Ishizaka Y, Ishizaka N, Tani M, Toda A, Toda EI, Koike K, Nagai R, Yamakado M.	Association between changes in obesity parameters and incidence of chronic kidney disease in Japanese individuals.	Kidney Blood Press Res	32	141-149	2009
Murata M, Matsuzaki K, Yoshida K, Sekimoto G, Uemura Y, Sakaida N, Fujisawa J, Seki T, Koike K, Okazaki K.	Hepatitis B virus X protein shifts hepatic Smad3-mediated signaling from tumor-suppression to oncogenesis in chronic hepatitis B.	Hepatology	49	1203-1217	2009
Yanagimoto S, Tatsuno K, Okugawa S, Kitazawa T, Tsukada K, Koike K, Kodama T, Kimura S, Shibasaki Y, Ota Y	A single amino acid of toll-like receptor 4 that is pivotal for its signaltransduction and subcellular localization.	J Biol Chem	284	3513-3520	2009
Ishizaka N, Ishizaka Y, Yamakado M, Toda E, Koike K, Nagai R.	Association between metabolic syndrome and carotid atherosclerosis in individuals without diabetes based on the oral glucose tolerance test.	Atherosclerosis	204	619-623	2009
Koike K	Steatosis, Liver injury and hepatocarcinogenesis in hepatitis C viral infection.	J Gastroenterol	44supl:	82-88	2009
Ichibangase T, Moriya K, Koike K, Imai K	Limitation of immunoaffinity column for the removal of abundant proteins from plasma in quantitative plasma proteomics.	Biomed Chromatogr	23	480-487	2009
Seronello S, Ito C, Wakita T, Choi J	Ethanol enhances hepatitis C virus replication through lipid metabolism and	J Biol Chem	285(2)	845-54	2010

	elevated NADH/NAD+				
Saeed M, Suzuki R, Kondo M, Aizaki H, Kato T, Mizuochi T, Wakita T, Watanabe H, Suzuki T	Evaluation of hepatitis C virus core antigen assays in detecting recombinant viral antigens of various genotypes.	J Clin Microbiol	47(12)	4141-3	2009
Angus AG, Dalrymple D, Boulant S, McGivern DR, Clayton RF, Scott MJ, Adair R, Graham S, Owsianka AM, Targett-Adams P, Li K, Wakita T, McLauchlan J, Lemon SM, Patel AH	The requirement of cellular DDX3 for Hepatitis C Virus replication is unrelated to its interaction with the viral core protein	J Gen Virol	91(1)	122-32	2010
Hara H, Aizaki H, Matsuda M, Shinkai-Ouchi F, Inoue Y, Murakami K, Shoji I, Kawakami H, Matsuura Y, Lai MM, Miyamura T, Wakita T, Suzuki T	Involvement of creatine kinase B in hepatitis C virus genome replication through interaction with the viral NS4A protein.	J Virol.	83(10)	5137-47.	2009
Park CY, Jun HJ, Wakita T, Cheong JH, Hwang SB.	Hepatitis C virus nonstructural 4B protein modulates sterol regulatory element-binding protein signaling via the AKT pathway.	J Biol Chem.	284(14)	9237-46.	2009
Akbar SM, Hiasa Y, Mishiro S, Onji M	Treatment of hepatitis B virus-infected patients: utility of therapeutic recommendations in developing countries	Expert Opin Pharmacother	10	1605-1614	2009
Akbar SM	Meetng Report: United States-Japan Cooperative	肝臓	50	681-682	2009

	Medical Science Program (USJCMPS) US-Japan Joint AIDS-Hepatitis Meeting Report, Portland, OR, USA, September 19-21, 2009				
Khan SI, Akbar SM, Hossain ST, Mahtab MA	Swine influenza (H1N1) pandemic: developing countries' perspective	Rural Remote Health.	9	1-4	2009
Akbar SM, Khan MS, Mishiro S.	Chronic microbial infection; manipulation of host immunity as interventional approach.	Bangladesh Liver Journal	1	13-19	2009
Noda I, Kitamoto M, Nakahara H, Hayashi R, Okimoto T, Monzen Y, Yamada H, Imagawa M, Hiraga N, Tanaka J, Chayama K	Regular surveillance by imaging for early detection and better prognosis of hepatocellular carcinoma in patients infected with hepatitis C virus.	J Gastroenterol	45	105-112	2010
Hiraga N, Imamura M, Hatakeyama T, Kitamura S, Mitsui F, Tanaka S, Tsuge M, Takahashi S, Abe H, Maekawa T, Ochi H, Tateno C, Yoshizato K, Wakita T, Chayama K.	Absence of viral interference and different susceptibility to interferon between hepatitis B virus and hepatitis C virus in human hepatocyte chimeric mice.	J Hepatol	51(6)	1046-54	2009
Ohira M, Ishiyama K, Tanaka Y, Doskali M, Igarashi Y, Tashiro H, Hiraga N, Imamura M, Sakamoto N, Asahara T, Chayama K,	Adoptive immunotherapy with liver allograft-derived lymphocytes induces anti-HCV activity after liver transplantation in humans and humanized mice.	J Clin Invest	119(11)	3226-35	2009
Abe H, Ochi H, Maekawa T, Hatakeyama T, Tsuge M, Kitamura S, Kimura T, Miki D, Mitsui F, Hiraga N, Imamura M, Fujimoto Y, Takahashi S, Nakamura Y, Kumada H, Chayama K.	Effects of structural variations of APOBEC3A and APOBEC3B genes in chronic hepatitis B virus infection.	Hepatol Res	39	1159-68	2009
Shindo M, Chayama K, Mochida S, Toyota J, Tomita E, Kumada H, Yokosuka O, Sata M, Hayashi N, Suzuki K, Okanoue T, Tsubouchi H, Ishikawa H, Seriu T, Omata M.	Antiviral activity, dose-response relationship, and safety of entecavir following 24-week oral dosing in nucleoside-naive Japanese adult patients with chronic hepatitis B: a randomized, double-blind, phase II clinical trial.	Hepatol Int	3	445-52	2009

Kodama H, Aikata H, Takaki S, Azakami T, Katamura Y, Kawaoka T, Hiramatsu A, Waki K, Imamura M, Kawakami Y, Takahashi S, Toyota N, Ito K, Chayama K.	Evaluation of portosystemic collaterals by MDCT-MPR imaging for management of hemorrhagic esophageal varices.	Eur J Radiol			In press
Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, Tanaka E, Onji M, Toyota J, Chayama K, Yoshioka K, Izumi N, Akuta N, Kumada H.	Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study.	J Gastroenterol	44(9)	952-63	2009
Tahara H, Tanaka Y, Ishiyama K, Ide K, Shishida M, Irei T, Ushitora Y, Ohira M, Banshodani M, Tashiro H, Itamoto T, Asahara T, Imamura M, Takahashi S, Chayama K, Ohdan H.	Successful hepatitis B vaccination in liver transplant recipients with donor-specific hyporesponsiveness.	Transpl Int.	22(8)	805-13.	2009
Yotsuyanagi H, Kikuchi Y, Tsukada K, Nishida K, Kato M, Sakai H, Takamatsu J, Hige S, Chayama K, Moriya K, Koike K.	Chronic hepatitis C in patients co-infected with human immunodeficiency virus in Japan: a retrospective multicenter analysis.	Hepatol Res	39(7)	657-63	2009
Kawaoka T, Aikata H, Takaki S, Katamura Y, Hiramatsu A, Waki K, Takahashi S, Hieda M, Toyota N, Ito K, Chayama K.	Transarterial infusion chemotherapy using cisplatin-lipiodol suspension with or without embolization for unresectable hepatocellular carcinoma.	Cardiovasc Intervent Radiol	32(4)	687-94	2009
Noguchi C, Imamura M, Tsuge M, Hiraga N, Mori N, Miki D, Kimura T, Takahashi S, Fujimoto Y, Ochi H, Abe H, Maekawa T, Tateno C, Yoshizato K, Chayama K.	G-to-A hypermutation in hepatitis B virus (HBV) and clinical course of patients with chronic HBV infection.	J Infect Dis	199(11)	1599-607	2009
Nabeshima Y, Tazuma S, Kanno K, Hyogo H, Chayama K.	Deletion of angiotensin II type I receptor reduces hepatic steatosis.	J Hepatol.	50(6)	1226-35.	2009
Matsumura T, Hu Z, Kato T, Dreux M, Zhang YY, Imamura M, Hiraga N, Juteau JM, Cosset FL, Chayama K, Vaillant A, Liang TJ.	Amphipathic DNA Polymers Inhibit Hepatitis C Virus Infection by Blocking Viral Entry.	Gastroenterolog	137(2)	673-81	2009
Kamatani Y, Wattanapokayakit S, Ochi H, Kawaguchi T, Takahashi A, Hosono N, Kubo M, Tsunoda T, Kamatani N, Kumada H, Puseenam A, Sura T, Daigo Y, Chayama K, Chantratita W, Nakamura Y, Matsuda K.	A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians.	Nat Ganet	41(5)	591-5.	2009

Katamura Y, Aikata H, Takaki S, Azakami T, Kawaoka T, Waki K, Hiramatsu A, Kawakami Y, Takahashi S, Kenjo M, Toyota N, Ito K, Chayama K.	Intra-arterial 5-fluorouracil/interferon combination therapy for advanced hepatocellular carcinoma with or without three-dimensional conformal radiotherapy for portal vein tumor thrombosis.	J Gastroenterol.	44(5)	492-502	2009
Mitsui F, Aikata H, Azakami T, Katamura Y, Kimura T, Kawaoka T, Saneto H, Takaki S, Hiraga N, Tsuge M, Waki K, Hiramatsu A, Imamura M, Kawakami Y, Takahashi S, Arihiro K, Chayama K.	The first Japanese case of COACH syndrome.	Hepatol Res.	39(3)	318-23.	2009
Tsuji K, Kawakami Y, Aimitsu S, Kohno H, Aisaka Y, Kimura S, Nagata S, Ohgoshi H, Kitamoto M, Hidaka T, Kawakami H, Nakanishi T, Chayama K.	Predicting the response to 48-week combination therapy with peginterferon alpha-2b plus ribavirin from the estimated HCV RNA load index after negative serum change in genotype 1b hepatitis C patients.	Hepatol Res.	39(6)	531-8	2009
Mori N, Imamura M, Kawakami Y, Saneto H, Kawaoka T, Takaki S, Aikata H, Takahashi S, Chayama K; Hiroshima Liver Study Group.	Randomized trial of high-dose interferon-alpha-2b combined with ribavirin in patients with chronic hepatitis C: Correlation between amino acid substitutions in the core/NS5A region and virological response to interferon therapy.	J Med Virol.	81(4)	640-9.	2009
Tsukada H, Ochi H, Maekawa T, Abe H, Fujimoto Y, Tsuge M, Takahashi H, Kumada H, Kamatani N, Nakamura Y, Chayama K; the Hiroshima Liver Study Group, and Toranomon Hospital.	A Polymorphism in MAPKAPK3 Affects Response to Interferon Therapy for Chronic Hepatitis C.	Gastroenterology.	136(5)	1796-805	2009
Kawaoka T, Aikata H, Takaki S, Uka K, Azakami T, Saneto H, Jeong SC, Kawakami Y, Takahashi S, Toyota N, Ito K, Hirokawa Y, Chayama K.	FDG positron emission tomography/computed tomography for the detection of extrahepatic metastases from hepatocellular carcinoma.	Hepatol Res.	39(2)	134-42.	2009
Katamura Y, Aikata H, Kimura Y, Azakami T, Kawaoka T, Takaki S, Waki K, Hiramatsu A, Kawakami Y, Takahashi S, Chayama K.	Successful treatment of pulmonary metastases associated with advanced hepatocellular carcinoma by systemic 5-fluorouracil combined with interferon-alpha in a hemodialysis patient.	Hepatol Res.	39(4)	415-20.	2009

Ohira M, Ishifuro M, Ide K, Irei T, Tashiro H, Itamoto T, Ito K, Chayama K, Asahara T, Ohdan H.	Significant correlation between spleen volume and thrombocytopenia in liver transplant patients: a concept for predicting persistent thrombocytopenia.	Liver Transpl.	15(2)	208-15.	2009
Kodama H, Aikata H, Takaki S, Takahashi S, Toyota N, Ito K, Chayama K.	Evaluation of patients with esophageal varices after endoscopic injection sclerotherapy using multiplanar reconstruction MDCT images.	AJR Am J Roentgenol.	192(1)	122-30.	2009
Seronello S, Ito C, Wakita T, Choi J	Ethanol enhances hepatitis C virus replication through lipid metabolism and elevated NADH/NAD+	J Biol Chem	285(2)	845-54	2010
Saeed M, Suzuki R, Kondo M, Aizaki H, Kato T, Mizuochi T, Wakita T, Watanabe H, Suzuki T	Evaluation of hepatitis C virus core antigen assays in detecting recombinant viral antigens of various genotypes.	J Clin Microbiol	47(12)	4141-3	2009
Angus AG, Dalrymple D, Boulant S, McGivern DR, Clayton RF, Scott MJ, Adair R, Graham S, Owsianka AM, Targett-Adams P, Li K, Wakita T, McLauchlan J, Lemon SM, Patel AH	The requirement of cellular DDX3 for Hepatitis C Virus replication is unrelated to its interaction with the viral core protein	J Gen Virol	91(1)	122-32	2010
Hara H, Aizaki H, Matsuda M, Shinkai-Ouchi F, Inoue Y, Murakami K, Shoji I, Kawakami H, Matsuura Y, Lai MM, Miyamura T, Wakita T, Suzuki T	Involvement of creatine kinase B in hepatitis C virus genome replication through interaction with the viral NS4A protein.	J Virol.	83(10)	5137-47.	2009
Park CY, Jun HJ, Wakita T, Cheong JH, Hwang SB.	Hepatitis C virus nonstructural 4B protein modulates sterol regulatory element-binding protein signaling via the AKT	J Biol Chem.	284(14)	9237-46.	2009

	pathway.				
E Mizukoshi, Y Nakamoto, K Arai, T Yamashita, N Mukaida, K Matsushima, O Matsui, S Kaneko.	Enhancement of tumor-specific T cell responses by transcatheter arterial embolization with dendritic cell infusion for hepatocellular carcinoma.	Int J Cancer			in press
S Ura, M Honda, T Yamashita, T Ueda, H Takatori, R Nishino, H Sunakozaka, Y Sakai, K Horimoto, S Kaneko	Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma.	Hepatology	49(4)	1098-1112	2009
T Yamashita, J Ji, A Budhu, M Forgues, W Yang, HY Wang, H Jia, Q Ye, LX Qin, E Wauthier, LM Reid, H Minato, M Honda, S Kaneko, ZY Tang, XW Wang	EpCAM-Positive Hepatocellular Carcinoma Cells Are Tumor-Initiating Cells With Stem/Progenitor Cell Features.	Gastroenterology	136(3)	1012-1024	2009
T Yamashita, M Honda, H Takatori, R Nishino, H Minato, H Takamura, T Ohta, S Kaneko	Activation of lipogenic pathway correlates with cell proliferation and poor prognosis in hepatocellular carcinoma.	J Hepatology	50(1)	100-110	2009

トピックス

ヒト肝細胞キメラマウスを用いた肝炎ウイルス研究

今村道雄*　茶山一彰**

はじめに

近年，ウイルス性肝炎に対する治療は飛躍的に進歩し，その治療成績は向上しつつある．しかし，耐性株の出現やインターフェロン（IFN）療法の限界など，大きな問題点が残されている．これらの問題を克服するためには，肝炎ウイルスが有効に感染する動物モデルを用いた基礎的研究が必要である．しかし有効な動物モデルはいまだ確立されておらず，その原因として肝炎ウイルスがヒトとチンパンジーにしか感染しないことがある．チンパンジーは優れた肝炎ウイルス感染モデルになりうるが，倫理的・経済的に大きな問題を抱えている．よって，チンパンジーに代わるマウスなどの小動物を用いたより実践的な感染モデルが必要である．肝炎ウイルスはヒト肝細胞に発現している受容体を介して感染すると考えられており，通常のマウスに肝炎ウイルスを投与しても感染は認められない．一方，ヒト肝細胞キメラマウスは肝臓が高度にヒト肝細胞に置換されたマウスであり，肝炎ウイルスの投与により，置換されたヒト肝細胞への感染が可能である．ここでは，ヒト肝細胞キメラマウスを用いた肝炎ウイルスの生物学的研究および今後の展望について述べる．

ヒト肝細胞キメラマウスの作製

ヒト肝細胞キメラマウスは肝臓がヒトの肝細胞で置換されたマウスであり，マウスにヒト肝細胞を移植して作製する．このマウスは，移植したヒト肝細胞が免疫反応によって拒絶されずに肝臓中で増殖可能である必要があり，またマウス自身の肝細胞は徐々に死滅する必要がある．そこで，免疫不全の SCID マウスと肝不全を有する uPA トランスジェニックマウスを掛け合わせて uPA/SCID マウスが作製された．この uPA/SCID マウスの脾臓にヒト肝細胞を注入することで，ヒト肝細胞キメラマウスを作製した（図1）．キメラマウスの肝臓のヒト肝細胞への置換率は，ヒト肝細胞に特異的な抗体であるヒトアルブミン抗体による免疫染色，およびマウス血中ヒトアルブミン濃度により算出した．その結果，90% 以上がヒト肝細胞で置換されたキメラマウスが作製された[1]．

B型肝炎ウイルス（HBV）感染マウス

ヒト肝細胞キメラマウスにB型肝炎患者血清を静脈内投与すると，投与2週後よりマウス血中 HBV DNA は陽性となる．その後，血中 HBV DNA は次第に上昇し，高 titer のウイルス血症が長期間にわたり認められる（図2）[2]．HBV 感染マウスの肝組織を免疫染色にて検討すると，ヒトアルブミン陽性のヒト肝細胞は HBc 抗原陽性であった．一方，マウス肝細胞は HBc 抗原陰性であり，置換されたヒト肝細胞に特異的に HBV が感染していることが確認された（図3）[2]．HBV 感染マウスに核酸アナログを経口投与すると，血中 HBV DNA の著明な低下が認められ（図2），HBV 感染マウスが抗ウイルス薬の評価に有用であることが確認された．

B型肝炎患者では核酸アナログ投与中，変異株の出現による薬剤耐性獲得が問題となっている．これらの問題を解決するためには，人工的に種々の変異を組み込んだウイルスに感染す

* 広島大学大学院医歯薬学総合研究科 分子病態制御内科学
** 同 教授

図1　uPA / SCID マウスおよびヒト肝細胞を用いたキメラマウスの作製法
(Hepatoday 10：3, 2005 より改変引用)

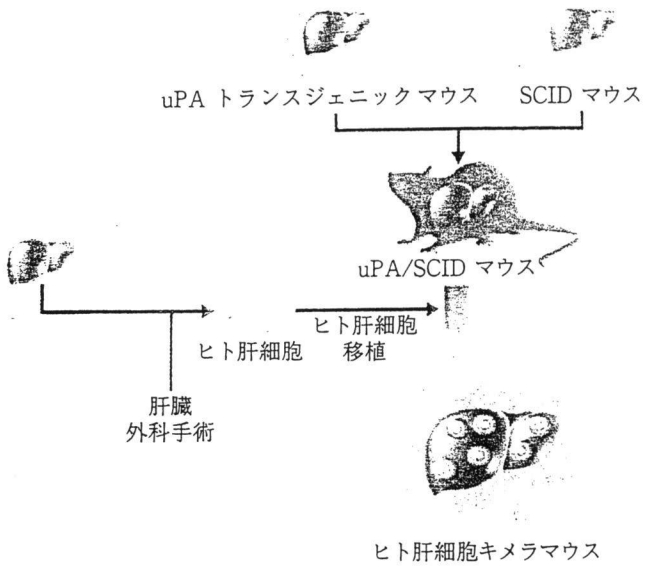
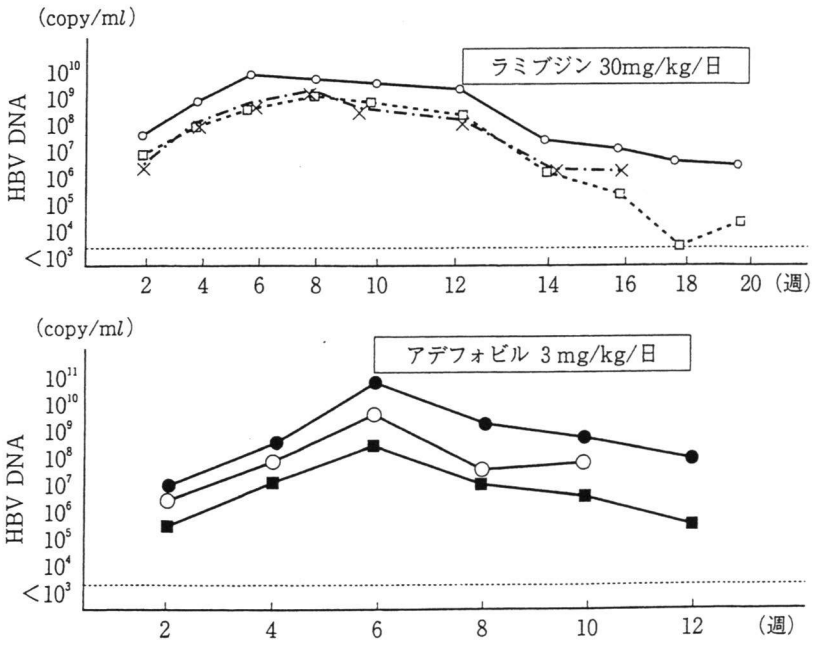


図2　血清を用いたマウスへの HBV 感染



るマウスが必要である．このため，1.4 倍長の HBV ゲノムを組み込んだプラスミドを作製し，HepG2 細胞に恒常発現させ，上清中に HBV を恒常的に産出する細胞を作製した．野生型の YMDD 株と同時に，ラミブジン耐性である YVDD 株を恒常的に産出する細胞も作製した．

図3 HBV 感染マウスの肝組織

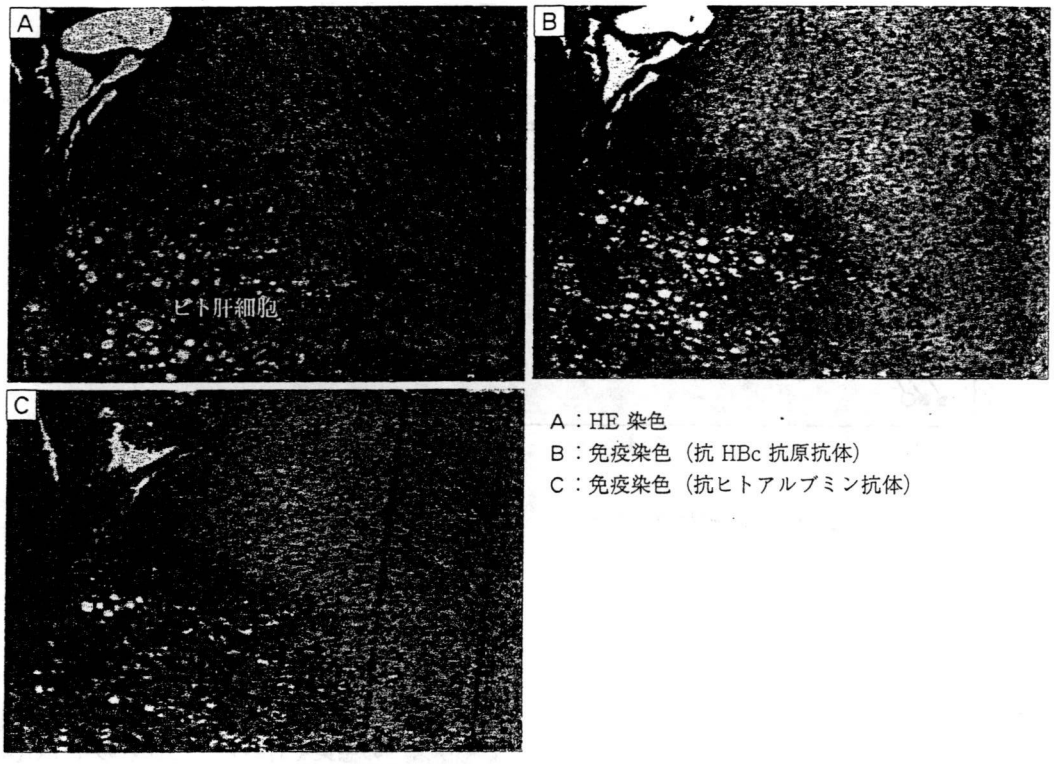
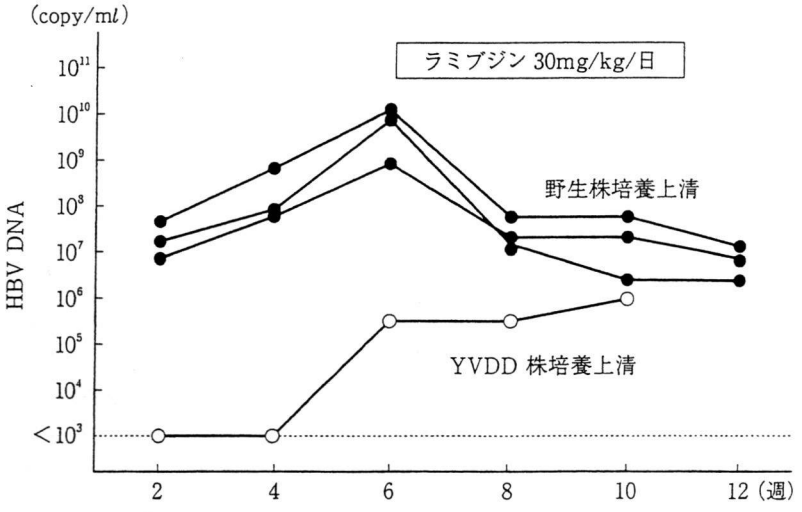
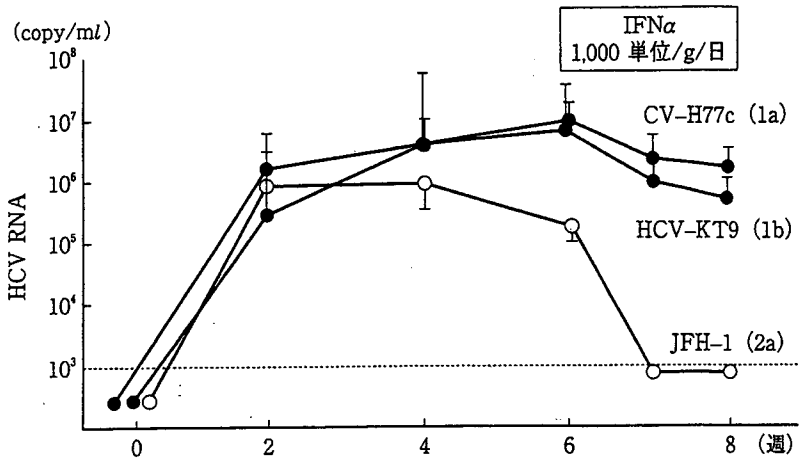


図4 培養上清を用いたマウスへの HBV 感染



これらの培養上清をキメラマウスへ投与すると，野生株および YVDD 株ともに HBV 感染が確認され，人工的に作製した変異ウイルスに感染したマウスが作製される（図4）．これらHBV 感染マウスにラミブジンを経口投与すると，野生株感染マウスでは血中 HBV DNA の低下が認められたが，YVDD 株感染マウスでは低下を認めなかった（図4）．このことは，

図5 クローンを用いたマウスへのHCV感染



感染性クローンを用いたHBV感染マウスが薬剤の効果判定に有用であることを示している．この手法を用いて新規のラミブジン耐性株の評価も可能であり[3]，HBV遺伝子型間でのウイルス感染・増殖，肝線維化の違いも報告されている[4)5)]．

C型肝炎ウイルス（HCV）感染マウス

ヒト肝細胞キメラマウスにHCV感染患者の血清を投与すると，マウス血中に高titerのHCV RNAが長期間検出される[6]．このHCV感染マウスにIFNを投与すると，マウス血中HCV RNAは低下する．これはHCV感染マウスが抗ウイルス薬の評価に有用であることを示しており，新規候補となる抗HCV薬の効果判定にも用いられている[7-10]．

HCVは，遺伝子型間でIFNの感受性が異なることが知られている．すなわち，遺伝子型1型は2型に比べてIFN抵抗性である．また，同じ遺伝子型であっても患者によってIFNの治療効果が異なっている．これらの原因としてHCVのアミノ酸配列が重要と考えられており，実際にHCVのCore[11]，E2[12]，NS5A[13]領域のアミノ酸変異とIFN感受性の違いが報告されている．しかし，これらの変異がなぜIFN抵抗性と関与しているのかはいまだ明らかではない．種々のアミノ酸変異を持つHCV感染マウスの作製が可能となれば，これらの解明の足掛かりになると思われる．そこで我々は，遺伝子型1a型クローンであるCV-H77C[14]，遺伝子型1b型クローンであるHCV-KT9[15]，および遺伝子型2a型クローンであるJFH-1[16]を用いてマウスへの感染を行った．これらのcDNAを発現するプラスミドより*in vitro*トランスクリプション法にてHCV RNAを合成した．マウスへの遺伝子型1aおよび1b型HCVの感染は，合成したRNAをマウス肝臓内に直接注入して行った．一方，遺伝子型2a型のJFH-1は細胞内で感染性ウイルス粒子を作製することが報告されており[16]，合成RNAをHuh7細胞にトランスフェクション後，ウイルス粒子を含む培養上清をマウスに静脈内投与した．いずれの手法においても，投与2週後に血中HCV RNAは定量可能となり，合成RNAを用いた遺伝子型1a, 1bおよび2a型のHCV感染マウスが作製された（図5）[6)11)]．これらウイルス感染マウスの血清をナイーブなマウスに投与するとHCV感染が確認され，マウス血中に感染性HCVが含まれていることが確認された．さらに，これらの感染マウスにIFNを連日投与すると，遺伝子型1a, 1b型の感染マウスでは投与2週後に血中HCV RNAはそれぞれ0.7

log，1.2 log 低下した．一方，遺伝子型 2a 型の感染マウスでは，投与1週後にすべて感度以下に低下した（図5）．これらの結果より，遺伝子型1型は2型に比べてIFN 抵抗性であることが確認された．

おわりに

ヒト肝細胞キメラマウスは，扱いが簡便であること，ウイルスの感染・複製がヒト肝細胞内で生じることなどの点より，優れた肝炎ウイルス感染モデルになりうると思われる．今後，肝炎ウイルス感染ヒト肝細胞キメラマウスを用いて，肝炎ウイルスの感染・増殖のメカニズムの解明，ウイルス性肝炎患者の病態解明，さらには新規抗ウイルス薬や感染予防薬の開発などに応用していきたいと考える．

文　献

1) Tateno C, et al: Near completely humanized liver in mice shows human-type metabolic responses to drugs. Am J Pathol 165 (3): 901–912, 2004.
2) Tsuge M, et al: Infection of human hepatocyte chimeric mouse with genetically engeneered hepatitis B virus. Hepatology 42 (5): 1046–1054, 2005.
3) Yatsuji H, et al: Emergence of a novel lamivudine-resistant hepatitis B virus variant with a substitution outside the YMDD motif. Antimicrob Agents Chemother 50 (11): 3867–3874, 2006.
4) Sugiyama M, et al: Influence of hepatitis B virus genotypes on the intra- and extracellular expression of viral DNA and antigens. Hepatology 44 (4): 915–924, 2006.
5) Sugiyama M, et al: Early dynamics of hepatitis B virus in chimeric mice carting human hepatocytes monoinfected or coinfected with genotype G. Hepatology 45 (4): 929–937, 2007.
6) Hiraga M, et al: Infection of human hepatocyte chimeric mouse with genetically engineered hepatitis C virus and its susceptibility to interferon. FEBS Lett 581 (10): 1983–1987, 2007.
7) Umehara T, et al: Serine palmitoyltransferase inhibitor suppresses HCV replication in a mouse model. Biochem Biophys Res Commun 346 (1): 67–73, 2006.
8) Kneteman N M, et al: Anti-HCV therapies in chimeric scid-Alb/uPA mice parallel outcomes in human clinical application. Hepatology 43 (6): 1346–1353, 2006.
9) Vanwolleghem T, et al: Ultra-rapid cardiotoxicity of the hepatitis C virus protease inhibitor BILN 2061 in the urokinase-type plasminogen activator mouse. Gastroenterology 133 (4): 1144–1155, 2007.
10) Inoue K, et al: Evaluation of a cyclophilin inhibitor in hepatitis C virus-infected chimeric mice *in vivo*. Hepatology 45 (4): 921–928, 2007.
11) Akuta N, et al: Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 46 (3): 403–410, 2007.
12) Chayama K, et al: Association of amino acid sequence in the PKR-eIF2 phosphorylation homology domain and response to interferon therapy. Hepatology 32 (5): 1138–1144, 2000.
13) Enomoto N, et al: Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 334 (2): 77–81, 1996.
14) Yanagi M, et al: Transcripts of a chimeric cDNA clone of hepatitis C virus genotype 1b are infectious *in vivo*. Virology 244 (1): 161–172, 1998.
15) Kimura T, et al: Establishment of an infectious genotype 1b hepatitis C virus clone in human hepatocyte chimeric mice. J Gen Virol 89 (9): 2108–2113, 2008.
16) Wakita T, et al: Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 11 (7): 791–796, 2005.

Hepatitis Virus-infected Animal Model by Using Human Hepatocyte Chimeric Mice

Michio Imamura, Kazuaki Chayama
Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University

動物モデルを用いたウイルス性肝炎研究　最前線

キメラマウス

今村道雄・柘植雅貴・茶山一彰

広島大学大学院医歯薬学総合研究科分子病態制御内科学／いまむら・みちお　つげ・まさたか　ちゃやま・かずあき

ヒト肝細胞キメラマウス●

肝炎ウイルスは，ヒトとチンパンジーにしか感染しない．そのため，生体を用いた肝炎ウイルス研究は困難であり，マウスなどの小動物を用いたより実践的な感染モデルが必要である．ヒト肝細胞キメラマウスは，肝臓が高度にヒト肝細胞に置換されたマウスであり，B型肝炎ウイルス(HBV)やC型肝炎ウイルス(HCV)の投与により置換されたヒト肝細胞への感染が可能である．

ヒト肝細胞キメラマウスは，マウスにヒト肝細胞を移植して作製する．免疫不全のSCIDマウスと，肝不全を有するurokinase-type plasminogen activator (uPA)トランスジェニックマウスを掛け合わせてuPA/SCIDマウスが作製される．このuPA/SCIDマウスの脾臓にヒト肝細胞を注入することでマウス肝臓の90%以上がヒトの肝細胞で置換されたヒト肝細胞キメラマウスが作製される(図1)[1]．

ヒト肝細胞キメラマウスを用いた肝炎ウイルス研究●

ヒト肝細胞キメラマウスにHBVあるいはHCV感染患者の血清を経静脈的に投与すると，10^6～10^8 copy/m*l*のウイルス血症が12週間以上継続する．HBVあるいはHCVを感染させたマウスに核酸アナログやインターフェロンなどの抗ウイルス薬を投与すると血中ウイルス量は低下することより，本マウスが抗ウイルス薬の効果判定

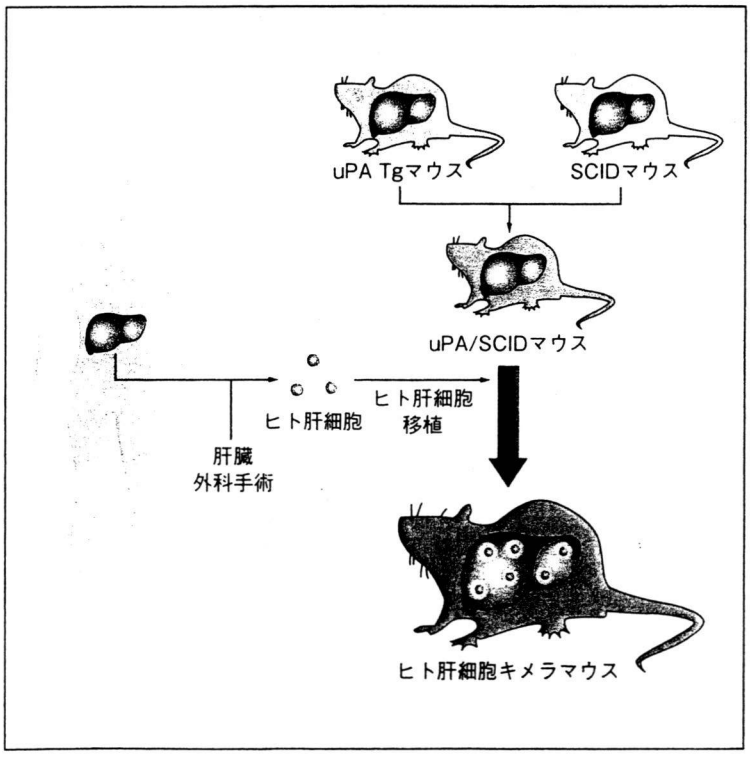


図1　uPA/SCIDマウスおよびヒト肝細胞を用いたキメラマウスの作製法
(茶山一彰：Hepatoday 10：3，2005，図1より引用)

0910-1551/10/￥100/頁/JCOPY

ヒト肝細胞キメラマウスに肝炎ウイルス患者血清や合成ウイルス核酸を投与することにより，肝炎ウイルスの感染が可能となる．肝炎ウイルス感染マウスは肝炎ウイルスの生物学的検討および抗ウイルス薬の効果判定に有用である．

に有用であると思われる[2,3]．本マウスに種々の抗ウイルス薬を組み合わせて投与し，より有効な治療法の開発が可能になり，また新規候補となる抗ウイルス薬や感染予防薬の効果判定にも有用である[4]．

近年，HCV の core 領域や NS5A 領域のアミノ酸変異とインターフェロン療法の治療効果の関係が明らかとなってきた．これらのアミノ酸変異がどのような原因でインターフェロンの効果と関係しているのか，あるいは難治性の HCV に対してどのような治療を行っていくべきかの検討は重要な課題である．そこで種々の HCV クローンから合成した全長 RNA をマウス肝臓内に直接投与することにより，モノクローナルな HCV 感染マウスを作製した[3,5]．さらに，最も課題となっている genotype 1b 型の HCV クローンにあらかじめ変異を導入することにより，種々の変異型 HCV 感染マウスの作製も可能である．これらマウスは HCV の変異と抗ウイルス薬の効果の関係の解明や，難治性 HCV に対する新規治療法の開発に有用であると思われる．

文　献

1) Tateno, C. et al. : Near completely humanized liver in mice shows human-type metabolic responses to drugs. Am J Pathol **165** : 901-912, 2004
2) Tsuge, M. et al. : Infection of human hepatocyte chimeric mouse with genetically engeneered hepatitis B virus. Hepatology **42** : 1046-1054, 2005
3) Hiraga, M. et al. : Infection of human hepatocyte chimeric mouse with genetically engineered hepatitis C virus and its susceptibility to interferon. FEBS Letts **581** : 1983-1987, 2007
4) Matsumura, T. et al. : Amphipathic DNA polymers inhibit hepatitis C virus infection by blocking viral entry. Gastroenterology **137** : 673-681, 2009
5) Kimura, T. et al. : Establishment of an infectious genotype 1b hepatitis C virus clone in human hepatocyte chimeric mice. J Gen Virol **89** : 2108-2113, 2008

Original Article

Impact of Changes in Waist Circumference and BMI over One-Year Period on Serum Lipid Data in Japanese Individuals

Nobukazu Ishizaka[1], Yuko Ishizaka[2], Ei-Ichi Toda[2], Kazuhiko Koike[3], Ryozo Nagai[1], and Minoru Yamakado[2]

[1]Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, Tokyo, Japan
[2]Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital, Tokyo, Japan
[3]Department of Gastroenterology, University of Tokyo Graduate School of Medicine, Tokyo, Japan

Aim: Loss or gain in obesity indexes, such as body mass index (BMI) and waist circumference (WC), may affect serum lipid parameters. We therefore analyzed the impact of changes in WC and BMI over a one-year period on serum levels of LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglycerides (TG).
Methods: We analyzed the data of 3,111 individuals who were not on lipid- lowering medication and who underwent general health screening two years running.
Results: The correlation between percent changes of WC (%dWC) and BMI (%dBMI) were both statistically significantly correlated with percent changes in LDL-C (%dLDL), HDL-C (%dHDL), and TG (%dTG) except that between %dWC and %dHDL in women. In multiple regression analysis, %dBMI, but not %dWC, was found to be an independent predictor of %dLDL, %dHDL, and %dTG. When %dBMI was excluded from the variables, %dWC was identified as an independent factor predicting %dLDL and %dTG; however, in individuals with %dBMI of ≥ 0, %dWC was not found to be a predictor of percent changes in any lipid parameters tested in this model.
Conclusion: Percent changes in BMI were found to be an independent predictor of adverse changes in lipid parameters in both genders. Although percent changes in WC (%dWC) also tended to confer adverse changes in lipid parameters, this relationship did not remain statistically significant after controlling for %dBMI. It is suggested that changes in obesity parameters are an important goal to avoid adverse lipid changes, although there might be some gender differences.

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Key words; Waist circumference, Body mass index, LDL-C, Health screening

Introduction

It is well known that obesity parameters, such as body weight, body mass index (BMI), and waist circumference (WC), may be related with values of serum lipid parameters, including low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TG) as well as other established cardiovascular risk factors[1, 2]. Although there are in general substantial correlations among various obesity parameters, some parameters may provide better prediction of insulin resistance than others[3, 4]. On the other hand, fewer studies have analyzed the effect of changes in obesity parameters on changes in these lipid parameters in the general population[5]. To this end, the aim of the current study was to investigate the relationship between changes in obesity parameters over a one-year period and changes in lipid parameters over the same period in Japanese individuals.

Address for correspondence: Nobukazu Ishizaka, Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, Hongo 7-3-1 Bunkyo-ku, Tokyo 113-8655, Japan
E-mail: nobuishizka-tky@umin.ac.jp
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Table 1a. Baseline Characteristics at the First Visit According to %dWC

Variables	%dWC quartiles				*p* for trend
	First (range: −21.2–−3.4)	Second (range: −3.4–−0.1)	Third (range: 0.0–3.2)	Fourth (range: 3.2–33.3)	
Women					
n	324	193	216	407	
Age, years	52.2 ± 10.2	51.4 ± 10.2	51.4 ± 9.8	50.4 ± 9.3	0.110
Height, cm	156 ± 5	159 ± 6	157 ± 6	158 ± 6	0.021
Weight, kg	51.3 ± 7.5	53.2 ± 8.6	53.7 ± 8.5	52.2 ± 6.9	0.002
WC, cm	78.9 ± 8.5	77.9 ± 9.4	76.8 ± 8.5	73.5 ± 7.9	< 0.001
BMI, kg/m^2	21.0 ± 2.9	21.6 ± 3.3	21.7 ± 3.2	21.0 ± 2.7	0.003
Systolic blood pressure, mmHg	117 ± 18	120 ± 20	117 ± 17	116 ± 19	0.242
Diastolic blood pressure, mmHg	73 ± 10	74 ± 12	73 ± 11	72 ± 12	0.225
Pulse rate, bpm	64 ± 8	64 ± 8	64 ± 9	63 ± 9	0.614
LDL-cholesterol, mg/dL	129 ± 32	130 ± 33	129 ± 33	123 ± 32	0.036
HDL-cholesterol, mg/dL	70 ± 14	68 ± 15	68 ± 14	69 ± 15	0.582
riglyceride (interquartile range), mg/dL	75 (55.5–98.5)	75 (55–108)	77 (54–103)	69 (54–90)	0.040
Uric acid, mg/dL	4.6 ± 1.0	4.5 ± 1.0	4.6 ± 0.9	4.4 ± 0.9	0.156
Fasting glucose, mg/dL	89 ± 15	92 ± 18	92 ± 22	90 ± 17	0.188
Haemoglobin A1C, %	5.1 ± 0.5	5.2 ± 0.5	5.2 ± 0.8	5.1 ± 0.6	0.602
Anti-hypertensive medication, n (%)	17 (5.3)	11 (5.7)	6 (2.8)	14 (3.4)	0.306
Anti-diabetic medication, n (%)	1 (0.3)	0	1 (0.5)	4 (1.0)	0.400
Blood urea nitrogen, mg/dL	13.3 ± 3.7	13.5 ± 3.4	13.3 ± 3.1	13.4 ± 3.2	0.928
Serum creatinine, mg/dL	0.66 ± 0.48	0.63 ± 0.09	0.62 ± 0.09	0.63 ± 0.09	0.368
Current smoker, n (%)	35 (10.8)	14 (7.3)	11 (5.1)	44 (10.8)	0.056
Men					
n	453	571	574	373	
Age, years	54.3 ± 10.2	53.2 ± 10.0	53.5 ± 10.5	51.8 ± 10.1	0.008
Height, cm	170 ± 6	169 ± 6	169 ± 6	169 ± 5	0.919
Weight, kg	68.7 ± 10.0	68.3 ± 9.2	68.2 ± 9.1	67.4 ± 8.8	0.246
WC, cm	88.0 ± 7.8	86.7 ± 7.1	85.3 ± 7.2	82.9 ± 7.5	< 0.001
BMI, kg/m^2	23.8 ± 3.0	23.8 ± 2.7	23.8 ± 2.8	23.5 ± 2.8	0.278
Systolic blood pressure, mmHg	129 ± 20	128 ± 20	126 ± 18	122 ± 16	< 0.001
Diastolic blood pressure, mmHg	82 ± 12	81 ± 12	80 ± 11	78 ± 10	< 0.001
Pulse rate, bpm	64 ± 10	63 ± 9	63 ± 9	62 ± 10	0.185
LDL-cholesterol, mg/dL	131 ± 30	130 ± 30	130 ± 30	127 ± 30	0.291
HDL-cholesterol, mg/dL	57 ± 14	55 ± 14	55 ± 13	57 ± 13	0.280
riglyceride (interquartile range), mg/dL	109 (76–154)	109 (79–157)	110 (77–160)	98 (73–143)	0.287
Uric acid, mg/dL	6.1 ± 1.3	6.1 ± 1.2	6.1 ± 1.2	6.1 ± 1.2	0.628
Fasting glucose, mg/dL	102 ± 24	99 ± 20	98 ± 18	99 ± 24	0.013
Haemoglobin A1C, %	5.5 ± 0.8	5.4 ± 0.8	5.3 ± 0.7	5.4 ± 0.9	0.022
Anti-hypertensive medication, n (%)	51 (11.3)	70 (12.6)	74 (12.9)	39 (10.5)	0.676
Anti-diabetic medication, n (%)	17 (3.8)	10 (1.8)	15 (2.6)	15 (4.0)	0.128
Blood urea nitrogen, mg/dL	14.7 ± 4.2	14.6 ± 3.5	14.3 ± 3.2	14.3 ± 3.0	0.144
Serum creatinine, mg/dL	0.87 ± 0.52	0.85 ± 0.13	0.86 ± 0.13	0.84 ± 0.11	0.429
Current smoker, n (%)	140 (30.9)	193 (33.8)	172 (30.0)	127 (34.0)	0.407

Methods

Study Population

The study was approved by the Ethics Committee of Mitsui Memorial Hospital. Between October 2005 and October 2006, 11,558 individuals underwent general health screening at our institute. Of these, 3,312 individuals (1,203 women, 2,109 men)

Table 1b. Baseline Characteristics at the First Visit According to %dBMI

Variables	%dBMI quartiles				*p* for trend
	First (range: −21.8–−1.8)	Second (range: −1.8–−0.2)	Third (range: −0.2–1.4)	Fourth (range: 1.4–15.6)	
Women					
n	267	263	290	320	
Age, years	51.9 ± 10.1	52.7 ± 9.9	51.2 ± 9.2	49.6 ± 9.9	0.001
Height, cm	156 ± 5	156 ± 6	158 ± 6	158 ± 6	0.005
Weight, kg	53.0 ± 7.7	52.7 ± 8.1	51.9 ± 7.4	52.2 ± 7.7	0.290
WC, cm	77.1 ± 8.6	77.1 ± 9.1	75.6 ± 9.0	75.9 ± 8.2	0.076
BMI, kg/m^2	21.6 ± 3.0	21.5 ± 3.1	20.8 ± 2.8	21.1 ± 3.0	0.002
Systolic blood pressure, mmHg	120 ± 20	118 ± 18	116 ± 18	116 ± 18	0.054
Diastolic blood pressure, mmHg	75 ± 12	74 ± 11	73 ± 11	72 ± 11	0.041
Pulse rate, bpm	64 ± 9	64 ± 8	63 ± 9	64 ± 9	0.171
LDL-cholesterol, mg/dL	132 ± 37	132 ± 30	125 ± 29	121 ± 31	< 0.001
HDL-cholesterol, mg/dL	68 ± 14	69 ± 15	70 ± 15	69 ± 14	0.293
riglyceride (interquartile range), mg/dL	78 (58–104)	75 (56–105)	72 (53–100)	67 (51.5–91)	< 0.001
Uric acid, mg/dL	4.5 ± 1.0	4.5 ± 0.9	4.6 ± 0.9	4.5 ± 1.0	0.470
Fasting glucose, mg/dL	90 ± 17	93 ± 27	89 ± 13	89 ± 12	0.038
Haemoglobin A1C, %	5.1 ± 0.5	5.2 ± 0.8	5.2 ± 0.5	5.1 ± 0.5	0.012
Anti-hypertensive medication, n (%)	11 (4.1)	13 (4.9)	12 (4.1)	12 (3.8)	0.913
Anti-diabetic medication, n (%)	0	3 (1.1)	1 (0.3)	2 (0.7)	0.315
Blood urea nitrogen, mg/dL	13.5 ± 3.0	13.3 ± 3.3	13.7 ± 3.9	13.1 ± 3.1	0.181
Serum creatinine, mg/dL	0.62 ± 0.09	0.62 ± 0.10	0.68 ± 0.51	0.62 ± 0.09	0.022
Current smoker, n (%)	19 (7.1)	22 (8.4)	23 (7.9)	40 (12.5)	0.095
Men					
n	510	515	488	458	
Age, years	54.0 ± 10.2	54.5 ± 10.0	53.4 ± 10.2	51.1 ± 10.3	< 0.001
Height, cm	169 ± 6	169 ± 6	170 ± 6	170 ± 6	0.190
Weight, kg	68.7 ± 9.7	67.5 ± 9.0	68.8 ± 9.5	67.7 ± 8.8	0.050
WC, cm	87.0 ± 7.6	85.5 ± 7.3	86.4 ± 7.7	84.9 ± 7.5	0.002
BMI, kg/m^2	23.9 ± 2.8	23.6 ± 2.9	23.9 ± 2.8	23.5 ± 2.7	0.020
Systolic blood pressure, mmHg	128 ± 19	126 ± 19	127 ± 18	124 ± 18	0.004
Diastolic blood pressure, mmHg	81 ± 12	80 ± 12	81 ± 11	79 ± 11	0.010
Pulse rate, bpm	63 ± 9	63 ± 9	64 ± 9	62 ± 9	0.231
LDL-cholesterol, mg/dL	132 ± 31	130 ± 29	129 ± 29	127 ± 31	0.026
HDL-cholesterol, mg/dL	55 ± 13	56 ± 14	56 ± 13	56 ± 14	0.774
riglyceride (interquartile range), mg/dL	111 (79–158)	107 (75–158)	110 (76–153)	102 (75–148)	0.253
Uric acid, mg/dL	6.2 ± 1.3	6.0 ± 1.2	6.0 ± 1.1	6.1 ± 1.2	0.312
Fasting glucose, mg/dL	100 ± 21	101 ± 23	99 ± 18	98 ± 23	0.088
Haemoglobin A1C, %	5.5 ± 0.8	5.4 ± 0.8	5.3 ± 0.7	5.4 ± 0.8	0.049
Anti-hypertensive medication, n (%)	67 (13.1)	70 (13.6)	42 (8.6)	55 (12.0)	0.065
Anti-diabetic medication, n (%)	16 (3.1)	14 (2.7)	11 (2.3)	16 (3.5)	0.692
Blood urea nitrogen, mg/dL	14.6 ± 4.1	14.5 ± 3.2	14.2 ± 3.2	14.5 ± 3.4	0.307
Serum creatinine, mg/dL	0.88 ± 0.50	0.86 ± 0.13	0.84 ± 0.12	0.85 ± 0.13	0.245
Current smoker, n (%)	162 (31.8)	163 (31.7)	151 (30.9)	156 (34.1)	0.758

underwent general health screening during this period (first visit) and again the following year (second visit). Among these 3,312 individuals, 3,111 (1,140 women, 1,971 men) who reported not taking anti-hyperlipidemic drugs at both visits were enrolled in the present study. The mean ± standard deviation (SD) of the interval between the two visits of the individuals enrolled was 355 ± 52 days. The percent difference in

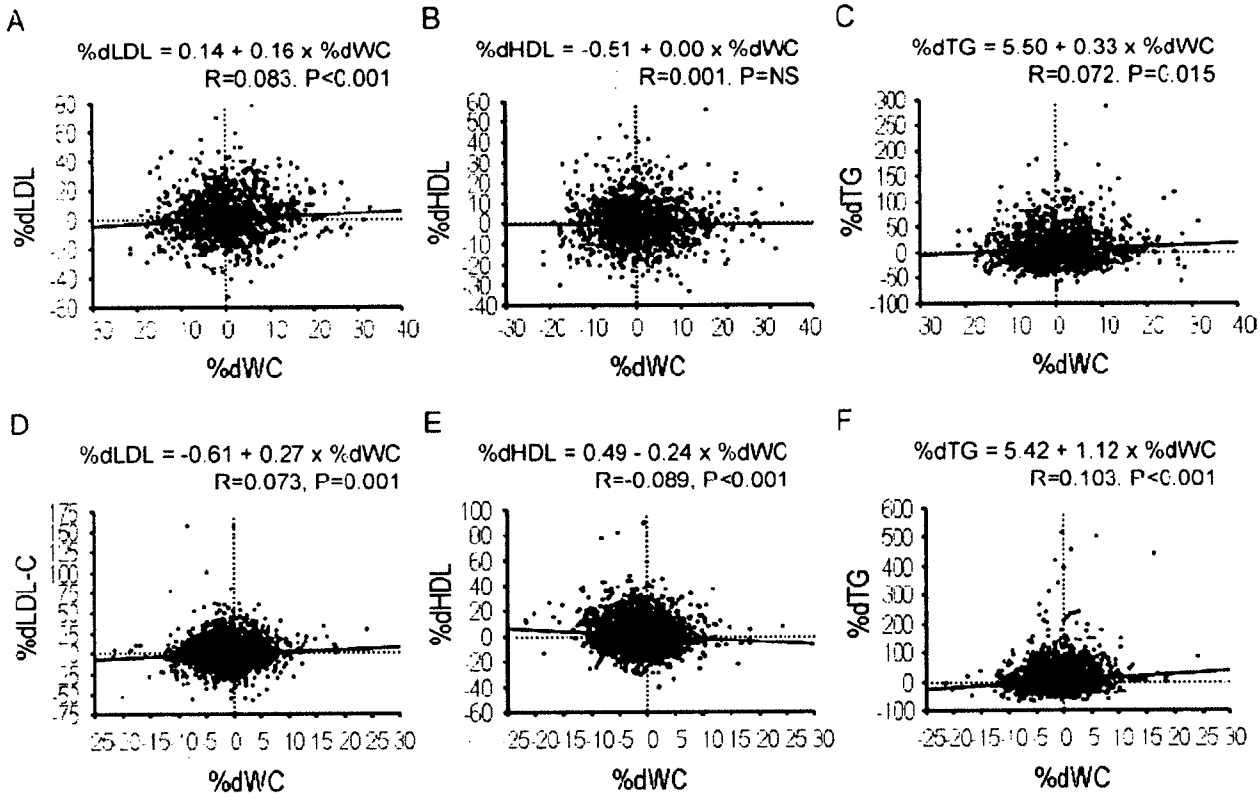

Fig. 1. Linear correlation between %dWC and %dLDL (A), %dHDL (B), and %dTG (C) in women and that between %dWC and %dLDL (D), %dHDL (E), and %dTG (F) in men.

the value of WC, BMI, serum levels of LDL-C, HDL-C, and TG between first and second visits was designated as %dWC, %dBMI, %dLDL, %dHDL, and %dTG respectively. All subjects were seen after an overnight fast. Height and weight were determined, and BMI was expressed as weight (in kilograms) divided by the square of the height (in meters). Waist circumference was measured at the umbilical level to the nearest 1 cm by trained physicians and technicians with the subject standing[6].

Laboratory Analysis

Blood samples were taken from the subjects after an overnight fast. Serum levels of TC, HDL-C, and TG were determined enzymatically. Serum uric acid was measured by the uricase-peroxidase method and hemoglobin A1C was determined using the latex agglutination immunoassay. Blood pressure was measured after about 10 min of rest using an automated sphygmomanometer.

Statistical Analysis

Data are expressed as the mean ± SD unless otherwise stated. Analyses of variance with trend analysis, linear regression analysis and stepwise multiple regression analysis were conducted as appropriate to assess the statistical significance of differences between groups using computer software, StatView ver. 5.0 (SAS Institute, NC) and Dr. SPSS II (SPSS Inc., Chicago, IL). A value of $p < 0.05$ was taken to be significant.

Results

Baseline Characteristics

The mean ± SD age of the individuals enrolled was 51.3 ± 9.8 years in women and 53.3 ± 10.2 years in men at the first visit. The sex-nonspecific ranges (min/max) was −21.2/−3.4, −3.4/−0.1, 0.0/3.2, and 3.2/33.3 for each %dWC quartile, and −21.8/−1.8, −1.8/−0.2, −0.2/1.4, and 1.4/15.6 for each %dBMI quartile. Baseline characteristics of the subjects according to %dWC quartile and %dBMI quartile are described in **Table 1a**, **1b**. There was no statistically

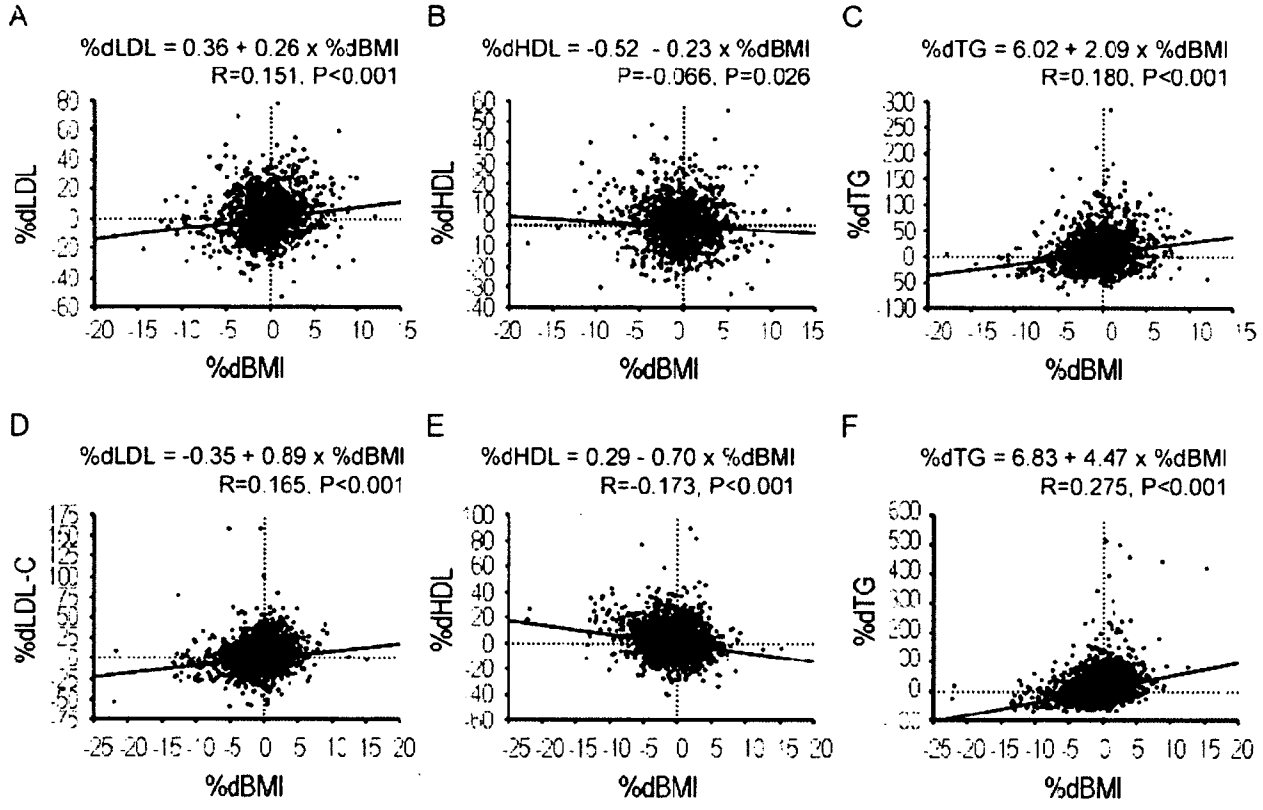

Fig. 2. Linear correlation between %dBMI and %dLDL (A), %dHDL (B), and %dTG (C) in women and that between %dBMI and %dLDL (D), %dHDL (E), and %dTG (F) in men.

significant trend in the rate of anti-hypertensive and anti-diabetic medications across four %dWC or %dBMI quartiles.

Association between Percent Changes in Obesity Parameters and Percent Changes in Lipid Parameters

Scatter plots of %dWC and percent changes in lipid parameters (**Fig. 1**) and %dBMI and percent changes in lipid parameters (**Fig. 2**) are shown. Except between %dWC and %dHDL in women, the correlation was found to be statistically significant between percent changes in obesity parameters and percent changes in lipid parameters; however, the coefficients of correlation were relatively small.

Table 2 describes the percent changes in lipid parameters by %dWC and %dBMI quartiles. In women, %dTG increased with increasing %dWC and with %dBMI. In men, %dLDL and %dTG increased and %dHDL decreased with increasing %dWC (**Table 2a**) and with %dBMI (**Table 2b**). Kappa coefficient between %dWC quartiles and %dBMI quartiles were found to be slight (women, 0.079, $p<0.001$; men, 0.171, $p<0.001$).

Stepwise Multiple Regression Analysis

The correlation coefficient between %dWC and %dBMI was 0.24 in women and 0.47 in men. The regression equation in each gender is as follows: $\%dBMI = -0.181 + 0.096 \times \%dWC$ (women), $\%dBMI = -0.287 + 0.319 \times \%dWC$ (men). We put both %dBMI and %dWC together with age into the statistical model of stepwise multiple regression analysis (**Table 3**, model 1) and it was found that %dBMI, but not %dWC, significantly predicts percent changes in all lipid parameters tested. When %dBMI was excluded from the independent variables, %dWC was identified as an independent factor predicting percent changes in lipid parameters, except for %dHDL in women (**Table 3**, model 2). In women or men with %dBMI of ≥ 0 (580 women, 890 men), %dWC was not found to be a predictor of percent changes in any lipid parameters tested (data not shown).