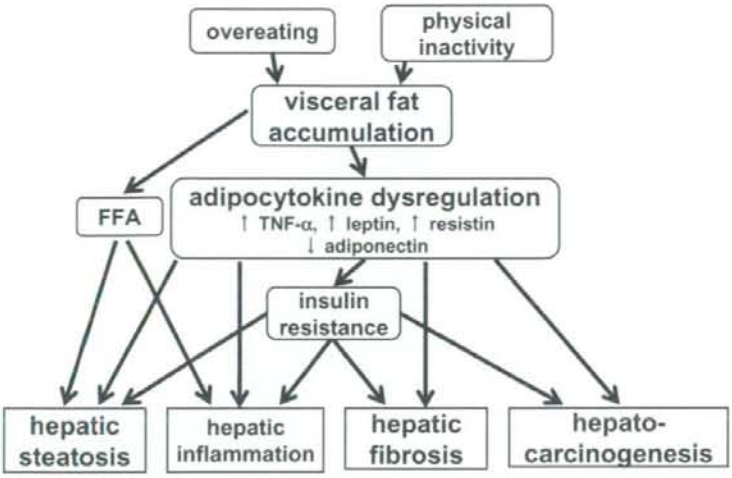

Fig. 3. Current hypothesis regarding the association between adipocytokines and liver diseases. Arrows, stimulatory effects

accumulation, a state of chronic low-grade inflammation. The inflammatory changes in obese adipose tissue induce adipocytokine dysregulation: an increase in offensive adipocytokines, TNF-α, IL-6, and resistin, and a decrease in the defensive adipocytokine adiponectin. Increased serum levels of TNF-α, resistin, and leptin, which are usually observed in obese subjects, may enhance steatosis, inflammation, fibrogenesis, or hepatocarcinogenesis in the liver. In addition, hypoadiponectinemia seems to enhance hepatic steatosis, inflammation, and fibrosis, as well as hepatocarcinogenesis (Fig. 3). Attenuation of proinflammatory adipocytokines or augmentation of the function of adiponectin might be an effective therapy for metabolic syndrome. Further clinical and experimental research should elucidate the relationship between adipocytokines and liver diseases.

References

1. Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. Cell 2001;104:531–43.
2. Friedman JM. Obesity in the new millennium. Nature 2000; 40:632–4.
3. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, et al. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. Nat Med 1996;2:800–3.
4. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun 1996;221: 286–9.
5. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–70.
6. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. Nature 2001;409:307–12.
7. Hotamisligil GS, Spiegelman BM. Tumor necrosis factor α: a key component of the obesity–diabetes link. Diabetes 1994;43: 1271–8.
8. Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. Am J Clin Nutr 2006;83: 461S–5S.
9. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006;116:1793–801.
10. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796–808.
11. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007;117:175–84.
12. Matsuzawa Y. The metabolic syndrome and adipocytokines. FEBS Lett 2006;580:2917–21.
13. Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, et al. Liver fibrosis in overweight patients. Gastroenterology 2000;118:1117–23.
14. McCullough AJ, Falck-Ytter Y. Body composition and hepatic steatosis as precursors for fibrotic liver disease. Hepatology 1999;29:1328–9.
15. Chitturi S, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. Semin Liver Dis 2001;21:27–41.
16. Naveau S, Giraud V, Borotto E, Aubert A, Capron F, Chaput JC. Excess weight is a risk factor for alcoholic liver disease. Hepatology 1997;25:108–11.
17. Hourigan LF, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology 1999;29:1215–9.
18. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 2001;33: 1358–64.
19. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348:1625–38.
20. Wolk A, Gridley G, Svensson M, Nyren O, McLaughlin JK, Fraumeni JF, Adam HO. A prospective study of obesity and cancer risk (Sweden). Cancer Causes Control 2001;12:13–21.
21. Crespo J, Cayon A, Fernandez-Gil P, Herandez-Guerra M, Mayorga M, Dominguez-Diez A, et al. Gene expression of

tumor necrosis factor alpha and TNF-receptors, p55 and p75 in nonalcoholic steatohepatitis patients. Hepatology 2001;34: 1158–63.
22. Yalniz M, Bahcecioglu IH, Ataseven H, Ustundag B, Ilhan F, Poyrazoglu OK, et al. Serum adipokine and ghrelin levels in nonalcoholic steatohepatitis. Mediators Inflamm 2006;2006: 34295.
23. Chitturi S, Farrell G, Frost L, Kriketos A, Lin R, Fung C, et al. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? Hepatology 2002;36: 403–9.
24. Pagano C, Soardo G, Pilon C, Milocco C, Basan L, Milan G, et al. Increased serum resistin in nonalcoholic fatty liver disease is related to liver disease severity and not to insulin resistance. J Endocrinol Metab 2006;91:1081–6.
25. Hui JM, Hodge A, Frost L et al. Beyond insulin resistance in NASH: TNFα or adiponectin? Hepatology 2004;40:46–54.
26. Jarrar MH, Baranova A, Collantes R, Stepanova M, Bennett C, Fang Y, et al. Adipokines and cytokines in non-alcoholic fatty liver disease (NAFLD). Aliment Pharmacol Ther 2008; 27:412–21.
27. Kaser S, Moschen A, Cayon A, Kaser A, Crespo J, Pons-Romero F, et al. Adiponectin and its receptors in non-alcoholic steatohepatitis. Gut 2005;54:117–21.
28. Shimizu A, Takamura T, Matsuzawa N, Nakamura S, Nabemoto S, Takeshita Y, et al. Regulation of adiponectin receptor expression in human liver and a hepatocyte cell line. Metabolism 2007;56:1478–85.
29. Musso G, Gambino R, Biroli G, Carello M, Faga E, Pacini G, et al. Hypoadiponectinemia predicts the severity of hepatic fibrosis and pancreatic beta-cell dysfunction in nondiabetic nonobese patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2005;100:2438–46.
30. Testa R, Franceschini R, Giannini E, Cataldi A, Botta F, Fasoli A, et al. Serum leptin levels in patients with viral chronic hepatitis or liver cirrhosis. J Hepatol 2000;33:33–7.
31. Jonsson JR, Moschen AR, Hickman IJ, Richardson MM, Kaser S, Clouston AD, et al. Adiponectin and its receptors in patients with chronic hepatitis C. J Hepatol 2005;43:929–36.
32. Petit JM, Minello A, Jooste V, Bour JB, Galland F, Duvillard L, et al. Decreased plasma adiponectin concentrations are closely related to steatosis in hepatitis C virus-infected patients. J Clin Endocrinol Metab 2005;90:2240–3.
33. Zografos TA, Liaskos C, Rigopoulou EI, Togousidis E, Makaritsis K, Germenis A, Dalekos GN. Adiponectin: a new independent predictor of liver steatosis and response to IFN-alpha treatment in chronic hepatitis C. Am J Gastroenterol 2008; 3:605–14.
34. Wang YY, Lin SY. Leptin in relation to hepatocellular carcinoma in patients with liver cirrhosis. Horm Res 2003;60: 185–90.
35. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425–32.
36. Muoio DM, Lynis Dohm G. Peripheral metabolic actions of leptin. Best Pract Res Clin Endocrinol Metab 2002;16: 653–66.
37. Haynes WG, Morgan DA, Walsh SA, Mark AL, Sivitz WI. Receptor-mediated regional sympathetic nerve activation by leptin. J Clin Invest 1997;100:270–8.
38. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 1997;26: 903–8.
39. Havel PJ, Kasim-Karakas S, Dubuc GR, Mueller W, Phinney SD. Gender differences in plasma leptin concentrations. Nat Med 1996;2:949–50.
40. Sandhofer A, Laimer M, Ebenbichler CF, Kaser S, Paulweber B, Patsch JR. Soluble leptin receptor and soluble receptor-bound fraction of leptin in the metabolic syndrome. Obes Res 2003;11:760–8.
41. Yang G, Ge H, Boucher A, Yu X, Li C. Modulation of direct leptin signaling by soluble leptin receptor. Mol Endocrinol 2004;18:1354–62.
42. Zhang Y, Scarpace PJ. The role of leptin in leptin resistance and obesity. Physiol Behav 2006;88:249–56.
43. Brabant G, Muller G, Horn R, Anderwald C, Roden M, Nave H. Hepatic leptin signaling in obesity. FASEB J 2005;19: 1048–50.
44. Enriori PJ, Evans AE, Sinnayah P, Jobst EE, Tonelli-Lemos L, Billes SK, et al. Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. Cell Metab 2007;5:181–94.
45. Kakuma T, Lee Y, Higa M, Wang Z, Pan W, Shimomura I, et al. Leptin, troglitazone, and the expression of sterol regulatory element binding proteins in liver and pancreatic islets. Proc Natl Acad Sci USA 2000;97:8536–41.
46. Javor ED, Ghany MG, Cochran EK, Oral EA, DePaoli AM, Premkumar A, et al. Leptin reverses nonalcoholic steatohepatitis in patients with severe lipodystrophy. Hepatology 2005;41: 753–60.
47. Ikejima K, Honda H, Yoshikawa M, Hirose M, Kitamura T, Takei Y, et al. Leptin augments inflammatory and profibrogenic responses in the murine liver induced by hepatotoxic chemicals. Hepatology 2001;34:288–97.
48. Cao Q, Mak KM, Ren C, Lieber CS. Leptin stimulates tissue inhibitor of metalloproteinase-1 in human hepatic stellate cells: respective roles of the JAK/STAT and JAK-mediated H2O2-dependant MAPK pathways. J Biol Chem 2004;279: 4292–304.
49. Saxena NK, Titus MA, Ding X, Floyd J, Srinivasan S, Sitaraman SV, et al. Leptin as a novel profibrogenic cytokine in hepatic stellate cells: mitogenesis and inhibition of apoptosis mediated by extracellular regulated kinase (Erk) and Akt phosphorylation. FASEB J 2004;18:1612–4.
50. Ikejima K, Takei Y, Honda H, Hirose M, Yoshikawa M, Zhang YJ, et al. Leptin receptor-mediated signaling regulates hepatic fibrogenesis and remodeling of extracellular matrix in the rat. Gastroenterology 2002;122:1399–410.
51. Angulo P, Alba LM, Petrovic LM, Adams LA, Lindor KD, Jensen MD. Leptin, insulin resistance, and liver fibrosis in human nonalcoholic fatty liver disease. J Hepatol 2004;41:943–9.
52. Chalasani N, Crabb DW, Cummings OW, Kwo PY, Asghar A, Pandya PK, et al. Does leptin play a role in the pathogenesis of human nonalcoholic steatohepatitis? Am J Gastroenterol 2003;98:2771–6.
53. Liu ZW, Zhang N, Han QY, Zeng JT, Chu YL, Qiu JM, et al. Correlation of serum leptin levels with anthropometric and metabolic parameters and biochemical liver function in Chinese patients with chronic hepatitis C virus infection. World J Gastroenterol 2005;11:3357–62.
54. Crespo J, Rivero M, Fabrega E, Cayon A, Amando JA, Garcia-Unzeta MT, et al. Plasma leptin and TNF-alpha levels in chronic hepatitis C patients and their relationship to hepatic fibrosis. Dig Dis Sci 2002;47:1604–10.
55. Romero-Gomez M, Castellano-Megias VM, Grande L, Irles JA, Cruz M, Nogales MC, et al. Serum leptin levels correlate with hepatic steatosis in chronic hepatitis C. Am J Gastroenterol 2003;98:1135–41.
56. Giannini E, Ceppa P, Botta F, Mastracci L, Romagnoli P, Comino I, et al. Leptin has no role in determining severity of steatosis and fibrosis in patients with chronic hepatitis C. Am J Gastroenterol 2000;95:3211–7.
57. Moller H, Mellemgaard A, Lindvig K, Olsen J. Obesity and cancer risk: a Danish record-linkage study. Eur J Cancer 1994;30A:344–50.
58. Saxena NK, Sharma D, Ding X, Lin S, Marra F, Merlin D, et al. Concomitant activation of the JAK/STAT, PI3K/AKT, and

ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells. Cancer Res 2007;67:2497–507.
59. Kitade M, Yoshiji H, Kojima H, Ikenaka Y, Noguchi R, Kaji K, et al. Leptin-mediated neovascularization is a prerequisite for progression of nonalcoholic steatohepatitis in rats. Hepatology 2006;44:983–91.
60. Yokota T, Meka CS, Medina KL, Igarashi H, Comp PC, Takahashi M, et al. Paracrine regulation of fat cell formation in bone marrow cultures via adiponectin and prostaglandins. J Clin Invest 2002;109:1303–10.
61. Corbetta S, Bulfamante G, Cortelazzi D, Barresi V, Cetin I, Mantovani G, et al. Adiponectin expression in human fetal tissues during mid- and late gestation. J Clin Endocrinol Metab 2005;90:2397–402.
62. Pineiro R, Iglesias MJ, Gallego R, Raghay K, Eiras S, Rubio J, et al. Adiponectin is synthesized and secreted by human and murine cardiomyocytes. FEBS Lett 2005;26:5163–9.
63. Wolf AM, Wolf D, Avila MA, Moschen AR, Berasain C, Enrich B, et al. Up-regulation of the anti-inflammatory adipokine adiponectin in acute liver failure in mice. J Hepatol 2006;44: 537–43.
64. Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, et al. Structure–function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. J Biol Chem 2003;278:9073–85.
65. Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, et al. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem 2003;278:40352–63.
66. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999;257: 79–83.
67. Tsao TS, Murrey HE, Hug C, Lee DH, Lodish HF. Oligomerization state-dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30). J Biol Chem 2002;277:29359–62.
68. Wang Y, Lam KS, Chan L, Chan KW, Lam JB, Lam MC, et al. Post-translational modifications of the four conserved lysine residues within the collagenous domain of adiponectin are required for the formation of its high molecular weight oligomeric complex. J Biol Chem 2006;281:16391–400.
69. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA 2001;98:2005–10.
70. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595–9.
71. Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, et al. Association of hypoadiponectinemia with impaired vasoreactivity. Hypertension 2003;42:231–4.
72. Ouchi N, Kihara S, Adrita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999; 100:1296–301.
73. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114: 1752–61.
74. Kondo H, Shimomura I, Matsukawa Y, Kumada M, Takahashi M, Matsuda M, et al. Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. Diabetes 2002;51:2325–8.
75. Ohashi K, Ouchi N, Kihara S, Funahashi T, Nakamura T, Sumitsuji S, et al. Adiponectin I164T mutation is associated with the metabolic syndrome and coronary artery disease. J Am Coll Cardiol 2004;43:1195–200.
76. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003;423:762–9.
77. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006;116: 1784–92.
78. Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, et al. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nat Med 2007;33:332–9.
79. Tsuchida A, Yamauchi T, Ito Y, Hada Y, Maki T, Takekawa S, et al. Insulin/Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. J Biol Chem 2004;279:30817–22.
80. Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS, Lodish HF. T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. Proc Natl Acad Sci USA 2004; 101:10308–13.
81. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002;123:134–40.
82. Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. Semin Liver Dis 2001;21:3–16.
83. Kamada Y, Matsumoto H, Tamura S, Fukushima J, Kiso S, Fukui K, et al. Hypoadiponectinemia accelerates hepatic tumor formation in a nonalcoholic steatohepatitis mouse model. J Hepatol 2007;47:556–64.
84. Koteish A, Diehl AM. Animal model. Semin Liver Dis 2001;21: 89–104.
85. Shklyaev S, Aslanidi G, Tennant M, Prima V, Kohlbrenner E, Kroutov V, et al. Sustained peripheral expression of transgene adiponectin offsets the development of diet-induced obesity in rats. Proc Natl Acad Sci USA 2003;100:14217–22.
86. Xu A, Wang Y, Keshaw H, Xu LY, Lam KSL, Cooper GJS. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver disease in mice. J Clin Invest 2003;112: 91–100.
87. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation 2000;102: 1296–301.
88. Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation 2003;107:671–4.
89. Matsubara M, Namioka K, Katayose S. Decreased plasma adiponectin concentrations in women with low-grade C-reactive protein elevation. Eur J Endocrinol 2003;148:657–62.
90. Tchernof A, Nolan A, Sites CK, Ades PA, Poehlman ET. Weight loss reduces C-reactive protein levels in obese postmenopausal women. Circulation 2002;105:564–9.
91. Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. Gut 2001;48:206–11.
92. Li Z, Yang S, Lin H, Huang J, Watkins PA, Moser AB, et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatology 2003; 37:343–50.
93. Yang SQ, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. Proc Natl Acad Sci USA 1997;94:2557–62.

94. Matsumoto H, Tamura S, Kamada Y, Kiso S, Fukushima J, Wada A, et al. Adiponectin deficiency exacerbates lipopolysaccharide/D-galactosamine-induced liver injury in mice. World J Gastroenterol 2006;12:3352–8.
95. Masaki T, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M, et al. Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-Ay obese mice. Hepatology 2004;40:177–84.
96. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, et al. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood 2000;96:1723–32.
97. Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. Biochem Biophys Res Commun 2004;323: 630–5.
98. Anania FA. Adiponectin and alcoholic fatty liver: is it, after all, about what you eat? Hepatology 2005;42:530–2.
99. Sennello JA, Fayad R, Morris AM, Eckel RH, Asilmaz E, Montez J, et al. Regulation of T cell-mediated hepatic inflammation by adiponectin and leptin. Endocrinology 2005;146:2157–64.
100. Takemura Y, Ouchi N, Shibata R, Aprahamian T, Kirber MT, Summer RS, et al. Adiponectin modulates inflammatory reactions via calreticulin receptor-dependent clearance of early apoptotic bodies. J Clin Invest 2007;117:375–86.
101. Vandivier RW, Ogden CA, Fadok VA, Hoffmann PR, Brown KK, Botto M, et al. Role of surfactant proteins A, D, and C1q in the clearance of apoptotic cells in vivo and in vitro: calreticulin and CD91 as a common collectin receptor complex. J Immunol 2002;169:3978–86.
102. Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. J Clin Invest 2004;114:147–52.
103. Motoshima H, Wu X, Mahadev K, Goldstein BJ. Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. Biochem Biophys Res Commun 2004;315:264–71.
104. Neumeier M, Weigert J, Schaffler A, Weiss TS, Schmidl C, Buttner R, et al. Aldehyde oxidase 1 is highly abundant in hepatic steatosis and is downregulated by adiponectin and fenofibric acid in hepatocytes in vitro. Biochem Biophys Res Commun 2006;350:731–5.
105. Fujita K, Nishizawa H, Funahashi T, Shimomura I, Shimabukuro M. Systemic oxidative stress is associated with visceral fat accumulation and the metabolic syndrome. Circ J 2006;70:1437–42.
106. Lieber CS. Cytochrome P-4502E1: its physiological and pathological role. Physiol Rev 1997;77:517–44.
107. Weltman MD, Farrell GC, Hall P, Ingelman-Sundberg M, Liddle C. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. Hepatology 1998;27:128–33.
108. Weltman MD, Farrell GC, Liddle C. Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. Gastroenterology 1996;111:1645–53.
109. Leclercq IA, Farrell GC, Field J, Bell DR, Gonzalez FJ, Robertson GR. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. J Clin Invest 2000;105:1067–75.
110. Tomita K, Tamiya G, Ando S, Kitamura N, Koizumi H, Kato S, et al. Tumour necrosis factor alpha signalling through activation of Kupffer cells plays an essential role in liver fibrosis of non-alcoholic steatohepatitis in mice. Gut 2006;55:415–24.
111. Koppe SW, Sahai A, Malladi P, Whitington PF, Green RM. Pentoxifylline attenuates steatohepatitis induced by the methionine choline deficient diet. J Hepatol 2004;41:592–8.
112. Kamada Y, Tamura S, Kiso S, Matsumoto H, Saji Y, Yoshida Y, et al. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. Gastroenterology 2003;125:1796–807.
113. Ding X, Saxena NK, Lin S, Xu A, Srinivasan S, Anania FA. The roles of leptin and adiponectin: a novel paradigm in adipocytokine regulation of liver fibrosis and stellate cell biology. Am J Pathol 2005;166:1655–69.
114. Caligiuri A, Bertolani C, Guerra CT, Aleffi S, Galastri S, Trappoliere M, et al. Adenosine monophosphate-activated protein kinase modulates the activated phenotype of hepatic stellate cells. Hepatology 2008;47:668–76.
115. Adachi M, Brenner DA. High molecular weight adiponectin inhibits proliferation of hepatic stellate cells via activation of adenosine monophosphate-activated protein kinase. Hepatology 2008;47:677–85.
116. Pagano C, Soardo G, Esposito W, Fallo F, Basan L, Donnini D, et al. Plasma adiponectin is decreased in nonalcoholic fatty liver disease. Eur J Endocrinol 2005;152:13–8.
117. Siagris D, Vafiadis G, Michalaki M, Lekkou A, Starakis I, Makri M, et al. Serum adiponectin in chronic hepatitis C and B. J Viral Hepat 2007;14:577–83.
118. Bach N, Thung SN, Schaffner F. The histological features of chronic hepatitis C and autoimmune chronic hepatitis: a comparative analysis. Hepatology 1992;15:572–7.
119. Goodman ZD, Ishak KG. Histopathology of hepatitis C virus infection. Semin Liver Dis 1995;15:70–81.
120. Castera L, Hezode C, Roudot-Thoraval F, Bastie A, Zafrani ES, Pawlotsky JM, et al. Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. Gut 2002;52:288–92.
121. Ohata K, Hamasaki K, Toriyama K, Matsumoto K, Saeki A, Yanagi K, et al. Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. Cancer 2003;97:3036–43.
122. Tietge UJ, Boker KH, Manns MP, Bahr MJ. Elevated circulating adiponectin levels in liver cirrhosis are associated with reduced liver function and altered hepatic hemodynamics. Am J Physiol Endocrinol Metab 2004;287:E82–9.
123. Tacke F, Wustefeld T, Horn R, Luedde T, Srinivas Rao A, Manns MP, et al. High adiponectin in chronic liver disease and cholestasis suggests biliary route of adiponectin excretion in vivo. J Hepatol 2005;42:666–73.
124. Housa D, Housova J, Vernerova Z, Haluzik M. Adipocytokines and cancer. Physiol Res 2006;55:233–44.
125. Otake S, Takeda H, Suzuki Y, Fukui T, Watanabe S, Ishihama K, et al. Association of visceral fat accumulation and plasma adiponectin with colorectal adenoma: evidence for participation of insulin resistance. Clin Cancer Res 2005;11:3642–6.
126. Wei E, Giovannucci E, Fuchs C, Willett W, Mantzoros CS. Low plasma adiponectin levels and risk of colorectal cancer in men: a prospective study. J Natl Cancer Inst 2005;97:1688–94.
127. Ishikawa M, Kitayama J, Kazama S, Hiramatsu T, Hatano K, Nagawa H. Plasma adiponectin and gastric cancer. Clin Cancer Res 2005;11:466–72.
128. Goktas S, Yilmaz MI, Caglar K, Sonmez A, Kilic S, Bedir S. Prostate cancer and adiponectin. Urology 2005;65:1168–72.
129. Petridou E, Mantzoros C, Dessypris N, Koukoulomatis P, Addy C, Voulgaris Z, et al. Plasma adiponectin concentrations in relation to endometrial cancer: a case–control study in Greece. J Clin Endocrinol Metab 2003;88:993–7.
130. Mantzoros C, Petridou E, Dessypris N, Chavelas C, Dalamaga M, Alexe DM, et al. Adiponectin and breast cancer risk. J Clin Endocrinol Metab 2004;89:1102–7.
131. Miyazaki T, Bub JD, Uzuki M, Iwamoto Y. Adiponectin activates c-Jun NH2-terminal kinase and inhibits signal transducer and activator of transcription 3. Biochem Biophys Res Commun 2005;333:79–87.
132. Wang Y, Lam JB, Lam KS, Liu J, Lam MC, Hoo RL, et al. Adiponectin modulates the glycogen synthase kinase-3beta/beta-catenin signaling pathway and attenuates mammary tumorigenesis of MDA-MB-231 cells in nude mice. Cancer Res 2006; 66:11462–70.

133. Konturek PC, Burnat G, Rau T, Hahn EG, Konturek S. Effect of adiponectin and ghrelin on apoptosis of Barrett adenocarcinoma cell line. Dig Dis Sci 2008;53:597–605.
134. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, et al. PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 2001;50:2094–9.
135. Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, et al. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. Diabetes 2003;52:1655–63.
136. Hiuge A, Tenenbaum A, Maeda N, Benderly M, Kumada M, Fisman EZ, et al. Effects of peroxisome proliferator-activated receptor ligands, bezafibrate and fenofibrate, on adiponectin level. Arterioscler Thromb Vasc Biol 2007;27:635–41.
137. Tsuchida A, Yamauchi T, Takekawa S, Hada Y, Ito Y, Maki T, et al. Peroxisome proliferator-activated receptor (PPAR)alpha activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: comparison of activation of PPARalpha, PPARgamma, and their combination. Diabetes 2005;54:3358–70.
138. Tonelli J, Li W, Kishore P, Pajvani UB, Kwon E, Weaver C, et al. Mechanisms of early insulin-sensitizing effects of thiazolidinediones in type 2 diabetes. Diabetes 2004;53: 1621–9.
139. Furuhashi M, Ura N, Higashiura K, Murakami H, Tanaka M, Moniwa N, et al. Blockade of the renin–angiotensin system increases adiponectin concentrations in patients with essential hypertension. Hypertension 2003;42:76–81.
140. Koh KK, Quon MJ, Han SH, Chung WJ, Ahn JY, Seo YH, et al. Additive beneficial effects of losartan combined with simvastatin in the treatment of hypercholesterolemic, hypertensive patients. Circulation 2004;110:3687–92.
141. Nagasawa A, Fukui K, Kojima M, Kishida K, Maeda N, Nagaretani H, et al. Divergent effects of soy protein diet on the expression of adipocytokines. Biochem Biophys Res Commun 2003;311:909–14.
142. Nagao K, Inoue N, Wang YM, Yanagita T. Conjugated linoleic acid enhances plasma adiponectin level and alleviates hyperinsulinemia and hypertension in Zucker diabetic fatty (fa/fa) rats. Biochem Biophys Res Commun 2003;310:562–6.
143. Shimada K, Kawarabayashi T, Tanaka A, Fukuda D, Nakamura Y, Yoshiyama M, et al. Oolong tea increases plasma adiponectin levels and low-density lipoprotein particle size in patients with coronary artery disease. Diabetes Res Clin Pract 2004;65: 227–34.
144. Narasimhan ML, Coca MA, Jin J, Yamauchi T, Ito Y, Kadowaki T, et al. Osmotin is a homolog of mammalian adiponectin and controls apoptosis in yeast through a homolog of mammalian adiponectin receptor. Mol Cell 2005;17:171–80.
145. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91.
146. Suganami T, Nishida J, Ogawa Y. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. Arterioscler Thromb Vasc Biol 2005;25:2062–8.
147. Suganami T, Tanimoto-Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S, et al. Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. Arterioscler Thromb Vasc Biol 2007;27:84–91.
148. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–21.
149. Manco M, Marcellini M, Giannone G, Nobili V. Correlation of serum TNF-alpha levels and histologic liver injury scores in pediatric nonalcoholic fatty liver disease. Am J Clin Pathol 2007; 127:954–60.
150. Tokushige K, Takakura M, Tsuchiya-Matsushita N, Taniai M, Hashimoto E, Shiratori K. Influence of TNF gene polymorphisms in Japanese patients with NASH and simple steatosis. J Hepatol 2007;46:1104–10.
151. Satapathy SK, Garg S, Chauhan R, Sakhuja P, Malhotra V, Sharma BC, et al. Beneficial effects of tumor necrosis factor-alpha inhibition by pentoxifylline on clinical, biochemical, and metabolic parameters of patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2004;99:1946–52.
152. Adams LA, Zein CO, Angulo P, Lindor KD. A pilot trial of pentoxifylline in nonalcoholic steatohepatitis. Am J Gastroenterol 2004;99:2365–8.
153. Banerjee RR, Rangwala SM, Shapiro JS, Rich AS, Rhhoades B, Qi Y, et al. Regulation of fasted blood glucose by resistin. Science 2004;303:1195–8.
154. Sato K, Kobayashi K, Inoguchi T, Sonoda N, Imamura M, Sekiguchi N, et al. Adenovirus-mediated high expression of resistin causes dyslipidemia in mice. Endocrinology 2005;146:273–9.
155. Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, et al. Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. Diabetes 2001;50:2199–202.
156. Curat CA, Wegner V, Sengenes C, Miranville A, Tonus C, Busse R, et al. Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. Diabetologia 2006;49:744–7.
157. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem 2004;50: 1511–25.

MUTATION IN BRIEF

Mutations in the Small Heterodimer Partner Gene Increase Morbidity Risk in Japanese Type 2 Diabetes Patients

Mayumi Enya[1§], Yukio Horikawa[1,2§*], Eiji Kuroda[1], Kayoko Yonemaru[1], Naoko Tonooka[2], Hideaki Tomura[2], Naohisa Oda[3], Norihide Yokoi[4], Kazuya Yamagata[5†], Nobuyuki Shihara[2], Katsumi Iizuka[2], Toshiji Saibara[6], Susumu Seino[4], and Jun Takeda[1]

From the [1]Department of Diabetes and Endocrinology Division of Molecule and Structure Gifu University School of Medicine, Gifu; the [2]Laboratory of Medical Genomics, Biosignal Genome Resource Center, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi; the [3]Department of Internal Medicine, Fujita Health University School of Medicine, Aichi; the [4]Department of Physiology and Cell Biology, Kobe University Graduate School of Medicine, Kobe; the [5]Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, Osaka, the [6]Department of Gastroenterology and Hepatology, Kochi Medical School, Nankoku, Japan

† present affiliation: *Department of Medical Biochemistry, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University*

§ These authors equally contributed to this work

*Correspondence to; Yukio Horikawa, MD, PhD
Department of Diabetes and Endocrinology, Division of Molecule and Structure, Gifu University School of Medicine, Gifu
TEL: +81-58-230-6564, FAX: +81-58-230-6376, E-mail: yhorikaw@gifu-u.ac.jp

Contract grant sponsor: This work was supported by a Health and Labor Science Research Grant for research on Human Genome and Tissue Engineering from the Japanese Ministry of Health, Labor and Welfare, a Grant-in-Aid for Scientific Research from the Japanese Ministry of Science, Education, Sports, Culture and Technology, and a New Energy and Industrial Technology Development Organization Grant.

Communicated by Michael Dean

Mutations in the small heterodimer partner gene (NR0B2; alias SHP) are associated with high birth weight and mild obesity in Japanese children. *SHP* mutations may also be associated with later obesity and insulin resistance syndrome that induces diabetes. To investigate this possibility, the prevalence of *SHP* mutations in Japanese with and without type 2 diabetes mellitus and the functional properties of the mutant proteins were evaluated. Direct sequencing of two exons and flanking sequences of *SHP* in 805 diabetic patients and 752 non-diabetic controls identified 15 different mutations in 44 subjects, including 6 novel mutations. Functional analyses of the mutant proteins revealed significantly reduced activity of nine of the mutations. Mutations with reduced activity were found in 19 patients (2.4%) in the diabetic group and in 6 subjects (0.8%) in the control group. The frequency difference between DM and control subjects adjusted for sex and age was statistically significant (P=0.029, odds ratio 2.67, 95% CI 1.05 – 6.81, 1-β=0.91). We conclude that *SHP* mutations associated with mild obesity in childhood increase susceptibility to type 2 diabetes in later life in Japanese. © 2008 Wiley-Liss, Inc.

KEY WORDS: SHP, type 2 diabetes, obesity, fatty liver, NASH

Received 16 April 2008; accepted revised manuscript 20 June 2008.

DOI: 10.1002/humu.20865

INTRODUCTION

Type 2 diabetes mellitus is characterized by defects of insulin secretion in pancreatic b-cells and insulin action in peripheral tissues. Failure of pancreatic β-cells to compensate for insulin resistance by increasing insulin secretion is thought to underlie the development of type 2 diabetes (Reaven, 1988, Polonsky, 2000).

We have previously shown that mutations in the gene encoding small heterodimer partner (*NR0B2*, alias *SHP*; MIM# 604630), an orphan nuclear receptor that interacts with a number of other receptors (Seol et al., 1996, Masuda et al., 1997, Seol et al., 1998, Johansson et al., 1999), are associated with high birth weight and mild obesity in Japanese children, although the molecular mechanisms by which the *SHP* mutations cause these disorders are unknown (Nishigori et al., 2001).

Nuclear receptors such as SHP and peroxisome proliferator-activated receptor (PPAR) α that regulate lipid metabolism in liver are potential contributors to fatty liver. It should be noted that the storage of lipids in liver can trigger inter-organ crosstalk systems that affect insulin sensitivity in muscle. Farnesoid X receptor (FXR)-null mice, with reduced levels of SHP, develop severe fatty liver and elevated circulating FFAs, which is associated with elevated serum glucose and impaired glucose and insulin tolerance resulting from attenuated inhibition of hepatic glucose production by insulin and reduced peripheral glucose disposal (Ma et al., 2006). Some patients with *SHP* mutations exhibit liver dysfunction due to fatty liver (Nishigori et al., 2001). Accordingly, mutations in *SHP* may be associated with insulin resistance due to both later obesity and also to fatty liver in Japanese subjects.

Nonalcoholic fatty liver disease (NAFLD) is a polygenic disease caused by a combination of environmental and genetic factors. Potential candidate genes contributing to NAFLD, a condition comprising a spectrum of pathological liver conditions ranging from steatosis alone to non-alcoholic steatohepatitis (NASH), include those involved in fat deposition, insulin sensitivity, and hepatic lipid oxidation, synthesis, storage, and export. NASH is believed to be a hepatic expression of metabolic syndrome (Ono and Saibara, 2006). In this regard, genetic abnormalities manifested in obesity and fatty liver might well act in concert to induce diabetes.

To evaluate the influence of *SHP* mutations on risk of later development of type 2 diabetes, we examined the frequencies of these mutations in Japanese subjects with and without type 2 diabetes mellitus as well as in patients with NASH.

MATERIALS AND METHODS

Patient populations

The ADA definitions of type 2 diabetes were used. Obesity is defined in these studies as BMI of >25 kg/m^2, in accord with the criteria of the Japan Society for the Study of Obesity (Japanese Society for the Study of Obesity, 2000) and the report by WHO (Western Pacific Region) and IASO/IOTF (International Association for the Study of Obesity/International Obesity Task Force) (WHO and IASO/IOTF, 2000). We evaluated the prevalence of SHP mutations in 805 Japanese patients with type 2 diabetes (male/female, 432/373: age, 60.3±11.8 yr.; BMI, 24.1± 4.0 kg/m^2) and 752 non-diabetic controls (male/female, 418/334; age, 59.7±13.3 yr.; BMI, 22.9±2.9 kg/m^2). Informed consent was obtained from all of the diabetic subjects and volunteer controls. NASH patients with nonalcoholic fatty liver disease underwent liver biopsy after signed informed consent and thorough clinical evaluation. Liver biopsy was analyzed by a pathologist (H.E.) and the diagnosis of NASH was based on Brunt's criteria (Brunt et al., 1999). Laboratory blood tests and BMI were analyzed in 93 biopsy-proved NASH patients (48 males and 45 females, age: 29.2 ± 5.4 years old, BMI: 29.2 ± 5.4 kg/m2, ALT: 102.6 ± 66.6 IU/L, T Chol: 5.25 ± 1.05 mmol/L, TG: 1.74 ± 0.88 mmol/L, HDL-C: 1.24 ± 0.35 mmol/L, HbA1c: 5.6 ± 1.0 %, FPG: 6.03 ± 1.79 mmol/L).

Mutation analysis

The two exons and flanking regions of the SHP gene were screened for mutations by direct DNA sequencing of the amplified polymerase chain reaction (PCR) products, using specific primer pairs and an ABI PRISM BigDye Terminator Cycle Sequencing FS ready Reaction Kit (Applied Biosystems, Foster City, CA). Primer pairs and PCR conditions used for screening of the SHP gene are as follows. Exon1: 5'-CATGACTTCTGGAGTCAAGG-3' and 5'-GTCCCTTTCAGGCAGGCATA-3',

5'-CATCCTTCTGGCAGCTGCCT-3' and 5'-TTAGAAGCTACCTTCCCTGGCT

GG-3' Exon2: 5'-CAGATCTTGGGCCAGTCTTG-3' and 5'-CTCCAGGAGCATTG GGTCAC-3'. Genomic DNA extracted from diabetic and control subjects was initially denatured at 95° C for 1 min, followed by 35 cycles of denaturation at 94° C for 30 sec, annealing at 60° C or 62° C for 30 sec, extension at 72° C for 30 sec, and a final extension step of 7 min. The sequencing reactions were analyzed by automatic DNA sequencers (Applied Biosystems models 3100 and 3700).

Mutation Nomenclature

The cDNA NM_021969.1 and protein NP_068804.1 sequences were used for mutation nomenclature, with DNA +1 corresponding to the A of the ATG translation initiation codon. Descriptions of all sequence variants were checked using the Mutalyzer program (http://www.LOVD.nl/mutalyzer/).

Functional analysis of SHP mutant proteins

Analysis of the functional properties of mutant and wild-type proteins was performed as described previously (Nishigori et al., 2001). Briefly, the *SHP* mutations newly identified in this study were generated by PCR-based site-directed mutagenesis and cloned in the expression pCMV-6b vector. The sequences for wild-type and mutant SHP proteins, and HNF-4α were cloned in pCMV-6b and pcDNA3.1 (Invitrogen, Groningen, The Netherlands), respectively. For luciferase reporter assays, the promoter region of the human HNF-1α gene was inserted into the pGL3-Basic Reporter vector (Promega, Madison, WI).

HepG2 cells ($1x10^5$) were grown in 6-well plates containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal calf serum. The cells were transfected with ExGen 500 solution (6.6 ml) (Fermentas, Ontario, Canada), 333 ng of HNF-1α-promoter/reporter construct, 100 ng of HNF-4α-expression plasmid, 0-125 ng of test DNA, and 17 ng of pRL (Renilla luciferase)-TK. Luciferase reporter activity was measured using a Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. Renilla luciferase activity was used to normalize transfection efficiencies among experiments.

Statistical analyses

Statistical difference in frequencies of *SHP* mutations between the diabetic and control groups was analyzed by logistic regression analysis, using a package of STATVIEW 5.0 (SAS Institute Inc., Cary, NC). Data obtained by luciferase reporter assay were analyzed by the Student's *t*-test.

RESULTS

Eight hundred five Japanese patients with adult-onset type 2 diabetes (T2DM), 752 non-diabetic controls, and 93 patients with NASH were examined. Screening of the SHP gene (*NR0B2*) by direct sequencing resulted in the identification of fifteen different mutations (c.100C>T [p.Arg34X], c.112C>T [p.Arg38Cys], c.134G>C [p.Arg45Pro], c.157_166del [p.His53AlafsX50], c.160C>T [p.Arg54Cys], c.169C>T [p.Arg57Trp], c.292_300delinsAC [p.Leu98ThyfsX6], c.314T>G [p.Val105Gly], c.512G>C [p.Gly171Ala], c.532G>A [p.Asp178Asn], c.566G>A [p.Gly189Glu], c.583G>T [p.Ala195Ser], c.618G>A [p.Trp206X], c.637C>T [p.Arg213Cys], and c.647G>A [p.Arg216His]) including six novel mutations in type 2 diabetic patients (Table 1),

eight of which were previously identified in obese children (Nishigori et al., 2001) and one of which, p.Gly171Ala, was reported as a polymorphism in a study of Caucasians (Hung et al., 2003, Echwald et al., 2004, Mitchell et al., 2003). In NASH patients, only one mutation, p.Arg45Pro, was identified. We could not find any variants in flanking sequences.

Table 1: Mutations identified in the human SHP gene (*NR0B2*).

Exon	Codon	Nucleotide change	Designation	Patients (n=805)	Controls (n=752)
Mutations with reduced activity					
1	34	c.100C>T	p.Arg34X [a)b)c)d)e)]	2	0
1	53	c.157_166del	p.His53AlafsX50 [a)b)c)e)]	2	0
1	54	c.160C>T	p.Arg54Cys*	0	1
1	57	c.169C>T	p.Arg57Trp [a)]	1	0
1	98	c.292_300 delinsAC	p.Leu98ThyfsX6 [a)c)e)]	6	1
1	105	c.314T>G	p.Val105Gly*	1	0
2	189	c.566G>A	p.Gly189Glu [a)]	3	0
2	195	c.583G>T	p.Ala195Ser [a)c)d)e)]	1	3
2	206	c.618G>A	p.Trp206X*	2	1
2	213	c.637C>T	p.Arg213Cys [a)b)c)e)]	1	0
			sum	19 (2.4%)	6 (0.8%)
Mutations with normal activity					
1	38	c.112C>T	p.Arg38Cys*	1	0
1	45	c.134G>C	p.Arg45Pro*	1	0
1	171	c.512G>C	p.Gly171Ala	0	1
1-2	178	c.532G>A	p.Asp178Asn*	1	0
2	216	c.647G>A	p.Arg216His	6	9
			sum	9 (1.1%)	10 (1.3%)

* indicates six novel variants identified in the present study.

To determine if the mutations alter the function of the SHP protein, the effect of the wild-type and mutant proteins on HNF-4α-mediated transactivation of HNF-1α gene transcription in HepG2 cells was examined by luciferase reporter assay (Fig. 1 and Nishigori et al., 2001). a) early-onset obesity, b) high birth weight, c) diabetes, d) fatty liver, e) decreased insulin sensitivity (Nishigori et al., 2001) Mutations were numbered according to GenBank NM_021969.1 and NP_068804.1. Nucleotide +1 is A of the ATG initiation codon.

Functional analyses of the novel mutant proteins showed significantly reduced activity of transcriptional regulation of HNF-4α, except in the case of p.Arg38Cys, p.Arg45Pro, and p.Asp178Asn. The results of functional analyses of the mutations newly identified in this study are shown in Fig. 1. The mutations with reduced activity were found in nineteen subjects (2.4%) in the diabetic group, six (0.8%) in the control group, and none in the NASH group (Table 1). The frequency difference between DM and control groups was statistically significant by logistic regression analysis considering gender and age (P=0.029; 1-β =0.91) with odds ratio of 2.67 [95% CI, 1.05-6.81]. This frequency difference between DM and control groups came to be not statistically significant by logistic regression analysis when considering gender, age, and BMI (P=0.078), with odds ratio of 2.26 [95% CI, 0.87-5.86]. Furthermore, subjects with mutations of reduced activity showed significantly higher BMI than subjects without the mutations (25.6±4.6 vs 23.5±3.6, P=0.0039 in combined subjects, and 24.0 ± 4.0 vs. 26.5 ± 5.0, P=0.012 in diabetic patients). In control subjects, those with mutations of reduced activity showed similar BMI to those without mutations (22.9 ± 2.9 vs. 23.1 ± 2.7, P=0.87).

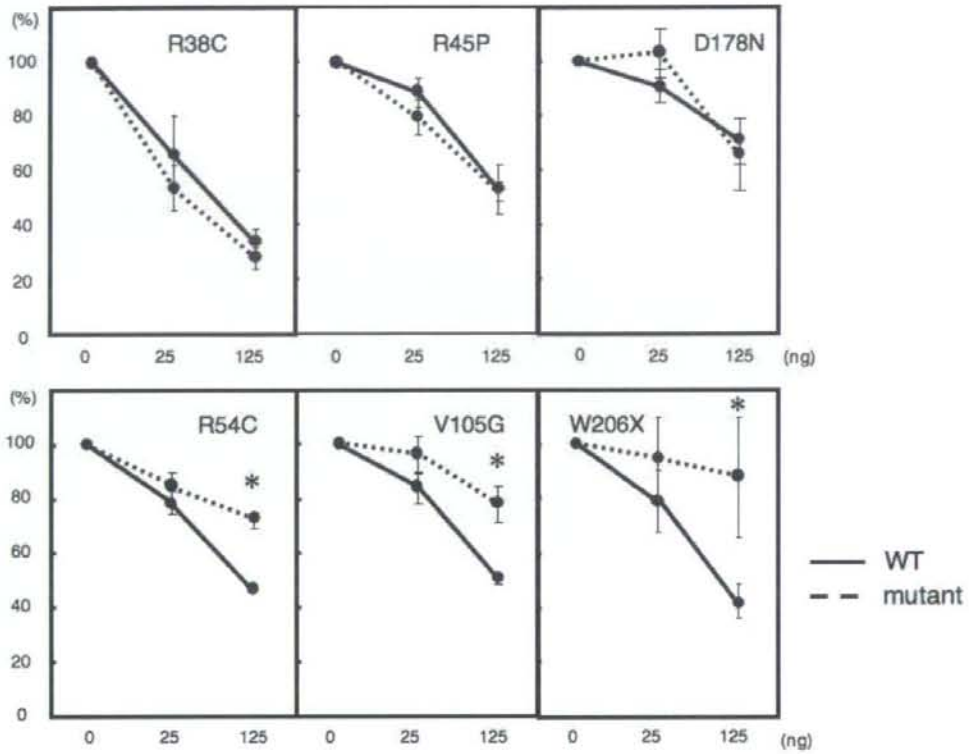

Figure 1: Inhibition of transactivation activity of HNF-4α by wild-type and mutant SHP proteins. It has been shown previously that expression of wild-type SHP significantly decreases HNF-4α transactivation of the HNF-1α gene prompter in HepG2 cells, indicating that SHP is a negative regulator of HNF-4α (Nishigori et al., 2001, Lee et al., 2000). Transcriptional regulation of the novel six mutations of p.Arg38Cys, p.Arg45Pro, p.Arg54Cys, p.Val105Gly, p.Asp178Asn and p.Trp206X was exaimined by luciferase reporter assay (n=3 in each experiment). Functional properties of the other mutations identified have been examined previously (Nishigori et al., 2001, Echwald et al., 2004). The relative luciferase activity (firefly/Renilla) of each construct at 0 ng, 25 ng, and 125 ng of wild-type and mutant SHP proteins was measured in HepG2 cells. Percent activity in relation to basic HNF-4 α activity is shown as mean ± SD. * $P<0.05$.

DISCUSSION

Mutations in the SHP gene have been shown to be associated with high birth weight and early-onset mild obesity in Japanese. Although the molecular mechanism by which these mutations increase body weight is unknown at present, one possibility is suggested by the fact that pancreatic β cells express SHP mRNAs at high levels. Since SHP inhibits HNF-4α (MODY1 protein) (Nishigori et al., 2001,Lee et al., 2000), functional defects of SHP might well increase the activity of HNF-4α and other downstream components of glycolytic signal transduction (Dukes et al., 1998), resulting in increased insulin secretory response to glucose (Wang et al., 2006). In addition, since insulin is a key hormone in fetal growth, high levels of fetal insulin may well be associated with high birth weight and postnatal obesity.

As adult-onset type 2 diabetes is a polygenic disorder requiring interaction of multiple genetic and environmental factors, and Japanese patients exhibit a lesser insulin secretory capacity due to pancreatic β–cell

dysfunction (Kosaka et al., 1977, Kosaka and Akanuma, 1980, Yoshinaga and Kosaka, 1999), the increased insulin secretory demand associated with *SHP* mutations might increase susceptibility to type 2 diabetes in this population. Since other nuclear receptors that interact with SHP in peripheral tissues (Seol et al., 1996, Masuda et al., 1997, Seol et al., 1998, Johansson et al., 1999) may be involved in the pathogenesis of insulin resistance due to obesity or fatty liver, the secondary demand for compensatory insulin secretion might also promote the development of overt diabetes.

FXR-null mice, which show reduced levels of SHP, exhibit elevated plasma cholesterol and triglyceride levels and excessive accumulation of fat in the liver (Ma et al., 2006). Fatty liver also was observed in some early-onset obesity patients with *SHP* mutations (Nishigori et al., 2001). In addition, increased insulin secretion derived from *SHP* mutations accelerates fat accumulation in the liver. Accordingly, we examined 93 NASH patients, and found one mutation. However, none of the mutations associated with reduced activity was found in the NASH group, suggesting that the effect of SHP on the accumulation of fat in the liver may be of little clinical importance.

While we cannot link the etiology of NASH to mutation of *SHP*, the finding that *SHP* mutations increase morbidity risk for type 2 diabetes due to mild obesity in later life in Japanese suggests a genetic link between obesity and type 2 diabetes in human. To clarify the complex relationship of type 2 diabetes with SHP deficiency, further genetic analysis and characterization of the diabetogenic factors involved is required.

According to previous epidemiological studies, low birth weight and fetal thinness are associated with insulin resistance syndrome and were therefore thought to be related to later risk of type 2 diabetes (Hales et al., 1991, Eriksson et al., 2003). In contrast, we demonstrate here an increased morbidity risk of type 2 diabetes due to *SHP* mutations associated with high birth weight and mild obesity in Japanese children. Further analysis of the functional properties of mutant SHP proteins in energy expenditure should provide new insight into the relationship between birth weight and the development of type 2 diabetes.

ACKNOWLEDGMENTS

We thank Tsuchida H., Yokoyama K., Uda I., and Ibe Y. for technical assistance.

REFERENCES

Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. 1999. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 94: 2467-2474

Dukes ID, Sreenan S, Roe MW, Levisetti M, Zhou YP, Ostrega D, Bell GI, Pontoglio M, Yaniv M, Philipson L, Polonsky KS. 1998. Defective pancreatic b-cell glycolytic signaling in hepatocyte nuclear factor-1α-deficient mice. J Biol Chem 273: 24457-24464

Echwald SM, Andersen KL, Sorensen TIA, Larsen LH, Andersen T, Tonooka N, Tomura H, Takeda J, Pedersen O. 2004. Mutation analysis of NR0B2 among 1545 Danish men identifies a novel c. 278 G>A (p. G93D) variant with reduced functional activity. Hum Mutat 24: 381-387

Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJP. 2003. Early adiposity rebound in childhood and risk of type 2 diabetes in adult life. Diabetologia 46: 190-194

Hales CN, Barker DJP, Clark PMS, Cox LJ, Fall C, Osmond C, Winter PD. 1991. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 303: 1019-1022

Hung CC, Farooqi IS, Ong K, Luan J, Keogh JM, Pembrey M, Teo GSH, Dunger D, Wareham NJ, O'Rahilly S. 2003. The contribution of variants in the small heterodimer partner gene to birthweight, adiposity and insulin levels: Mutational analysis and association studies in multiple populations. Diabetes 52:1288-1291

Japanese Society for the Study of Obesity. 2000. Committee Report. J Jpn Soc Study Obes 6: 18-28

Johansson L, Thomsen JS, Damdimopoulos AE, Spyrou G, Gustafsson J-A, Treuter E. 1999. The orphan nuclear receptor SHP inhibits agonist-dependent transcriptional activity of estrogen receptors ERα and ERβ. J Biol Chem 274: 345-353

Kosaka K, Hagura R, Kuzuya T. 1977. Insulin responses in equivocal and definite diabetes, with special reference to subjects who had mild glucose intolerance but later developed definite diabetes. Diabetes 26: 944-952

Kosaka K, Akanuma Y. 1980. Heterogeneity of plasma IRI responses in patients with IGT. Diabetologia 18: 347-348

Lee Y-K, Dell H, Dowhan DH, Hadzopoulou-Cladaras M, Moore DD. 2000. The orphan nuclear receptor SHP inhibits hepatocyte nuclear factor 4 and retinoid X receptor transactivation: Two mechanisms for repression. Mol Cell Biol 20: 187-195

Ma K, Saha PK, Chan L, Moore DD. 2006. Farnesoid X receptor is essential for normal glucose homeostasis. J Clin Invest 116: 1102-1109

Masuda N, Yasuno H, Tamura T, Hashiguchi N, Furusawa T, Tsukamoto T, Sadano H, Osumi T. 1997. An orphan nuclear receptor lacking a zinc-finger DNA-binding domain: interaction with several nuclear receptors. Biochim Biophys Acta 1350: 27-32

Mitchell SM, Weedon MN, Owen KR, Shields B, Wilkins-Wall B, Walker M, McCarthy MI, Frayling TM, Hattersley AT. 2003. Genetic variation in the small heterodimer partner gene and young-onset type 2 diabetes, obesity, and birth weight in U.K. subjects. Diabetes 52: 1276-1279

Nishigori H, Tomura H, Tonooka N, Kanamori M, Yamada S, Sho K, Inoue I, Kikuchi N, Onigata K, Kojima I, Kohama T, Yamagata K, Yang Q, Matsuzawa Y, Miki T, Seino S, Kim M-Y, Choi H-S, Lee Y-K, Moore DD, Takeda J. 2001. Mutations in the small heterodimer partner gene are associated with mild obesity in Japanese subjects. Proc Natl Acad Sci USA 98: 575-580

Ono M, Saibara T. 2006. Clinical features of nonalcoholic steatohepatitis in Japan: evidence from the literature. J Gastroenterol 41: 725-732

Polonsky KS. 2000. Dynamics of insulin secretion in obesity and diabetes. Int J Obes Relat Metab Disord (Suppl.) 2: S29-31

Reaven GM. 1988. Role of insulin resistance in human disease. Diabetes 37: 1595-1607

Seol W, Choi H-S, Moore DD. 1996. An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors. Science 272: 1336-1339

Seol W, Hanstein B, Brown M, Moore DD. 1998. Inhibition of Estrogen receptor action by the orphan receptor SHP (short heterodimer partner). Mol Endo 12: 1551-1557

Wang L, Huang J, Saha P, Kulkarni RN, Hu M, Kim YD, Park KG, Chan L, Rajan A, Lee I, Moore DD. 2006. Orphan receptor SHP is an important mediator of glucose homeostasis. Mol Endocrinol 20: 2671-81

WHO (Western Pacific Region) and IASO (The International Association for the Study of Obesity) /IOTF (The International Obesity Task Force). 2000. The Asia-Pacific perspective: Redefining obesity and its treatment. Web site URL; http://www.diabetes.com.au/pdf/obesity_report.pdf

Yoshinaga H, Kosaka K .1999. Heterogeneous relationship of early insulin response and fasting insulin level with development of non-insulin-dependent diabetes mellitus in non-diabetic Japanese subjects with or without obesity. Diabetes Res Clin Pract 44: 129-136

臨床

NASHの治療

宇都浩文**　上村修司*　坪内博仁***

要　旨

NASH は今後日本で増加することが予想されるが，NASH に対する薬物療法に関してはエビデンスレベルの高い報告は少なく，日本人を対象とした検討も少ない．NASH は内臓脂肪蓄積を基盤とするメタボリックシンドロームを背景に，インスリン抵抗性・耐糖能異常，糖尿病，脂質異常症，高血圧などを合併することが多く，その対策が NASH 治療の基本である．つまり，日常生活・生活習慣の是正や肥満の改善に加え，合併する疾患に対する治療が NASH の病態改善にも有効と考えられる．

はじめに

非アルコール性脂肪肝炎（NASH）は，非アルコール性脂肪性肝疾患（NAFLD）の中で，肝細胞の変性・壊死に伴う炎症や線維化を伴う進行性の疾患である．NASH の成因機序として，Day らの提唱した“two hit theory”が広く知られている．すなわち，NASH の発生には，正常肝から単純性脂肪肝（simple steatosis）となる“1st hit”と，単純性脂肪肝から NASH へ進行する“2nd hit”が必要と言われている[1]．“1st hit”には，肥満，脂質異常症，糖尿病などの生活習慣病や遺伝子多型，薬剤などが原因として考えられ，“2nd hit”には，高インスリン血症，TNFα などのサイトカイン，鉄沈着などに起因する酸化ストレスなどが重要な因子として挙げられる．このことから，NASH の治療は肥満，糖尿病，脂質異常症，高血圧といった生活習慣病に対する食事・運動療法を主体とした生活習慣の改善が中心となる．しかし，NASH に対する薬物治療に関してはエビデンスレベルの高い報告は少なく，推奨度の高い治療法は十分確立していない[2]．本稿では，NASH の治療に関して最近の知見を含めて紹介する．

日常生活指導

1．食事・運動療法

NASH は，肝における生活習慣病やメタボリックシンドロームの表現型と言われており，生活習慣の改善が必須である．そのため，NASH ではメタボリックシンドロームの治療に則した食事・運動療法を行うことが重要である[3]．食事指導の目安としては，1日当たりのエネルギーは標準体重当たり 25～35 kcal/kg 程度，タンパク質は 1.0～1.5g/kg，

*　鹿児島大学大学院医歯学総合研究科
消化器疾患・生活習慣病学
** 同 講師　*** 同 教授

キーワード：非アルコール性脂肪性肝炎（NASH），治療，インスリン抵抗性

表1 NASHに対する薬物治療

治療薬	対象患者	結果（文献 No.）
インスリン抵抗性改善薬		
ピオグリタゾン	NASH 55例	ALT値低下，組織学的改善（6）
ロシグリタゾン	NASH 63例	ALT値低下，組織学的改善，アディポネクチン上昇（7）
メトホルミン	NAFLD 110例	ALT値低下，評価可能患者の組織学的改善（8）
糖尿病治療薬		
ナテグリニド	NASH 10例	ALT値低下，組織学的改善（9）
抗酸化薬		
ビタミンE	NASH 12例	ALT値低下，組織学的改善（10）
ビタミンE＋C	NASH 49例	ALT値変化なし，組織学的線維化改善（11）
ベタイン	NASH 10例	ALT値低下，組織学的改善（12）
UDCA	NASH 166例	ALT値低下あるもコントロール群と有意差なし（13）
UDCA＋ビタミンE	NASH 48例	ALT値低下，組織学的改善（14）
脂質異常症治療薬		
ゲムフィブロジル	NASH 46例	ALT値低下（16）
アトルバスタチン	NASH 27例	ALT値低下（17）
プロブコール	NASH 30例	ALT値低下（18）
高血圧薬		
ロサルタン	NASH 7例	ALT値低下，組織学的改善（22）

略語：巻末の「今月の略語」参照

脂肪は総エネルギーの20%以下とする[4]．また，散歩などの有酸素運動により筋肉や脂肪細胞における代謝が改善し，脂肪細胞より分泌されるTNFαが減少し，インスリン抵抗性の改善やトリグリセリド（TG），VLDLの減少，HDLコレステロール（HDL-C）の増加など脂質代謝も改善する[5]．この際，体重は1週間に1.6kg未満の割合で緩徐に減量することが肝要である[2]．

薬物療法

NASHを含めたNAFLDに肥満，糖尿病，脂質異常症，高血圧などの生活習慣病を合併する場合には，これらの合併症に対する薬物治療がまず必要である．NASHの病態進展にはインスリン抵抗性や糖代謝異常，酸化ストレス，脂質代謝異常，高血圧などが重要な因子と考えられ，それぞれを分子標的とした薬物治療が行われている（表1）．

1．インスリン抵抗性改善薬

NASH患者の8割に認められるインスリン抵抗性はNASHの基本病態と考えられ，まずその治療がNASHの治療ターゲットとして挙げられる．インスリン抵抗性は，脂肪細胞からのアディポサイトカイン分泌異常によって引き起こされると考えられており，それらの受容体や細胞内シグナル伝達に作用する薬剤が，治療薬として用いられている．PPARγはインスリン感受性の作用増強にかかわる核内受容体であり，脂肪細胞の分化，脂質代謝調節にもかかわっている．チアゾリジン系誘導体はPPARγのリガンドとして作用する薬剤であり，全身のインスリン抵抗性改善や，肝局所において微小炎症の改善や線維化進展抑制などの作用を有することも報告されており，最近，ピオグリタゾンとロシグリタゾンはそれぞれ二重盲検試験でNASHに対する有効性が報告されている[6][7]．本邦では，ピオグリタゾンはインスリン抵抗性改善

薬としてすでに臨床応用されており，インスリン抵抗性を呈する糖尿病を合併しているNASHには試みるべき治療法である．その他，ビグアナイド系薬剤であるメトホルミンもインスリン抵抗性改善薬であり，肝での糖新生を抑制し，肝での糖取り込みを亢進させることによって血糖を調節する．腎機能障害や呼吸循環不全の患者には禁忌であるが，糖尿病患者に有効であるだけでなくNASHに対しても，トランスアミナーゼの改善，肝内脂肪化，線維化の改善作用を有することが示されている[8]．また，ナテグリニドはインスリン分泌能がある程度保たれた2型糖尿病に用いられるが，NASHに対する有効性も報告されている[9]．

2．抗酸化療法

酸化ストレスとは，生体内の酸化還元状態を維持する機構が破綻し，種々の活性酸素種（ROS）が過剰となった状態である．NASHでは，過剰の脂肪酸負荷に対しROSが過剰産生状態となっており，NF-κBを活性化し，TNFαやインターロイキンの転写亢進が認められ，肝細胞死が誘導される．また酸化ストレスは肝星細胞の増殖促進，活性化作用も有しているため，肝線維化も進展させ，NASHの増悪に促進的に作用する．ビタミンE，ビタミンC，ベタイン，ウルソデオキシコール酸（UDCA）などは抗酸化作用を有し，NASHの治療薬の候補である．ビタミンE単独投与やビタミンCとの併用により，NASHにおけるトランスアミナーゼや肝線維化の改善が報告されている[10][11]．コリン代謝産物であるベタインはメチオニンの代謝に関与し，グルタチオンなどの抗酸化物質の供給に重要であり，小胞体のストレスを軽減する可能性があり，NASHのトランスアミナーゼや肝組織の炎症，線維化を改善する[12]．

UDCAは抗酸化作用のほか，利胆作用，肝細胞膜保護作用，免疫調節作用などさまざまな機能を有しており，NASHに対するUDCAの有効性も期待されている．しかしLindorらが行った二重盲検試験では，UDCA投与により肝機能検査値，肝組織所見の改善は認められたが，プラセボ投与群と有意差はなかった[13]．一方Dufourらは無作為比較試験で，UDCAとビタミンEの併用はUDCA単独やコントロールと比較して有意にALTの低下および組織学的改善を認めたと報告している[14]．このような結果から，NASHに対するUDCAの効果は十分ではなく，ビタミンEなどの併用がより効果的であると考えられる．また，肝内に沈着した鉄はフェントン反応を介して酸化力の強いヒドロキシルラジカルを生成し，酸化ストレスをもたらしている．NASH患者では肝組織中の鉄沈着や血清フェリチンの上昇が高頻度に認められ，瀉血療法による生体からの適切な鉄の除去は抗酸化療法の1つと考えられる[15]．

3．脂質異常症治療薬

NAFLD，特にNASHにはメタボリックシンドロームを伴うことが多い．メタボリックシンドロームの診断基準の1つである高TG血症と低HDL-C血症などの脂質代謝異常に対する治療は，NASHの治療にも有用である可能性がある．PPARαアゴニストであるフィブラート系薬剤は，肝臓や骨格筋に作用して脂肪酸燃焼を促進し，組織内脂肪量を低下させ，血中TGは低下，HDL-Cは上昇させ，インスリン抵抗性も改善するため，肝脂肪化に対して有効な薬剤と期待されている[16]．またスタチン製剤は，コレステロール生合成の律速酵素であるHMG-CoA還元酵素を阻害することにより，肝でのコレステロール合成を抑制する．さらに，血中コレステロール，血中TGの低下作用を有しているだけでなく，レニン・アンジオテンシン系抑制，抗炎症作

用やマトリックスメタロプロテアーゼ阻害作用など多彩な作用を有しており，NASH の治療に有用と期待されている．脂質異常症を伴った NASH 患者にスタチン製剤の1つであるアトルバスタチンを，脂質異常症のない NASH 患者には UDCA を投与した Kiyici らの前向き試験では，アトルバスタチン群において肝の脂肪化が有意に改善している[17]．一方，プロブコールは肝でのコレステロール合成抑制作用とともに抗酸化作用を有することが知られているが，NASH に対する ALT 低下作用も報告されている[18]．脂質異常症治療薬のトランスアミナーゼや肝脂肪化に対する作用機序は不明な点も多く，NASH に対する治療に関しては今後の検討が待たれる．

4．高血圧治療薬

NAFLD の約 40% 近くに高血圧が認められ[19]，血圧調整をつかさどる種々の因子が NASH の発症・増悪に関与し，特にレニン・アンジオテンシン系の活性亢進やアディポサイトカイン分泌異常の関与が重要と考えられている．脂肪組織由来のアディポサイトカインにはアディポネクチン，TNFα などが含まれ，インスリン抵抗性を介して血圧上昇に関連している．アンジオテンシンⅡはレニン・アンジオテンシン系の主要な因子の1つであり，昇圧反応や血管リモデリングにも重要な役割を担っている．肝星細胞の表面にはアンジオテンシンⅡタイプ1受容体が発現しており，肝星細胞自体もアンジオテンシンⅡを産生し，オートクリン的およびパラクリン的に肝星細胞自身を活性化・増殖させ，肝線維化の促進に重要な役割を果たしているが，アンジオテンシンⅡにはインスリン抵抗性，酸化ストレスの増強作用もある[20][21]．実際，アンジオテンシンⅡタイプ1受容体拮抗薬（ARB）には降圧効果，抗動脈硬化，インスリン抵抗性改善などの多彩な作用があり，ARB は NASH において抗線維化作用のみならずインスリン抵抗性改善，抗炎症作用も期待できる．つまり，アンジオテンシンⅡをターゲットとした治療には，血圧管理，インスリン抵抗性改善，肝線維化予防の効果が期待でき，NASH の治療に有用である可能性がある[22]．

外科治療法

NASH の病態進行・悪化には肥満が大きく影響するが，体格指数（BMI）が 40kg/m^2 以上の重症肥満で体重の自己コントロールが困難な場合には，外科的治療法も考慮される．外科的治療の基本的な戦略は摂取エネルギーの抑制であり，術式として消化吸収能抑制術と胃縮小手術に分けられる．消化吸収能抑制術には，小腸バイパス術，胆膵バイパス術，十二指腸転換術などが挙げられ，胃縮小手術には，胃バイパス術，胃形成術，胃バンディング術などがある[23]．胃バンディング術は胃上部に巻きつけたバンドにより胃の容積を減らして食事摂取量を減らす術式で，腹腔鏡下にて治療が可能である．

おわりに

本邦においてはメタボリックシンドロームの罹患者の増加が危惧されており，NASH の患者数も今後増加していくと予想される．NASH は進行性の比較的予後不良な疾患であり，EBM に基づいた治療法の早期確立が望まれる．

文　献

1) Day CP, et al: Steatohepatitis: a tale of two "hits"? Gastroenterology 114: 842-845, 1998.
2) Nugent C, et al: Evaluation and management of obesity-related nonalcoholic fatty liver disease. Nat Clin Pract Gastroenterol Hepatol 4: 432-441, 2007.
3) Ueno T, et al: Therapeutic effects of restricted diet and exercise in obese patients with fatty

liver. J Hepatol 27: 103–107, 1997.
4) 日本肝臓学会 編: NASH, NAFLD の診療ガイド. 文光堂, 東京, 2006.
5) Mulcahy K, et al: Diabetes self-management education core outcomes measures. Diabetes Educ 29: 768–803, 2003.
6) Belfort R, et al: A placebo–controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med 355: 2297–2307, 2006.
7) Ratziu V, et al: Rosiglitazone for nonalcoholic steatohepatitis: One–year results of the randomized placebo–controlled Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT) Trial. Gastroenterology 135: 100–110, 2008.
8) Bugianesi E, et al: A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. Am J Gastroenterol 100: 1082–1090, 2005.
9) Morita Y, et al: Nateglinide is useful for nonalcoholic steatohepatitis (NASH) patients with type 2 diabetes. Hepatogastroenterology 52: 1338–1343, 2005.
10) Hasegawa T, et al: Plasma transforming growth factor–beta1 level and efficacy of alpha–tocopherol in patients with non–alcoholic steatohepatitis: a pilot study. Aliment Pharmacol Ther 15: 1667–1672, 2001.
11) Harrison S A, et al: Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. Am J Gastroenterol 98: 2485–2490, 2003.
12) Abdelmalek M F, et al: Betaine, a promising new agent for patients with nonalcoholic steatohepatitis: results of a pilot study. Am J Gastroenterol 96: 2711–2717, 2001.
13) Lindor K D, et al: Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: Results of a randomized trial. Hepatology 39: 770–778, 2004.
14) Dufour J F, et al: Randomized placebo–controlled trial of ursodeoxycholic acid with vitamin E in nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol 4: 1537–1543, 2006.
15) Sumida Y, et al: Effect of iron reduction by phlebotomy in Japanese patients with nonalcoholic steatohepatitis: A pilot study. Hepatol Res 36: 315–321, 2006.
16) Basaranoglu M, et al: A controlled trial of gemfibrozil in the treatment of patients with nonalcoholic steatohepatitis. J Hepatol 31: 384, 1999.
17) Kiyici M, et al: Ursodeoxycholic acid and atorvastatin in the treatment of nonalcoholic steatohepatitis. Can J Gastroenterol 17: 713–718, 2003.
18) Merat S, et al: Probucol in the treatment of non–alcoholic steatohepatitis: a double–blind randomized controlled study. J Hepatol 38: 414–418, 2003.
19) Hamaguchi M, et al: The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann Intern Med 143: 722–728, 2005.
20) Togashi N, et al: Effect of TNF–alpha–converting enzyme inhibitor on insulin resistance in fructose–fed rats. Hypertension 39: 578–580, 2002.
21) Folli F, et al: Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. J Clin Invest 100: 2158–2169, 1997.
22) Yokohama S, et al: Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis. Hepatology 40: 1222–1225, 2004.
23) 川村 功: 外科治療法. 別冊「医学のあゆみ」NAFLD のすべて (西原利治 編), p121–126. 医歯薬出版, 東京, 2006.

1 血液検査

非アルコール性脂肪肝炎 nonalcoholic steatohepatitis（NASH）の診断のための血液検査のひとつは，NASHの基盤となる非アルコール性脂肪性肝疾患 non-alcoholic fatty liver disease（NAFLD）の診断における血液検査である．これには，脂肪肝に特徴的な血液検査に加えて，NASHへの病態の進展を評価する血液検査が含まれる．また，NASHの診断には，アルコール性脂肪肝だけでなく，各種肝炎ウイルス，自己免疫，先天的肝代謝異常など他の原因による肝疾患を除外する必要がある．したがって，除外診断のための血液検査も必要である．さらにNAFLDやNASHの確定診断には肝組織所見が不可欠であるが，NAFLDの頻度は高く，肝生検は侵襲的であるため，血液検査は，肝生検を行う患者を選択するためにも重要である．

スクリーニング検査でNAFLDが考えられる症例のうち，特に以下のような血液検査所見から，単純性脂肪肝よりNASHを疑い（表5-1），肝生検による確定診断を行う．さらに，NASHは，Dayらが提唱したtwo hit theoryによると，肥満，高脂血症，糖尿病などの1st hitによる脂肪肝に，インスリン抵抗性や酸化ストレス，エンドトキシンなどの2nd hitが加わって発症する．したがって，このtwo hit theoryに関連する因子に関する血液検査は，NAFLDやNASHの診断にも有用であると考えられる．

表5-1　肝生検を考慮すべきNAFLD患者の血液検査所見

1. 血液・生化学検査
   - トランスアミナーゼの持続高値（特にALT100以上が持続）
   - AST/ALT比の経時的上昇
   - コリンエステラーゼの経時的低下
   - 肝線維化マーカー（ヒアルロン酸，Ⅳ型コラーゲン，PⅢP）高値
   - 血小板数の低下
   - 血糖コントロール不良
   - 血清フェリチン高値
   - HOMA-IRの上昇（2以上）
2. 特殊検査
   - 高感度CRP高値
   - 血清チオレドキシン高値
   - TNF-α高値
   - アディポネクチン低値
   - レプチン高値
   - 血清CK18断片高値
   - DHEA-S高値

A. NAFLD および NASH 診断に有用な血液生化学検査

AST, ALT の軽度上昇が NAFLD ではみられ，さらに NASH では高い傾向にある（表 5-2）[1]．しかし正常の 5 倍以上になることはほとんどなく，NASH の 2/3 は正常値まで変動することがある[2]．NASH は ALT 優位で AST/ALT 比は 1 未満であり，1 以上となるアルコール性脂肪肝などと鑑別される．さらに，コリンエステラーゼも NAFLD や NASH で上昇する．しかし，ウイルス性慢性肝疾患と同様に NASH でも AST/ALT 比は肝線維化の進行とともに上昇し，肝硬変では 1 以上になることから，経過観察には有用なマーカーの一つである．コリンエステラーゼもアルブミンやプロトロンビン時間と同様に肝硬変に進展すると低下する．また，NAFLD のなかで肝線維化進展例は肝線維化非進展例と比較してトランスアミナーゼは高値であることも報告されており[3]，トランスアミナーゼが持続高値であれば，NASH を疑う根拠となる．ALP，γGTP は NAFLD で上昇することがあるが，軽度であり，NASH と単純性脂肪肝の鑑別には有用ではない．

肝線維化マーカーであるヒアルロン酸，Ⅳ型コラーゲン，procollagen Ⅲ polypeptide（PⅢP）は単純性脂肪肝より NASH で高値となると考えられ，血小板数は低値となる．また，肝合成能の指標であるアルブミン，プロトロンビン時間は NASH の病態進展とともに低下するが，軽度の肝線維化を呈する NASH では単純性脂肪肝とほとんど変わらない．

耐糖能異常，脂質代謝異常，高血圧などの生活習慣病は NASH の発症や病態進展と関連することから，血糖，HbA_{1c}，総コレステロール，LDL コレステロール，中性脂肪などは高値となることが多い．また，NASH は単純性脂肪肝と比較して肝組織中の鉄沈着の程度が高度であり，血清フェリチンは NASH では高値となる．高フェリチン血症と肝組織炎症や線維化重症度との関連もあるといわれる[4]．細胞内で過剰となった鉄がフリー鉄となり，Fenton 反応により ROS の産生を亢進させ，肝細胞障害や肝線維化を惹起すると考えられている．

表 5-2　NASH と単純性脂肪肝患者の生化学検査値の比較*

	steatosis patients	NASH patients	*P* value
AST（U/m*l*）	33.3 ± 12.8	53.0 ± 35.5	0.01
ALT（U/m*l*）	57.0 ± 32.3	80.6 ± 60.0	0.08
FBS（m*l*/m*l*）	108.4 ± 16.4	123.6 ± 36.8	0.06
IRI（μ*l*/m*l*）	11.4 ± 7.8	14.1 ± 10.6	0.28
HOMA-IR	3.10 ± 2.30	4.07 ± 3.73	0.26
HDL cholesterol（mg/d*l*）	49.9 ± 14.4	47.9 ± 11.5	0.50
LDL cholesterol（mg/d*l*）	115.1 ± 31.0	129.3 ± 38.5	0.13
triglycerides（mg/d*l*）	170.7 ± 82.3	172.6 ± 98.5	0.94
iron（ng/m*l*）	116.1 ± 46.9	110.1 ± 40.2	0.59
ferritin（ng/m*l*）	146.6 ± 96.1	264.2 ± 245.4	0.05
hyaluronic acid（ng/d*l*）	27.3 ± 26.2	51.2 ± 52.2	0.05
type Ⅳ col.7s（ng/d*l*）	4.21 ± 0.93	4.67 ± 1.23	0.12

（* means ± SD．文献 1 を改変）

B. 他の肝疾患除外のための血液検査

まず，ウイルス性肝炎の除外のためにHBs抗原とHCV抗体を測定し，自己免疫性肝疾患の可能性を否定するために，抗ミトコンドリア抗体と抗核抗体を測定する．ただし，NAFLDでは抗核抗体陽性者が存在することから，検査結果の解釈には注意が必要である．特徴的な臨床経過や症状などから，先天的肝代謝異常が疑われる場合には，セルロプラスミン，尿中銅，α_1アンチトリプシン，トランスフェリン飽和度などそれらに特異的な検査を行う（図5-1）．

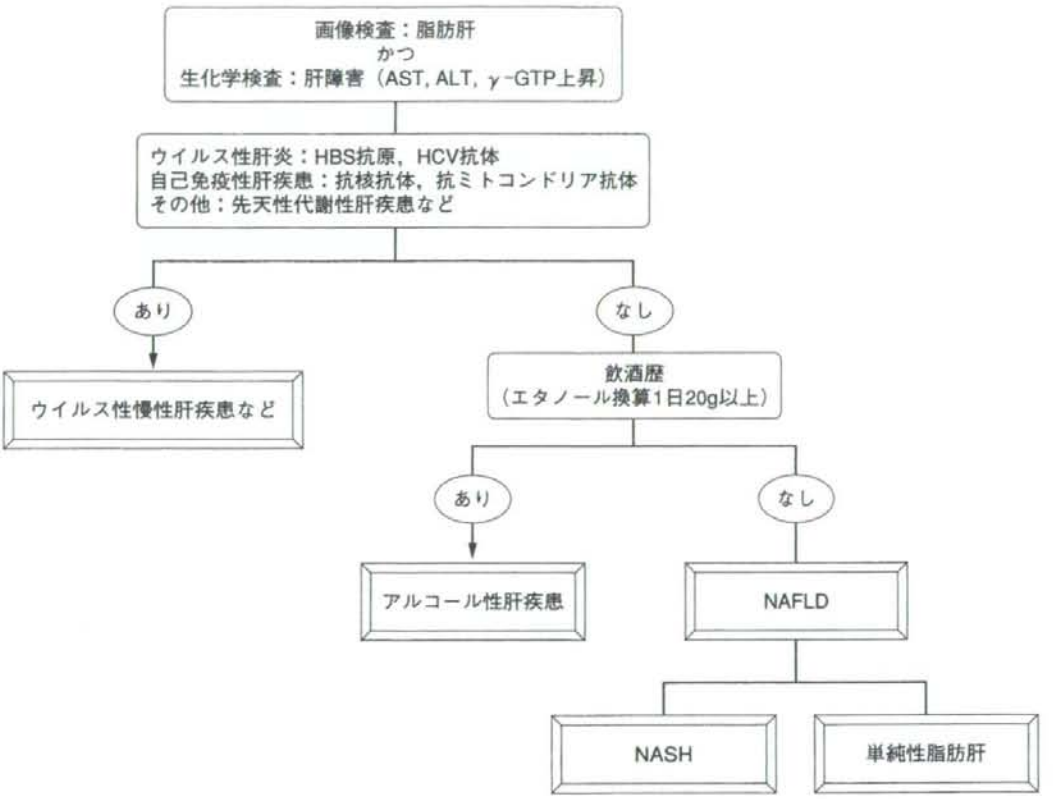

図5-1　NASH/NAFLDの診断

C. NAFLDおよびNASHの病態に関連する血液検査

1. インスリン抵抗性

内臓脂肪蓄積はNAFLDの基盤として重要であり，それに伴うインスリン抵抗性はNASHの基盤となる病態である．インスリン抵抗性の評価法はいくつかあるが，現在最も応用されているのは，HOMA-IR（homeostasis model assessment insulin resistance index）で，1回の採血で評価できることから汎用されている．HOMA-IRは空腹時血糖（mg/dl）×空腹時インスリン（μU/ml）÷405の計算式で求められる．一般に，HOMA-IRが2未満の場合は正常，2以上はイ