Letter to the Editor

Absorption of Unconjugated Bile Acids from the Perfused Jejunum of the Anesthetized Rat: Structure-Activity Relationships and Rate Limiting Steps

Short title: Unconjugated bile acid absorption

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Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; UCA, ursocholic acid norCDCA, 24nor-chenodeoxycholic acid; norUDCA, 24nor-ursodeoxycholic acid; CDC-gly (glycochenodeoxycholic; CDC-tau, taurochenodeoxycholic; P_{uwl} , permeability coefficient of the unstirred water layer; P_m, permeability coefficient for the membrane; P_{app}, permeability observed experimentally.

Disclosures: None of the authors have any conflict of interest. Author contributions: Jean-Louis Dupas performed the experiments and drafted the initial manuscript; Dr. Norman Ho provided the method for data analysis and generated the values in Table 1. Dr. Alan Hofmann planned the experimental design and revised the draft manuscript prepared by Jean-Louis Dupas. All authors have read and approved this version of the manuscript.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi:

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Unconjugated bile acids are not only formed in the mammalian intestine by bacterial enzymes, but are also used as therapeutic agents for the treatment of cholestatic liver disease and for cholesterol gallstone prevention and dissolution. Ursodeoxycholic acid (UDCA) is used for the treatment of primary biliary cholangitis (formerly termed primary biliary cirrhosis) where it decreases the need for liver transplantation (Borg, 2006). UDCA is also used for the prevention of gallstone formation in obese patients who have rapid weight loss after bariatric surgery (Sugerman,1995). Recently, 24-norUDCA (a homologue of UDCA with one less carbon atom in the side chain) has been shown to improve liver tests in primary sclerosing cholangitis. (Fickert, 2017). A semi-synthetic bile acid, obeticholic acid, is the 6α-ethyl derivative of CDCA and a potent agonist of the nuclear receptor FXR (Farnesoid X Receptor). Obeticholic acid has been shown to improve liver tests in patients with primary biliary cholangitis who have not responded to treatment with UDCA (Nevens, 2017) and is being tested in other liver diseases

Bile acids used for therapy are formulated in the form of the protonated acid. This physical form has extremely low aqueous solubility (< 100 μM) and crystal dissolution is generally considered to be rate limiting in their absorption. Nonetheless, it seems important to define the rate of uptake of unconjugated bile acids from an aqueous solution in which the bile acid is largely present in the form of the water-soluble bile acid anion. We report here ~~have determined~~ the rate of passive intestinal absorption of six polyhydroxy unconjugated bile acids from a perfused jejunal segment of the anesthetized rat. We determined the influence of the unstirred water layer by including d-glucose in the perfusate, a solute whose rate of uptake is determined solely by the thickness of the unstirred water layer (Levitt, 1990).

Male Wister rats weighing 350-400 g were used. A jejunal segment of the anesthetized rat was perfused using a bidirectional pump to minimize the unstirred water layer (Doluisio,1969). The perfusate (pH 7.3) contained phosphate buffer, bile acid, glucose and a polymeric dye (Polycyanine) as a non-absorbable marker to correct for water movement into or out of the perfused segment (Dupas,1985). Samples (25 μL) of the perfusate were obtained by means of a fine catheter inserted through the intestinal wall into the lumen, enabling calculation of the first order disappearance rate.

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. Bile acids labeled with ^{14}C or ^{3}H, were synthesized in this laboratory and were radiopure by zonal scanning. The bile acid concentration in perfusates was 0.1 mM, a concentration at which only bile acid monomers are present. At least 4 studies were made for each unconjugated bile acid and pairwise comparisons for rates of absorption were tested for statistical significance using Student's T-test). Concentrations of labeled bile acids were determined by liquid scintillation counting or in some instances by a sensitive enzymatic assay (Roda,1982). The study was approved by the Committee on Animal Care and Protection of the University of California San Diego.

The influence of nuclear substitution on membrane permeability was determined for four natural C_{24} bile acids chenodeoxycholic (CDCA), ursodeoxycholic (UDCA) cholic (CA), and ursocholic (UCA). Their structure is given in Table 2. The influence of side chain length was assessed using two C_{23} bile acids (24-norCDCA and 24-norUDCA) that have 4 rather than 5 carbon atoms in the side chain. The effect of conjugation on absorption was defined using the glycine and taurine conjugates of CDCA. Values for the permeability of the unstirred layer and for the lipid membrane were calculated for individual experiments as described (Ho, shown in Table 1.

Calculated values for the permeability of the membrane and the lipid membrane were obtained using the equations shown in Table 1 (Ho, 1974), and are summarized in Table 2.. Nuclear hydroxylation had a marked effect on membrane permeation as dihydroxy bile acids were absorbed more rapidly than trihydroxy bile acids (CDCA>CA; UDCA>UCA ($p<0.05$ for each pair). In addition, changing the orientation of the hydroxyl group at C-7 from α to β greatly decreased the rate of absorption: CDCA>UDCA; CA>UCA) ($p<0.05$ for all comparisons). Side chain length had a variable effect (CDCA<norCDCA) but absorption of norUDCA did not differ from that of UDCA. Uptake of CDCA was so rapid that it was entirely controlled by the thickness of the unstirred water layer. The glycine and taurine conjugates of CDCA showed negligible absorption, in agreement with previous studies (Wilson, 1981). Based on the work of Marcus et al (Marcus,1991), it is reasonable to equate disappearance from the lumen as being equivalent to absorption from the small intestine. Anesthesia has been shown to increase the thickness of the unstirred layer (Levitt, 1990), so that the calculated P_{uwl} values are only valid for the

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experimental conditions of this study. The values for P_m (membrane permeability) should be rather similar across species as they are normalized to epithelial area.

Values for membrane permeability and fraction of uptake attributable to diffusion through the unstirred water layer were calculated as described in Table 1 (Ho, 1974), and are summarized in Table 1. Our measurements of net absorption are in general agreement with previous studies of unconjugated bile acid absorption in humans (Krag, 1974; Berge Henegouwen, 1977), in the rabbit (Aldini, 1996) and in the rat (Schiff,1972; Wilson, 1981), but provide new information on the effect of side chain length. Such information may be of interest as norUDCA is now in clinical trials for the treatment of cholestatic liver disease (Fickert, 2017).

We conclude that the rate of unconjugated bile acid absorption from the jejunum is influenced markedly by the pattern of bile acid hydroxylation and to a lesser extent by side chain length. CDCA is absorbed so rapidly that its uptake is largely determined by the thickness of the unstirred water layer. Changing the C-7 hydroxy group from an α to a β configuration greatly decreases the rate of absorption. C_{23} 24-NorCDCA and 24-norUDCA were well absorbed from the jejunum, presumably also by passive means.

Acknowledgements: Dr. Joseph H. Steinbach aided in manuscript preparation, and printing of the equations.

References

1. Aldini, R., Montagnani, M., Roda, A., Hrelia, S., Biagi, P.L., & Roda, E. (1996). Intestinal absorption of bile acids in the rabbit: different transport rates in jejunum. Gastroenterology **110**:459-68.
2. Anderson, B.W., Levine, A.S., Levitt, D.G., Kneip, J.M., & Levitt, M.D. (1968). Physiological measurement of luminal stirring in perfused rat jejunum. American Journal of Physiology **254**:G843-8.
3. van Berge Henegouwen, G.P., & Hofmann, A.F. (1977). Pharmacology of

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chenodeoxycholic acid. II. Absorption and metabolism. Gastroenterology **73**:300-309.

4. ter Borg, P.C., Schalm, S.W. Hansen, B.E., van Buuren, H.R., & Dutch PBC Study Group. (2006). Prognosis of ursodeoxycholic acid-treated patients with primary biliary cirrhosis. Results of a 10-yr cohort study involving 297 patients. American Journal of Gastroenterology **101**:2044-50.
5. Doluisio, J.T., Billips, N.F., Dittert, L.W., Sugita, E.T., & Swintosky J.V. (1969). Drug absorption I: An *in situ* rat gut technique yielding realistic absorption rates. Journal of Pharmaceutical Science **5**8:1196-1200.
6. Dupas, J-L., Moreau, M., & Hofmann, A.F. (1985) Polymeric dyes: ideal non-absorbable reference markers for intestinal absorption studies. Journal of Pharmaceutical Science **74**:328-330.
7. Fickert, P., Hirschfield, G.M., Denk, G., Marschall ,H.U., Altorjay; I., 'Farkkila.. M., Schramm, C., Spengler, U., Chapman, R., Bergquist, A., Schrumpf, E., Nevens, F., Trivedi, P., Reiter, F.P., Tornai, I., Halilbasic, E., Greinwald, R., Prols, M., Manns, M.P., & Trauner, M. (2017) NorUrsodeoxycholic acid improves cholestasis in primary sclerosing cholangitis. Journal of Hepatology 2017;**67**:549-58.
8. Ho, N.F., & Higuchi W.I. (1974) Theoretical model studies of intestinal absorption IV. Bile acid transport at premicellar concentrations across diffusion layer – membrane barrier. Journal of Pharmaceutical Science **63**:686-90.
9. Krag, E., & Phillips, S. (1974) Active and passive bile acid absorption in man: Perfusion studies of the ileum and jejunum. Journal of Clinical Investigation **53**:1686-94.

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10. Levitt, M.D., Fume, J.K., Strocchi, I., Anderson, B.W., & Levitt, D.G. (1990) Physiological measurements of luminal stirring in the dog and human small bowel Journal of Clinical Investigation **86**:1540-7.
11. Marcus, S.N., Schteingart ,C.D., Marquez, M.L., Hofmann, A.F., Xia,Y., Steinbach, J.H., Ton-Nu, H-T., Lillienau, J., Angellotti, M.A., & Schmassmann, A. (1991) Active absorption of conjugated bile acids in vivo: kinetic parameters and molecular specificity of the ileal transport system in the rat. Gastroenterology **100**:212-221.
12. Nevens, F., Andreone, P., Mazzella, G., Strasser, S.I., Bowlus, C., Invernezzi, P., Drenth, J.P., Pockros, P.J., Regula, J., Beuers, U., Trauner, M., Jones, D.E., Floreani, A., Hohenester, S., Luketic, V., Shiffman, M., van Erpecum, K.J., Vargas, V., Vincent, C., Hirschfield, G.M., Shah, H., Hansen, B., Lindor, K.D., Marschall, H.U., Kowdley, K.V., Hooshman-Rad, R., Marmon, T., Sheeron, S., Pencek, R., MacConell, L., Pruzanski, M., Shapiro D., & POISE Study group. (2016). A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. New England Journal of Medicine **375**:631-43.
13. Roda, A., Kricka, L.J., DeLuca, M., & Hofmann, A.F. (1982) Bioluminescent measurement of primary bile acids using immobilized 7α-hydroxysteroid dehydrogenase: application to serum bile acids. Journal of Lipid Reseach **23**:1354-1361.
14. Schiff, E.R., Small, N.C., & Pietsch, J., M. (1972) Characterization of the kinetics of the passive and active transport mechanisms for bile acid absorption in the small intestine and colon of the rat Journal of Clinical Investigation **51**:1351-62.
15. Sugerman,H.J., Brewer, W.H., Shiffman, M.L., Brolin, R.E., Fobi, M.A., Linner, J.H., Mac/Donald, K.G., MacGregor, A.M., Martin, L.F., Oram-Smith, J.C., Popoola, D.A., Schirmer, B.D., & Vickers, F.F. (1995) A multicenter placebo-

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controlled randomized, double-blind prospective trial of prophylactic ursodiol for the prevention of gallstone formation following gastric-bypass-induced rapid weight loss. American Journal of Surgery 169:91-6.

16. Wilson F. A. (1981) Intestinal transport of bile acids. American Journal of Physiology **241**:G83-G92.

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Table 1 **Calculation of Permeability Coefficients.**

From the first equation:

$$(1)\quad K = \frac{A}{V} \cdot \frac{1}{\frac{1}{P_{UWL}} + \frac{1}{P_M + P_P}}$$

Where

K = first order rate constant given by the absorption of glucose or a given bile acid

A= geometric area of perfused jejunal segment of length L

V = volume of solution in the perfused segment

$$\frac{A}{V} = 2\sqrt{\frac{\pi L}{V}}$$

P_{UWLB}= undisturbed water layer permeability of bile acid

P_{uwlG} = undisturbed water layer permeability of glucose

P_{MB} = membrane permeability of bile acid

P_P = pore permeability

* In the case of bile acids P_P = insignificant because the size of the bile acid molecules precludes paracellular absorption

$$(2)\quad \frac{1}{P_{UWLB}} + \frac{1}{P_{MB}} = \frac{A}{V \cdot K_B}$$

* and for glucose (unstirred water layer rate controlled) wherein $P_{UWL} <<< P_M$,

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$$(3) \quad \frac{1}{P_{UWLG}} = \frac{A}{V \cdot K_G}$$

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(B) Aqueous diffusion relationships, accounting for the molecular sizes of glucose and the bile acids, lead to the following ratio,

$$(4) \quad \frac{P_{UWLB}}{P_{UWLG}} = \left(\frac{M_G}{M_B}\right)^{\frac{1}{3}}$$

where M is the molecular weight.

From equations 2, 3, 4 we arrive at the following equation.

$$(5) \quad P_{MB} = \frac{A}{V} \cdot \frac{1}{\frac{1}{K_B} - \frac{1}{K_G}\left(\frac{M_B}{M_G}\right)^{\frac{1}{3}}} = \frac{1}{\frac{1}{P_{EXPB}} - \frac{1}{P_{UWLB}}}$$

P_{MB} values were calculated for each experiment

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Table 2. Permeability Coefficients of bile acids (x 10^4) cm/sec (Mean±Standard Deviation) in the perfused rat jejunum at pH 7.3[§].

Unconjugated						
	Bile acid	Structure	P_{app}	P_{uwl}	P_m	% P_{uwl} controlled*
	CDCA	C_{24}, 3αOH,7αOH	1.03±0.18	1.08±0.19	22.2±10.0	95%
	norCDCA	C_{23}, 3αOH,7αOH	0.78±.19	0.91±0.13	5.46±3.52	85%
	UDCA	C_{24}, 3αOH,7βOH	0.67±0.05	1.20±0.52	1.52±1.47	56%
	norUDCA	C_{23}, 3αOH,7βOH	0.64±0.15	0.99±0.14	1.81±0.82	65%
	CA	C_{24}, 3αOH,7αOH,12αOH	0.52±0.24	0.68±0.27	1.25±0.48	76%
	UCA	C_{24}, 3αOH,7βOH,12αOH	0.24±0.08	0.87±0.06	0.33±0.16~	27%
Conjugated						
	CDC-tau	C_{24} – CDC-taurine	0.05		~0.05	<10%
	CDC-gly	C_{24} – CDC-glycine	0.1-0.2		~0.05	<10%

[§]Abbreviations: $P_{app,}$ Apparent permeability; $P_{uwl,}$ Permeability of the unstirred water layer; $P_{m,}$ permeability of the membrane; CDC-tau, taurochenodeoxycholate; CDC-gly, glycochenodeoxycholate

*Calculated as P_{app} / P_{uwl} (x 100)

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