血漿 ADAMTS13 活性とインヒビターの動態から鑑別することはできず，本酵素活性が著減し，IgG インヒビターを有する症例は，TTP 類似状態あるいは "subclinical TTP" と言っても過言ではないと考えられる[8]。

また，ADAMTS13 に対するインヒビターは AH の 2 割，SAH の 8 割，急性肝炎の 2 割，急性肝不全の 7 割に検出され，その力価は多数例で 0.5～1.5 BU/mL と比較的低値であった。アルコール性肝炎では，血漿 IL-6，IL-8 および TNFα が上昇するに従って ADAMTS13 活性の低下，VWF 抗原の増加が観察され，血漿 UL-VWFM 出現率がより高率となった[10]。ヒト臍帯静脈由来の内皮細胞を用いた *in vitro* の系において，IL-6 が ADAMTS13 の作用を抑制し，IL-8，TNFα が内皮細胞からの UL-VWFM の放出を増強させることが報告されており[20]，炎症性サイトカインと ADAMTS13，VWF との関連が注目されている。最近，健常人に ET を静注した際，急性炎症反応とともに血漿 ADAMTS13 活性の低下，VWF 抗原の増加に加えて UL-VWFM が出現することが確認されており[21]，ET 自身がサイトカインとともに血漿 ADAMTS13 活性を低下させる可能性が論じられている。

今後，慢性・急性肝不全における ADAMTS13 活性低下の機序について，ADAMTS13 抗体依存性および非依存性インヒビターの有無を，ET，サイトカインとの関連のもとにさらに検討していく必要があろう。

おわりに

ADAMTS13 は TTP の発症との関連で注目されてきたが，その主な産生部位が肝星細胞であることが判明して以来，本酵素活性と基質の不均衡は，慢性・急性肝不全[7~10]のみならず造血幹細胞移植後の VOD[7,11]，肝移植後早期のグラフト機能不全[7,12]，重症急性膵炎[22]など微小循環障害，多臓器不全をもたらす多くの病態形成にかかわっていることを示唆する成績が得られてきている。一方，急性・慢性肝不全では高率に ET 血症が招来し，多臓器不全を含めた臓器障害の病態形成に関与していることが明らかにされている[1,2,10]。今後肝不全における ADAMTS13 活性と ET の臨床的意義をさらに解明していくことは，新たな治療戦略に繋がる可能性があろう。

文　献

1) Nolan JP : The role of intestinal endotoxin in liver injury : a long and evolving history. Hepatology 52 : 1829-1835, 2010
2) Fukui H : Relation of endotoxin, endotoxin binding proteins and macrophages to severe alcoholic liver injury and multiple organ failure. Alcohol Clin Exp Res 29 : 172S-179S, 2005
3) Moake JL : Thrombotic microangiopathies. N Engl J Med 347 : 589-599, 2002
4) Fujimura Y, Matsumoto M, Yagi H, et al. : von Willebrand factor-cleaving protease and Upshaw-Schulman syndrome. Progress in Hematology. Int J Hematol 75 : 25-34, 2002
5) Uemura M, Tatsumi K, Matsumoto M, et al. : Localization of ADAMTS13 to the stellate cells of human liver. Blood 106 : 922-924, 2005
6) Moschcowitz E : Hyaline thrombosis of the terminal arterioles and capillaries : a hitherto undescribed disease. Proc NY Pathol Sac 24 : 21-24, 1924
7) Uemura M, Fujimura Y, Ko S, et al. : Pivotal role of ADAMTS13 function in liver diseases. Int J Hematol 91 : 20-29, 2010
8) Uemura M, Fujimura Y, Matsumoto M, et al. : Comprehensive analysis of ADAMTS13 in patients with liver cirrhosis. Thromb Haemost 99 : 1019-1029, 2008
9) Uemura M, Fujimura Y, Matsuyama T, et al. : Potential role of ADAMTS13 in the progression of alcoholic hepatitis. Curr Drug Abuse Rev 1 : 188-1196, 2008
10) Ishikawa M, Uemura M, Matsuyama T, et al. : Potential role of enhanced cytokinemia and plasma inhibitor on the decreased activity of plasma ADAMTS13 in patients with alcoholic hepatitis : relationship to endotoxemia. Alcohol Clin Exp Res 34 : S25-S33, 2010
11) Matsumoto M, Kawa K, Uemura M, et al. :

Prophylactic fresh frozen plasma may prevent development of hepatic VOD after stem cell transplantation via ADAMTS13-mediated restoration of von Willebrand factor plasma levels. Bone Marrow Transplant 40 : 251-259, 2007

12) Ko S, Okano E, Kanehiro H, et al. : Plasma ADAMTS13 activity may predict early adverse events in living donor liver transplantation : observations in 3 cases. Liver Transpl 12 : 859-869, 2006
13) Amitrano L, Guardascione MA, Brancaccio V, et al. : Coagulation disorders in liver disease. Semin Liver Dis 22 : 83-96, 2002
14) Northup PG, Sundaram V, Fallon MB, et al. : Hypercoagulation and thrombophilia in liver disease. J Thromb Haemost 6 : 2-9, 2008
15) Rake MO, Flute PT, Pannell G, et al. : Intravascular coagulation in acute hepatic necrosisi. Lancet 14 : 533-537, 1970
16) Fujiwara K, Ogata I, Ohta Y, et al. : Intravascular coagulation in acute liver failure in rats and its treatment with antithrombin Ⅲ. Gut 29 : 1103-1108, 1988
17) Knittel T, Neubauer K, Armbrust T, et al. : Expression of von Willebrand factor in normal and diseased rat livers and in cultivated liver cells. Hepatology 21 : 470-476, 1995
18) Fukui H, Brauner B, Bode JC, et al. : Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease : reevaluation with an improved chromogenic assay. J Hepatol 12 : 162-169, 1991
19) Fujiwara K, Mochida S, Matsui A, et al. : Fulminant hepatitis and late onset hepatic failure in Japan. Hepatol Res 38 : 646-657, 2008
20) Bernardo A, Ball C, Nolasco L, et al. : Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. Blood 104 : 100-106, 2004
21) Reiter RA, Varadi K, Turecek PL, et al. : Change in ADAMTS13 (von-Willebrand-factor-cleaving protease) activity after induced release of von Willebrand factor during acute systemic inflammation. Thromb Haemost 93 : 554-558, 2005
22) Morioka C, Uemura M, Matsuyama T, et al. : ADAMTS13 activity parallels the APACHE Ⅱ score, reflecting an early prognostic indicator for patients with severe acute pancreatitis. Scand J Gastroenterol 43 : 1387-1396, 2008

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IgG4 関連硬化性胆管炎

2 次性硬化性胆管炎

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要約：一次検診の普及や画像診断の進歩に伴い，肝内・肝外の胆管における硬化性変化に遭遇する機会が増加している。硬化性胆管病変には主に炎症性変化と腫瘍性変化があり，両者の病因・病態が異なり，おのおのに適切に診療されなければならない。しかし，それらの鑑別は容易ではなく，それゆえ予後も良好とはいえないのが現状である。本稿では硬化性胆管病変に対する迅速かつ適切な診療を遂行することを目的として，硬化性胆管炎の分類とその病因・病態・臨床像について，2 次性硬化性胆管炎を中心に解説した。

Key words：原発性硬化性胆管炎，IgG4 関連硬化性胆管炎，2 次性硬化性胆管炎

はじめに

画像診断の進歩とともに診断機会が増加している硬化性胆管病変は，炎症性変化と腫瘍性変化とに大別され，それらの病因・病態を精査して適切に診療する必要がある[1)]。硬化性胆管炎は，①原因不明の進行性病変として原発性硬化性胆管炎（primary sclerosing cholangitis：PSC），②IgG4 関連疾患に随伴する硬化性胆管病変（IgG4-SC）と，③2 次性硬化性胆管炎（Secondary SC：SSC）に分類され，その鑑別診断が必要である。2 次性硬化性胆管炎は明らかな原因に基づく硬化性胆管炎と解釈されることから，それらが除外された場合に PSC と IgG4-SC を鑑別することになる。本稿では厚生労働科学研究費補助金・難治性疾患克服研究事業による難治性の肝・胆道疾患に関する調査研究（坪内班）における『硬化性胆管炎・診断基準 WG』で進められた硬化性胆管炎診断基準作成の試みを踏まえて，2 次性硬化性胆管炎について解説する[2)]。

Ⅰ．硬化性胆管炎の分類

硬化性胆管炎は疾患概念や臨床像から PSC，IgG4 関連硬化性胆管炎，二次性硬化性胆管炎に分類される。PSC は原因不明の肝内・肝外胆管に線維性狭窄を生じる進行性慢性炎症疾患であり，他疾患に基づく二次性（続発性）胆管炎や全身性疾患の局所変化（自己免疫性膵炎を含む IgG4 関連疾患などの胆管病変）は除外される。ステロイドには不応性の非可逆的変化で免疫抑制剤も奏効しない[3)]。一方，IgG4 関連硬化性胆管炎は自己免疫性膵炎を含む IgG4 関連疾患に伴う胆管病変であり，ステロイド反応性の可逆性病変である[4,5)]。また，他疾患に基づく続発性変化として硬化性胆管変化を呈するものを 2 次性硬化性胆管炎として取り扱い，感染症，胆管悪性腫瘍に伴う胆管硬化性変化，胆道の手術や外傷，総胆管結石ならびにその再発・併発慢性炎症，先天性胆道異常，腐食性硬化性胆管炎，胆管の虚血性狭窄，動注化学療法による胆管障害や狭窄に伴うものが含まれる[3)]。ただ，PSC でも IgG4 高値を示すものもあり[6)]，それらの鑑別は必ずしも容易ではないのが現状である。

Secondary Sclerosing Cholangitis

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表 1　PSC 診断基準に基づく 2 次性硬化性胆管炎（文献 7）

1．胆道造影による典型的な胆管の異常所見
2．臨床像，血液生化学所見
　胆汁うっ滞による症状
　ALP の正常上限 2～3 倍以上の増加が 6ヵ月以上持続
3．基礎疾患と背景病態
　AIDS に伴う胆道病変
　胆道の腫瘍性病変（PSC の診断が先行する場合は除く）
　胆道の手術，外傷
　胆道結石
　胆道の先天性異常
　腐食性硬化性胆管炎
　虚血に伴う狭窄性変化
　Floxuridine（5-FU）の動脈内投与に伴う胆道狭窄

Ⅱ．2 次性硬化性胆管炎の概要と診断基準のあり方

1．概念

他疾患に基づく続発性変化として硬化性胆管変化を呈するものであり，①感染症（AIDS そのほかの慢性胆道感染症），②胆管悪性腫瘍（PSC 診断後および早期癌は例外）に伴う胆管硬化性変化，③胆道の手術や外傷，④総胆管結石ならびにその再発・併発慢性炎症，④先天性胆道異常，⑤腐食性硬化性胆管炎，⑥胆管の虚血性狭窄，⑦ Floxuridine などの特定薬物動注による胆管障害や狭窄に伴うものである[3,7]（表 1）。ただ，自己免疫性膵炎に伴う硬化性胆管炎は前述の IgG4-SC に組み入れられているため SSC からは除外される。

2．臨床症状と診断

臨床症状は，発熱，黄疸，腹痛など，基礎疾患により症状は多彩である。臨床検査においても原疾患に基づく血液検査値異常（白血球増多，肝機能異常，胆道系酵素上昇，血清ビリルビン高値，炎症反応）を認める。各種画像（US，CT，ERCP，MRCP，EUS，IDUS および血管造影）が，ほかの硬化性胆管炎同様に診断上重要である。

3．治療と予後

基礎疾患の治療（薬物治療，内視鏡治療，肝移植を含む外科手術）が主体である。病態改善については抗菌薬，利胆剤，代謝改善薬（脂質異常症など）が投与される。予後も基本的には原因疾患の予後に準じる。

4．診断基準のあり方

2 次性硬化性胆管炎の診断基準は現在のところ明確なものは見当たらないが，他項にある PSC や IgG4 関連硬化性胆管炎の診断基準との補完的な位置づけにある。後述の基礎疾患に基づくものと定義するとともに，自己免疫性膵炎に伴う胆管病変を除いてこれを IgG4-SC に組み入れ，それらすべてを除く硬化性胆管炎を PSC とすることが現状ではコンセンサスが得られやすいであろう。試案を表 1 に示す。

Ⅲ．2 次性硬化性胆管炎の基礎疾患と背景病態

1．後天性免疫不全症候群（AIDS）

AIDS 患者では ERCP により広範な胆管硬化性変化のほかに膵管閉塞を認めることがあり，膵炎を示す場合もある。AIDS による胆管障害の主体は感染であるが，その病変は，①肝内胆管に限局する病変，②肝内・肝外胆管の両方に及ぶびまん性病変，③乳頭狭窄症，④総胆管の膵内部分狭窄症，⑤膵管病変，とさまざまである。そのほかの関連病原体としては *Cytomegalovirus* の頻度が高い。さらに，*Crtptosporidium*, *Mycobacterium avium-intracellulare*, *Microsporidia*, *Isospora* も比較的頻度が高い。AIDS には無石胆嚢炎が 10%にみられる。

2．胆管悪性腫瘍

胆管悪性腫瘍（PSC 診断後および早期癌は例外）に伴う胆管硬化性変化も 2 次性硬化性胆管炎のカテゴリーに属する。画像上は不均一で不整な胆管壁肥厚を呈することが多く IDUS や MDCT が有力な手掛かりとなるが診断は決して容易ではない。胆管生検や胆汁細胞診も重要な判断材料であるが正診率は必ずしも高くはない。機転としては，壁内進展や胆汁うっ滞，続発性胆管炎に伴う硬化性胆管病変である。

3．胆道の手術や外傷

胆道の手術や外傷に引き続く治癒過程での硬化性変化も少なくない。近年は肝移植後の胆管狭窄の発生率が高くなっており，それに伴う慢性炎症による硬化性胆管病変の発症頻度も増加している。

4．総胆管結石ならびにその再発・併発慢性炎症

胆道結石による慢性炎症は硬化性胆管病変を惹起する。一般に胆道結石は内視鏡的に除去されるが，経乳頭的あるいは経皮経肝的にせよ再発を繰り返すことから胆管硬化は避けられない場合が多い。したがって結石除去後に利胆剤を長期投与する試みもあるがその有用性については確定的なエビデンスが得られていない。

5．胆道の先天異常

胆道閉鎖，膵・胆管合流異常などでは，胆汁中構成成分や逆流膵液およびそれによる分解産物による慢性刺激による炎症に硬化性変化をきたす。胆道腫瘍の発生とも関連するため臨床的には介入の要否を的確に判

断する必要がある。

6．抗癌剤の肝動脈内投与や肝動脈塞栓療法後の胆管硬化

Floxuridine などの特定薬物動注による胆管障害や狭窄，肝動脈塞栓療法後の胆管硬化に伴うものである。医原性の硬化であり狭窄の程度によっては胆管ステントの留置などの介入を行う必要がある。

7．その他

そのほか，好酸球性胆管炎，腐食性硬化性胆管炎，胆管の虚血性狭窄なども硬化性胆管病変の原因となる。

Ⅳ．PSC と IgG4-SC

1．PSC

PSC は原因不明であるが，炎症性腸疾患（IBD）の合併が多いことから類似の病因が示唆されている。2003 年の我が国の集計[8]では，その頻度は男性にやや多く，発症年齢は 20 歳代と 60 歳代の 2 峰性である。肝内肝外胆管両方の罹患例が多く，潰瘍性大腸炎の合併を 37%に認めている。臨床症状は黄疸，掻痒感が主体であり，血液生化学検査では主に ALP，ALT，ビリルビン高値を認める。また好酸球増多，抗核抗体陽性も認められるが，ERCP や MRCP による特徴的な胆管像（数珠状変化など）が診断上重要である。併存疾患として潰瘍性大腸炎と非典型腸炎の頻度が高い。現在，PSC の診断には Lindor ら[7]の提示した診断基準が広く用いられている。すなわち典型的な胆管画像診断が主体である一方，欧米では IBD の合併が重要な要素となっている。ただ，我が国では IBD の合併が比較的少なく，健診の普及に伴う画像診断による拾い上げ診断が少なくない実情に即した診断基準が考慮されるべきであろう。また，PSC は胆管癌合併リスクが高く[9]，PSC 診断時に早期胆管癌が認められる場合に，これを除外することは議論の余地がある。

2．IgG4-SC

自己免疫性膵炎を含む IgG4 関連疾患などの特徴的な全身性疾患に伴う硬化性胆管病変はステロイド治療に反応することが多い。2007 年に厚生労働科学研究費補助金（難治性疾患克服研究事業）による難治性の肝・胆道疾患に関する調査研究（大西班）と難治性膵疾患調査研究（大槻班）の合同ワーキング調査により，『原因不明の硬化性胆管炎の分類と治療の指針』が報告された[10]。その要旨は，自己免疫性膵炎（AIP）に合併する硬化性胆管炎（Type 2）は古典的 PSC（Type 1）に比較して高齢・男性に好発し顕性黄疸の出現率が高いことと，IgG4 値 135 mg/dl 以上をカットオフ値とすると診断精度が高まること，ステロイド剤に対する反応性が良好であること，UDCA やベザフィブレートの有効性が低いこと，内視鏡的胆管拡張術は有用性が低いことが特徴的であったとしている。本疾患は AIP に高率に合併するが，涙腺・唾液腺炎，後腹膜線維症を併存することもあり，AIP を含む IgG4 関連疾患などの免疫異常に基づく自己免疫疾患がかかわると推定される。臨床像としては閉塞性黄疸を示すことが多く，上腹部痛，掻痒感なども認める。血液検査所見では血清総ビリルビン，IgG および IgG4 が上昇することが多いのが特徴である。画像では下部（膵内）胆管の病変を認める頻度が高い。病理組織所見では胆管における IgG4 陽性形質細胞浸潤が特徴的な所見である。ステロイド治療が奏効して予後は比較的良好である。自己免疫性膵炎を合併する場合，高頻度に糖尿病を併発する。IgG4-SC については最近その診断基準がまとめられたが[11]，詳細は本特集の他項を参照されたい。

おわりに

以上，硬化性胆管炎を疾患概念から原発性硬化性胆管炎，IgG4 関連硬化性胆管炎，二次性硬化性胆管炎の分類とその診断基準に関する概要を述べた。疾患の特性からそれらの鑑別は決して容易ではなく実地診療の現場では治療指針ともなる診療ガイドラインが求められる場合もあり，厚生労働科学研究費補助金（難治性疾患克服研究事業）『難治性の肝・胆道疾患に関する調査研究（坪内班）』での硬化性胆管炎診断基準に向けた一層の臨床研究進展が期待される。

参考文献

1）田妻　進：硬化性胆管病変をどうみるか．日消誌 **103**：1119-1126，2006.
2）田妻　進：硬化性胆管炎・診断基準 WG 報告書．厚生労働科学研究費補助金難治性疾患克服研究事業・難治性の肝・胆道疾患に関する調査研究，平成 22 年度総括・分担報告書，159-161.
3）LaRusso NF, Wiesner RH, Ludwig J, et al.：Current concepts. Primary sclerosing cholangitis. N Engl J Med **310**：899-903, 1984.
4）Kamisawa T, Okamoto A：Autoimmune pancreatitis：proposal of IgG4-related sclerosing disease. J Gastroenterol **41**：613-625, 2006.
5）Hamano H, Kawa S, Uehara T, et al.：Immunoglobulin G4-related lymphoplasmacytic sclerosing cholangitis that mimics infiltrating hilar cholangiocarcinoma：part of a spectrum of autoimmune pancreatitis? Gastrointest Endosc **62**：152-157, 2005.

6) Mendes FD, Jorgensen R, Keach J, et al.：Elevated serum IgG4 concentration in patients with primary sclerosing cholangitis. Am J Gastroenterol **101**：2070-2075, 2006.
7) Lindor KD, LaRusso NF：Primary sclerosing cholangitis. In：Schiff's disease of the liver, Schiff L, Schiff ER, editors. 9th edition, 673-684, JB Lippincott, Philadelphia, 2003.
8) Takikawa H：Characteristics of primary sclerosing cholangitis in Japan. Hepatol Res **37**：S470-S473, 2007.
9) 小道大輔，田妻　進：原発性硬化性胆管炎と胆道癌―発癌メカニズムとマネジメント―．胆と膵 **31**：193-197，2010.
10) 西森　功：自己免疫性膵炎に合併する硬化性胆管炎の病態の解明．厚生労働科学研究費補助金（難治性疾患克服研究事業）難治性膵疾患調査研究班，平成19年度総括・分担報告書，122-132.
11) IgG4関連硬化性胆管炎臨床診断基準作成ワーキンググループ：IgG4関連硬化性胆管炎臨床診断基準2012. 胆道 **26**：59-63，2012.

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—— 第49回大会 ——

特別講演

検診における胆道疾患の取り扱い
～胆石症診療ガイドラインに基づく胆道がんへの取り組み～

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〔要　旨〕

胆石症と胆道がんの関わりは古くより議論されているが，胆道がんリスクファクターを一次検診システムにて拾い上げるとともに，積極的な二次精査と診療ガイドラインに基づくフォローアップによって胆道がんの早期診断に取り組むことが求められる。本稿では，第49回日本消化器がん検診学会大会において著者が行った特別講演をもとに，①検診における胆道疾患頻度，②胆石症診療ガイドラインと胆道がん診療，③胆道がん検診におけるPETの実用性の概要を述べて，胆道がんへの取り組みについて私見を含めて概説した。

キーワード　胆石症，診療ガイドライン，胆道がん

はじめに

胆道がんは予後不良である。従って，その早期診断の可否が診療のあり方に大きく関わるため，消化器がん検診における拾い上げ診断が生命予後に影響を与える。本稿では，検診における拾い上げ診断の現状を紹介したうえで，背景疾患として話題にあがることの多い胆石症診療における胆道がん診療のあり方を解説するとともに，PETによる胆道がんスクリーニングの有用性を検証した。

1．検診における胆道疾患の拾い上げの現状

教室の関連検診施設における最近５年間の腹部超音波検診57,489名（男：女，33,656：23,833）を対象に（表１）胆道疾患拾い上げ診断の実際を示すと，その中の7,286名12.7％に何らかの胆道疾患が認められた。その内訳としては，胆嚢ポリープ5,571名9.7％（男：女，4,133：1,458）が最も多く（表２），50歳代で12.0％，60歳以上では10.7％であった。次いで胆石が多く（表３），1,645名2.9％（男：女，1,047：598）であった。胆石は50歳代で4.1％，60歳以上で5.3％という年齢分布であった。続いて胆嚢腺筋症70例0.1％（男：女，63：７）の順で，本研究の実施施設では胆道疾患はいずれも男性優位であった（胆嚢ポリープ12.3％ vs 6.1％，胆石3.1％ vs 2.5％，胆嚢腺筋症0.2％ vs 0.03％）。一方，同検診における胆道がん拾い上げは肝内胆管癌２例（0.003％）であった。

２．胆石症診療ガイドラインと胆道がん

最近５年間に広島大学病院で診療した胆石症は1,276例（図１）で，年次的には1.6～3.4％，胆石保有者の61％が女性，62％が60歳以上であった。リスクファクターとして，肥満33％，脂質異常症39％，糖尿病40％，その両者21％であった。一方，胆嚢癌は135例であったが胆石症患者の経過観察中の胆嚢癌合併は認められなかった。

2009年に発表された日本消化器病学会・胆石症診療ガイドライン[1)]では1983年以降のエビデンスを基に胆嚢結石症と胆嚢癌の因果関係には否定的な立場である。疫学や病態・成因に関するエビデ

年齢	M	F	Total
＜40	6,716	6,656	13,372
40 ～ 49	9,974	6,789	16,763
50 ～ 59	10,424	6,443	16,867
＞60	6,542	3,945	10,487
	33,656	23,833	57,489

表1　腹部超音波検査による胆道疾患の拾い上げ(1)
（広島県・健康クリニック，2006 ～ 2010）

胆嚢ポリープ発見率（%）

年齢	M	F	Total
＜40	8.8	2.6	5.7
40 ～ 49	12.5	6.0	9.9
50 ～ 59	14.3	8.4	12.0
＞60	12.1	8.5	10.7
	12.2	6.1	9.7

表2　腹部超音波検査による胆道疾患の拾い上げ(2)
～胆嚢ポリープ発見率～（広島県・健康クリニック，2006 ～ 2010）

胆石・発見率（%）

年齢	M	F	Total
＜40	1.0	0.4	0.7
40 ～ 49	1.7	1.6	1.8
50 ～ 59	4.1	3.9	4.1
＞60	5.2	5.3	5.3
	3.1	2.5	2.9

表3　腹部超音波検査による胆道疾患の拾い上げ(3)
～胆石発見率～（広島県・健康クリニック，2006 ～ 2010）

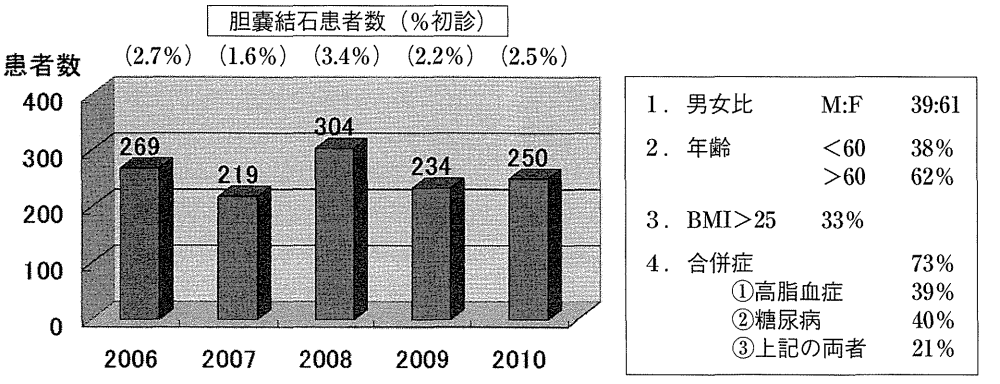


図1　最近5年間の胆石保有率（広島大学病院2006 ～ 2010）

(1) PETによる“がん検診”における胆道所見の指摘状況

2005～2011/09/22のPET件数［件］	8,996		
	男	女	計
胆道所見あり・件数［件］	115	97	212

(2) 胆道所見の内訳（単位［件］）

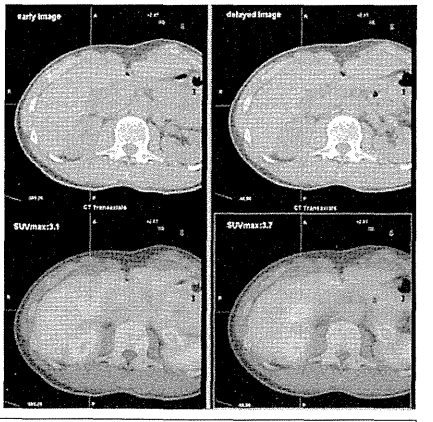
	男	女	合計
胆石・石灰化	86	54	140
● 胆嚢への軽度集積（SUVmax＜4.0）	5	7	12
● 胆嚢への高集積（SUVmax≧4.0）	1	2	3
胆嚢周囲への集積	1	0	1
胆摘	3	1	4
胆管の拡張	2	5	7
胆道系に異常集積なし・アーチファクト	19	20	39
● 胆管癌	3	0	3
壁の肥厚	0	3	3
腫大（胆道気腫・水腫）	0（0）	4（2）	4（2）
計	120	98	218＊

＊重複あり

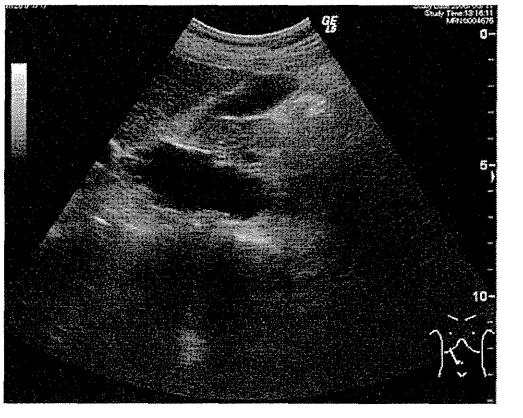
表4　PETがん検診における胆道所見(1)（広島平和クリニック，2005～2011）

（胆道所見の内訳（単位［件］））

	男	女	合計
胆嚢への軽度集積（SUVmax＜4.0）	5	7	12



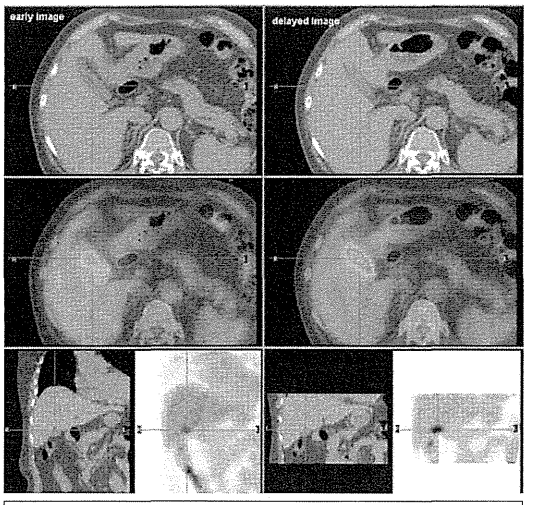
胆嚢にFGDの軽度集積。Delayed Imageにて SUVmax3.1→3.7（集積亢進），PET上，悪性パターン



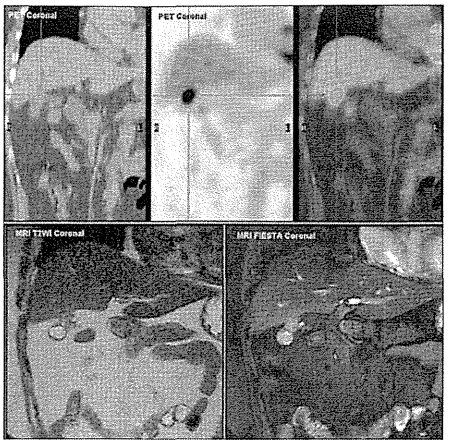
USでは異常を指摘できない。

図2　PETがん検診における胆道所見(2)（広島平和クリニック，2005～2011）

	男	女	合計
胆嚢への高集積（SUVmax≧4.0）	1	2	3



胆嚢にFGDの高集積。Delayed Imageにて
SUVmax : early 4.0→delayed 6.6（悪性パターン）



胆嚢にFDGの限局した高集積（early
SUVmax : 10.8→80min SUVmax : 13.3）
（悪性パターン）

図３　PETがん検診における胆道所見(3)（広島平和クリニック，2005～2011）

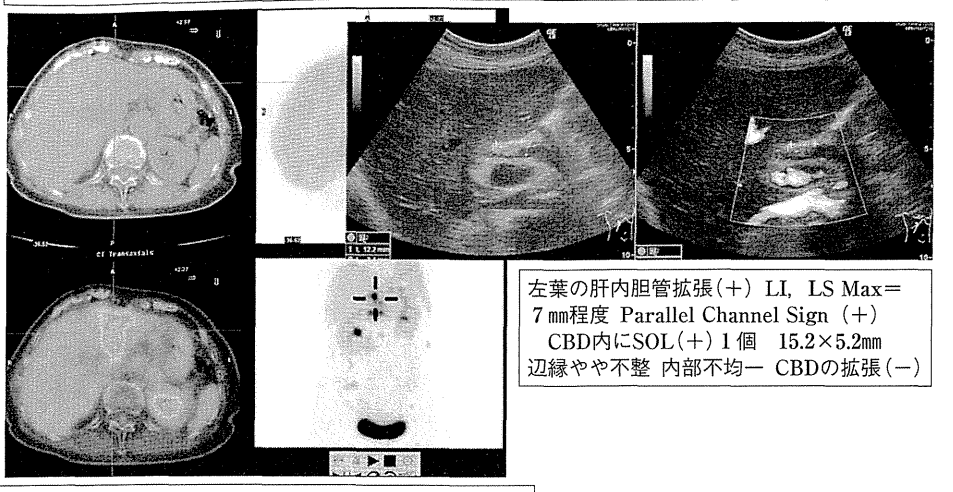
ンスも十分ではない。2003年から2010年の広島大学病院総合内科・総合診療科におけるWalk-in初診10,852例中の胆石保有率は3.2%（346例）であった。2010年の年間診療実績（対象105例）は，１）男女比41：64，２）年齢40歳未満２，40～49歳18，50～59歳20，60～69歳34，70～79歳22，80歳以上９名，３）肥満（BMI＞25）35例（33.3%），４）無症状胆石（経過観察）17例（16.1%），５）胆嚢摘出術23例（21.9%），６）UDCA治療にて無症状化47例（44.8%），溶解消失６例（5.7%），７）ESWL施行12例（11.4%）であった。無症状経過観察例では６カ月毎に腹部超音波でフォローアップしている。

日本消化器病学会・胆石症診療ガイドラインでは無症状胆石は経過観察を推奨しているが，巨大胆石（３cm径以上）や胆嚢壁肥厚，無機能胆嚢（胆嚢造影陰性）は発癌リスクとしている。胆道癌の多くも無症状に進行するため，無症状胆石や膵胆管合流異常，陶器様胆嚢などの胆道異常は画像診断にて定期的に形状変化を評価することが望ましい。

３．PETによる胆道がん検診

胆道がんの早期診断はUSによるスクリーニングが主体であるが，PETも有用なModalityの一つである。2005年から2011年９月22日までの広島平和クリニックにおけるPETがん検診8,996例中，胆道所見を指摘されたものは212例であった。内訳としては胆石（石灰化）が140例と最も多かった（表４）。胆嚢への軽度集積（SUVmax＜4.0）12例の中には，USで異常を指摘されていない症例があり悪性パターンを示すものがあった(図２)が，胆嚢への高集積（SUVmax≧4.0）３例の中には，悪性パターンを示す胆嚢炎が含まれた(図３)。一方，胆管癌は３例で，隆起型胆道がんではPETにおける異常集積を認め，USでも同部に描出された（図４）。すなわち，他のModality同様に隆起型病変の診断能は高いと思われた。

	男	女	合計
胆管癌	3	0	3



左葉の肝内胆管拡張(＋) LI, LS Max＝
7㎜程度 Parallel Channel Sign (＋)
CBD内にSOL(＋)1個 15.2×5.2㎜
辺縁やや不整 内部不均一 CBDの拡張(－)

胆嚢にFDGの異常集積 (SUVmax : early 4.0→delayed 6.6)

図4 PETがん検診における胆道所見(4) (広島平和クリニック, 2005～2011)

4．肝内結石症と肝内胆管癌

2005年以降の厚生労働省難病対策事業(跡見班, 坪内班) における疫学研究から, 結石除去治療のみは肝切除に比較して肝内胆管癌の発生リスクが相対的に高いこと, さらにUDCA治療によりその発生リスクが低下することが示唆されている。今後の調査継続によりさらに詳細が明らかにされることが期待される。

おわりに

胆道がん診療における検診のあり方を解説した。拾い上げ診断のModalityとしてはUS, CTが汎用されるが, PET検診も含めた包括的な検診システムの構築による画像診断に加えて新たなバイオマーカーを組み合わせた早期診断と, それに基づく早期治療の実現が期待される。

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文 献

1) 日本消化器病学会編：胆石症 診療ガイドライン, 南江堂, 東京, 2009.

Biliary diseases screening: An approach to biliary cancer management based upon the guideline for biliary stone treatment

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The causal relationship of gallstone diseases to biliary cancer has long been controversial. In this regard, it is necessary to develop a primary screening system associated with the following diagnostic strategy for early biliary cancer, and thus, the guideline for gallstone treatment recently developed by the Japanese Society of Gastroenterology will be beneficial for screening cancer risk factors and complications in the follow-up system. In this review, an approach to biliary cancer management based upon the guideline for biliary stone treatment is discussed as a part of biliary disease screening in light of the following aspects: 1) the prevalence of biliary diseases, 2) the benefit of guidelines, and 3) the benefit of PET.

Keywords : gallstone, guideline, biliary cancer

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Increased serum liver X receptor ligand oxysterols in patients with non-alcoholic fatty liver disease

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Abstract
Background This study is a post-hoc analysis of a subset of patients who participated in our multi-institutional case-control study that evaluated the effects of pitavastatin in patients with non-alcoholic fatty liver disease (NAFLD) with hypercholesterolemia.
Methods Serum samples of fifteen patients with biopsy-proven NAFLD with dyslipidemia were investigated. Serum markers of lipid metabolism were quantified by liquid chromatography-mass spectrometry (LC–MS)/MS. These data were then compared with those of 36 sex- and age-matched healthy controls. In addition, changes in these markers produced by treatment with pitavastatin were evaluated.
Results Serum non-cholesterol sterols, reflecting intestinal cholesterol absorption, were significantly lower in the NAFLD patients compared to the controls, and the cholesterol synthesis marker, the ratio of lathosterol to cholesterol, was not significantly different between the two groups. Serum proportions of liver X receptor α (LXRα) ligand oxysterols (ratios to cholesterol) were significantly elevated in the NAFLD patients compared to the controls. The sum of oxysterols relative to cholesterol and the homeostasis model assessment as an index of insulin resistance (HOMA-IR) were significantly correlated. The marker representing cholesterol synthesis was significantly suppressed by pitavastatin treatment, from 3 months after initiation of the treatment, and the suppression remained significant during the observation period. The markers representing cholesterol absorption were unchanged at 3 months, but had significantly increased at 12 months. Serum oxysterol levels relative to cholesterol maintained high values and did not change significantly during the 12-month period of treatment.
Conclusions: We speculate that serum LXRα ligand oxysterol levels (relative to cholesterol) could be surrogate markers of insulin resistance, and that high oxysterol levels in the circulation may play an important role in the development of hepatic and peripheral insulin resistance followed by NAFLD.

Keywords NAFLD · Cholesterol metabolism · Oxysterol · Bile acids

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by an increase in intra-hepatocellular tryglycerides that is not due to alcohol or other known causes [1]. NAFLD could be called "insulin resistance-associated steatosis" because all components of the metabolic syndrome correlate with liver fat independent of the body mass index (BMI) [2]. In addition, hepatic insulin resistance is also closely correlated with the amount of fat in the liver both in non-diabetic [3] and type 2 diabetic [4] subjects. Recent studies have implicated several important hepatic cellular processes and signaling pathways that are affected by abnormal lipid metabolism, resulting in the specific biochemical, histological, and clinical changes associated with NAFLD.

Biological samples contain a large number of oxysterols [5] and some of them are important molecules to preserve lipid homeostasis in the body [6]. In particular, 4β-hydroxycholesterol, 22*R*-hydroxycholesterol, 24*S*-hydroxycholesterol, 24*S*,25-epoxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol are reported to be endogenous ligands of a nuclear receptor, liver X receptor α (LXRα; NR1H3) [7, 8]. When LXRα is activated by these oxysterols, the fatty acid biosynthetic pathway is stimulated through the up-regulation of sterol regulatory element-binding protein 1c (SREBP1c) [9], and an up-regulated SREBP1c and fatty acid biosynthetic pathway has actually been observed in NAFLD [10, 11]. Furthermore, a recent report by Kotronen et al. [12] showed that diacylglycerols might contribute to hepatic insulin resistance in NAFLD. Thus, oxysterols appear to play an important role in the development of hepatic insulin resistance and NAFLD.

Oxysterols are synthesized from cholesterol, and the total body pool of cholesterol is enlarged by endogenous synthesis or by dietary absorption [13]. The use of plasma sterol biomarkers for cholesterol synthesis and fractional absorption [14] clarified that obesity [15, 16], the metabolic syndrome [17], type 2 diabetes [18], and NAFLD [19] were all characterized by low efficiency of dietary cholesterol absorption and high cholesterol biosynthesis.

These data suggest that inhibition of the cholesterol biosynthetic pathway by statins, which are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, may be an effective way to reduce LXRα ligand oxysterols in the bodies of NAFLD patients. In fact, statins have already been used in patients with NAFLD and/or non-alcoholic steatohepatitis (NASH) complicated with dyslipidemia or metabolic syndrome [20–22]. Most reports have demonstrated certain advantages of statin therapy in NAFLD/NASH patients with dyslipidemia, but their effectiveness is still controversial. For instance, in a randomized placebo-controlled trial using simvastatin in the treatment of NASH, Nelson et al. [22] concluded that simvastatin did not seem to be an effective treatment for NASH. We conducted a multi-institutional case-control study to evaluate the efficacy of pitavastatin, a newly developed statin, for the treatment of NAFLD with hypercholesterolemia [23]. In that study, we demonstrated that the alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) levels, and serum lipid profiles (including total cholesterol, low-density lipoprotein [LDL]-cholesterol and triglyceride), were significantly improved by 12-month treatment with pitavastatin. However, there was no significant difference in insulin resistance before and after pitavastatin treatment in this cohort [23].

The present study is a post-hoc analysis of a subset of patients who participated in the multi-institutional case-control study mentioned above, in which we evaluate the effects of pitavastatin in NAFLD patients with hypercholesterolemia. The aims of the study were to identify the characteristic features of serum oxysterol profiles, which could be a clue to an understanding of their biological roles in the cholesterol metabolism of NAFLD patients. We found that NAFLD patients had a significantly elevated level of certain LXRα ligand oxysterols in their serum, and pitavastatin did not reverse this elevation in spite of its strong reducing effect on serum cholesterol levels.

Subjects, materials, and methods

Subjects

In this prospective study we evaluated 15 patients diagnosed with biopsy-proven NAFLD with hypercholesterolemia between 2006 and 2009 . Written informed consent was obtained from each enrolled patient, and the study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the ethics and research committees of each institute participating in this study (Tokyo Medical University Ibaraki Medical Center, Hiroshima University, Tokyo Women's Medical University, and Fujita Health University School of Medicine). In all patients, current and past daily alcohol consumption was less than 20 g per week; detailed information regarding alcohol consumption was obtained independently by at least 2 physicians and confirmed by close family members. Exclusion criteria other than alcohol consumption of more than 20 g per week were: evidence of pregnancy, treatment with corticosteroid, and hormone replacement therapy. Subjects using lipid-lowering medication or food enriched with functional plant stanols or sterols were excluded from the study. Subjects with positive test results for the following disorders were also excluded: secondary causes of

steatohepatitis and drug-induced liver injury, viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, α-1-antitrypsin deficiency, hemochromatosis, Wilson's disease, and biliary obstruction. After registration of the study, all patients received 2 mg/day of pitavastatin (Livalo; Kowa Pharmaceuticals, Tokyo, Japan) for 12 months. In addition, all patients were given standard weight-loss counseling and encouraged to follow a low-fat and low-carbohydrate diet before and during treatment. Venous blood samples were taken in the morning(following a 12-h overnight fast) at baseline and 3 and 12 months after the initiation of pitavastatin treatment. Some serum samples were utilized for various laboratory tests, and the remaining sera were stored at −20 °C until later analysis.

Fasting sera of 60 healthy volunteers without obesity, hyperlipidemia, diabetes, or liver dysfunction (obtained for another study group [courtesy of Professor T. Teramoto, Teikyo University, with written informed consent from the healthy volunteers) were obtained, and samples were selected from 36 sex- and age-matched subjects, and used as the control group. The control serum samples were stored and handled as mentioned above.

Quantification of serum lipid biomarkers

Serum non-cholesterol sterols (lathosterol, campesterol, and sitosterol) and LXRα ligand oxysterols were quantified by liquid chromatography-mass spectrometry (LC–MS)/MS as described in our previous papers [24–26]. Briefly, coprostanol and deuterated oxysterols were added to 10 μl of serum as internal standards, and alkaline hydrolysis was carried out in 1 N ethanolic KOH with butylated hydroxytoluene at 37 °C for 1 h. Sterols were extracted with *n*-hexene, derivatized to the picolinyl esters, and injected into an LC–electron spray ionization (ESI)–MS/MS system consisting of a TSQ Vantage triple stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an HESI-II probe and a Prominence ultra fast liquid chromatography (UFLC) system (Shimazu, Kyoto, Japan).

Serum concentrations of 7α-hydroxy-4-cholesten-3-one (C4), a biomarker of CYP7A1 activity, were determined by LC–MS/MS without alkaline hydrolysis [27]. Deuterium-labeled C4 was added to 20 μl of serum and C4 was extracted with acetonitrile. After derivatization into the picolinyl ester, it was injected into the LC–ESI–MS/MS system described above.

Serum malonic acid (MA), a marker of lipogenesis, was quantified by our previously described method [28]. After the addition of [$^{13}C_3$]MA as an internal standard, MA was extracted with acetonitrile from 20 μl of serum, derivatized into di-(1-methyl-3-piperidinyl)-MA and determined by the LC–ESI–MS/MS system described above.

Serum acetylcarnitine (ACT), a marker of fatty acid β-oxidation, was quantified by the method of Ghoshal et al. [29] with some modifications. In brief, 50 ng of [$^2H_3$]ACT HCl was added to 10 μl of serum and ACT was extracted with 100 μl of acetonitrile–water (19:1, v/v). The extract was evaporated to dryness and redissolved in 150 μl of water, and an aliquot (2 μl) was analyzed by LC–ESI–MS/MS. Chromatographic separation was performed using a Hypersil GOLD aQ column (150 × 2.1 mm, 3 μm; Thermo Fisher Scientific) at 40 °C. The mobile phase was comprised of 0.2 % formic acid in water and was used at a flow rate of 200 μl/min.

Determination of serum fibroblast growth factor 19 (FGF19)

Serum FGF19 levels were determined by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine Human FGF19 Immunoassay; R&D systems, Minneapolis, MN, USA), following the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed either by one-way analysis of variance or with the two-tailed Student's *t*-test, using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Results are shown as means ± SEM, and *P* values of less than 0.05 were considered statistically significant.

Results

Characteristics of the study groups

The sera of 15 NAFLD patients were available for the determination of lipid biomarkers. The mean ages and ratios of male/female subjects were not significantly different between the control and NAFLD groups. In the control group, none of the subjects were obese (BMI >25), and none had hypercholesterolemia (total cholesterol >220 mg/dl), hypertriglycemia (triglyceride [TG] >150 mg/dl), hypertension (systolic blood pressure [BP] >120 mmHg), or diabetes, while in the NAFLD group there were 3 subjects with hypertension, 3 with diabetes, and 10 of the 15 were obese (BMI >25) (Table 1).

Cholesterol, bile acid, and fatty acid metabolism in NAFLD patients

As shown in Table 2, serum total cholesterol concentrations were significantly higher in the patients with with

Table 1 Characterization of the study population

Characteristics	Control ($n = 36$)	NAFLD ($n = 15$)
Sex (male/female)	20:16	10:05
Age, years (range)	42.2 (20–49)	43.7 (25–53)
BMI (kg/m^2)		
<25	36 (100 %)	5
25–29	0	7
>30	0	3
Hypercholesterolemia	0	15
Hypertension	0	3
Diabetes mellitus	0	3

NAFLD non-alcoholic fatty liver disease, *BMI* body mass index

Table 2 Serum biomarkers representing lipid metabolism

	Control ($n = 36$)	NAFLD ($n = 15$)	P
Total cholesterol (mg/dl)	177.7 ± 4.46	242.8 ± 13.3	<0.001
Cholesterol absorption			
Sitosterol (μg/mg Chol)	1.90 ± 0.08	0.73 ± 0.06	<0.0001
Campesterol (μg/mg Chol)	2.34 ± 0.11	0.75 ± 0.07	<0.0001
Cholesterol synthesis			
Lathosterol (μg/mg Chol)	3.29 ± 0.17	3.03 ± 0.31	NS
Bile acid synthesis			
C4 (ng/mg Chol)	14.68 ± 1.65	13.68 ± 2.91	NS
Bile acid absorption			
FGF19 (pg/ml)	345.8 ± 48.6	195.1 ± 21.1	NS
Fatty acid synthesis			
Malonic acid (ng/ml)	63.0 ± 22.6	68.7 ± 4.7	NS
Fatty acid β-oxidation			
Acetylcarnitine (ng/ml)	1059.0 ± 195.3	1011.0 ± 134.4	NS

Chol cholesterol, *C4* 7α-hydroxy-4-cholesten-3-one, *NS* not significant, *FGF19* fibroblast growth factor 19

NAFLD than in controls. Serum non-cholesterol sterols, reflecting intestinal cholesterol absorption (ratio of sitosterol or campesterol to cholesterol), were significantly lower in NAFLD patients compared to controls ($P < 0.0001$), and the cholesterol synthesis marker, the ratio of lathosterol to cholesterol, was not significantly different between the two groups.

Bile acid metabolism was investigated by determination of the concentrations of a biomarker reflecting bile acid synthesis (ratio of C4 to cholesterol) and FGF19. FGF19 is an enterokine that is synthesized and released when bile acids are taken up into the ileum. Serum FGF19 inhibits the expression of hepatic CYP7A1, the rate-limiting enzyme in the major bile acid biosynthetic pathway. In NAFLD patients, neither serum C4 levels nor FGF19 concentrations were significantly different from those in controls (Table 2).

Fatty acid synthesis and β-oxidation in the subjects with NAFLD were studied by the quantification of serum MA and ACT levels, respectively. However, these markers did not show any differences between NAFLD patients and controls.

Table 3 Serum LXRα ligand oxysterol levels

	Control ($n = 36$)	NAFLD ($n = 15$)	P
4βOH-Chol (ng/mg Chol)	27.3 ± 1.1	34.5 ± 3.5	<0.05
22*R*OH-Chol (ng/mg Chol)	Trace	Trace	
24*S*OH-Chol (ng/mg Chol)	34.0 ± 0.9	36.8 ± 2.5	NS
24*S*,25-epoxy-Chol (ng/mg Chol)	Trace	Trace	
25OH-Chol (ng/mg Chol)	7.9 ± 0.6	23.9 ± 2.6	<0.0001
27OH-Chol (ng/mg Chol)	73.1 ± 2.3	101.1 ± 7.7	<0.0005

LXRα liver X receptor α

Serum LXRα ligand oxysterol levels in NAFLD patients

The serum levels of LXRα ligand oxysterols (ratios to cholesterol) are shown in Table 3. The levels of 4β-hydroxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol were significantly elevated, at 26 % ($P < 0.05$), 303 % ($P < 0.0001$), and 38 % ($P < 0.0005$), respectively, in NAFLD patients compared with controls, although the 24*S*-hydroxycholesterol level in NAFLD patients was not significantly different from that in the controls. 22*R*-hydroxycholesterol and 24*S*, 25-epoxycholesterol are reported to be effective ligands of LXRα, but only trace amounts of these oxysterols were detected by our present method. The absolute concentrations of these oxysterols were also elevated in the NAFLD patients. Not only 4β-hydroxycholesterol (+58 %, $P < 0.001$), 25-hydroxycholesterol (+200 %, $P < 0.001$), and 27-hydroxycholesterol (+80 %, $P < 0.001$), but also 24*S*-hydroxycholesterol was increased (33 %, $P < 0.05$) in NAFLD patients.

Effects of pitavastatin treatment on the serum markers

The marker representing cholesterol synthesis (lathosterol to cholesterol) was significantly suppressed by pitavastatin treatment, from 3 months after the initiation of the treatment ($P < 0.01$) and its level remained significantly suppressed during the observation period (12 months after

Table 4 Changes in serum biomarkers produced by pitavastatin treatment

	Pre-treatment	3 Months	12 Months
Total cholesterol (mg/dl)	242.8 ± 13.3	188.3 ± 12.1*	182.7 ± 9.29*
Cholesterol absorption			
Sitosterol (μg/mg Chol)	0.73 ± 0.06	0.91 ± 0.08	1.16 ± 0.12*
Campesterol (μg/mg Chol)	0.75 ± 0.07	0.94 ± 0.09	1.24 ± 0.15*
Cholesterol synthesis			
Lathosterol (μg/mg Chol)	3.03 ± 0.31	1.44 ± 0.27*	1.72 ± 0.23*
Bile acid synthesis			
C4 (ng/mg Chol)	13.68 ± 2.91	10.57 ± 2.27	17.23 ± 3.44
Bile acid absorption			
FGF19 (pg/ml)	195.1 ± 21.1	172.4 ± 36.3	166.3 ± 23.1
Fatty acid synthesis			
Malonic acid (ng/ml)	68.7 ± 18.4	82.0 ± 5.6	61.3 ± 5.9
Fatty acid β-oxidation			
Acetylcarnitine (ng/ml)	1011.0 ± 134.4	914.2 ± 98.2	1318.0 ± 172.9

Chol cholesterol, *C4* 7α-hydroxy-4-cholesten-3-one

*$P < 0.01$ compared to pre-treatment

Table 5 Changes in serum LXRα ligand oxysterol levels produced by pitavastatin treatment

	Pre-treatment	3 Months	12 Months
4βOH-Chol (ng/mg Chol)	34.5 ± 3.5	33.8 ± 4.3	32.9 ± 3.0
22*R*OH-Chol (ng/mg Chol)	Trace	Trace	Trace
24*S*OH-Chol (ng/mg Chol)	36.8 ± 2.5	31.9 ± 3.3	36.1 ± 3.2
24*S*,25-epoxy-Chol (ng/mg Chol)	Trace	Trace	Trace
25OH-Chol (ng/mg Chol)	23.9 ± 2.61	22.3 ± 3.2	16.2 ± 1.7
27OH-Chol (ng/mg Chol)	101.1 ± 7.7	94.8 ± 11.1	105.0 ± 6.4

initiation of treatment, $P < 0.05$) (Table 4). On the other hand, the markers representing cholesterol absorption (sitosterol or campesterol to cholesterol) were unchanged at 3 months after the initiation of pitavastatin administration, but were significantly increased at 12 months ($P < 0.01$). However, the markers for bile acid and fatty acid metabolism and the levels of oxysterols (relative to cholesterol) were not changed significantly by pitavastatin treatment (Tables 4, 5).

Figure 1 shows a comparison of the effects of pitavastatin treatment on serum absolute concentrations of total cholesterol, lathosterol, and oxysterols. Total cholesterol and lathosterol concentrations were markedly decreased at 3 months, and no further reduction was observed at 12 months. In contrast, the concentrations of oxysterols, except for 24*S*-hydroxycholesterol, were significantly decreased at 12 months after treatment initiation, but not at 3 months. The 24*S*-hydroxycholesterol concentration tended to be decreased by pitavastatin treatment, but the difference from the pre-treatment level was not statistically significant.

Serum oxysterol levels and insulin resistance

The association between serum oxysterol levels and insulin resistance, calculated by the homeostasis model assessment as an index of insulin resistance; HOMA-IR (=fasting serum insulin (μU/ml) × fasting blood glucose (mg/dl)/405), was determined. As shown in Fig. 2, the sum of oxysterols (27-hydroxycholesterol, 25-hydroxycholesterol, and 4β-hydroxycholesterol: relative to serum cholesterol) and HOMA-IR were significantly correlated ($r^2 = 0.2762$, $P < 0.001$). Among the oxysterols determined in the present study, 24*S*-hydroxycholesterol was excluded in this calculation because a significant increase of 24*S*-hydroxycholesterol relative to cholesterol was not observed in the NAFLD patients, as mentioned above. Pitavastatin treatment did not improve the insulin resistance over the treatment period, similar to results reported in our previous study [23].

Discussion

This is the first report that demonstrates a significant elevation of serum concentrations of LXRα ligand oxysterols in NAFLD patients. Most serum oxysterols are found in the LDL and high-density lipoprotein (HDL) fractions [30], suggesting that oxysterols are transported in serum with cholesterol. Accordingly, the hypercholesterolemia found in NAFLD patients may lead to the overestimation of oxysterol production in their body. However, we found that 4β-hydroxycholesterol, 25-hydroxycholesterol,

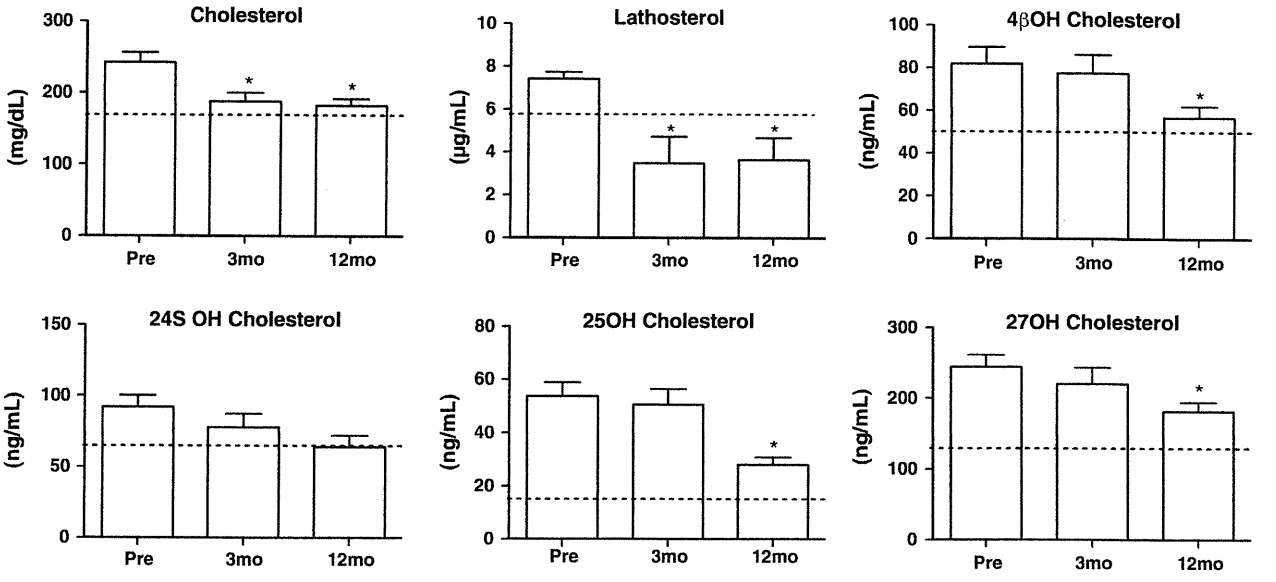

Fig. 1 Effects of pitavastatin treatment on serum absolute concentrations of total cholesterol, lathosterol, and oxysterols. *Pre* basal level before pitavastatin treatment, *3mo* serum concentrations at 3 months from initiation of therapy, *12mo* serum concentrations at 12 months from initiation of therapy, *OH* hydroxy. *Dotted lines* represent the mean value of each sterols in control subjects. $^*P < 0.01$

and 27-hydroxycholesterol levels expressed relative to cholesterol, but not those of 24*S*-hydroxycholesterol,were significantly increased in NAFLD patients compared with controls (Table 3). Thus, the increased production of LXRα ligand oxysterols appears to be a characteristic feature of NAFLD. Because most oxysterols are formed from cholesterol by enzymatic oxidation or autoxidation [25], it may be reasonable to assume that oversaturation of the tissue cholesterol concentration results in augmented oxysterol production. It was intriguing that only 24*S*-hydroxycholesterol levels expressed relative to cholesterol were not elevated in NAFLD patients. While serum 4β-hydroxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol are produced by ubiquitously expressed CYP3A4 [31] and CYP27A1 [32], 24*S*-hydroxycholesterol is synthesized by a brain-specific CYP46A1 [33]. Therefore, our results suggest that, in NAFLD, the cholesterol metabolism in the brain is not affected as much as that in other organs.

Cholesterol balance in our NAFLD patients was studied by the determination of serum markers for intestinal absorption (sitosterol/cholesterol and campesterol/cholesterol), biosynthesis (lathosterol/cholesterol), and catabolism to bile acids (C4/cholesterol). The results showed that cholesterol absorption was significantly reduced, while cholesterol and bile acid syntheses were not altered in NAFLD patients compared to controls. The reduced cholesterol absorption in NAFLD was consistent with the findings of a previous Finnish study [19], but the unchanged cholesterol synthesis was not consistent with the findings of that study. There are no definitive data to explain this inconsistency; however, differences in the patients' backgrounds in the two studies should be noted. First, the severity of liver damage in the study subjects needs to be considered. In the Finnish study, although subjects were recruited based on strict exclusion and inclusion criteria, the diagnosis of NAFLD was based on the measurement of liver fat content by [1] proton magnetic resonance spectrometry (H-MRS) without liver biopsy. In contrast, all NAFLD patients in our cohort were diagnosed by liver biopsy, and elevation of ALT was greater in our patient cohort (average ALT level at baseline in the NAFLD group was 102.1 U/l in our study, while the level was 39.5 U/l in the Finnish study [19]). Although severe fibrosis was not seen in any of our enrolled subjects, it is possible that sustained inflammation acted upon cholesterol synthesis. Second, the inclusion of patients with hypercholesterolemia in the present study may explain the differences in the cholesterol synthesis findings. Because of the use of pitavastatin, NAFLD patients with hypercholesterolemia were enrolled in the present cohort. It is possible that, in the present study cohort, cholesterol synthesis was already suppressed due to increased tissue cholesterol concentration. Third, differences in genetic background between Finnish and Japanese may also be discussed. However, it is assumed that increased cholesterol synthesis is not a major observation in hypercholesterolemic NAFLD patients in Japan.

The reason for the reduced cholesterol absorption in NAFLD has not been clarified. However, LXRα ligand oxysterols may up-regulate the expression of the

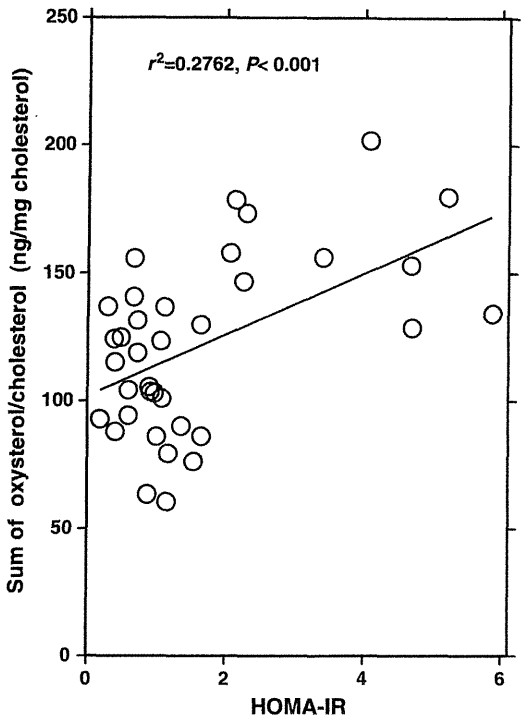

Fig. 2 The association between serum oxysterol levels and insulin resistance, calculated by the homeostasis model assessment as an index of insulin resistance (*HOMA-IR*; =fasting serum insulin (μU/ml) × fasting blood glucose (mg/dl)/405), was determined. The sum of oxysterol levels relative to cholesterol (25-hydroxycholesterol, 27-hydroxycholesterol, and 4β-hydroxycholesterol) were plotted against HOMA-IR

ATP-binding cassette transporters G5 and G8 (ABCG5/G8) through the activation of LXRα [34]. These transporters are present in the ileal brush-border membrane as well as in the hepatic apical membrane, and are responsible for the efflux of cholesterol into the intestinal lumen and bile duct. Net cholesterol absorption from the intestine depends on the competing activities of three membrane proteins: Niemann-Pick C1-like 1 (NPC1L1), ABCG5, and ABCG8 [35–37]. Pharmacological induction or overexpression of ABCG5/G8 in mice decreases fractional cholesterol absorption from the intestine [38, 39]. In addition, Nakamuta et al. [10] have reported the overexpression of LXRα and ABCG5 genes in the livers of NAFLD patients. Thus, up-regulation of ABCG5/G8 due to the activation of LXRα could contribute to the decreased cholesterol absorption in NAFLD, as demonstrated in the schematic figure shown in Fig. 3. We have previously reported the importance of the serum 27-hydroxycholesterol level (relative to cholesterol) for predicting the effects of a high-cholesterol diet on plasma LDL cholesterol concentrations [40]. In subjects with high serum 27-hydroxycholesterol levels (more than 80 ng/mg cholesterol), serum LDL cholesterol concentrations tended to increase after cholesterol ingestion (750 mg/day for 4 weeks) compared with findings in those with low serum 27-hydroxycholesterol levels (<80 ng/mg cholesterol). These results suggest that ABCG5/G8 proteins were fully up-regulated before cholesterol loading in the subjects with high 27-hydroxycholesterol levels, so that they might have been unable to adapt to a high-cholesterol diet.

The above cholesterol loading study showed that the relative 27-hydroxycholesterol levels were quite stable and were not influenced by the cholesterol loading itself or by the change of serum cholesterol concentrations after the cholesterol loading [40]. Stability of oxysterol levels was also observed during the treatment with pitavastatin in the present study. In NAFLD patients, pitavastatin markedly reduced serum cholesterol concentrations in parallel with the inhibition of cholesterol biosynthesis (Table 4), and serum total cholesterol concentrations became normal and were not significantly different from those in untreated control subjects after 3 months of treatment. However, levels of LXRα ligand oxysterol expressed relative to cholesterol remained high and did not change significantly during the 12-months period of pitavastatin treatment (Table 5). As shown in Fig. 1, the stability of oxysterol levels is due to the extremely slow reduction of absolute oxysterol concentrations compared with cholesterol and lathosterol. This finding also supported our hypothesis that the increase of oxysterol found in NAFLD is not only due to an increase of tissue cholesterol but is also due to other factors. All enrolled NAFLD patients in the present study cohort had insulin resistance according to HOMA-IR, and pitavastatin treatment did not improve the insulin resistance over the treatment period [23]. Plots of each determined oxysterol/cholesterol ratio and HOMA-IR demonstrated the significant associations between serum oxysterols and insulin resistance (Fig. 2). We speculate that serum LXRα ligand oxysterol levels (relative to cholesterol) could be a surrogate marker of insulin resistance, and that high oxysterol levels in the circulation may play an important role in the development of hepatic and peripheral insulin resistance followed by NAFLD. A study by Biddinger et al. [41] demonstrated the increased expression of ABCG5/G8 in the insulin-resistant liver, associated with increased biliary cholesterol excretion, and increased susceptibility to cholesterol gallstones. This finding is also suggestive of the involvement of oxysterol in increasing the expression of ABCG5/G8 in conditions with insulin resistance. The precise mechanism of how insulin resistance and oxysterol are linked should be elucidated in future studies.

In the present study, the C4-to-cholesterol ratio (C4/Chol; a biomarker representing bile acid synthesis) and FGF19 were not significantly different between the NAFLD patients and controls. In addition, the administration of pitavastatin exerted no effect on C4/Chol despite

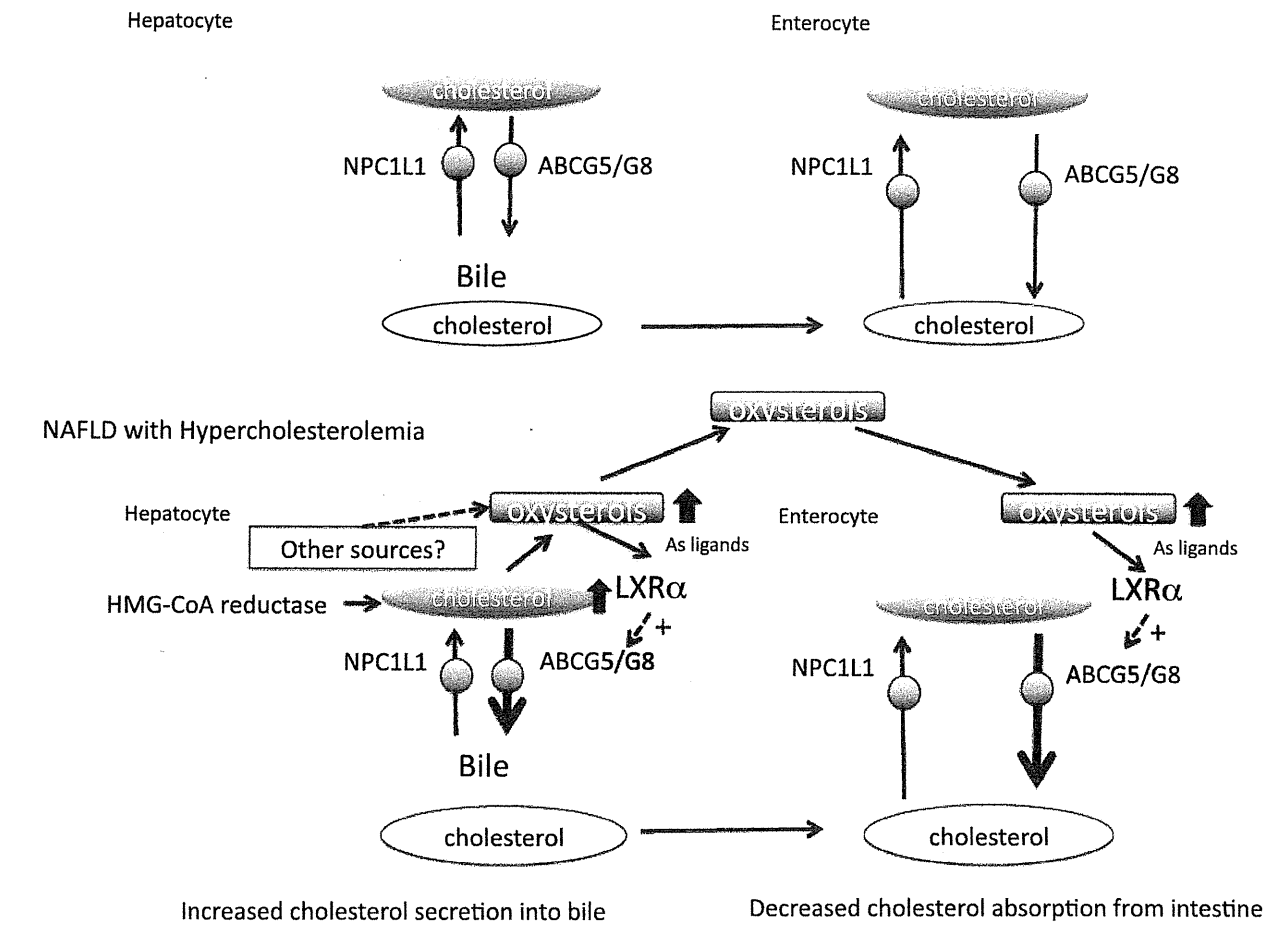

Fig. 3 Schematic figure demonstrating the hypothesis that up-regulation of ABCG5/G8 due to the activation of liver X receptor α (*LXRα*) could contribute to the decreased cholesterol absorption in non-alcoholic fatty liver disease (*NAFLD*). *HMG-CoA* 3-hydroxy-3-methylglutaryl-coenzyme A

producing a marked decrease in the serum cholesterol level. These findings suggest a lack of enhanced bile acid synthesis and secretion in NAFLD patients with hypercholesterolemia despite their increased cholesterol concentrations. A recent report also suggests that the hepatic response to FGF19 is impaired in patients with NAFLD and insulin resistance [42]. As poor adjustment of the bile acid synthesis system for an increased cholesterol level in humans may be a potential risk for metabolic syndrome, it has been suggested in recent studies that the alteration of bile acid signaling and/or hepatic flux may contribute to the pathogenesis of NAFLD and metabolic disorders [43]. This idea is consistent with the report by Yang et al., which demonstrated that the levels of FXR protein and mRNA were decreased in patients with NAFLD, whereas those of LXR were increased [44]. Hence, further clinical studies of bile acid metabolism in NAFLD should also be performed.

In conclusion, NAFLD patients showed significantly elevated levels of LXRα ligand oxysterols, 4β-hydroxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol in their sera. The reduced intestinal cholesterol absorption in NAFLD seemed to be caused by the upregulation of ABCG5/8 through the activation of LXRα by the oxysterols. The inhibition of cholesterol biosynthesis by pitavastatin normalized serum cholesterol concentrations in 3 months, but the abnormal oxysterol levels (relative to cholesterol) had not recovered by the end of the 12 months of treatment.

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Conflict of interest All authors declare that they have no conflict of interest.