ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2017 June 15; 8(6): 230-310

Published by Baishideng Publishing Group Inc

World Journal of Diabetes

Contents

Monthly Volume 8 Number 6 June 15, 2017

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

EDITORIAL OFFICE
Xiu-Xia Song, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
June 15, 2017

World Journal of Diabetes

Submit a Manuscript: http://www.f6publishing.com

World J Diabetes 2017 June 15; 8(6): 230-234

DOI: 10.4239/wjd.v8.i6.230

ISSN 1948-9358 (online)

FIELD OF VISION

Statins redux: A re-assessment of how statins lower plasma cholesterol

Rajendra Raghow

Rajendra Raghow, Department of Veterans Affairs Medical Center, Memphis, TN 38104, United States

Rajendra Raghow, Department of Pharmacology, University of Tennessee Health Science Center, Memphis, TN 38163, United States

Author contributions: Raghow R solely wrote this paper.

Conflict-of-interest statement: Raghow R declares that there is neither a conflict of interest with regard to the publication discussed in this FOV communication nor with respect to a commercial entity.

Manuscript source: Invited manuscript

Correspondence to: Rajendra Raghow, PhD, Professor, Department of Veterans Affairs Medical Center, 1030 Jefferson Avenue, Memphis, TN 38104, United States. rraghow@uthsc.edu
Telephone: +1-901-5238990
Fax: +1-901-5237274

Received: October 26, 2016
Peer-review started: October 31, 2016
First decision: December 1, 2016
Revised: February 7, 2017
Accepted: April 23, 2017
Article in press: April 24, 2017
Published online: June 15, 2017

Abstract

Obesity associated dyslipidemia and its negative effects on the heart and blood vessels have emerged as a major healthcare challenge around the globe. The use of statins, potent inhibitors of hydroxyl-methyl glutaryl (HMG) Co-A reductase, a rate-limiting enzyme in cholesterol biosynthesis, has significantly reduced the rates of cardiovascular and general mortality in patients with coronary artery disease. How statins lower plasma cholesterol levels presents a mechanistic conundrum since persistent exposure to these drugs *in vitro* or *in vivo* is known to induce overexpression of the HMG Co-A reductase gene and protein. In an attempt to solve this mechanistic puzzle, Schonewille *et al*, studied detailed metabolic parameters of cholesterol synthesis, inter-organ flux and excretion in mice treated with 3 common statins, rosuvastatin, atorvastatin or lovastatin, each with its unique pharmacokinetics. From the measurements of the rates of heavy water (D_2O) and [^{13}C]-acetate incorporation into lipids, the authors calculated the rates of whole body and organ-specific cholesterol synthesis in control and statin-treated mice. These analyses revealed dramatic enhancement in the rates of hepatic cholesterol biosynthesis in statin-treated mice that concomitantly elicited lower levels of cholesterol in their plasma. The authors have provided strong evidence to indicate that statin treatment in mice led to induction of compensatory metabolic pathways that apparently mitigated an excessive accumulation of cholesterol in the body. It was noted however that changes in cholesterol metabolism induced by 3 statins were not identical. While sustained delivery of all 3 statins led to enhanced rates of biliary excretion of cholesterol and its fecal elimination, only atorvastatin treated mice elicited enhanced trans-intestinal cholesterol excretion. Thus, blockade of HMGCR by statins in mice was associated with profound metabolic adaptations that reset their cholesterol homeostasis. The findings of Schonewille *et al*, deserve to be corroborated and extended in patients in order to more effectively utilize these important cholesterol-lowering drugs in the clinic.

Key words: Statins; Atorvastatin; Lovastatin; Rosuvastatin; Cholesterol synthesis; Hydroxyl-methyl glutaryl-CoA reductase

Core tip: Schonewille *et al*, comprehensively studied cholesterol metabolism (*de-novo* synthesis of cholesterol and its inter-organ flux, and fecal elimination) in mice treated with rosuvastatin, atorvastatin or lovastatin. These analyses revealed that the rates of whole body and organ-specific cholesterol synthesis were boosted by all three statins. Mice treated with statins also elicited enhanced rates of biliary excretion of cholesterol and its fecal elimination. Remarkably, the process of trans-intestinal cholesterol excretion was augmented by only atorvastatin. These data shed mechanistic light on how statin treatment led to organ-specific metabolic adaptations in mice to reset their cholesterol homeostasis.

Raghow R. Statins redux: A re-assessment of how statins lower plasma cholesterol. *World J Diabetes* 2017; 8(6): 230-234 Available from: URL: http://www.wjgnet.com/1948-9358/full/v8/i6/230.htm DOI: http://dx.doi.org/10.4239/wjd.v8.i6.230

INTRODUCTION AND COMMENTARY ON HOT TOPICS

Statins are among the most commonly prescribed drugs across the globe[1,2]. Statins were discovered in the 1970s, as competitive inhibitors of hydroxyl-methyl glutaryl coenzyme-A reductase (HMGCR), the rate limiting enzyme in cholesterol biosynthesis[3]. Soon after their discovery, statins were shown to be relatively safe drugs that caused lowering of serum cholesterol and reduction of fatty streaks in the blood vessels of laboratory animals. The positive outcomes of statin therapy in experimental animals were corroborated and extended to patients in large-scale clinical studies that established that statin treatment was correlated with significantly reduced rates of mortality in patients with atherosclerosis[1,2].

In addition to eliciting salutary effects in the cardiovascular system that includes improved endothelial function and neo-angiogenesis, statins exert pleiotropic actions in various tissues including the central nervous system. For instance, statins appear to exert anti-inflammatory actions in diseases such as rheumatoid arthritis, Alzheimer and Parkinson's disease, and multiple sclerosis[4]. It is worth mentioning however, that statins are far from innocuous since a sub-set of patients taking these drugs develop a range of adverse reactions that include muscle pain or myalgia, and in rare cases, rhabdomyolysis. Additionally, statin therapy has been shown to predispose some patients to develop type 2 diabetes[5].

In spite of decades of research in cultured cells and experimental animals[6], the mechanistic details underlying diverse actions of statins *in vivo* are poorly understood. According to the prevailing mechanism of action, statins competitively block HMGCR enzyme that leads to reduced biosynthesis of cholesterol. Statin-induced depletion of cholesterol in the endoplasmic reticulum (ER) activates the sterol response element binding proteins (SREBP-1 and SREBP-2) cleavage and activation protein, SCAP, that chaperones the full-length transcription factor SREBP2 from ER to the Golgi. The nascent SREBP-2 is sequentially cleaved by regulated intra-membrane proteolysis catalyzed by two proteases, named S1P and S2P. The truncated nSREBP-2 then moves into the nucleus to activate transcription of its downstream targets that include the gene encoding the low-density lipoprotein receptor (LDLR). The LDLRs are the primary regulators of cholesterol homeostasis and their increased abundance on plasma membranes is inversely coupled to levels of LDL-cholesterol in circulation[7,8].

This relatively straightforward relationship between statin-induced blockade of HMGCR and reduced concentration of LDL-cholesterol in plasma fails to reconcile a key observation that persistent exposure to statins leads to enhanced expression of HMG Co-A reductase gene and its enzyme, both in cells in culture and in intact animals[6]. This behavior of statins is not unusual since targeted inhibition of enzymatic pathways often leads the emergence of adaptive mechanisms to cope with such metabolic blockades. The compensatory up regulation of HMGCR expression in patients in response to statins does not hinder their clinical utility since doses of statins are invariably adjusted to achieve the desired range of plasma cholesterol.

Although the pharmacology of statins has been extensively studied, attempts to decipher whether and to what extent these drugs modulate rates of cholesterol biosynthesis and its tissue-specific sequestration and excretion *in vivo* have yielded conflicting results. For example, based on measurements of serum concentration of surrogates of cholesterol (*e.g.*, mevalonic acid, squalene, cholestenol, lathosterol and desmosterol), some investigators have claimed that statin therapy was associated with reduced rates of cholesterol biosynthesis[9,10]. Similarly, the results of direct quantification of cholesterol have also yielded apparently conflicting conclusions. Thus, while one study[11] reported that rates of cholesterol synthesis remained unaltered in patients taking pravastatin or lovastatin, two other reports claimed that statin treatment led to reduced rates of cholesterol biosynthesis[12,13] and, yet another study reached an exactly opposite conclusion[14]. We believe that a clear-cut interpretation of earlier studies has been partially confounded by relative paucity of experiments that were designed to directly measure the rates cholesterol biosynthesis *in vivo*. Additionally, most earlier studies did not rigorously address the relationship between mechanisms involved in the biosynthesis of cholesterol and its inter-organ flux in statin-treated animal or patients. A number of studies have shown the inadequacy of molecular surrogates of cholesterol

to accurately reflect the rates of cholesterol synthesis *in vivo* (discussed in detail in the publication of Schonewille *et al*[15], which is the subject of the current Commentary). Finally, studies to date have not undertaken a sufficiently comprehensive analysis of cholesterol metabolism to specifically reconcile the paradoxical observation that statin therapy was associated with increased expression of HMGCR messenger RNA and protein in the livers of laboratory rodents and patients while concomitantly leading to lower levels of serum cholesterol[16-22].

In a recent paper (Schonewille M, Freark de Boer J, Mele L, Wolters H, Bloks VW, Wolters JC, Kuivenhoven JA, Tietge UJ, Brufau G, Groen AK. Statins increase hepatic cholesterol synthesis and stimulate fecal cholesterol elimination in mice. *J Lipid Res* 2016; 57: 1455-1464), Schonewille *et al*[15], have attempted to re-examine the mechanistic paradox of how statins affect cholesterol metabolism *in vivo*. To achieve this goal, the authors undertook a comprehensive analysis of putative pathways involved in *de novo* biosynthesis of cholesterol and its elimination in mice that were fed rosuvastatin, atorvastatin or lovastatin, mixed with regular rodent chow. The authors chose these 3 common statins because they have unique pharmacokinetics properties. Thus, lovastatin, a pro-drug that needs to be metabolically activated in the enterohepatic circulation, was given to a group of mice; a parallel cohort of mice received rosuvastatin which is a more hydrophilic and liver-selective statin. The third group of mice were fed chow mixed with atorvastatin. Both atorvastatin and lovastatin are more lipophilic and have longer half-lives than rosuvastatin. After 5 d of statin treatment, rates of cholesterol synthesis, sequestration and elimination were assessed in various tissues of control and statin-treated mice.

The authors used two complementary, stable isotope-labeling techniques to compare the rates of whole body and organ-specific cholesterol synthesis. Using the classic method[23], based on the incorporation of deuterium-labeled water (D_2O) into newly synthesized lipids, Schonewille *et al*[15], assessed *de novo* cholesterol synthesis. Since deuterium-enriched body water becomes uniformly distributed in all organs, this technique allows determination of absolute rates of cholesterol synthesis from quantification of deuterium incorporated into lipids. Schonewille *et al*[15], also studied cholesterol metabolism in mice that drank water spiked with 2% [^{13}C]-acetate. Under these conditions, acetate was transported into the liver *via* the portal vein which continuously fluxes acetate from the gastrointestinal (GI) tract to the liver[24]. As documented earlier, the majority of the exogenously supplied [^{13}C]-acetate enters the liver in an intermittent, yet highly efficient manner and gets assimilated into acetyl-CoA and newly synthesized lipids[25,26]. This technique enabled the authors to calculate fractional rates of cholesterol synthesis in control and statin-treated mice. It is noteworthy that although the two techniques measured different facets of *de novo* lipid synthesis, the profiles of absolute rates of cholesterol synthesis derived from incorporation of H^3-enriched drinking water into newly synthesized lipids or fractional rates of hepatic synthesis of cholesterol, as assessed by rates of incorporation of a bolus of exogenous [^{13}C]-acetate, showed similar qualitative and quantitative trends in statin-treated mice that were significantly different from untreated animals.

Based on the kinetics of incorporation of either [^{13}C]-acetate or D_2O water into lipids, the authors surmised that atorvastatin and lovastatin treatments led to a robust increase in *de novo* cholesterol synthesis in the liver. The authors simultaneously measured changes in organ-specific expression of genes relegated to the biosynthesis of cholesterol and molecular pathways that were putatively involved in the accumulation of cholesterol in various tissues. Concomitant quantification of rates of lipid synthesis and gene expression analyses led to three important insights. Thus, Schonewille *et al*[15], observed that: (1) treatment with rosuvastatin, atorvastatin and lovastatin was associated with 6-, 15-, and 11-fold greater expression of hepatic HMGCR protein, respectively; (2) treatment of all 3 statins also led to greater expression of *SREBP-2* gene and some of its downstream targets (*e.g.*, mevalonate kinase, phosphomevalonate kinase, farnesyl-diphosphate farnesyltransferase 1 and squalene epoxidase); and (3) plasma concentrations of 3 cholesterol surrogates (*e.g.*, lathosterol, lanosterol and desmosterol) failed to accurately reflect the rates of cholesterol synthesis in statin-treated mice.

To seek an explanation for the discrepancy between enhanced rates of synthesis of hepatic cholesterol and its lower concentration in the plasma of statin-treated mice, Schonewille *et al*[15], undertook additional experiments aimed at discovering putative pathways involved in inter-organ flux of cholesterol and its elimination. The authors found that hepatic expression of genes encoding LDLR and two ATP-binding cassettes-containing cholesterol transporters, ABCG5 and ABCG8, was greatly enhanced in mice receiving statin treatment. The authors also experimentally probed inter-organ fluxes of cholesterol to determine if excessive cholesterol was removed from the liver and plasma *via* biliary secretion or *via* fecal elimination. Since accumulation of cholesterol in the feces represents 3 potential sources, *i.e.*, unabsorbed dietary cholesterol, cholesterol secreted from the gall bladder, and trans-intestinal cholesterol elimination (TICE), Schonewille *et al*[15], sought evidence for all three sources of cholesterol in the feces of statin-treated mice. Based on these analyses, the authors concluded that while statins did not alter intake of dietary cholesterol or its absorption, statin-mediated inhibition of HMGCR was associated with increased cholesterol in the bile acids; with regard to biliary secretion, lovastatin was found to be particularly potent. It was noted however that the metabolic adaptations induced by 3 statins were not identical. For instance, the atorvastatin-treated mice uniquely elicited increased rates of TICE. These data were consistent with the conclusion that

treatment with either rosuvastatin and lovastatin was associated with robustly increased rates of secretion of hepatobiliary cholesterol and its fecal elimination, atorvastatin treatment also impinged on the mechanism of TICE. Thus, although enhanced cholesterol catabolism might have partially contributed to increased cholesterol synthesis in atorvastatin-treated mice, apparently, this was not the case in mice treated with rosuvastatin or lovastatin. Whether or not statin treatment is associated with enhanced rates of TICE in humans remains to be experimentally established.

Although the question of how statins lower plasma cholesterol has been studied in many laboratories, the report of Schonewille *et al*[15], is remarkable with regard to the comprehensive nature of their experiments aimed at answering this question. Thus, the authors not only explored the molecular mechanisms of biosynthesis of cholesterol (stable isotope-labeling of newly synthesized lipids, gene and protein expression) and its inter-organ flux but also the processes of biliary and fecal elimination of cholesterol in statin-treated mice. A key insight of experiments reported by Schonewille *et al*[15], is that sustained presence of statins *in vivo* leads to significant inter-organ metabolic interactions that profoundly altered cholesterol homeostasis in statin-treated mice. These data also revealed that all statins were not alike with respect to their ability to alter the mechanisms of cholesterol homeostasis.

While the report of Schonewille *et al*[15], contains novel data that bear directly on the actions of statins on whole body and organ-specific cholesterol homeostasis, it raises a number of key mechanistic questions that need to be addressed in follow-up studies. For example, it is not clear from the data of Schonewille *et al*[15], if age of the mice or their gender played a significant role in altered cholesterol metabolism in response to statin treatment. In a similar vein, additional investigations are needed to reveal if effects of statins on the biosynthesis and inter-organ flux of cholesterol are dose-dependent. Finally, future studies need to address if the effects of statins on lipoprotein profiles (HDL *vs* LDL) are species-specific, and if so, what are the underlying molecular mechanisms that explain variable responses of rodents and humans to statin treatment. These caveats notwithstanding, the findings of Schonewille *et al*[15], in mice deserve to be corroborated and extended in other animals, and in patients. Such investigations ought to be launched with an aim to decipher the cellular and molecular mechanisms involved in species-specific differences in statin-induced alterations in the pathways of *de novo* biosynthesis of cholesterol and its inter-organ flux[21,27,28].

CONCLUSION

To sum up, the tantalizing discovery of a novel mechanism of action of statins in mice, reported by Schonewille *et al*[15], has important implication for patients receiving statin therapy. In my opinion, analogous investigations in patients have generally lacked the experimental rigor and scope of the present study, that have perhaps contributed to apparently conflicting data[6,29]. This situation can only be mitigated by more comprehensive studies aimed at directly measuring the rates of *de novo* cholesterol synthesis, inter-organ cholesterol flux and the processes of sequestration and elimination of neutral sterols in humans taking statins. Accomplishing this mission will be predicated on the development of innovative, noninvasive methods to study the mechanisms of cholesterol homeostasis in humans in greater detail. Such analyses are likely to yield new insights that will enable a more judicious use of statins in millions of patients worldwide.

REFERENCES

1 **Baigent C**, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J, Collins R. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010; **376**: 1670-1681 [PMID: 21067804 DOI: 10.1016/S0140-6736(10)61350-5]

2 **Stulc T**, Ceška R, Gotto AM. Statin Intolerance: the Clinician's Perspective. *Curr Atheroscler Rep* 2015; **17**: 69 [PMID: 26490078 DOI: 10.1007/s11883-015-0552-3]

3 **Endo A**. The discovery and development of HMG-CoA reductase inhibitors. *J Lipid Res* 1992; **33**: 1569-1582 [PMID: 1464741]

4 **Liao JK**, Laufs U. Pleiotropic effects of statins. *Annu Rev Pharmacol Toxicol* 2005; **45**: 89-118 [PMID: 15822172 DOI: 10.1146/annurev.pharmtox.45.120403.095748]

5 **Sattar N**, Preiss D, Murray HM, Welsh P, Buckley BM, de Craen AJ, Seshasai SR, McMurray JJ, Freeman DJ, Jukema JW, Macfarlane PW, Packard CJ, Stott DJ, Westendorp RG, Shepherd J, Davis BR, Pressel SL, Marchioli R, Marfisi RM, Maggioni AP, Tavazzi L, Tognoni G, Kjekshus J, Pedersen TR, Cook TJ, Gotto AM, Clearfield MB, Downs JR, Nakamura H, Ohashi Y, Mizuno K, Ray KK, Ford I. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. *Lancet* 2010; **375**: 735-742 [PMID: 20167359 DOI: 10.1016/S0140-6736(09)61965-6]

6 **Gazzerro P**, Proto MC, Gangemi G, Malfitano AM, Ciaglia E, Pisanti S, Santoro A, Laezza C, Bifulco M. Pharmacological actions of statins: a critical appraisal in the management of cancer. *Pharmacol Rev* 2012; **64**: 102-146 [PMID: 22106090 DOI: 10.1124/pr.111.004994]

7 **Shao W**, Espenshade PJ. Expanding roles for SREBP in metabolism. *Cell Metab* 2012; **16**: 414-419 [PMID: 23000402 DOI: 10.1016/j.cmet.2012.09.002]

8 **Soyal SM**, Nofziger C, Dossena S, Paulmichl M, Patsch W. Targeting SREBPs for treatment of the metabolic syndrome. *Trends Pharmacol Sci* 2015; **36**: 406-416 [PMID: 26005080 DOI: 10.1016/j.tips.2015.04.010]

9 **Naoumova RP**, Marais AD, Mountney J, Firth JC, Rendell NB, Taylor GW, Thompson GR. Plasma mevalonic acid, an index of cholesterol synthesis in vivo, and responsiveness to HMG-CoA reductase inhibitors in familial hypercholesterolaemia. *Atherosclerosis* 1996; **119**: 203-213 [PMID: 8808497]

10 **Miettinen TA**, Gylling H. Synthesis and absorption markers of cholesterol in serum and lipoproteins during a large dose of statin treatment. *Eur J Clin Invest* 2003; **33**: 976-982 [PMID: 14636301]

11 **Goldberg IJ**, Holleran S, Ramakrishnan R, Adams M, Palmer RH, Dell RB, Goodman DS. Lack of effect of lovastatin therapy on the parameters of whole-body cholesterol metabolism. *J Clin Invest* 1990; **86**: 801-808 [PMID: 2394831 DOI: 10.1172/JCI114777]

12 **Vanhanen H**, Kesäniemi YA, Miettinen TA. Pravastatin lowers serum cholesterol, cholesterol-precursor sterols, fecal steroids, and cholesterol absorption in man. *Metabolism* 1992; **41**: 588-595 [PMID: 1640845]

13 **Gylling H**, Miettinen TA. Effects of inhibiting cholesterol absorption and synthesis on cholesterol and lipoprotein metabolism in hypercholesterolemic non-insulin-dependent diabetic men. *J Lipid Res* 1996; **37**: 1776-1785 [PMID: 8864962]

14 **Kallien G**, Lange K, Stange EF, Scheibner J. The pravastatin-induced decrease of biliary cholesterol secretion is not directly related to an inhibition of cholesterol synthesis in humans. *Hepatology* 1999; **30**: 14-20 [PMID: 10385633 DOI: 10.1002/hep.510300119]

15 **Schonewille M**, Freark de Boer J, Mele L, Wolters H, Bloks VW, Wolters JC, Kuivenhoven JA, Tietge UJ, Brufau G, Groen AK. Statins increase hepatic cholesterol synthesis and stimulate fecal cholesterol elimination in mice. *J Lipid Res* 2016; **57**: 1455-1464

16 **Morand OH**, Aebi JD, Dehmlow H, Ji YH, Gains N, Lengsfeld H, Himber J. Ro 48-8.071, a new 2,3-oxidosqualene: lanosterol cyclase inhibitor lowering plasma cholesterol in hamsters, squirrel monkeys, and minipigs: comparison to simvastatin. *J Lipid Res* 1997; **38**: 373-390 [PMID: 9162756]

17 **Bergstrom JD**, Bostedor RG, Rew DJ, Geissler WM, Wright SD, Chao YS. Hepatic responses to inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase: a comparison of atorvastatin and simvastatin. *Biochim Biophys Acta* 1998; **1389**: 213-221 [PMID: 9512650]

18 **Fujioka T**, Nara F, Tsujita Y, Fukushige J, Fukami M, Kuroda M. The mechanism of lack of hypocholesterolemic effects of pravastatin sodium, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, in rats. *Biochim Biophys Acta* 1995; **1254**: 7-12 [PMID: 7811749]

19 **Roglans N**, Verd JC, Peris C, Alegret M, Vázquez M, Adzet T, Díaz C, Hernández G, Laguna JC, Sánchez RM. High doses of atorvastatin and simvastatin induce key enzymes involved in VLDL production. *Lipids* 2002; **37**: 445-454 [PMID: 12056585]

20 **Verd JC**, Peris C, Alegret M, Díaz C, Hernández G, Vázquez M, Adzet T, Laguna JC, Sánchez RM. Different effect of simvastatin and atorvastatin on key enzymes involved in VLDL synthesis and catabolism in high fat/cholesterol fed rabbits. *Br J Pharmacol* 1999; **127**: 1479-1485 [PMID: 10455299 DOI: 10.1038/sj.bjp.0702668]

21 **Yamauchi S**, Linscheer WG, Beach DH. Increase in serum and bile cholesterol and HMG-CoA reductase by lovastatin in rats. *Am J Physiol* 1991; **260**: G625-G630 [PMID: 2018137]

22 **Reihnér E**, Rudling M, Ståhlberg D, Berglund L, Ewerth S, Björkhem I, Einarsson K, Angelin B. Influence of pravastatin, a specific inhibitor of HMG-CoA reductase, on hepatic metabolism of cholesterol. *N Engl J Med* 1990; **323**: 224-228 [PMID: 2114543 DOI: 10.1056/NEJM199007263230403]

23 **Rittenberg DS**. Deuterium As An Indicator In The Study Of Intermediary Metabolism: Viii. Hydrogenation Of Fatty Acids In The Animal Organism. *J Biol Chem* 1937; **117**: 485

24 **Faix D**, Neese R, Kletke C, Wolden S, Cesar D, Coutlangus M, Shackleton CH, Hellerstein MK. Quantification of menstrual and diurnal periodicities in rates of cholesterol and fat synthesis in humans. *J Lipid Res* 1993; **34**: 2063-2075 [PMID: 8301227]

25 **Neese RA**, Faix D, Kletke C, Wu K, Wang AC, Shackleton CH, Hellerstein MK. Measurement of endogenous synthesis of plasma cholesterol in rats and humans using MIDA. *Am J Physiol* 1993; **264**: E136-E147 [PMID: 8430782]

26 **Lee WN**, Byerley LO, Bergner EA, Edmond J. Mass isotopomer analysis: theoretical and practical considerations. *Biol Mass Spectrom* 1991; **20**: 451-458 [PMID: 1768701 DOI: 10.1002/bms.1200200804]

27 **Parker RA**, Garcia R, Ryan CS, Liu X, Shipkova P, Livanov V, Patel P, Ho SP. Bile acid and sterol metabolism with combined HMG-CoA reductase and PCSK9 suppression. *J Lipid Res* 2013; **54**: 2400-2409 [PMID: 23614904 DOI: 10.1194/jlr.M038331]

28 **Bodmer M**, Brauchli YB, Krähenbühl S, Jick SS, Meier CR. Statin use and risk of gallstone disease followed by cholecystectomy. *JAMA* 2009; **302**: 2001-2007 [PMID: 19903921 DOI: 10.1001/jama.2009.1601]

29 **Pasha MK**, Muzeeb S, Basha SJ, Shashikumar D, Mullangi R, Srinivas NR. Analysis of five HMG-CoA reductase inhibitors--atorvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin: pharmacological, pharmacokinetic and analytical overview and development of a new method for use in pharmaceutical formulations analysis and in vitro metabolism studies. *Biomed Chromatogr* 2006; **20**: 282-293 [PMID: 16143964 DOI: 10.1002/bmc.561]

P- Reviewer: Francis KR, Poirot M, Rosa-Bray M
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Wu HL

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com