Applied Pharmacology of Retinoids

DISCLAIMER

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INTRODUCTION

Retinol and its derivatives have often been described as essential vitamins, but the retinoids are also known to cause severe toxicity and teratogenicity at clinically relevant steady-state concentrations (Roche Laboratories Inc. 2010).

Recent experimental findings have potentially suggested a link between non-alcoholic fatty liver disease (NAFLD) and retinol consumption (Raja Gopal Reddy, Pavan Kumar et al. 2016). Additionally, a comprehensive meta-analysis of transcriptome data from human liver biopsies comparing the expression of thousands of genes in patients with alcoholic liver disease, NAFLD, and control, was recently published (Wruck and Adjaye 2017). The raw data, published separately in Supplementary Table 2 (Wruck and Adjaye 2017), will be referenced extensively in the present summary. For example, “GPR88 (-1.578; -1.2)” denotes that the background-corrected $\log_2$ ratio between GPR88 gene expression in NAFLD patients and control was -1.578. The second number is the $\log_2$ ratio for the same gene as as determined by (Hoang, Oseini et al. 2019), for additional corroboration. Positive values indicate that a gene is overexpressed, negative values indicate that a gene is underexpressed. For the vast majority of genes, this value does not exceed ± 0.2, a threshold for a significant value might be ± 0.5, and out of the over 10,000 genes analyzed, only 27 exceeded ± 1 compared to control.

Ethanol has also been described extensively in this summary, due to the author’s initial hypothesis that retinol toxicity might proceed in a similar manner. The genome analysis has shown, however, that in many cases where a gene was significantly

upregulated in NAFLD, the same gene was significantly downregulated in ALD and vice versa. In fact, it was rare for a gene to be up- or downregulated in both ALD and NAFLD. Nonetheless, the author's original comments on ethanol have been kept (where accurate) for educational value.

The goal of this summary is to both highlight recent developments in retinoid research, and to integrate data from different fields like pharmacokinetics, organic chemistry and genomics, in order to formulate a better framework to describe the effects retinoids have on the human body.

Note: If data is provided without any attribution it was either taken from Wikipedia or UniProt.

Toxic Effects of Retinoids

Adapted from the prescribing information for 13-cis-RA (Accutane).

Reproduction: Birth defects, spontaneous abortion, sexual dysfunction, erectile dysfunction, reduced libido, vaginal dryness

Psychiatric: Depression, anxiety, aggressive behavior, mood swings, psychosis, suicidal thoughts, attempted suicide, suicide

Skin: very dry lips, skin and nose; nosebleeds, acne, increased susceptibility to sunburn, severe weakening of skin (patients must avoid waxing treatments for 6 months after end of treatment due to the risk of pulling off skin instead of hair), erythema multiform, Steven-Johnson syndrome, toxic epidermal necrolysis, hoarseness, itchiness, hair loss, increased sweating

Eyes: dry eyes, corneal opacities, night blindness, keratitis, eye reactions with contact lenses, conjunctivitis, blurred vision, photophobia

Musculoskeletal: Muscle pain, joint pain, increased blood creatine kinase that can lead to rhabdomyolysis (patients are advised to avoid high intensity exercise), dark urine, weakness, premature epiphyseal closure, excessive growth of bone, calcium deposits on tendons, back pain, arthritis, reduced bone density

Nervous system: idiopathic intracranial hypertension resulting in headache, nausea, vomiting, vision changes, papilloedema; seizures, drowsiness, hearing loss

Metabolism: increased free fatty acids in plasma, elevated plasma triglycerides, reduced HDL, pancreatitis, acute pancreatitis resulting in death, diabetes

GI tract: Crohn's disease, colitis, severe diarrhea, gastrointestinal bleeding,

Blood and liver: thrombocytosis, anemia, neutropenia, hyperuricemia, vasculitis, hepatitis

CHEMICAL STRUCTURE

Alcohol, Aldehyde and Acid

Retinol (Vitamin A, ROL) is a primary alcohol, characterized by the functional trailing OH (hydroxy) group. Retinol is mostly stored in liver stellate cells, and metabolized in the same way as ethanol: reversible oxidation to retinaldehyde (retinal, RAL) by alcohol dehydrogenases (ADH) and irreversible oxidization to retinoic acid (RA) by aldehyde dehydrogenases (ALDH) (Kohlmeier 2015).

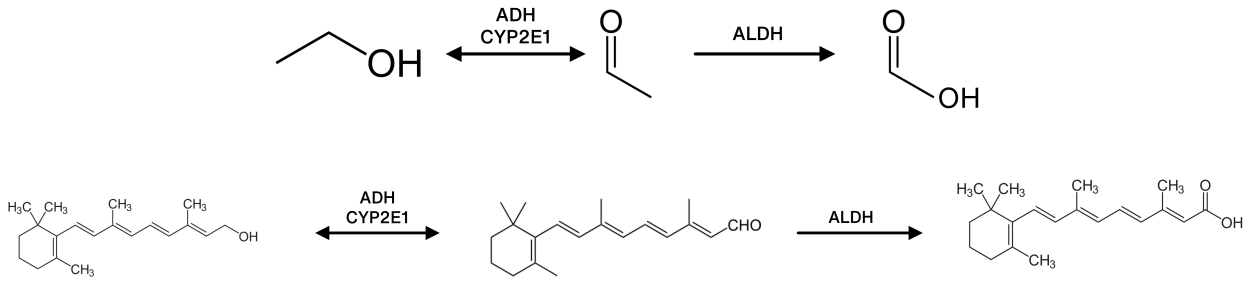

Figure: Metabolic pathways for the alcohol, aldehyde and carboxylic acid forms of retinol (bottom) and ethanol (top).

Unlike all other pharmaceutic drugs (Dettli 1977), ethanol metabolism is capacity-limited by the amount of ADH in the body. Once the elimination capacity of the enzyme system is reached, the elimination rate of ethanol becomes independent of the ethanol concentration (Holford 1987). At very high concentrations, however, elimination again begins to follow first-order kinetics, leading to speculation of another enzyme system involved in the metabolism, described as the microsomal ethanol oxidizing system (MEOS). After ethanol overdose, the clearance of MEOS was found to be ten times higher than that of ADH, and it has been said to contribute 25–35% to elimination at typical doses (Holford 1987).

Retinoic Acid as an Oxidant

Retinoic acid has been proposed to cause structural damage to cell membranes through nonenzymatic isomerization catalyzed by sulfhydryl compounds in the cell membranes. Such a reaction has been shown in vitro, and effective catalysts given were amino acids including L-cysteine methyl ester, glutathione, N-acetyl-L-cysteine, the thiol-containing protein apoferritin, and even native microsomes. Intact cells were also described as capable of catalyzing the isomerization of all-trans-RA, 9-cis-RA, 13-cis-RA and 9,13-dicis-RA, the process inhibited to an unknown extent by a sulfhydryl-specific reagent (Shih, Lin et al. 1997).

Pharmacologically active RA undergoes about 50% photochemical isomerization to 13-cis-RA (Samokyszyn, Chang et al. 1997). Metabolization to 4-OH-RA requires both CYP and prostaglandin H synthase (PTGS1; 0.31; 0.547) enzymes. C4 radical formation is preferred in the 13-cis isomer compared to the all-trans isomer.

RA is a strong oxidant, and is known to auto-oxidize in the presence of high-pressured molecular oxygen (O_2), free radicals like hydrogen peroxide, and even other C4-retinoic acid radicals, as part of the following process:

(1) Initial radical formation: A proton is taken from the cyclohexene ring component of atRA at the 4th carbon (C4), which was shown to be the most energetically beneficial for such a reaction (Samokyszyn and Marnett 1987), producing a C4-retinoic acid radical.

   i. Substances catalyzing initial radical formation were described as -$H^{\bullet}$ (Samokyszyn and Marnett 1987),

   ii. or as -$OO^{\bullet}$ (Clark, Howard et al. 1997).

(2) The C4-RA radical reacts with molecular oxygen (O_2) to produce a superoxide radical, R-$CHOO^{\bullet}$ at the fourth carbon, 4-hydroperoxyl-retinoic acid

i. A radical chain autoxidation is initiated with another non-radical retinoic acid or $RH^{\bullet}$, producing the major products 5,6-epoxy-5,6-dihydroretinoic acid, 5,8-dihydroretinoic acid, and 5-hydroxy-8-oxo-6,7-dihydroretinoic acid in one experiment

ii. Additionally, the following theoretical reaction, capable of producing the major atRA metabolites detected in vivo, has been postulated:

4-hydroperoxyl-retinoic acid + Retinoic acid → 5,6-expoxyretinoic acid + 4-hydroxy-retinoic acid

These findings suggest that the major metabolites of atRA, 4-OH-RA and 5-epoxy-RA, could theoretically be produced via radical chain autoxidation in vivo, raising questions over the extent of enzymatic metabolism and isomerization versus spontaneous chemical reactions.

RETINOL METABOLISM

Retinol Dehydrogenase

Retinyl esters from the diet are hydrolyzed (split into retinol and the fatty acid) in the intestinal lumen and absorbed into enterocytes (cells of the intestinal lining). After absorption, the retinol is re-esterified with a long-chain fatty acid and incorporated into chylomicrons (droplets of fat that enter the blood after absorption from the small intestine). Most of the retinyl esters in the blood are hydrolyzed by functional liver cells. Most (80–90%) of the resulting retinol is bound to retinol binding protein (RBP) and transferred to stellate cells where it is re-esterified and stored, while 10–20% remains in lipid globules of hepatocytes (Kohlmeier 2015). 80% of the body's total retinol content is found in the liver, which amounts to 450 mg or 50 µg/g of wet tissue, and accumulates over time.

Many different retinol dehydrogenase enzymes (RDH, a subset of ADH) catalyze retinol and retinal metabolism, with a very strong preference for retinal over retinol as substrate (i.e. most enzymes show a much higher affinity for detoxification of retinal than for metabolizing retinol). They can be classified into distinct groups (suggested by the author):

- aldehyde-reducing enzymes (RDH11–14), which specifically either oxidize retinol (with $NADP^+$ as a co-factor) or reduce retinal (with NADPH as a co-factor), with a 5–50x greater preference for catalyzing the reduction than the oxidation reaction,
- steroid-metabolizing enzymes that also metabolize (with a much lower affinity) retinol, with a slightly higher affinity for oxidation of retinol including RBP-bound retinol to (RBP-bound) retinal than for reduction of retinal (RDH5, RDH16, HSD17B6, DHRS9), and
- steroid-metabolizing enzymes that do not show activity towards retinoids (HSD17B-family except HSD17B6); oxidation of steroids occurs in the presence of NAD^+ and

generally produces less potent metabolites (e.g. estradiol to estrone) whereas reduction of steroids occurs in the presence of NADH; these enzymes often exhibited similar preferences for oxidation and reduction reactions, indicating that the direction of the reactions are predominantly determined by substrate/product and co-factor availability rather than enzyme substrate affinity.

RDH10, located in *FIXME* and RDH11 catalyze reactions with the same substrates, but RDH11 is significantly increased in NAFLD (0.696; 0.141), whereas RDH10 is reduced (-0.302; -0.1). Unlike RDH10, RDH11 and RDH13 which are found in the ER, RDH12 (-0.454; -0.771) is located in the cytoplasm, and was shown to additionally metabolize nonanal and nonenal in humans. Nonenal is a product of omega-7 unsaturated fatty acid degradation and associated with body odor alterations during aging.

RDH16 (also known as RoDH4) is also upregulated in NAFLD (0.314; -0.11), and additionally metabolizes 3α-hydroxysteroids in the presence of NAD^+. The significance of this is not yet known. Since 7α-hydroxycholesterol is a precursor of bile acids, created by CYP7A1, RDH16 could play a role in bile acid synthesis. Potentially also relevant could be the significantly increased AKR1B10 expression (0.837; 2.97), which catalyzes (in the presence of $NADP^+$) the reduction of retinal to retinol and the detoxification of 4-hydroxynonenal (4-HNE), a substance thought to be carcinogenic produced during the frying or burning of food, to 4-hydroxynonen-1-ol.

While research has extensively shown the toxicity of aldehydes, nonetheless an aldose reductase (aldehyde reductase) inhibitor has been marketed as Alredase (tolrestat), and it was believed to inhibit RDH16. The drug was discontinued in 1997 due to the risk of severe liver toxicity and death. A retracted study found that hydrogen peroxide and 4-HNE induced aldose reductase mRNA (Spycher, Tabataba-Vakili et al. 1997). A specific, non-toxic RDH16 inducer could be beneficial to treat NAFLD.

It will be noted that none of the ADH enzymes were significantly impacted by NAFLD, with the exception of ADHFE1, suggesting they do not play a signifiant role in retinol metabolism. ADHFE1 (-0.561; -0.611) catalyzes the reversible

oxidation of GHB to succinic semialdehyde. [Note: ADH1B (-0.062; -0.358) and ADH6 (-0.051; -0.313) were statistically significantly changed in (Hoang, Oseini et al. 2019)]

Enzyme	Expression	V_{max} / K_M (pmol/min/pmol enzyme)/μM	Co-factor	Location
RDH11	0.696; 0.141	36 (atRAL), 0.7 (atROL)	NADPH	ER
RDH12	-0.454; -0.771	atRAL (K_M = 0.04 μM, V_{max} n.d.) > 11-cis-RAL > 9-cis-RAL > 11-cis-ROL = 9-cis-ROL > atROL > (Z)-non-6-enal > nonanal > (E)-non-2-enal	NADPH	ER
RDH13	0.002; 0.052	72 (atRAL), 1.6 (atROL)	NADPH	Mitochondria
RDH14	n.d.; 0.232	338 (atRAL), 78 (atROL)	NADPH	Cytosol, ER
RDH16	0.314; -0.109	androsterone > androstanediol > dihydrotestosterone > CRBP-atROL > atRAL	NAD^+	ER
HSD17B6	0.304; -0.056	152 (androsterone), 127 (3α-androstanediol), 61 (allopregnanolone), 7 (dehydroepiandrosterone), 0.4 (atROL), n.d. (CRBP-atROL)	NAD^+	ER, early endosome (EE) membrane
DHRS9	0.219; 1.261	Allopregnanolone > 3α-androstanediol > dihydrotestosterone, n.d. (atROL)	NAD^+	ER
RDH5	n.d.; -0.21	n.d. (atROL, androsterone)	NAD^+	ER
HSD17B11	-0.726; -0.481	n.d. (17β-estradiol to estrone, 3α-androstanediol to androsterone)	NAD^+	ER, lipid droplets
HSD17B12	0.388; 0.16	n.d. (estrone to estradiol)	NADH	ER
HSD17B2	-0.402; 0.485	181 (estradiol to estrone), 123 (dihydrotestosterone to androstanedione), 115 (testosterone to androstenedione)	NAD^+	Cell membrane
HSD17B7	0.478; 0.019	Dihydrotestosterone > estrone, n.d. (cholestenol)	NADPH	ER

Enzyme	Expression	V_{max} / K_M (pmol/min/pmol enzyme)/µM	Co-factor	Location
HSD17B7P2	0.537; 0.113	n.d.	n.d.	n.d.

Table: Enzymatic constants for RDH and RDH-like enzymes; for the third column: higher values indicate a greater efficiency for the (substrate) listed in parentheses; if V_{max} or K_M are unknown substrates are listed in order of highest to lowest rate of metabolism (V_{max}) or substrate affinity (K_M).

Cytochrome P450

With most RDH enzymes exhibiting a clear preference for the reduction of retinal versus the oxidation of retinol, cytochrome P450 (CYP) enzymes likely play a more significant role in the metabolism of retinol. Retinol is only oxidized by RDH in situations involving a large retinol or NAD^+ oversupply (imbalance). CYP enzymes nonspecifically metabolize various xenobiotics and are found in the plasma membrane and cytosol of many different kinds of cells. While the most prominent isoforms are found in the liver, CYP2J2 for example is most expressed in the heart while CYP2U1 is mostly found in the brain.

The general purpose of xenobiotic (a substance that is foreign to the body) metabolism in the body is to increase their aqueous solubility. Xenobiotics must be lipophilic to traverse the blood-brain-barrier, but lipophilic substances aren't readily eliminated from the body due to resorption. The phase I reactions are oxidative, reductive or hydrolytic and lead to the introduction of a functional group (OH, SH, NH_2 or COOH) and a modest increase in solubility. In Phase II reactions, the molecule is conjugated by modifying the functional group to form mostly more hydrophilic products such as O- and *N*- glucuronides, sulfates and glutathionyl adducts (Kedderis 2018).

Nonspecific Cytochrome P450 Enzymes

Retinol causes a severe reduction in some nonspecific oxidative cytochrome P450 enzymes, most notably

Table 1

	(1)	(2)	Substrate	Product	K_M (μM)	V_{max} (pmol/min/pmol enzyme)	V_{max}/K_M	Co-factor	Location	Expression	Inducers	Inhibitors
CYP1A1	-1.245	-1.563	atROL	atRAL	8	0.507	0.063375	$NADP^+$	Cytosol, ER, Mitochondria	Lungs, lymphocytes, placenta		
			atRAL	atRA								
CYP1A2	-0.323	-1.119	atROL	atRAL	9	0.491	0.0545555555555	$NADP^+$	ER membrane	Liver	Nicotine, phenobarbital	
			atRAL	atRA								
CYP1B1	0.023	0.374	atROL	atRAL	14.75	0.493	0.0334237288135	$NADP^+$	ER membrane, mitochondria	Heart, brain, lung, many others	PAH, TCDD	
			atRAL	atRA	8.5			$NADP^+$				
CYP2C9	0.215	-0.065							ER membrane	Liver, binary system, intestine	Rifampicin	
CYP2C18	0.292	0.199	atRA	4-OH-RA	16	0.251	0.0156875	$NADP^+$	ER membrane	Small intestine,		
CYP2C19	-0.94	-1.827						$NADP^+$	ER membrane	Liver	Drugs, pesticides, carcinogens	
CYP2J2	-0.285	-0.023							ER membrane	Heart		
CYP3A4	-0.497	-0.573	atROL	atRAL	25	0.225	0.009	$NADP^+$	ER membrane	Prostate, liver	Rifampicin	
			atRA	4-OH-RA	34	0.195	0.00573529411764	$NADP^+$				
CYP3A43	-0.408	-0.378						$NADP^+$	ER membrane	Prostate		
CYP4F2	0.336	-0.036						$NADP^+$	ER membrane	Liver, kidney	Dietary sesamin	
CYP4F12	-0.354	-0.638						$NADP^+$	ER membrane	Small intestine, liver, colon, heart		
CYP4F22	0.668	0.873						$NADP^+$	ER membrane	Skin		
CYP4V2	0.293	-0.077						$NADP^+$	ER membrane	Broadly expressed		
CYP4X1	-0.481	-0.596						$NADP^+$	ER membrane	Brain, heart, kidney, skin		
CYP4Z1	-0.466	-0.758						$NADP^+$	ER membrane	Breast		
CYP4Z2P	-0.527								Membrane	Mammary gland		
CYP7A1	1.15	1.13						$NADP^+$	ER membrane	Liver	Glucose, cholestyramine	Chenodeoxycholic acid
CYP7B1	-0.309	-0.176						$NADP^+$	ER membrane	Widely expressed		Voriconazole, metyrapone
CYP11A1	0.053	0.376							Mitochondrion membrane	Adrenal gland, reproductive		
CYP17A1	-0.094	-0.469						$NADP^+$	ER membrane	Adrenal gland		
CYP20A1	-0.226	-0.06								Leukocyte membranes		
CYP26A1	-0.93	0.066	atRA	4-OH-RA	50.1	9.5	0.189620758483033	$NADP^+$	ER membrane	Most adult tissues		
			atRA	18-OH-RA	48.9	5	0.102249488752555	$NADP^+$				
CYP26B1	-0.003	-0.638	atRA	4-OH-RA				$NADP^+$	ER membrane	Brain		
CYP27B1	-0.038	0.607						$NADP^+$	Mitochondrion membrane	Kidney	Cardiolipin, DOPE	High substrate concentrations

- CYP1A1 (-1.245; -1.563; oxidizes retinol, retinal, and atRA to 4-OH-RA)
- CYP2C19 (-0.94; -1.827; likely not directly related to retinol but could potentially epoxigenate the double bonds or play a role in 5-epoxy-RA production)
- CYP3A4 (-0.497; -0.573; oxidizes retinal, and atRA to 4-OH-RA)

CYP enzymes, specifically CYP1A1, have been shown to auto-regulate their own expression. Reactive oxygen species (ROS), notably hydrogen peroxide (H_2O_2), produced as byproducts of CYP-catalyzed oxidation reactions, inhibit their expression (Morel, Mermod et al. 1999). Hydrogen peroxide induces downregulation of nuclear factor I (NFI). However, none of NFIA (0.049; -0.226), NFIB (0.06; -0.212) and NFIC (0.048; -0.234) are significantly affected in NAFLD. More likely, CYP1A1 is reduced due to a decrease in the transcription factors AHR (-0.206; -0.167), which was shown to be reduced by 41% in vitro after treatment with 1 μM atRA (Fallone, Villard et al. 2004), and ARNTL (-0.403; -0.269), both of which are required to induce CYP1A1 transcription. ARNTL has not specifically been reported to induce transcription, but ARNT (0.173; -0.244) was shown to be required, and ARNTL is reportedly also capable of forming heterodimers with AHR. Interestingly, the relatively unexplored RAI1 (retinoic acid-induced protein 1) was shown to activate ARNTL transcription in mice, however data about the human form RAI2 (-0.188; 0.28) is inconclusive.

Another pathway is via NCOR2 (0.167; -0.123) which represses CYP1A1 and is mildly upregulated in NAFLD. NCOR2 may also cause downregulation of AHR.

Therapeutically, a CYP inducer does not seem beneficial due to the additional production of reactive oxygen species (ROS) and atRA (from ROL) which can't be readily eliminated due to disruptions in other systems; conversely a CYP inhibitor would affect metabolism of various xenobiotics and fatty acids.

A promising therapeutic avenue could be an inducer of catalase (CAT; 0.137; -0.155), which catalyzes the following reaction:

$2\ H_2O_2 + NADP^+ \rightarrow NADPH + 2\ H_2O + O_2$

Induction would be beneficial due to the reduction of oxidative stress through neutralization of ROS, and would additionally induce CYP1A1 production. Though a specific catalase inducer (apart from hydrogen peroxide) has not been described in humans, catalase is sold as a supplement and apparently sourced from the saw palmetto palm tree. The carotenoid content of such supplements remains to be determined, and may fluctuate significantly between formulations. Fatty acid composition of different Serenoa repens extract products was shown to fluctuate significantly (Habib and Wyllie 2004).

Specific Cytochrome P450 Enzymes

Retinol effects a complex change by inducing some cytochrome P450 enzymes and inhibiting others, notably

- CYP2C18 (0.292; 0.199; oxidizes atRA to 4-OH-RA)
- CYP2E1 (-0.05; no data; decreased in ALD and thought to be a major contributor to toxic effects in ALD but does not appear to play a significant role in NAFLD)
- CYP26A1 (-0.93; 0.066 oxidizes atRA to 4-OH, 16-OH and 18-OH-RA)
- CYP26B1 (-0.003; -0.638; an RA-metabolizing enzyme like CYP26A1)
- CYP26C1 (no data; no data; oxidizes atRA to 16-OH-RA)

Conjugation

Retinol can be enzymatically conjugated to retinyl glucuronide, which is eliminated in the feces. It is unknown whether retinyl gucuronide is bioactive. Retinoyl beta-glucuronide, which is bioactive, is formed through the conjugation of retinoic acid with the glucuronic acid component of UDP glucuronic acid. UDP glucuronic acid is formed by UGDH (-0.195; 0.203) in the following reaction:

$H_2O + 2\ NAD^+ + UDP\text{-}\alpha\text{-}D\text{-}glucose \rightarrow 3\ H^+ + 2\ NADH + UDP\text{-}\alpha\text{-}D\text{-}glucuronate$

UDP glucuronic acid levels are presumably regulated by UXS1 (0.063; 0.33), which catalyzes (in the presence of NAD^+) the production of the UGDH inhibitor UDP xylose from UDP glucuronic acid. There are currently no known UXS1 inhibitors, however UXS1 has been reported to interact with zinc transporter ZIP4 (SLC39A4; -0.129; 0.026). Of note is also zinc transporter SLC39A7 (0.263; 0.464) which transports Zn^{2+} out of the endoplasmic reticulum and Golgi network into the cytosol. UGDH is found in the cytosol and elsewhere whereas UXS1 is only found in the Golgi network membrane, with functional and substrate-binding sites facing outward.

Hypothetical Mechanism for UGDH Downregulation

When cargo enters a cell it is first transported to early endosomes (EE), which forward the cargo to either (a) the plasma membrane (fast recycling pathway), (b) late endosomes (LE) and lysosomes for degradation or (c) inwards, including towards the Golgi network (O'Sullivan and Lindsay 2020). Cargo returned from the Golgi network for outward travel is transported to the plasma membrane by recycling endosomes (RE) in the slow recycling pathway.

SLC39A4 proteins transport zinc into cells through the plasma membrane, depending on availability. If no zinc is available to transport in, or more zinc is available for transport out than in, they migrate to the recycling endosomes, where they take up zinc from the Golgi network for transport to the plasma membrane and out of the cell, potentially in a pH-dependent manner. When SLC39A7 is overexpressed, indicating that unusually high amounts of zinc are being transported into they cytoplasm, out of the nucleus and/or/via [the endoplasmic reticulum (ER) and Golgi network], an explanation for the downregulation of SLC39A4 could be that the cell is trying to prevent excessive loss of zinc (there is currently no data available to support or reject this theory).

To suggest an additional mechanical explanation, when SLC39A4 proteins are stationary in the plasma membrane, they can achieve a higher throughput than

when they are lodged in the membrane of transiting REs. If SLC39A4 is induced by zinc in the same way as SLC39A7 was shown to be, then SLC39A4 transporting zinc intra-cellularly would by means of throughput be induced more often than SLC39A4 transporting zinc inter-cellularly.

Nonetheless, even though downregulated, SLC39A4 will transport zinc from the Golgi network outwards (in REs) more often than from the plasma membrane inwards, due to increased zinc efflux (evident by SLC39A7 overexpression in NAFLD). If SLC39A4 is an activator of UXS1 (which has not been demonstrated, only that there is an interaction), or if USX1 is activated by zinc, or requires zinc as a co-factor, this could lead to excess production of UDP xylose by UXS1, increased inhibition of UGDH, less production of UDP glucuronic acid, and ultimately less conjugation of retinoic acid with glucuronic acid.

UGT Enzymes

“If drug undergoes Phase II with UGT1A1 [-0.022; 0.957], it will compete with bilirubin metabolism conjugation, leading to reduced bilirubin elimination and build up in plasma.” (Lipid solubility and the brain uptake of compounds)

One study found UGT2B7 (no data; 0.099) to show higher enzymatic activity for retinoic acid (275 and 1,236 pmol/mg × min vs 13 and 23 pmol/mg × min UGT1A3 [no data; -1.095] for atRA and 4-OH-RA, respectively) (Samokyszyn, Gall et al. 2000). These were the only two enzymes assayed in that study. Another study (Rowbotham, Illingworth et al. 2010) proposed UGT1A9 (no data; 0.108) to be the most important enzyme.

Substrate Enzyme (host cell kind)	K_m (μM)	V_{max} (pmol/mg × min)	V_{max}/K_M
atRA UGT2B7 (HLM15)[a]	1.5	764	509
atRA UGT2B7 (HepG2)[a]	1.3	523	402
4-OH-RA UGT2B7 (HLM15)[a]	273	2176	8

Substrate Enzyme (host cell kind)	K_m (μM)	V_{max} (pmol/mg × min)	V_{max}/K_M
4-OH-RA UGT2B7 (HepG2)[a]	221	1709	8
4-Oxo-13-cis-RA UGT1A9 (recombinant)[b]	18		
4-Oxo-13-cis-RA UGT1A9 (HLM)[b]	95		
13-cis-RA UGT1A9 (recombinant)[b]	14		
13-cis-RA UGT1A9 (HLM)[b]	974		

(a) (Samokyszyn, Gall et al. 2000)

(b) (Rowbotham, Illingworth et al. 2010)

Even UGT2B7 showed the highest affinity for atRA, atRA and 9-cis-RA were also found to downregulate UGT2B7 mRNA expression in a dose-dependent manner (Lu, Bratton et al. 2008). This effect was observed in Caco-2 cells (which model the intestinal epithelial barrier) but not in HepG2 cells (liver cells). The catabolic products 4-OH-, 4-oxo-, and 5,6-epoxy-RA, showed no effect on UGT2B7. The same study also summarized previous research that showed retinol was conjugated by UGT2B7 to retinoyl-β-glucuronide in the intestinal mucosa, which can apparently be retained in the body even though it is more polar than ROL, and may be biologically active. Furthermore, the bile acid Lithocholic acid (LCA) “dramatically” decreased UGT2B7 mRNA expression.

Colestipol and cholestyramine are two bile acid binding resins that decreased serum cholesterol levels.

UGT2B17 (-0.919; 0.986) is the major isozyme for clearance of anabolic steroids, and has greater than double the activity of the next most active form UGT2A1 (Jenkinson, Petroczi et al. 2013). UGT2B17 was highly down-regulated in NAFLD (-0.919), even more so than in ALD (-0.737). Two NSAIDs (diclofenac and ibuprofen) were found to be non-competitive inhibitors, green and white tea were found to inhibit UGT2B17 by 20% and 30%, respectively, attributed to the competitive inhibitor epigallocatechin gallate (EGCG) found in tea, with an affinity

similar to that of diclofenac. In another study, white and green tea were found to inhibit UGT2B17 activity by 70% and 90%, respectively. Caffeine did not significantly affect UGT2B17 (-2.7%). The flavonol quercetin reduced activity by 72% and was suggested as the primary cause for inhibition caused by red wine.

DISRUPTION OF FATTY ACID CYCLE

Fatty Acid Oxidation

ACSL4

Retinol severely disrupts the fatty acid cycle, and it remains to be determined whether this disruption is the underlying cause of many of its deleterious effects on the body. In fatty acid oxidation, only two genes are significantly affected by NAFLD: ACSL4 (1.317; 1.32) and ACAT2 (0.567; -0.089). ACSL4 catalyzes the reaction

Palmitic acid + ATP + CoA → Palmitoyl-CoA + AMP + Diphosphate

While ACSL4 is severely upregulated, the next step in fatty acid metabolism, the rate-limiting uptake of fatty acids into mitochondria for beta-oxidation, is catalyzed by CPT1A (-0.263; 0.263) which is unexpectedly downregulated in NAFLD, indicating that high amounts of fatty acids may get diverted before oxidation. ACSL4, like most of the RDH enzymes, is found in the endoplasmic reticulum. Additionally, retinol is known to extensively form esters in the presence of the activated acyl-CoA form of a fatty acid but not in the presence of the fatty acid itself (Törmä and Vahlquist 1987).

Esterification by an acyl-CoA:Retinol acyltransferase enzyme has been experimentally demonstrated in mouse epidermal microsomes (Törmä and Vahlquist 1987) with a K_M of 6 μM for retinol. Esterification of retinol in humans is still not completely understood, but the DGAT1 (0.123; 0.086), DGAT2 (0.276; -0.014), AWAT2 (no data; no data) and LRAT (no data; -0.143) genes have been proposed to encode proteins with retinol acyltransferase activity. DGAT1 catalyzes the reaction

atROL + Palmitoyl-CoA → atRetinyl palmitate + CoA

Of the fatty acids, DGAT1 has highest affinity for the acyl-CoAs of 16:1 palmitoleic acid (K_M = 6.2 μM), 16:0 palmitic acid (6.4 μM), 18:0 stearic acid (8.6 μM) and 18:1 oleic acid (14.6 μM), while its affinity for atROL is 25.9 μM. Substrate affinities for DGAT2 could not be found in literature.

AWAT2 catalyzes esterification, especially of the cis-isomers, of ROL with high efficiency (V_{max} / K_M = 169 pmol/min/mg enzyme/μM for 11-cis-ROL, 51 for 9-cis-retinol and 6 for atROL). Lecithin retinol acyltransferase (LRAT) has been shown to catalyze the esterification of RBP-bound atROL, producing an atROL ester and apo-RBP. DGAT1, DGAT2, AWAT2 and LRAT are all found in the ER.

An analysis of the different retinol esters formed in monkeys given 50,000 RE/kg[1] showed the area under the curve (AUC) of different retinyl esters was (16:0 + 18:1) > 18:2 > 18:0 ≈ atROL in a monkey which efficiently esterified retinol (MR+), compared to atROL > (16:0 + 18:1) > 18:0 > 18:2 in a monkey with impaired esterification (MR-) (Collins, Eckhoff et al. 1992). Monkeys given atROL in polysorbate 20 (MP20) showed minor differences in atROL AUC and additionally formed 14:0 myristic acid and 12:0 lauric acid, but not 18:3 α-linolenic acid, compared to monkeys given atROL in oil. Total fatty acid AUC was 80,869 (ng · hr)/ml for MR+, 34,807 for MR-, and ~91,000 for MP20, whereas atROL AUC was 9,610; 41,535; and ~9,500, respectively.

While this data does not conclusively show that polysorbate 20 significantly affected AUC due to the low sample size, it shows that ester concentrations roughly correlated with substrate specificity of DGAT1. Acetates (retinol acetate and retinol acetate in polysorbate 20) were also examined, and evidence for an increased total AUC compared to atROL and [atROL and polysorbate 20] was strong.

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[1] The study did not explicitly define retinol equivalents (RE), but assuming RE is synonymous with international units (IU), 50,000 RE would be equivalent to 15 mg atROL.

ACAT2

ACAT2 catalyzes the reaction

AcetoacetylCoA + CoA → AcetylCoA

This reaction concludes the β-oxidation of (even-length) fatty acids. Increased expression of ACAT2 in NAFLD is not unexpected due to its involvement in other pathways including ketogenesis, and primarily in cholesterol synthesis.

Toxicity due to Fatty Acid Cycle Disruptions

To provide a brief, high level summary, all retinol in the body must undergo metabolism to either retinoic acid (which produces localized, acute damage), or to a retinyl ester (which causes no immediate harm but likely leads to diseases like NAFLD and diabetes in the long term). The human body appears to favor the latter pathway, since enzymes that favor the production of RA are downreguated in NAFLD and enzymes that favor the production of ROL are upregulated. Additionally, a single orally administered retinol molecule is hydrolyzed and re-esterified at least three times before arriving at liver stellate cells. If palmitoyl-CoA is in fact diverted before entering the fatty acid oxidation pathway, because it is needed to esterify retinol, as a side effect the body would perform fatty acid synthesis for longer periods of time than necessary and perform fatty acid oxidation for shorter periods of time than necessary, leading to a buildup of fatty acids, and ultimately weight gain and metabolic disease, as well as a buildup of NADPH (a product of fatty acid synthesis).

Since NADPH is a co-factor for the detoxification of RAL by RDH11–16, retinol may even auto-regulate its own metabolism, since an abundance of NADPH would cause RAL to preferably be reduced to ROL, and CYP enzymes capable of oxidizing RAL to RA require $NADP^+$ as a co-factor. The fact that NADP+ is also a factor for some steroid metabolizing enzymes from the HSD17B family could be an explanation for retinol-induced gynecomastia, and the general effects of retinol on steroid metabolism could help explain the 2-fold greater incidence of autoimmune diseases in women than men.

Fatty Acid Biosynthesis

Some enzymes enzymes involved in the biosynthesis of up to 16:0 saturated fatty acids are upregulated including ACACA (0.415; -0.105), FASN (0.963; 0.365):

1. FASN (0.963; 0.365)
2. FASN/MCAT (-0.017; 0.041)
3. FASN/OXSM (-0.265; 0.131)
4. FASN
5. EHHADH (0.114; 0.088)
6. FASN

The rate-limiting enzymes in fatty acid elongation are ELOVL2 (0.175; 0.711) ELOVL6 (0.009; 0.612).

Citric Acid Cycle

ACLY (0.478; 0.541) catalyzes the initial conversion from Acetyl-CoA to citrate. IDH1 (0.126; -0.102) and IDH2 (0.316; 0.278) are upregulated whereas IDH3A is downregulated (-0.27; -0.08).

ETHANOL TOXICITY

Many of the toxic effects attributed to long-term retinol exposure are also present in alcoholics, for example: increased risk of Alzheimer's and dementia; Alcohol-related brain damage (ARBD)/brain imaging alterations caused by RA (Bremner, Fani et al. 2005), skin deterioration, depression increased risk of stroke, breast cancer and some other kinds cancers, liver problems and cirrhosis. Additionally, ethanol is known to trigger Inflammatory Bowel Syndrome (IBS) and Crohn's flareups. Therefore, an investigation into ethanol's mechanism of action, which "has proven elusive and remains not fully understood" (Wikipedia), is warranted.

CYP2E1 (-0.05; no data) is an enzyme thought to be a primary or at least significant component of the microsomal ethanol oxidizing system. Most of CYP2E1 is found in the liver, but it has also been found in the small and large intestine, lungs and brain (Seitz and Wang 2013). Chronic alcohol consumption results in a “striking” induction of CYP2E1 in different tissue which correlated with the amount of alcohol consumed, and CYP2E1 is also elevated in NAFLD (according to Seitz and Wang but not corroborated by transcriptome analysis). Free fatty acids and acetone also induce CYP2E1.

Ethanol metabolism through CYP2E1 generates various reactive oxygen species (ROS) including H_2O_2, OH- and carbon centered OH-. Carcinogenic etheno-DNA adducts were found to significantly correlate with CYP2E1 and 4-hydroxynonenal (a product of lipid peroxidation thought to be caused by ROS) levels. In a different study (Millonig 2011) CYP2E1 was demonstrated to be a causal factor for the generation of carcinogenic DNA adducts and further correlated with the amount of ethanol consumed. Etheno-DNA adducts were also found to correlate significantly with hepatic fat and CYP2E1.

Clomethiazole (CMZ) has been shown to inhibit CYP2E1 at 1.3–2.3 g/day. Distraneurin® 192 mg tablets is a prescription medication in Germany for delirium and severe insomnia in elderly that did not respond to other treatment: for insomnia take 384–768 mg, do not exceed 1,152–1,536 mg within 2 hrs. Taurine has been proposed to inhibit CYP2E1 (Manley and Ding 2015), whereas the benzodiazepine Tranxene (Clorazepate) is a prodrug for desmethyldiazepam and has been shown to induce CYP2E1.

In rats with non-alcoholic steatohepatitis (NASH, a type of fatty liver disease) additional alcohol consumption even at moderate levels further enhanced CYP2E1 expression and DNA adduct formation. Chronic ethanol consumption results in a significant depletion of retinol in the liver of patients with alcoholic liver disease (ALD) (Seitz and Wang 2013).

Liver fibrosis (scarring) occurs when hepatic stellate cells (HSC) are activated and become collagen type I-producing cells. HSCs play a crucial role in alcoholic steatosis via production of endocannabinoid and retinol metabolites (Suh and Jeong 2011). During HSC activation, retinol is oxidized to retinoic acid. Activated HSC cells express a natural killer (NK) cell ligand known as retinoid acid early inducible-1 (RAE1; -0.061; 0.011), which is not present in quiescent HSCs. This would imply that the oxidation of atROL to atRA allows natural killer cells to bind to the host cell.

Apart from oxidation, about 5% of total ethanol consumed is metabolized through esterification with fatty acids to form fatty acid ethyl esters (FAEE) such as linolenic acid ethyl ester (LAEE) (Li, Hu et al. 2003). LAEE has activating effects on HSCs, but the mechanisms of these actions are not known. The highest concentrations of FAEEs were found in the liver, where values were up to 569 pmol/mg.

Ethanol Metabolism

(Adapted from *Ethanol Metabolism*, Wikipedia)

Dehydration

$\text{Ethanol} + \text{NAD}^+ \rightarrow \text{Acetaldehyde} + \text{NADH} + \text{H}^+$

This reaction is endergonic and the Gibbs free energy is 47.2 kJ/mol (excluding the reduction of NAD^+)

Carboxylation

$\text{Acetaldehyde} + \text{NAD}^+ \rightarrow \text{Acetic Acid} + \text{NADH} + \text{H}^+$

This reaction is exergonic and the Gibbs free energy is -262.3 kJ/mol

Activation

$\text{Acetic Acid} + \text{CoA} + \text{ATP} \rightarrow \text{Acetyl-CoA} + \text{AMP} + \text{PP}_i$

This reaction is exergonic and the Gibbs free energy is -1,110.5 kJ/mol

Acetyl-CoA can be used for synthesis of fatty acids (fatty acid synthesis) or cholesterol. If acetyl-CoA accumulates, NADH, FADH2 and ATP are increased to inhibit fatty acid oxidation. Furthermore, in alcoholism, NADH accumulates as a product of ethanol metabolization. Eventually, the citric acid cycle is stalled due to the NAD+/NADH imbalance in favor of NADH, since three reactions in the cycle require NAD+ and produce NADH. Due to the oversupply of acetic acid the equilibrium in the carboxylation reaction is shifted back towards acetaldehyde, which accumulates and forms toxic adducts with DNA, proteins, and reacts with thiol groups causing the lipid bilayer in cell walls to disintegrate.

The NADH buildup also causes the liver to move away from fatty acid oxidization and towards fatty acid synthesis, which consumes NADH. In lipogenesis, the acetyl-CoA is converted to triglycerides which are stored in fat cells. Fatty acids (such as palmitic acid) are activated by (catalyzed by ATP) reaction with CoA-SH to form an acyl-CoA (for example Palmitoyl-CoA). These activated fatty acids can react with retinol to form retinyl esters (for example retinyl palmitate) and CoA.

Retinoic acid can also bind to CoA (in the presence of ATP) in rats and human kidney cells, producing Retinoyl-CoA. Retinoyl-CoA is formed rapidly and is rapidly turned over. Retinoyl-CoA is a substrate for the retinoylation of certain proteins (Renstrom and DeLuca 1989).

Both Retinoyl-CoA and a metabolite of retinoic acid, 4-hydroxy-RA, have been proposed to bind to proteins in the cell membrane (Breitman and Takahashi 1996). 4-OH-RA is believed to be produced by CYP26 through oxidation of RA, and glucuronidated by UGT2B7. 4-OH-RA and 4-OH-RA acetate were conjugated with 6-fold and 3-fold higher activity, respectively, than atRA, and were found to be to be the main glucoronides, as opposed to 4-oxo-RA and 5,6-epoxy-RA (Samokyszyn, Gall et al. 2000).

Elimination of Glucuronates Through Bile

Bile acids are detergents required for disposal of lipid-soluble vitamins, toxic metabolites and xenobiotics. Bile acid synthesis involves multiple hydroxylation reactions, where the rate-limiting first step in the synthetic pathway is hydroxylation of cholesterol by 7α-hydroxylase (CYP7A1) (Chiang 2014). CYP7A1 is induced by the statin atorvastatin (atorvastatin decreased bile acids in the intestines, suggesting that CYPA7A1 is upregulated in response to this imbalance) and up-regulated by glucose and colestyramine. Colestyramine is an ion exchange polymer which binds bile acids in the GI tract to prevent their reabsorption; it is used to treat high cholesterol and is known to interfere with absorption of fat-soluble vitamins.

The final products in bile acid metabolism are deoxycholic acid (DCA) produced from cholic acid (CA), which is reabsorbed, and lithocholic acid (LCA) produced from chenodeoxycholic acid (CDCA), which is eliminated through feces. The main pathways for LCA are:

- Cholesterol $\xrightarrow{\text{CYP7A1}}$ 7α-Hydroxycholesterol $\xrightarrow{\text{CYP27A1}}$ CDCA $\xrightarrow{\text{7α-Dehydroxylase}}$ LCA
- 27-Hydroxycholesterol $\xrightarrow{\text{CYP7B1}}$ CDCA $\xrightarrow{\text{7α-Dehydroxylase}}$ LCA
  - CDCA $\xrightarrow{\text{7β-HSDH}}$ UDCA $\xrightarrow{\text{7β-Dehydroxylase}}$ LCA (Chiang and Ferrell 2020)
- LCA is further catabolized by CYP3A11 in gut bacteria to hyodeoxycholic acid (HDCA) and ? (MDCA)

The secondary bile acids are highly insoluble and toxic, and are secreted into feces. Notably, the 7α-dehydroxylase enzyme is found in bacterial flora and also converts CA to DCA. DCA is reabsorbed in the colon while most of LCA is secreted (Chiang 2014). NADH is reduced to NAD^+ in LCA synthesis by 7α-dehydroxylase.

7α-dehydroxylase activity is believed to occur mainly in Clostridium cluster XIVa (Chiang and Ferrell 2020), which (together with cluster IV) are the predominant

bacteria in the gut and account for 10–40% of the total bacteria. Additionally, the group Pathogenic clostridium spp., of which C. butyricum is a member, includes some non-pathogenic strains. C. butyricum has the ability to actively scavenge reactive oxygen species, and furthermore possesses NADH/NADPH peroxidases which catalyze the reaction $NAD(P)H + H^+ + H_2O_2 \rightleftarrows NAD(P)^+ + 2\ H_2O$. NADH peroxidase is structurally similar to glutathione reductase, which catalyzes the reduction of GSSG to GSH while oxidizing NADPH to $NADP^+$ (Fagan and Palfey 2010). An active site histidine is proposed to protect the enzyme from irreversible oxidation leading to inactivation.

In general, Clostridium bacteria prefer dietary carbohydrates, especially non-starch polysaccharides. Arabinoxylan-oligosaccharides, which are found in rye, wheat, oats and rice, and inulin-type fructants, which are found in wheat, onion, banana, garlic and leek, are thought to promote the growth and reproduction of Clostridium. Conversely, fecal Clostridium Cluster IV and XIVa species were found to decrease following highly digestible casein and soybean meal (Guo, Zhang et al. 2020).

Flavonoids, which are the most abundant polyphenols in plants, can also be metabolized by Clostridium bacteria, notably flavonifractor plautii (C. Orbiscindens) (Yang, Hong et al. 2021), which is believed to reduce flavonoids in a reaction catalyzed by NADH. A literature search for “retinol Clostridium” and “carotene Clostridium” produced no meaningful results.

Zinc Supplements

Zinc supplements are sold in many different forms, the most common being zinc acetate and zinc gluconate. Philosophically, the choice of zinc supplement is between the do-no-harm approach with zinc acetate (where the acetate enters the citric acid cycle) and the combination therapy approach, where the anion may provide benefits in addition to zinc. The zinc formulation containing gluconic acid, which was shown to protect against oxidative stress in vitro (Kolodziejczyk, Saluk-Juszczak et al. 2011), was reported to be the most effective form of zinc

supplement for treatment of the common cold. Also noteworthy is zinc histidine, where the histidine could help protect glutathione reductase in gut bacteria. Furthermore, patient information for zinc histidine claims that zinc deficiency can be deleterious for skin (contributing to acne), hair and nails, and that diabetics are more susceptible to zinc deficiency.

Bile Acid Fanesoid X Receptor

The bile acid farnesoid X receptor (FXR, NR1H4; -0.117; -0.115) has been identified as a nuclear receptor that suppresses the production of several key enzymes involved in bile salt homeostasis (Cai, He et al. 2010). Activation of FXR upregulates the expression of small heterodimeric partner (SHP, NR0B2; -0.453; -0.668) and fibroblast growth factor 19 (FGF19; -0.352; no data). FGF19 represses CYP7A1 (1.15; 1.13) expression, and the FXR-FGF19 pathway is suggested to play a more important role than the FXR-SHP pathway. FXR directly regulates the expression of CYP7A1, CYP27A1, UGT2B4 and other genes involved in lipid and glucose metabolism, induces bile salt export pump (BSEP, ABCB11; -0.467; -0.82) to stimulate bile acid efflux to bile and inhibits sodium/bile acid cotransporter (NTCP, SLC10A1; -0.028; -0.177) to inhibit bile acid uptake into hepatocytes (Chiang 2014). The endogenous ligand for FXR is considered to be CDCA, however FXR can also be activated by the retinoid X receptor (RXR) ligand, which is 9-cis-RA.

atRA and the synthetic retinoid TTNPB were found to activate FXR in mice. atRA at concentrations from 1–5 μM reduced CYP7A1 mRNA levels in HepG2 cells by 79% and 87%, respectively, while CDCA reduced levels by 91.7% (Cai, He et al. 2010). The combination of atRA and CDCA was synergistic and reduced CYP7A1 mRNA expression by 95.3%. atRA showed significantly greater induction of FCF19 (313x vs 2x) in primary human hepatocytes than in HepG2 cells, and achieved significantly greater repression of CYP7A1 (99.3% vs 87%). atRA moderately repressed CYP27A1 and NTCP expression (by about 50% for both 1

and 5 μM). atRA alone apparently did not stimulate the mRNA expression of BSEP of OSTα.

Furthermore, atRA decreased mRNA expression of UGT2B7 (no data; 0.099) by 10%, 25%, 50% and 95% at concentrations of .1, 1, 10 and 50 μM, respectively, whereas it had no effect on UGT2B15 (0.181; 0.432) and UGT1A6 (no data; 0.288) (Lu, Bratton et al. 2008). The most significantly impacted enzymes in NAFLD as determined through transcriptome analysis were UGT1A1 (-0.022; 0.957), UGT1A3 (no data; -1.095), UGT2A3 (0.362, 0.587), UGB2B17 (-0.919; 0.986), UGT2B28 (-0.004; -0.444) and UGT3A2 (no data; 0.736). The cause for the significant fluctuations between the two data sources remains to be determined.

NAFLD patients have increased CA, CDCA and bile acid synthesis, and increased ratio of primary bile acids to secondary bile acids. NASH patients also have increased circulating conjugated primary bile acids, an increased ratio of conjugated CA to CDCA and decreased secondary bile acids.

These findings introduce a paradox: atRA, believed to be the cause of NAFLD, is a potent inducer of FXR, and should therefore reduce CYP7A1 expression and bile acid synthesis. However, both pathways proposed to mediate FXRs effects, FGF19 and SHP, were found to be significantly repressed, whereas CYP7A1 was significantly upregulated. Furthermore, even though CDCA levels are elevated in NAFLD, and CDCA is the natural ligand for FXR, a decrease in FXR activation was observed. Additionally, the UGT enzymes known to not be inhibited by atRA were significantly upregulated while UGT2B7, which is inhibited by atRA, appeared relatively unchanged.

The steroid dexamethasone was found to induce CYP27A1 while the immunosuppressant cyclosporin A was found to inhibit CYP27A1 (Chiang and Ferrell 2020). Mutations in the CYP7B1 gene lead to cirrhosis and liver failure, while mutations in the CYP7A1 gene are believed to cause more mild metabolic conditions.

Surprisingly, in chronic alcoholism, FXR receptors were found to be inactivated by acetylation, and FGF15 in mice was found to be down regulated, while acute ethanol administration increased FGF15 levels (You, Zhou et al. 2017) While the underlying mechanism is not well-understood, CYP7A1 was shown to be reduced in ALD and up-regulated in NAFLD (Wruck and Adjaye 2017).

Fatty acid oxidation

- Squalene monooxygenase (SQLE; 1.53): Catalyzes the reaction

  Squalene + NADPH + H^+ + O_2 → (S)-2,3-epoxysqualene + NADP+ + H_2O

  - Squalene is a triterpene (six isoprene units), and the product of this reaction is an intermediate in the synthesis of cholesterol in humans and potentially saponins, which have detergent effects
  - The stereoisomer (R)-2,3-epoxysqualene is an inhibitor of cholesterol synthesis
  - Beta carotene is synthesized from eight isoprene units
- ACSL4 (1.317): Catalyzes the reaction

  20 carbon-tetraunsaturated fatty acid + CoA + ATP → fatty acid CoA ester + AMP + PP_i

  - Expression is regulated by SHP2 activity

Fatty Acid Cycle

When fed, glucose stimulated insulin release from pancreatic beta cells. Insulin activates a pathway that stimulates glycogen synthesis by inhibiting GSK beta and activating GS. Glycogens are stored in the liver. Insulin stimulates glycolysis to produce acetyl-CoA, which is utilized for synthesis of fatty acids and cholesterol. Fatty acids are esterified to glycerol to form TG and stored in lipid droplets. TGs are assembled to VLDL, which are secreted into circulation for energy metabolism or storage in adipocytes. Excess acetyl-CoA is used for synthesis of ketone bodies, which are used by muscle and brain during starvation. Excess acetyl-CoA is also converted in the liver to cholesterol. When acetyl-CoA is accumulated, nicotinamide adenosine diphosphate (NADH), FADH2, and ATP increase to inhibit fatty acid oxidation.

Fatty Acid Metabolism

There is some evidence that dietary ROL especially affects metabolism of 20-carbon polyunsaturated (especially tetra-unsaturated) fatty acids, since

- ALOX5 (0.272) and its co-factor ALOX5AP (0.488), which metabolize arachidonic acid, are significantly up-regulated in NAFLD
- ALOX15 (-0.07), which metabolizes other polyunsaturated acids with higher affinity for 19-carbon 2/4x-unsaturated acids than for eicosatetraenoic acid, with 2x higher Vmax and 4x lower Km, is not significantly affected in NAFLD
- ACSL4 (1.317), which binds long-chain fatty acids to CoA, with a distinguished preference for arachidonic acid, is highly overexpressed in NAFLD
- FABP4 (1.778), which transports long-chain fatty acids including arachidonic acid, as well as retinoic acid, into adipocytes, and (by a factor of 1000) less frequently into macrophages, is severely overexpressed in NAFLD
  - The affinity for specific ligands of FABP4 is not known

Furthermore, SCD (1.6), which rate-limits synthesis of unsaturated fatty acids, is severely overexpressed in NAFLD. The quantitatively most important product of SCD is oleic acid, which is the most abundant fatty acid in human adipose tissue. Oleic acid can be cleaved at the double bond in the presence of ozone (O3) and oxygen with the reaction:

Oleic acid → Azelaic acid + Nonanoic acid

The human body isn't capable of synthesizing fatty acids longer than 18-carbon, and it is uncertain if humans can convert lineolic acid to arachidonic acid. However, both enzymes involved in the metabolism of 20-carbon fatty acids and blood plasma levels of 20-carbon fatty acids

VITAMIN A-DEFICIENT MICE

A recent study (Raja Gopal Reddy, Pavan Kumar et al. 2016) with a small sample size (n = 8 for for control, n = 7 for VAD, n = 7 for VADHFr) compared mice which, shortly after birth, were fed a control diet, a vitamin A-deficient diet (VAD) and a vitamin A-deficient diet with large amounts of high fructose corn syrup added (VADHFr). While an analysis of the methods shows significant differences between the control and VAD diets, including unusually high amounts of some nutrients in the VAD group (for example 13.2 g/Kg vs 1.6 g/Kg calcium pantothenate and 19.8 g/Kg vs 3 g/Kg niacin), the VAD diet appears to be free of retinoids, which was achieved by not adding retinol palmitate (0.15 g/Kg) in addition to replacing casein with the ingredient 'Casein, "Vitamin-Free" Test'.

After 16 weeks mice in the VAD group achieved a 23% reduction in body weight, 43% reduction in retroperitoneal white adipose tissue (WAT) and 60% reduction in epididymal WAT compared to control. Average daily food intake (-5%) and liver weight as a percentage of body weight (+0%) did not differ significantly between the groups. Mice in the VADHFr group achieved a 28% reduction in body weight, 39% reduction in retroperitoneal WAT and 40% reduction in epididymal WAT compared to control. Average daily food intake decreased significantly by 15% while liver weight as % of body weight did not differ significantly between the VADHFr and control groups (+7%).

Plasma AST to ALT ratio was significantly improved, plasma TNFα levels (a marker for inflammation) were slightly lower, and C-reactive protein levels decreased by more than 50% in mice in the VAD group compared to control. Additionally, plasma triglycerides (TGs) decreased by about 50% after 8 weeks in the VAD group and by almost 2/3 after 16 weeks whereas liver TGs decreased significantly only after 16 weeks by a minor amount. mRNA expression of SCD1 decreased by 2/3, expression of CPT1 increased by 75% in the VAD group. Hepatic fatty acid oxidation did not significantly differ between VAD mice and control.

In general, even though adding high fructose corn syrup showed similar or even greater improvements in body weight, adipose tissue and TGs than VAD (compared to control), VADHFr did not have the same positive impact on inflammatory markers as VAD and was similar to control in both TNFα and C-reactive protein levels. Nonetheless, these results indicate that sugar may not be as detrimental to human health, especially body weight, as previously thought. Statistically significant differences between VAD and VADHFr were also seen in ALT levels (2x higher in VAD than both VADHFr and control) and plasma TG levels after 16 weeks (2x higher in VADHFr than VAD and similar to control). Liver TG levels did not differ significantly while SCD1 mRNA expression increased dramatically (2.5x higher in VADHFr than control and 5x higher than in VAD).

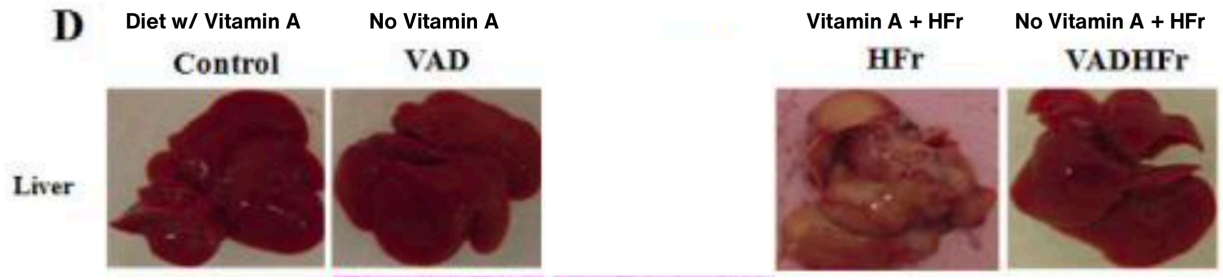

Figure: Mouse liver after 16 weeks on the described diets, adapted from (Raja Gopal Reddy, Pavan Kumar et al. 2016)

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