The role of thrombospondin (TSP)-1 in obesity and diabetes

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The extracellular matrix does not only provide mechanical support, but also transduces key homeostatic signals and regulates cellular responses to a wide range of injurious stimuli. In vertebrates, evolution of the biological properties of the matrix network resulted in emergence of complex matrix macromolecules that contain multiple functional domains and are capable of modulating signaling cascades through binding with cellular receptors. The interactions between the matrix and tissue cells are particularly important in injury and repair, where dynamic alterations in the matrix network drive the cellular responses. A hallmark of tissue remodeling is the induction of matricellular proteins, a family of structurally unrelated extracellular macromolecules that become transiently incorporated into the structural matrix and modulate a diverse range of signaling pathways and cellular responses, by serving as a molecular bridge between the matrix and the cellular elements.1,2 Deposition of matricellular proteins into the matrix confers plasticity to remodeling tissues and ensures tight regulation of inflammatory, reparative, fibrogenic, and angiogenic responses.

**Introduction: The Matricellular Concept**

As a prototypical matricellular protein, thrombospondin (TSP)-1 exhibits all the major features of the family.3 First, TSP-1 is expressed at very low levels in normal adult mammalian tissues, but is markedly upregulated following injury. Second, TSP-1 does not play a direct structural role; mice lacking TSP-1 do not have significant phenotypic abnormalities in the absence of injury.4 Third, when incorporated into the matrix, TSP-1 exerts de-adhesive actions stimulating adipocyte proliferation. Am J Physiol Endocrinol Metab 2013; 305:E439-50; PMID:23757408; http://dx.doi.org/10.1152/ajpendo.00006.2013

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activation. Many functions of the TSP-1 molecule are mediated through the type 1 domains.7

**TSP-1 in Obesity and Diabetes**

Expression of TSP-1 is markedly upregulated in tissues from diabetic and obese subjects. In diabetic patients and in animal models of obesity and diabetes, TSP-1 expression is increased in many organs, including the kidney, the adipose tissue,9 the heart,10 and blood vessels.11 The basis for accentuated TSP-1 synthesis remains unclear; however, extensive evidence suggests that hyperglycemia potently induces TSP-1 synthesis by vascular cells12 through pathways that may involve glucose-mediated activation of hexosamine.13,14 Leptin stimulation also upregulates TSP-1 in vascular smooth muscle cells through activation of JAK-2 and MAPK signaling.15

**TSP-1 in the Adipose Tissue**

The continuous expansion of adipose tissue during the development of obesity requires dynamic alterations in the extracellular matrix network.16 Collagen deposition in the adipose tissue is observed in both animal models of obesity and in obese individuals.17,18 Alterations in the adipose extracellular matrix are not limited to deposition of structural matrix proteins, but also involve secretion of matricellular macromolecules that become incorporated into the remodeling fat and modulate phenotype of both adipocytes and non-adipocytes. Several matricellular proteins are upregulated in expanding and remodeling adipose tissue. SPARC (secreted protein acidic and rich in cysteine)/osteonectin, one of the prototypical members of the matricellular family is overexpressed in obese subjects19,20 and may attenuate mitotic expansion of pre-adipocytes,21 while inhibiting adipocyte differentiation.22 Osteopontin expression is also increased in obese individuals23 and may exert macrophage-mediated pro-inflammatory actions causing metabolic dysfunction.24 TSP-1, perhaps the best-studied matricellular protein in obesity and diabetes,9,25 is consistently overexpressed in adipose tissue harvested from obese subjects.26-28 In obese patients, adipose TSP-1 expression is localized in both adipocytes and macrophages and TSP-1 expression levels are associated with inflammatory activity and metabolic dysfunction.29

Our recent work systematically studied the regulation of TSP-1 in diet-induced obesity. Mice fed a high-fat diet (HFD) had significant TSP-1 induction in perigonadal, but not in subcutaneous fat, predominantly localized in interstitial and perivascular areas. Differentiated adipocytes were not a major source of TSP-1 in vivo and in vitro. Our in vitro studies showed that undifferentiated 3T3-L1 pre-adipocytes, but not differentiated adipocytes, expressed high levels of TSP-1 suggesting that adipocyte maturation may be associated with a reduction in expression of matricellular proteins. Moreover, adipocyte TSP-1 synthesis does not appear to be regulated by inflammatory cytokines. In contrast, non-adipocytes (such as endothelial cells, fibroblasts, and vascular smooth muscle cells) exhibit intense TSP-1 upregulation in the presence of a high concentration of glucose.11 Thus, accentuation of TSP-1 synthesis by non-adipocytes in response to hyperglycemia, or increased numbers of immature adipocyte progenitors capable of TSP-1 expression may be the basis for increased adipose tissue TSP-1 synthesis in obesity.

What is the role of TSP-1 in the pathogenesis of weight gain and metabolic dysfunction? In our recent study9 we found that mice with genetic disruption of TSP-1 fed with a HFD (60% fat content) or a high-carbohydrate low-fat diet (HCLFD) (10% fat content) exhibit reduced weight gain and significantly attenuated adiposity when compared with WT animals. TSP-1 loss also improved metabolic dysfunction, reducing glucose levels, insulin levels, and HOMA I/R. The findings identified TSP-1 as a mediator critically involved in diet-induced weight gain and adipose tissue growth.

Our findings suggested that TSP-1 does not act by affecting food intake or energy expenditure, but may have direct modulatory effects on the phenotype of adipocytes and non-adipocytes. Our in vitro experiments suggested that TSP-1 stimulation exerts proliferative effects on adipocytes. Moreover, TSP-1 may also affect adipocyte fatty acid uptake; this possibility was suggested by increased serum free fatty acid (FFA) and triglyceride levels in TSP-1 knockouts. Lack of CD36, a major TSP-1 ligand, also results in defective adipocyte uptake of FFAs.30,31 Thus, incorporation of TSP-1 in the adipose interstitial matrix may facilitate CD36-mediated fatty acid uptake. In addition to its potential effects on adipocytes, TSP-1 may also regulate phenotype and function of the inflammatory and vascular cells that infiltrate the expanding adipose tissue. In our study, TSP-1 loss was associated with attenuated adipose tissue inflammatory activity evidenced by reduced macrophage infiltration and decreased expression of tumor necrosis factor (TNF)-α. These findings are consistent with the association between adipose TSP-1 expression and inflammation observed in obese human subjects39 and may suggest pro-inflammatory actions of TSP-1.

**TSP-1 as a Mediator of Organ Dysfunction in Obesity and Diabetes**

A growing body of evidence suggests that, in addition to its role in adipose tissue inflammation, TSP-1 is implicated in the pathogenesis of organ dysfunction in diabetes and obesity (Fig. 1). Olerud and coworkers have suggested that in the pancreas, TSP-1 is expressed by endothelial cells and regulates β-cell function through activation of TGF-β.32,33 In the diabetic kidney, TSP-1-dependent activation of TGF-β may promote nephropathy.34 In the vasculature, increased TSP-1 expression may mediate the pro-atherogenic effects of glucose35 and may explain the delayed reendothelialization of diabetic vessels following percutaneous interventions.35 Our recent work suggested an important role for TSP-1 in diabetic cardiomyopathy. In obese diabetic db/db mice, TSP-1 expression was markedly upregulated in the myocardium and was localized in the perivasular and interstitial space. db/db mice lacking TSP-1 exhibited mild chamber dilation and modest non-progressive systolic dysfunction reflecting increased MMP activation.
presumably due to loss of TSP-1-induced protease inhibition. TSP-1 was also implicated in the age-associated vascular rarefaction observed in db/db animals; the angiostatic actions of TSP-1 were mediated through upregulation of angiopoietin-2. These findings suggest that TSP-1 upregulation in the myocardium may be a key mediator in diabetes-associated impairment of angiogenesis.

**Targeting TSP-1 in Diabetes and Obesity**

Due to its direct involvement in metabolic dysregulation and in organ dysfunction associated with obesity and diabetes, TSP-1 may be a promising therapeutic target for patients with metabolic disease. As a modular matricellular protein, TSP-1 exerts its actions through specific functional domains. Peptide-based strategies inhibiting specific TSP-1-mediated actions may be effective therapeutic approaches for treatment of diabetic complications. For example, because the angiostatic effects of TSP-1 are mediated through interactions with CD36 or CD47, selective inhibition of the anti-angiogenic actions of TSP-1 may correct the impaired angiogenesis in diabetic hearts.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**

1. Bornstein P, Sage EH. Matricellular proteins: extracellular modulators of cell function. Curr Opin Cell Biol 2002; 14:608-16; PMID:12281357; http://dx.doi.org/10.1016/S0955-0674(02)00361-7
2. Frangogiannis NG. Matricellular proteins in cardiac adaptation and disease. Physiol Rev 2012; 92:635-88; PMID:22535894; http://dx.doi.org/10.1152/physrev.00008.2011
3. Adams JC. Thrombospondin: multifunctional regulators of cell interactions. Annu Rev Cell Dev Biol 2001; 17:25-51; PMID:11687483; http://dx.doi.org/10.1146/annurev.cellbio.17.1.25
4. Lawler J, Sunday M, Thibert V, Duquette M, George EL, Rayburn H, Hyynes RO. Thrombospondin-1 is required for normal murine pulmonary homeostasis and its absence causes pneumonia. J Clin Invest 1998; 101:982-92; PMID:9486968; http://dx.doi.org/10.1172/JCI1684
5. Crawford SE, Stellmach V, Murphy-Ullrich JE, Ribeiro SM, Lawler J, Hyynes RO, Bovin GP, Bouck N. Thrombospondin-1 is a major activator of TGF-beta in vivo. Cell 1998; 93:1159-70; PMID:9657149; http://dx.doi.org/10.1016/S0092-8674(00)81460-9
6. Jiménez B, Volpert OV, Crawford SE, Febbraio M, Silverstein RL, Bouck N. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. Nat Med 2000; 6:41-8; PMID:10613822; http://dx.doi.org/10.1038/71517
7. Lawler J. Thrombospondin-1 as an endogenous inhibitor of angiogenesis and tumor growth. J Cell Mol Med 2002; 6:1-12; PMID:12003605; http://dx.doi.org/10.1111/j.1582-4934.2002.tb00307.x
8. Wahab NA, Schaef er L, Weston BS, Yiannikouris O, Wright A, Babelova A, Schaefer R, Mason RM. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. Nat Med 2000; 6:41-8; PMID:10613822; http://dx.doi.org/10.1038/71517
9. Kong P, Gonzalez-Quesada C, Cavalera M, Biernacka A, Kong P, Lee DW, Saxena A, Frunza O, Dobaczewski M, Shinde AV, Frangogiannis NG. Thrombospondin-1 Induction in the Diabetic Myocardium Stabilizes the Cardiac Matrix, While Promoting Vascular Rarefaction Through Angiopoietin-2 Upregulation. Circ Res 2013; (Forthcoming); PMID:24081879; http://dx.doi.org/10.1161/CIRCRESAHA.113.302939
10. Stenina OI, Krakovets I, Wang K, Zhou Z, Forudi F, Penn MS, Topol EJ, Blow EF. Increased expression of thrombospondin-1 in vessel wall of diabetic Zucker rat. Circulation 2003; 107:3209-15; PMID:12810612; http://dx.doi.org/10.1161/01.CIR.0000074223.56882.97
11. Lawler J, Hynes RO, Wright A, Babelova A, Schaefer R, Mason RM. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. Nat Med 2000; 6:41-8; PMID:10613822; http://dx.doi.org/10.1038/71517
12. Raman P, Harry C, Weber M, Krukovets I, Stenina OI. A novel transcriptional mechanism of cell type-specific regulation of vascular gene expression by glucose. Arterioscler Thromb Vasc Biol 2011; 31:634-42; PMID:21148424; http://dx.doi.org/10.1161/ATVBAHA.110.309675
13. Raman P, Krukovets I, Marinic TE, Bornstein P, Stenina OI. Glycosylation mediates up-regulation of a potent antiangiogenic and proatherogenic protein, thrombospondin-1, by glucose in vascular smooth muscle cells. J Biol Chem 2007; 282:5704-14; PMID:17178709; http://dx.doi.org/10.1074/jbc.M609092000

**Figure 1.** Thrombospondin (TSP)-1 upregulation in diabetes and obesity is involved in the pathogenesis of metabolic dysregulation and organ dysfunction. TSP-1 induction in adipose tissue mediates inflammation and may enhance adipocyte proliferation while promoting fatty acid uptake. In the pancreas, TSP-1 expression may regulate β-cell function. TSP-1 may also be involved in the pathogenesis of diabetic nephropathy, atherosclerosis, and may explain the impaired angiogenesis in diabetic myocardium and the defective re-endothelialization following vascular injury. TSP-1 actions are mediated through activation of TGF-β, through angiostatic actions, through matrix metalloproteinase inhibition and through direct stimulation of CD36 signaling.
14. Dabir P, Marinic TE, Krukovs I, Stenina OI. Aryl hydrocarbon receptor is activated by glucose and regulates the thrombospondin-1 gene promoter in endothelial cells. Circ Res 2008; 102:1558-65; PMID:18515748; http://dx.doi.org/10.1161/CIRCRESAHA.108.176090

15. Chavez RJ, Haney RM, Cuadra RH, Ganguly R, Adapala KA, Thodier CK, Raman P. Upregulation of thrombospondin-1 expression by leptin in vascular smooth muscle cells via JAK-2 and MAPK-dependent pathways. Am J Physiol Cell Physiol 2012; 303:C379-91; PMID:22592401; http://dx.doi.org/10.1152/ajpcell.00008.2012

16. Sun K, Kusminski CM, Scherer PE. Adipose tissue: composition, distribution, and link with metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. Mol Cell Biol 2009; 29:1575-91; PMID:19114551; http://dx.doi.org/10.1128/MCB.01300-08

17. Khan T, Muise ES, Iyengar P, Wang ZV, Chandalia A, Divoux A, Tordjman J, Lacasa D, Veyrie N, Hugol C, Gautier N, Van Obberghen E. The matricellular protein SPARC/osteonectin as a newly identified factor up-regulated in obesity. J Biol Chem 2001; 276:22231-7; PMID:11294850; http://dx.doi.org/10.1002/jbc.219011

18. Ramas J, Franssen-van Hal NL, Kramer E, Llado I, Boullaud F, Palou A, Keijer J. Carboxypeptidase E and thrombospondin-1 are differentially expressed in subcutaneous and visceral fat of obese subjects. Cell Mol Life Sci 2002; 59:1960-71; PMID:12530526; http://dx.doi.org/10.1007/s00018-002-0851-8

19. Varma Y, Yao-Borengasser A, Bodles AM, Rasouli N, Phanavanh B, Nolen GT, Kern EM, Nagarajan R, Spencer HJ 3rd, Lee MJ, et al. Thrombospondin-1 deficiency reduces obesity-associated inflammation and improves insulin sensitivity in a diet-induced obese mouse model. PLoS One 2011; 6:e226655; PMID:22039525; http://dx.doi.org/10.1371/journal.pone.0022665

20. Voros G, Marqux E, Demeulemeester D, Clerx N, Collen D, Lijnen HR. Modulation of angiogenesis during adipose tissue development in murine models of obesity. Endocrinology 2005; 146:4545-54; PMID:16020476; http://dx.doi.org/10.1210/en.2005-0532

21. Hida K, Wada J, Zhang H, Hiragushi K, Tsuchiyama Y, Shikata K, Makino H. Identification of genes specifically expressed in the accumulated visceral adipose tissue of OLETF rats. J Lipid Res 2004; 45:1615-22; PMID:15082850; http://dx.doi.org/10.1194/jlr.M013032

22. Nie J, Sage EH. SPARC inhibits adipogenesis by its enhancement of beta-catenin signaling. J Biol Chem 2009; 284:1279-90; PMID:18990699; http://dx.doi.org/10.1074/jbc.M808028200

23. Gómez-Ambrós J, Catalin V, Ramirez B, Rodrigue A, Colina E, Silva C, Rotellar F, Muñuera C, Gil MJ, Castfuegos JA, et al. Plasma osteopontin levels and expression in adipose tissue are increased in obesity. J Clin Endocrinol Metab 2007; 92:3719-27; PMID:17959250; http://dx.doi.org/10.1210/jc.2007-0349

24. Nomiyama T, Perez-Tilve D, Ogawa D, Gizard F, Zhao Y, Heywood EB, Jones KL, Kawamori R, Casis LA, Tischöp MH, et al. Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. J Clin Invest 2007; 117:2877-88; PMID:17823662; http://dx.doi.org/10.1172/JCI31986

25. Li Y, Tong X, Rumala C, Clemons K, Wang S. Thrombospondin1 deficiency reduces obesity-associated inflammation and improves insulin sensitivity in a diet-induced obese mouse model. PLoS One 2011; 6:e226656; PMID:22039525; http://dx.doi.org/10.1371/journal.pone.0022665

26. Voros G, Marqux E, Demeulemeester D, Clerx N, Collen D, Lijnen HR. Modulation of angiogenesis during adipose tissue development in murine models of obesity. Endocrinology 2005; 146:4545-54; PMID:16020476; http://dx.doi.org/10.1210/en.2005-0532

27. Hida K, Wada J, Zhang H, Hiragushi K, Tsuchiyama Y, Shikata K, Makino H. Identification of genes specifically expressed in the accumulated visceral adipose tissue of OLETF rats. J Lipid Res 2004; 45:1615-22; PMID:15082850; http://dx.doi.org/10.1194/jlr.M013032

28. Ramas J, Franssen-van Hal NL, Kramer E, Llado I, Boullaud F, Palou A, Keijer J. Carboxypeptidase E and thrombospondin-1 are differentially expressed in subcutaneous and visceral fat of obese subjects. Cell Mol Life Sci 2002; 59:1960-71; PMID:12530526; http://dx.doi.org/10.1007/s00018-002-0851-8

29. Varma Y, Yao-Borengasser A, Bodles AM, Rasouli N, Phanavanh B, Nolen GT, Kern EM, Nagarajan R, Spencer HJ 3rd, Lee MJ, et al. Thrombospondin-1 is an adipokine associated with obesity, adipose inflammation, and insulin resistance. Diabetes 2008; 57:432-9; PMID:18057990; http://dx.doi.org/10.2337/db07-0840

30. Hajj T, Han XX, Bonen A, Aburomad NA. Defective fatty acid uptake modulates insulin responsiveness and metabolic responses to diet in CD36-null mice. J Clin Invest 2002; 109:1381-9; PMID:12021254

31. Hajj T, Hall AM, Jensen DR, Pierka TA, Drover VA, Tao H, Eckel R, Aburomad NA. CD36-facilitated fatty acid uptake inhibits lepin production and signaling in adipose tissue. Diabetes 2007; 56:1872-80; PMID:17440173; http://dx.doi.org/10.2337/db06-1699

32. Olerud J, Mohktari D, Johansson M, Christofferson G, Lawler J, Welsh N, Carlsson PO. Thrombospondin-1: an islet endothelial cell signal of importance for β-cell function. Diabetes 2011; 60:1946-54; PMID:21617177; http://dx.doi.org/10.2337/db11-0277

33. Dowt CJ, Olerud J, Emanuelsson H, Christofferson G, Carlsson PO. Sustained beta-cell dysfunction but normalized islet mass in aged thrombospondin-1 deficient mice. PLoS One 2012; 7:e47163; PMID:23094049; http://dx.doi.org/10.1371/journal.pone.0047451

34. Daniel C, Schaub K, Amann K, Lawler J, Hugo C. Thrombospondin-1 is an endogenous activator of TGF-beta in experimental diabetic nephropathy in vivo. Diabetes 2007; 56:2582-9; PMID:17878288; http://dx.doi.org/10.2337/db07-0349

35. Li M, Takenaka H, Asai J, Ibuki K, Mizukami Y, Maruyama Y, Koon YS, Wecker A, Luedemann C, Eaton E, et al. Endothelial progenitor thrombospondin-1 mediates diabetes-induced delay in reendothelialization following arterial injury. Circ Res 2006; 98:697-704; PMID:16484619; http://dx.doi.org/10.1161/01.RES.0000209948.59043.aa

36. Henkin J, Volpert OV. Therapies using anti-angiogenic peptide mimetics of thrombospondin-1. Expert Opin Ther Targets 2011; 15:1369-86; PMID:217147282; 22.011.640319

37. Lawler PR, Lawler J. Molecular basis for the regulation of angiogenesis by thrombospondin-1 and -2. Cold Spring Harb Perspect Med 2012; 2:a006627; PMID:22534394; http://dx.doi.org/10.1101/cshperspect.a006627