflammatory stress in both young and aged mice. A limitation of this study is that we did not further elucidate which cell types among the SVF, contribute most to the observed gene expression changes. We did, however, provided evidence that it is the resident SVF cells that are likely responsible for high inflammatory and coagulation factor gene expression within the adipose tissue and that infiltration of inflammatory cells from the circulation is minimal. Based on our data that the gene expression of CD11c (infiltrating M1 macrophage marker) decreases or does not significantly change in response to LPS, it is highly unlikely that infiltration of these macrophages are responsible for the age-associated upregulation of inflammatory/coagulant genes. Our data also showed that expression of CD206

(resident macrophage marker) significantly decreases after LPS injection, without age-associated change, indicating that resident M2 macrophages are also not likely contributing to age-associated inflammation; this conclusion is reasonable as resident macrophages are thought to have a more anti-inflammatory function (Gordon & Taylor, 2005) and the capacity for macrophage activation in general is known to decline with age (Sebastian *et al.*, 2009). It should be noted that our data represent gene expression changes, not protein levels. Further studies are warranted to further elucidate specific cell types expressing these genes and confirm protein levels.

A newly identified subtype of adipose tissue macrophages, double-negative $CD206^{-}/CD11c^{-}$ (Zeyda *et al.*, 2010), was recently found to be increased in numbers by aging (Lumeng *et al.*, 2011). Further work is needed to verify whether these cells account for some of the age-dependent upregulation of cytokines/coagulation factors in adipose tissue under inflammatory stress. Lumeng *et al.* (Lumeng *et al.*, 2011) also reported that T cell-enriched immune cell aggregates (fat-associated lymphoid clusters) are present in young and old mice at a similar density, but that the size of the clusters are larger in old mice, thus potentially accounting for increased age-associated inflammation. We also noted the presence of immune cell aggregates which were not increased in number by aging or LPS treatment. Expansion of T cells within these clusters is another candidate for further study on age-dependent inflammation in adipose tissue (Yang *et al.*, 2010; Lumeng *et al.*, 2011). Our results and that of Lumeng *et al.* indicate that inflammation in aged adipose tissue occurs by a different mechanism than what is established for obese adipose tissue, where the SVF, mainly infiltrating macrophages, is thought to be the major source of adipose tissue inflammation (Gustafson *et al.*, 2009; Kanneganti & Dixit, 2012). Our results are also in contrast to a previous study which reported that, whereas adipocytes express IL-6 and TNFα in an age-dependent manner, SVF cells from young and aged mice express similar levels of these cytokines (Wu *et al.*, 2007); however, experimental methods in this study were different from ours. For example Wu *et al.* treated isolated adipocytes and SVF cells *in vitro* with a low dose of LPS (0.2 μg mL^{-1}) as a model of low-grade chronic inflammation, whereas we utilized an *in vivo* model of acute systemic inflammation by first injecting mice with a strong dose of LPS and then isolating adipocytes and SVF cells. Assuming the total blood volume of an adult male C57BL/6 mouse is approximately 2–2.5 mL, the dose of LPS used in our study is several thousand times greater than that used by Wu *et al.*; thus the two models are entirely different and could cause distinct physiological changes in adipocytes or SVF cells. Within the resident SVF cells, preadipocytes may constitute an important source of adipose tissue inflammation (Chung *et al.*, 2006; Tchkonia *et al.*, 2007, 2010; Cartwright *et al.*, 2010). Further studies are warranted to identify specific cell types in the SVF that express proinflammatory and procoagulant factors in an age-dependent fashion.

Changes in adiposity with age, in addition to aging itself are possibly responsible for some of the observed gene expression differences; however, measures of adiposity in young vs. aged mice suggest that this is not likely the case. Hemmeryckx (Hemmeryckx *et al.*, 2010) *et al.* reported that although aged mice are significantly heavier than young mice, percentage of total body fat and abdominal fat volume are not significantly different. We also previously reported that aged mice having smaller epididymal fat pads than matured young mice, still expressed higher levels of IL-6 in the adipose tissue (Starr *et al.*, 2009). A clear limitation of this study is that we did not directly compare differences in adiposity between young and aged mice or how those potential differences might affect gene expression changes.

Another limitation of this study is that we used a small sample size ($n = 3$) for each time point. This small sample size was a necessary compromise as we chose to include multiple time points in our analysis. The inclusion of only three mice per time point allowed us to investigate four time points which increased the likelihood of identifying as many gene expression changes as possible. Indeed, as mentioned in the Results section over 50% of gene expression changes would have been overlooked had we only investigated the 12-h time point. The consequence of using a small n in this study is that statistical analyses were not as powerful; however, most significant differences detected with $n = 3$ would remain significant with a larger n.

In conclusion, our results demonstrate that, upon inflammatory stress, visceral white adipose tissue expresses many different types of genes including those involved in inflammation, coagulation, immune response, and metabolism, among other categories. In addition, many adipose-derived factors, particularly those involved in acute inflammatory and procoagulant process, are differentially regulated by aging. The adipose tissue was determined to be the major organ responsible for these changes highlighting the significance of adipose-derived factors to the clinical condition. In light of our previous work demonstrating the association of age-dependent mortality with inflammation and coagulation, we believe this data adds evidence that changes to adipose tissue with aging may be responsible for a more profound response and increased mortality in the aged.

Experimental procedures

Animals

Young (4 months old) and aged (24 months old) male C57BL/6 mice were obtained from colonies of the National Institute on Aging, housed in a temperature controlled environment at 22°C and maintained in a 12-h light/dark cycle. All mice were acclimated for at least 14 days with free access to food (LabDiet, Brentwood, MO, USA) and water. All animal procedures were approved by the Institutional Animal Care and Use Committee.

LPS model of systemic inflammation

Acute systemic inflammation was induced by intraperitoneal injection with bacterial endotoxin lipopolysaccharide (LPS) derived from *Pseudomonas aeruginosa* (Sigma-Aldrich, St. Louis, MO, USA), as previously described (Ueda *et al.*, 2008; Starr *et al.*, 2009, 2010). Body temperature, as a measure of the severity of hypothermia (Saito *et al.*, 2003), was monitored throughout the study. See

supporting information for additional details regarding animal sacrifice and tissue harvesting.

Enzyme-linked immunosorbent assays (ELISA)

Plasma samples were obtained by centrifugation of blood samples at 3000× g for 10 min and used in ELISA kits (see supporting information for kit details).

Sample preparation and labeling

Frozen epididymal adipose tissue, liver, and kidney were homogenized with TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and total cellular RNA was isolated as we previously described (Starr *et al.*, 2009). The concentration of the RNA was determined by measuring the absorbance at 260 and 280 nm using a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA). The quality of the RNA was determined through visualization of 18S and 28S RNA bands using an Agilent 2100 Bioanalyzer (Aligent Technologies, Santa Clara, CA, USA). Total RNA (500 ng) was prepared for microarray analysis using the *Ambion* MessageAmp Premier RNA Amplification Kit (Austin, TX, USA). Eighteen chips were run in total: $n = 3$ for each of three time points in two age groups. See supporting information regarding additional methods for RNA labeling and hybridization.

Pathway analysis

The Gene Map Annotator and Pathway Profiler (GenMAPP, www.genmapp.org) software package, a computer program designed for analyzing genome-scale experiments was used to organize gene expression data into biological pathways (Dahlquist *et al.*, 2002; Doniger *et al.*, 2003; Salomonis *et al.*, 2007). See supporting information for additional details.

Histological analysis

Formalin-fixed epididymal adipose tissues were embedded in paraffin, and sections (5 μm) were stained with hematoxylin and eosin (H&E). Photomicrographs were taken using a Nikon Eclipse E200 microscope and Nikon Digital Sight DS-U3/DSFi1 digital camera system with NIS Elements F3.2 Imaging Software.

Adipocyte and stromal vascular fraction cell separation and RNA isolation

Perfused epididymal adipose tissues harvested from LPS-injected mice were cut into 30 mg (approximate size) pieces and digested with 1 mg mL^{-1} collagenase (Type I, Sigma-Aldrich, St. Louis, MO, USA) dissolved in 2% BSA-HBSS + 5 mM glucose for 1 h with agitation at 37°C. Digested cells were then passed through a 100-μm cell strainer and the strainer was washed with HBSS, followed by centrifugation for 10 min. Floating adipocytes and buffer were transferred to a new tube, followed by a second centrifugation. The pellet containing the SVF was resuspended in HBSS and transferred to a new tube, followed by centrifugation. All centrifugation steps were performed at 4°C and 450× g. Total RNA was isolated using RNeasy® Lipid Tissue Mini Kit (Qiagen, Valenica, CA, USA).

Real-time quantitative reverse transcriptase–polymerase chain reaction (qRT–PCR)

Reverse transcription was performed on 1 μg of total RNA from adipose tissue, liver, and kidney and 500 ng of RNA from isolated adipocytes and SVF cells, after repurification with RNAqueous (Ambion, Austin, TX, USA), using random primers and TaqMan® reverse transcription reagents (Applied Biosystems, Foster City, CA, USA). See supporting information regarding additional methods for qRT–PCR.

Acknowledgments

This research was funded by the National Institutes of Health Grant R01-AG025908, R01-AG039732, and R36-AG038547. Additional support came from the American Foundation for Aging Research Graduate Fellowship Award. This study was presented in part at the 39th Annual Meeting of the American Aging Association, and M. E. S. was named runner-up for the 2010 Walter R. Nicolai Award for Meritorious Research by a Predoctoral Candidate in Biomedical Gerontology. The authors gratefully acknowledge the pathological expertise of Dr. Eun Y. Lee and the illustrative expertise of Ms. Donna A. Gilbreath of the Markey Cancer Center at the University of Kentucky. In addition, the authors wish to thank Ms. Deborah J. Prusak and Ms. Jill K. Thompson of the University of Texas Medical Branch Molecular Genomics Core for their thoughtful advice and technical assistance, and Dr. George E. Taffet of Baylor College of Medicine for his guidance and critique of the data.

Author contributions

M. E. S. designed the research, performed the experiments, analyzed and interpreted data, ran the pathway analysis, and prepared the manuscript; A. J. S. and Y. H. provided statistical expertise and performed statistical analysis; J. R. C and T. G. W. oversaw microarray analysis and real-time qPCR assays, and J. R. C. performed principal components analysis; B. M. E reviewed results and edited the manuscript; and H. S. designed the research, analyzed and interpreted data, and prepared the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Data S1 Experimental Procedures.

Fig. S1 Principal components analysis.

Fig. S2 Method of classification and statistical analysis to create filtered gene lists.

Table S1 An alphabetical gene list of 6025 genes whose expression level was significantly changed by LPS-induced inflammation (overall, $P < 0.01$) with a fold change greater than 2.0. Mean fold change for each age and time point is listed separately, although aging was not a criterion for statistical evaluation.

Table S2 An alphabetical gene list of 667 genes whose expression level was significantly changed by LPS-induced inflammation (overall, $P < 0.01$) with a mean fold change greater than 2.0 and whose change in expression was significantly altered by aging (*t*-test, $P < 0.01$) with a fold change greater than 1.5.

Table S3 An alphabetical gene list of 303 genes whose expression level was significantly changed by aging alone (*t*-test, $P < 0.01$) with a mean fold change greater than 1.5. This list was compiled by evaluating only 0-h control (no LPS injection) samples. Overall *P* value was included in the table to indicate which genes expressions are also later changed by LPS injection ($P < 0.01$), although LPS was not a criterion for statistical evaluation.

Table S4 Raw data file of gene expression levels of each sample determined by Affymetrix Mouse Genome 430 2.0 gene chips.

Table S5 List of Genes that are significantly upregulated by LPS within the selected pathways from Table 2.

Table S6 List of genes in the adipose tissue with the highest fold change by aging, *per se* (basal levels).

Table S7 List of biological pathways that are significantly upregulated in the adipose tissue by aging.

Table S8 List of genes that are significantly upregulated by aging within the selected pathways from Table 4.