DILIsym®

DILIsym Services

A SIMULATIONS PLUS COMPANY

Please note: this presentation, including questions from the audience, is being recorded

DILIsym Review Session 24:

Lipotoxicity in DILIsym

July 19, 2018

Scott Q Siler

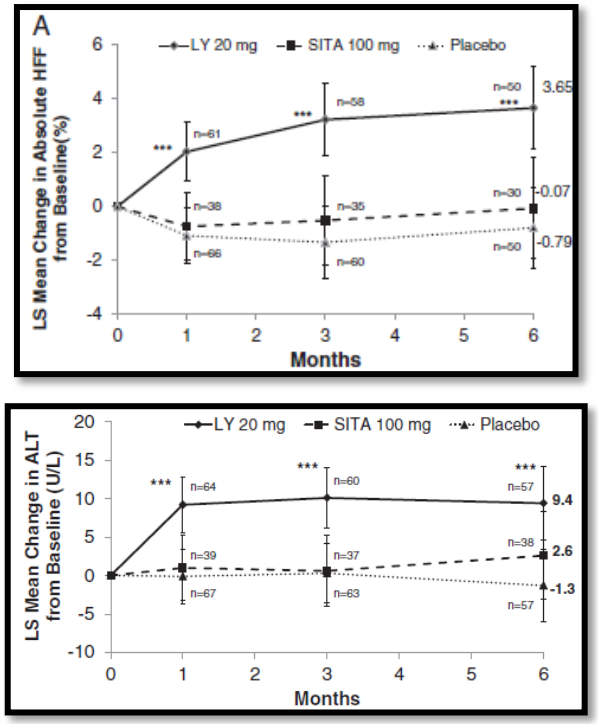
DILIsym Review Session Agenda

- Brief lipotoxicity overview
- Representation of lipotoxicity in DILIsym
- Adjusting DILIsym parameters to simulate lipotoxicity
- Predicted toxicity risk of elevated DNL due to lipotoxicity

Lipotoxicity Suspected to Be Responsible for Liver Signals for Several Compounds

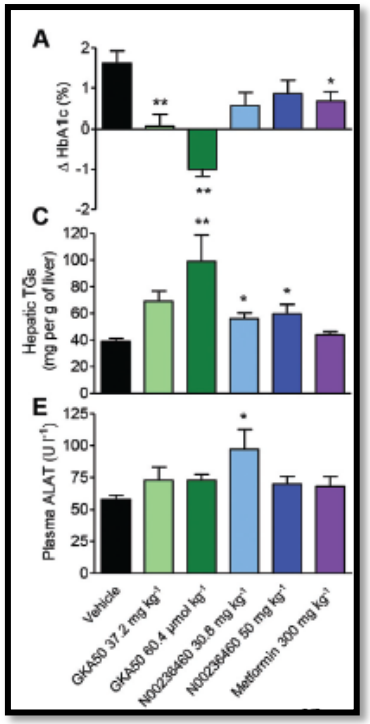
- VLDL-TG release inhibitors
  - Juxtapid
  - Kynamro
- Glucagon receptor antagonists (GRA)
  - LY2409021
- Glucokinase activators (GKA)
  - GKA50
  - Piragliatin
- Fatty acid oxidation inhibitors
  - Etomoxir

GRA, Humans



Guzman 2017

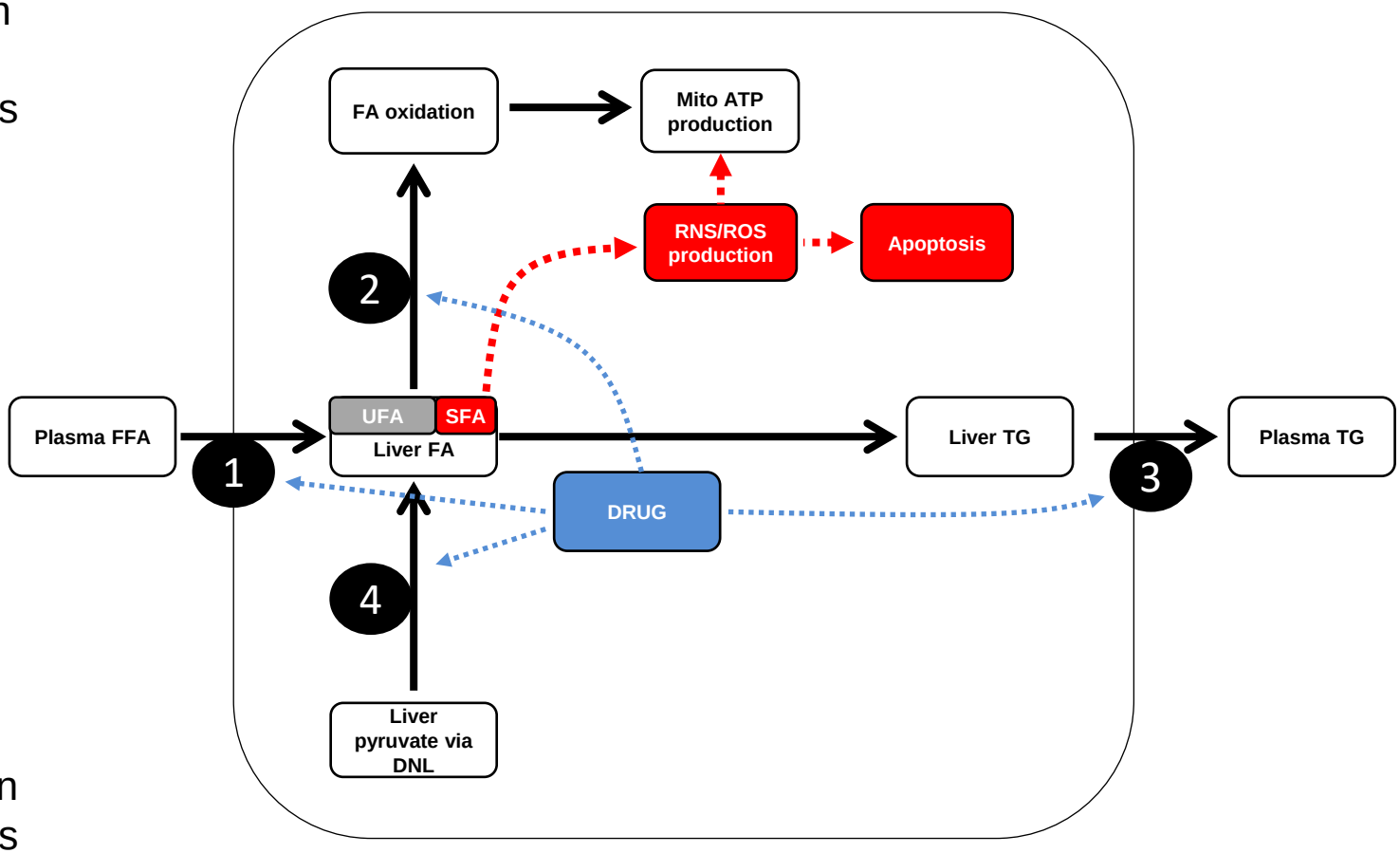
GKA, Mice



De Ceuninck 2013

Steatosis and Lipotoxicity Can Result From Drug-Induced Dysregulation of Lipid Partitioning in Liver

1. Adipose insulin resistance can cause steatosis
2. Reductions in FA oxidation can cause steatosis
3. Disrupted VLDL-TG release can cause steatosis
4. De novo lipogenesis can cause steatosis
5. Lipotoxicity due to SFA

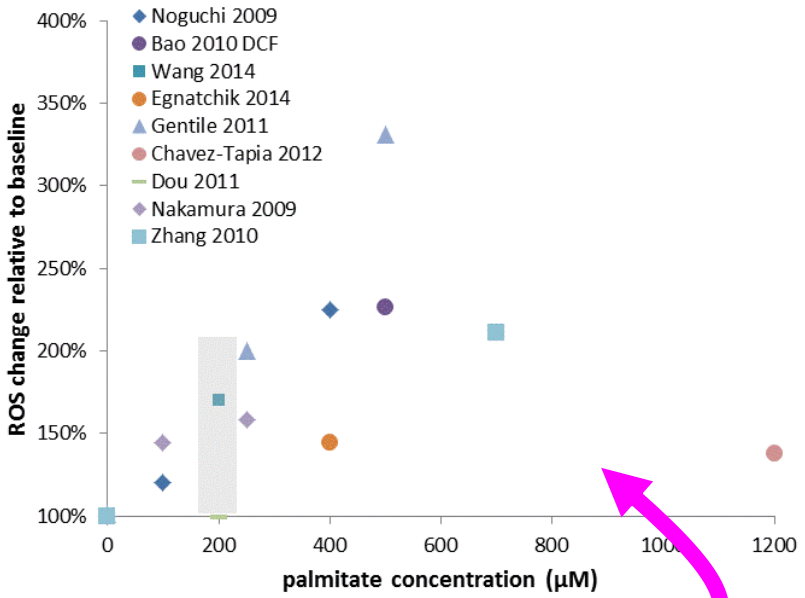


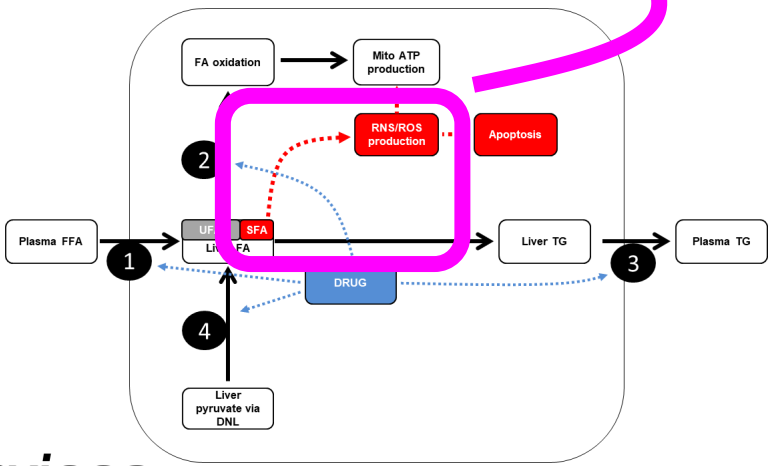
DILIsym Services

A SIMULATIONS PLUS COMPANY

ROS Increases with Exposure to Increasing Amounts of Saturated Fatty Acids

- Aggregated results from multiple experiments show that ROS levels are increased in cells cultured with increasing amounts of palmitate
  - Palmitate is most abundant saturated fatty acid (SFA)
  - Gray box indicates normal range of hepatocyte palmitate concentration
  - Results from HepG2, H4IIEC3, primary HC
  - Exposure times from 6-24 h
  - Range of ROS increase for a given level of palmitate





Preclinical Data

DILIsym Services

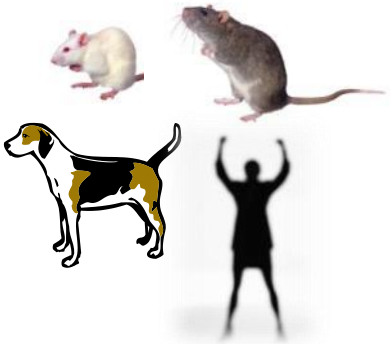
A SIMULATIONS PLUS COMPANY

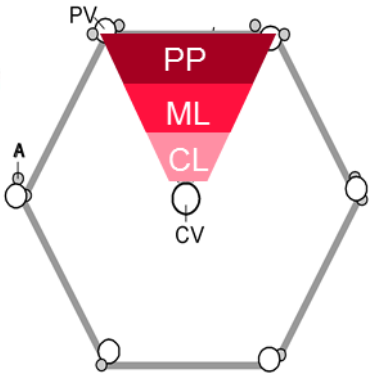
DILIsym Review Session Agenda

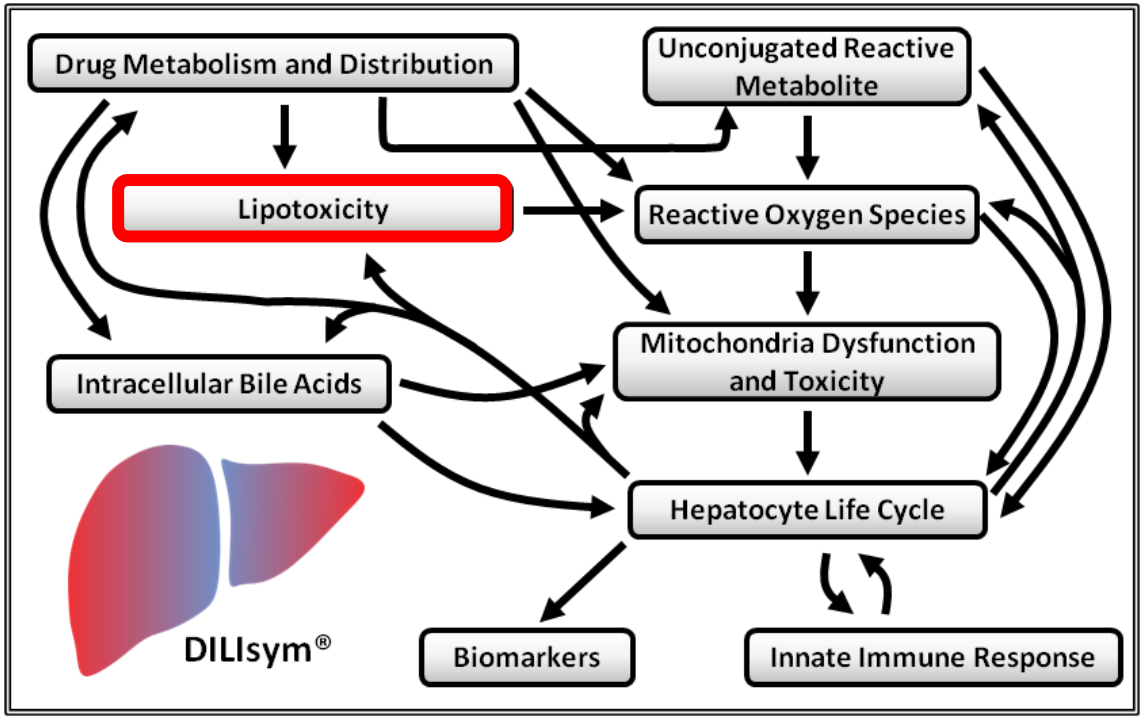
- Brief lipotoxicity overview
- Representation of lipotoxicity in DILIsym
- Adjusting DILIsym parameters to simulate lipotoxicity
- Predicted toxicity risk of elevated DNL due to lipotoxicity

Lipotoxicity Mechanism Is Included in DILIsym

- **Multiple species: human, rat, mouse, and dog**
  - Population variability
- **The three primary acinar zones of liver represented**
- **Essential cellular processes represented to multiple scales in interacting sub-models**
  - Pharmacokinetics
  - Dosing (IP, IV, Oral)
  - Transporter Inhibition
  - Drug metabolism
  - GSH depletion
  - Injury progression
  - Mitochondrial dysfunction, toxicity, DNA depletion
  - Bile acid mediated toxicity
  - **Steatosis and lipotoxicity**
  - Cellular energy balance
  - Hepatocyte apoptosis and necrosis, and proliferation
  - Macrophage, LSEC life cycles
  - Immune mediators
  - Caloric intake
  - Biomarkers





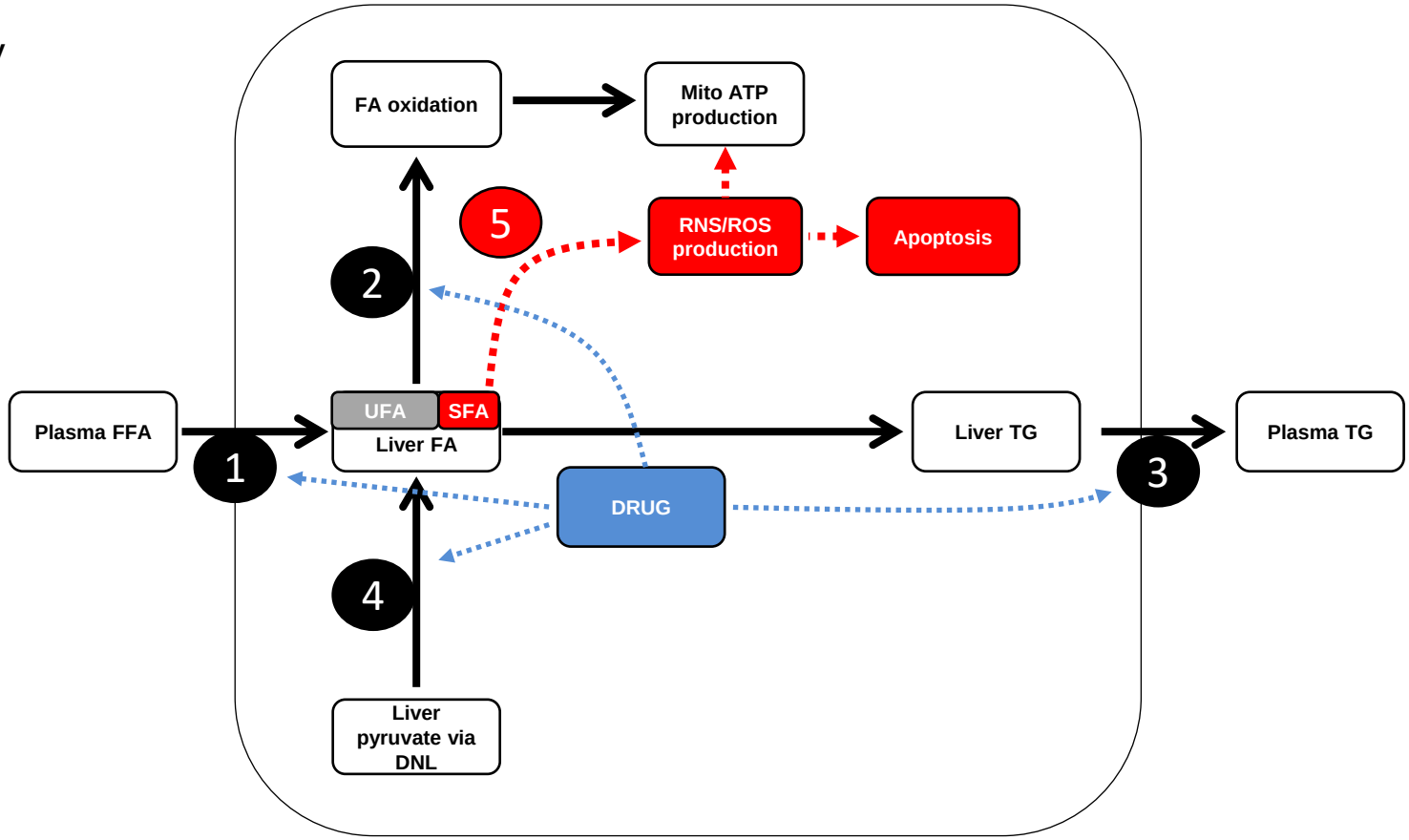


DILIsym Services

A SIMULATIONS PLUS COMPANY

Lipid Dynamics and Lipotoxicity Representation in DILIsym Based on Clinical Data

1. Adipose release of fatty acids
2. Hepatic FA oxidation
3. VLDL-TG release
4. De novo lipogenesis
5. Lipotoxicity due to SFA

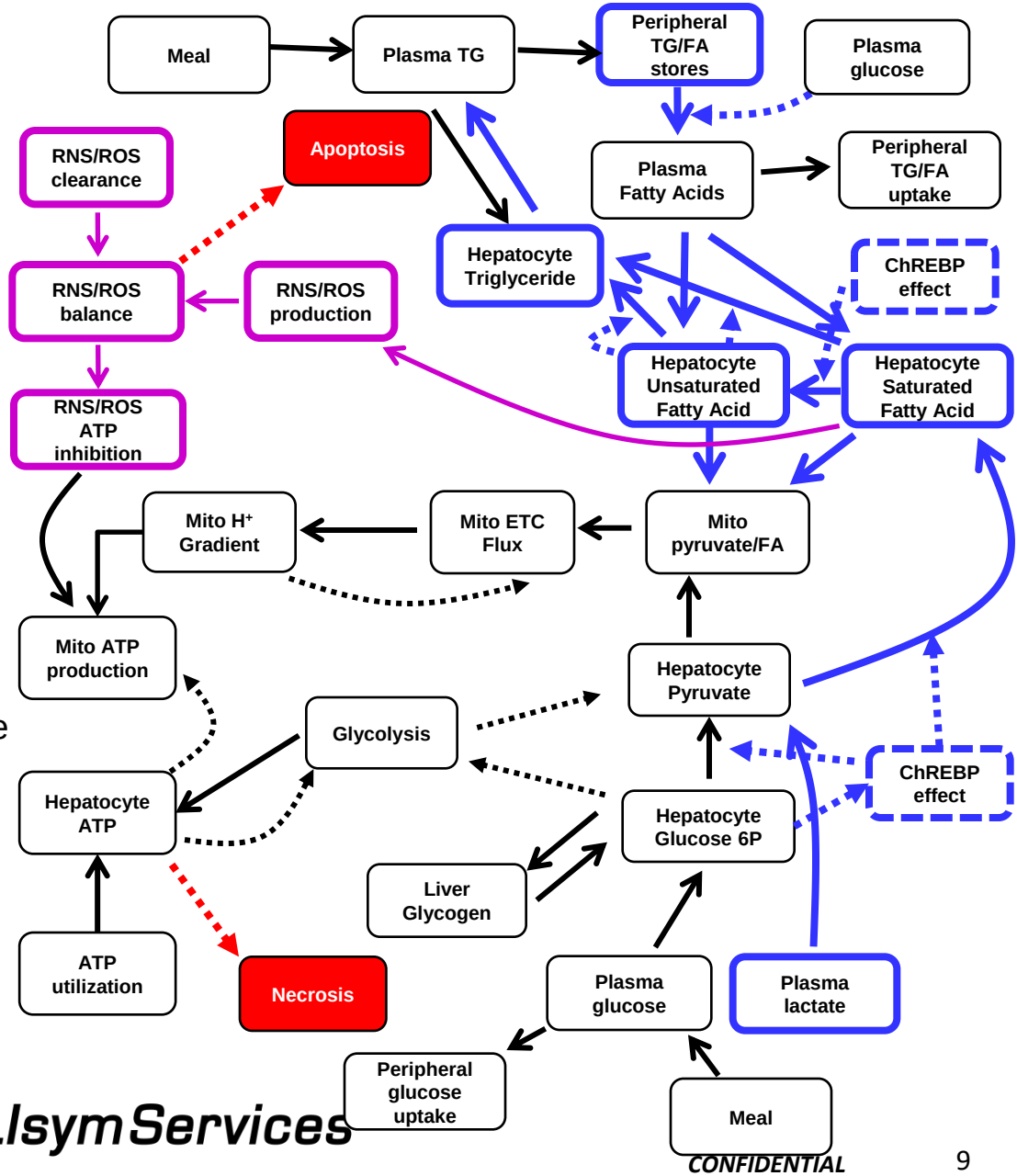


DILIsym Services

A SIMULATIONS PLUS COMPANY

Mitochondria Sub-Model: Lipotoxicity

- *De novo* lipogenesis (DNL)
  - Plasma lactate provides mass to support DNL in fasting state (when there is zero glucose uptake)
  - Pyruvate from glucose uptake supports DNL in fed state
  - ChREBP increases expression of enzymes for DNL, glycolysis, desaturation
- Adipose FA release
  - Dependent upon fat mass
- Liver VLDL-TG release
  - Dependent upon liver TG
- Liver FA include SFA and UFA
  - Primary output from DNL is palmitate and stearate, saturated fatty acids
  - Desaturation also generates some oleate and palmitoleate
  - SFA represents ~45% of FA pool, although there is variability
- SFA increases ROS production
  - Increases in SFA can disturb mitochondria function



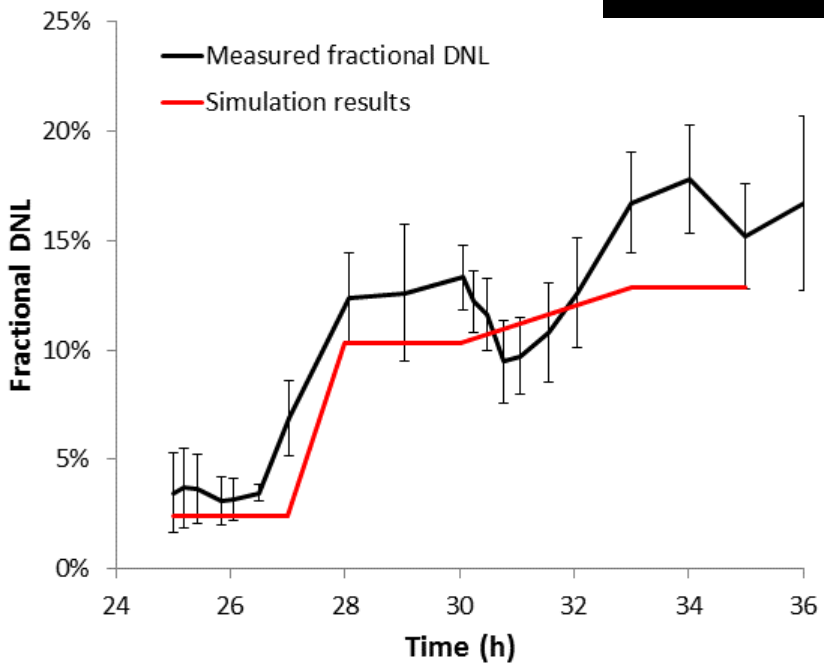
DILIsym Services

A SIMULATIONS PLUS COMPANY

Good Agreement between Measured and Simulated Fasting and Post-Prandial DNL

- Timlin 2005 reported post-prandial increases in fractional DNL in healthy volunteers
  - Quantitatively significant fate for glucose taken up by liver after meals in normal feeding conditions
  - Fractional DNL = fraction of fatty acids in VLDL that have been newly synthesized
  - Used isotopic tracers to estimate rates
- Relatively low fractional DNL in overnight fasted state under normal conditions
  - Somewhat higher in patients with type 2 diabetes, NAFLD, and hypertriglyceridemia
- Plasticity of DNL pathway in response to feeding conditions and pharmaceutical intervention
  - Can substantially increase DNL
  - Potentially affect pathophysiology and/or hepatotoxicity

HUMANS

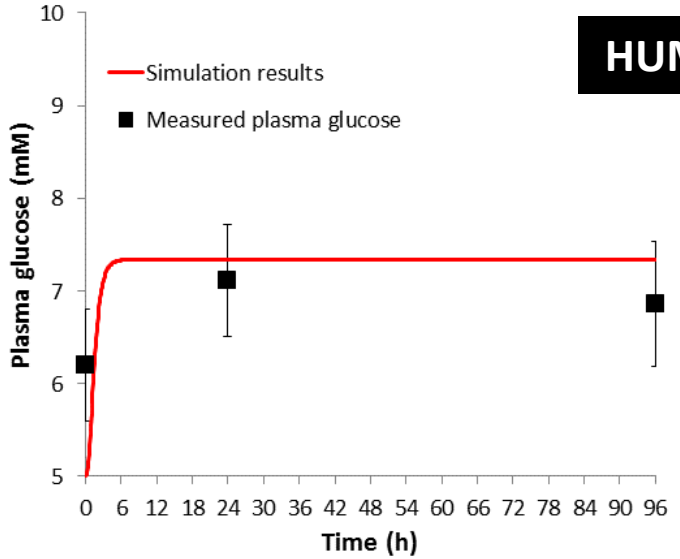


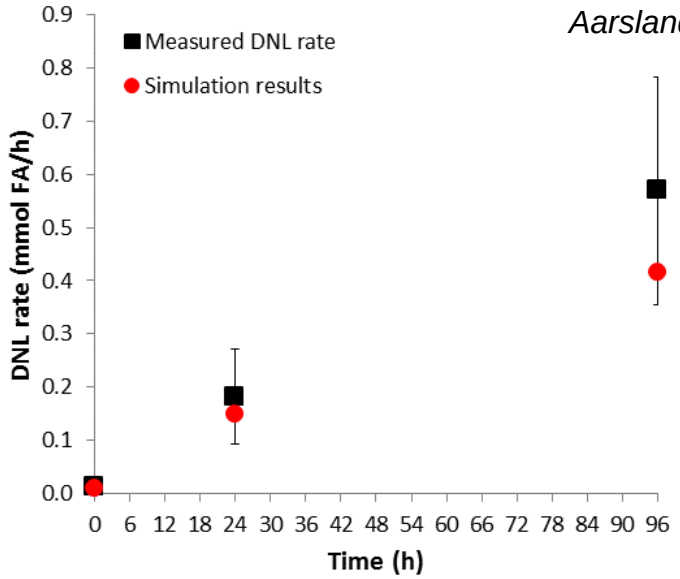
Timlin 2005

DILIsym Consistent with DNL Rates from Extreme Overfeeding Studies

- Aarsland 1996 reported substantial increases in DNL rates in healthy volunteers with sustained hyperglycemia
  - High carbohydrate meals + intravenous glucose
  - Used isotopic tracers to estimate rates
  - Extremely lipogenic conditions
- DNL increased markedly within 24 hours
  - Represents incorporation of hepatic glucose uptake into DNL pathway
- Additional DNL increase after 24 hours
  - Represents increased expression of DNL and glycolysis enzymes via ChREBP
- DILIsym simulations include both substrate and enzyme induction-related increases in DNL

HUMANS





Aarsland 1996

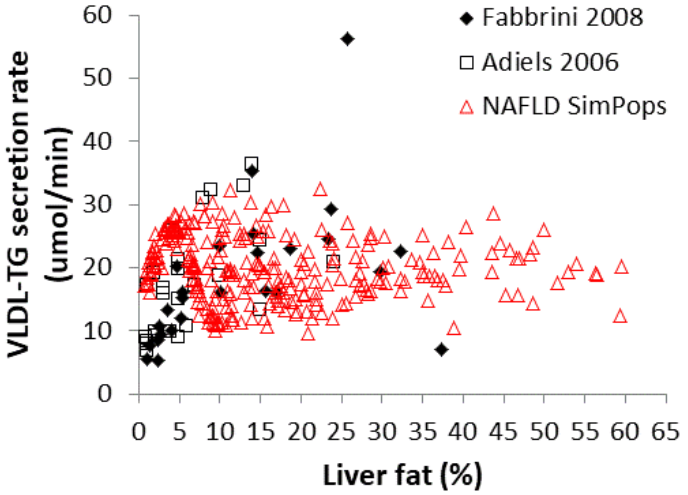
Clinical Data and Simulation Results

DILIsym Services

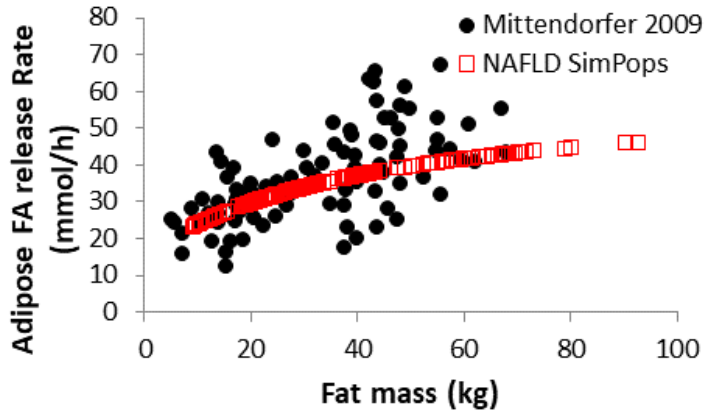
A SIMULATIONS PLUS COMPANY

NAFLD SimPops Include A Range of VLDL-TG and Adipose FA Release Rates Consistent with Clinical Data

- Simulated patients have wide range of VLDL-TG release that is consistent with clinical data
  - Generally greater VLDL-TG release rates with higher degrees of steatosis
- Adipose fatty acid release rates dependent upon fat mass in clinical and simulated patients
  - Consistent with Mittendorfer 2009 clinical data



Fabbrini 2008, Adiels 2006

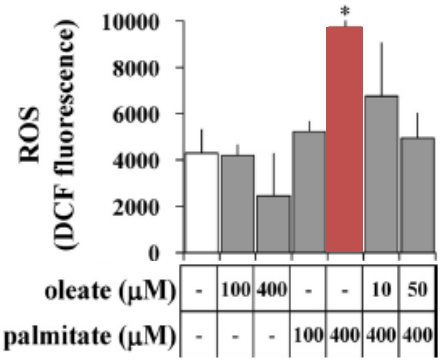


Mittendorfer 2009

Approach for Modeling Lipotoxicity within DILIsym

- Utilized hepatocyte experimental data showing that saturated fatty acids (SFA) can elicit increases in reactive oxygen species (ROS)
- Clinical data from pharmaceutical interventions used to further optimize and validate lipotoxicity sub-model
  - BMS-201038 clinical data (Cuchel 2007) used to optimize SFA-ROS relationship
    - MTP inhibitor restricts hepatocyte TG from being packaged into VLDL
    - ↓ VLDL synthesis and ↑ liver TG are consequence
    - ↑ ALT reported coincident with ↑ liver TG
  - Mipomersen clinical data (FDA filing documents) used as validation
    - Antisense oligonucleotide (ASO) used to ↓apoB100 synthesis
    - Restricts VLDL assembly and release
    - ↓VLDL synthesis and ↓ liver TG accumulation
    - ↑ ALT coincident with ↑ liver TG
    - ↑ cleaved CK18 correlated with ↑ liver TG

SFA only

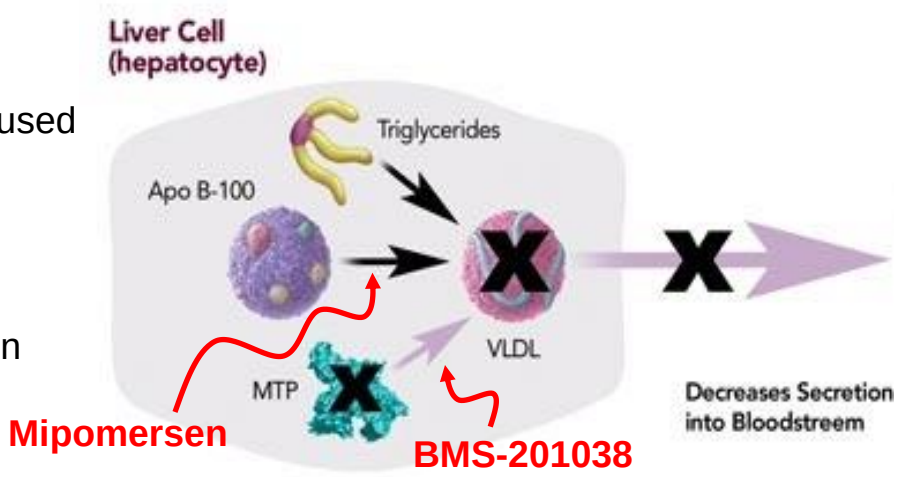


Human HC

HepG2

Rat HC

Noguchi 2009



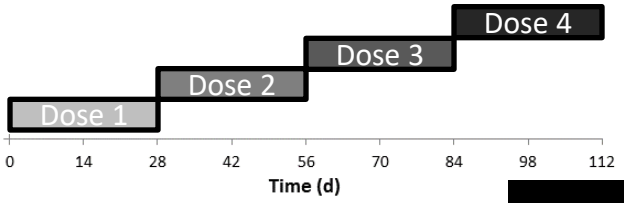
Preclinical Data

DILIsym Services

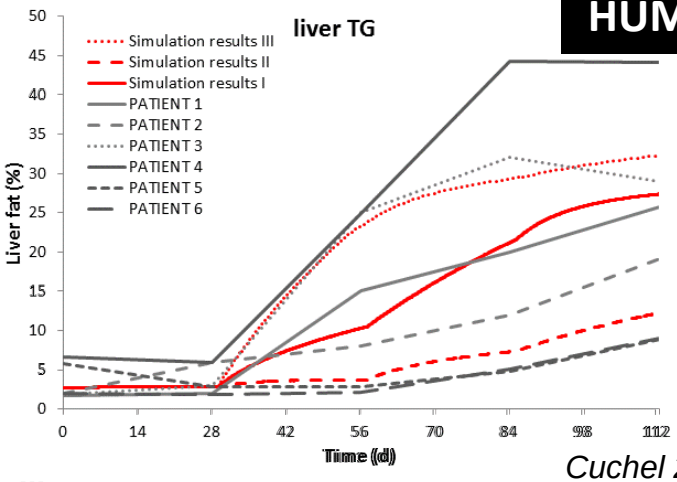
A SIMULATIONS PLUS COMPANY

Juxtapid MTP Inhibitor Established Quantitative Lipotoxicity Relationship

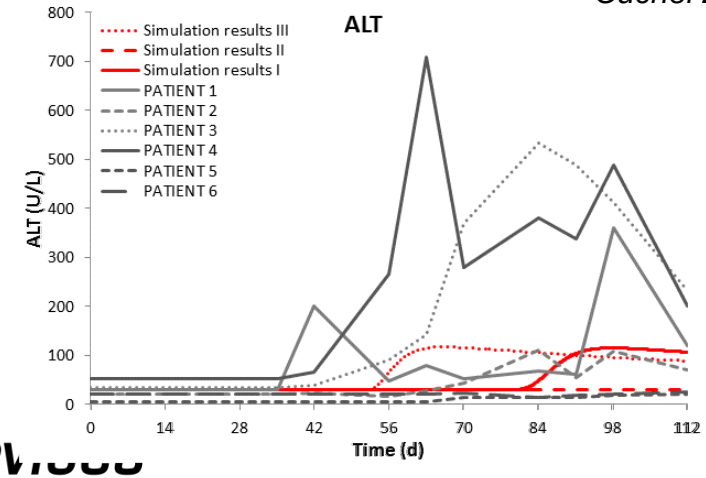
- Juxtapid is a microsomal transfer protein (MTP) inhibitor that is available to treat homozygous familial hypercholesterolemia
  - Safety concerns highlighted by key trial with dose escalation protocol; doses were increased every 4 weeks; n=6 patients
- Liver TG increased in all patients, substantially in some
  - Due to inability to package and secrete VLDL
- ALT increased coincident with liver TG
  - Indicative of lipotoxicity
- Simulation results consistent with measured data
  - Three simulations performed to capture range of liver TG accumulation
  - Accompanying ALT increases within range observed for n=6 FH patients
  - Lipotoxicity due to increases in SFA and resultant changes in ROS
  - By design—optimization phase
  - SFA-ROS quantitative relationship decreased relative to summarized *in vitro* data



HUMANS



Cuchel 2007



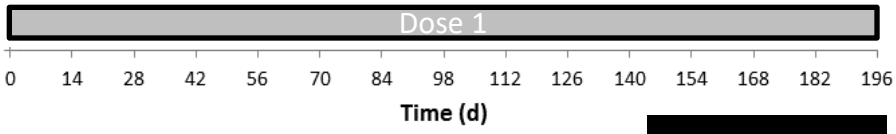
Clinical Data and Simulation Results

DILIsym Services

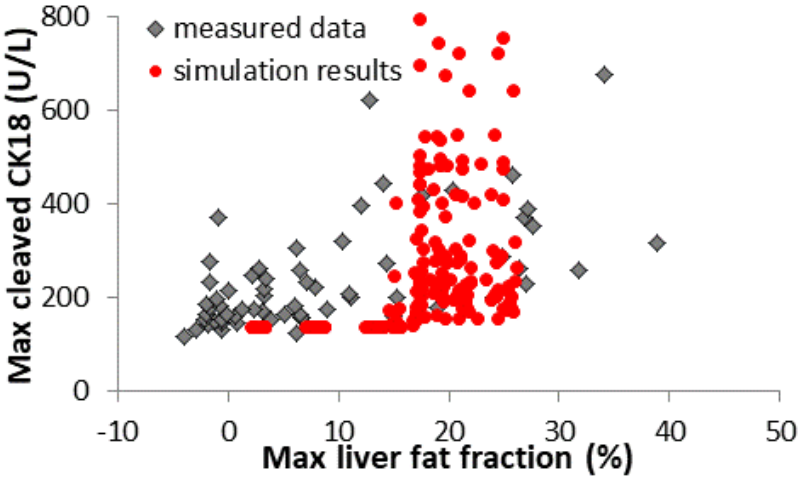
A SIMULATIONS PLUS COMPANY

Kynamro-Induced Increases In Apoptosis Included in DILIsym

- Kynamro (Mipomersen) is an apoB100 ASO that is available to treat homozygous familial hypercholesterolemia (Stein 2013)
  - Safety concerns raised from clinical trial data
  - Example trial: consistent dosing over 26 weeks
- High fraction of patients had increased liver TG
  - Median increase was 5%--clinical steatosis
  - Liver TG increased in >60% of patients
- Cleaved CK18 reported to increase
  - Indicative of lipotoxicity-induced apoptosis
  - Correlation between steatosis and cleaved CK18
  - ALT increases also observed in a number of patients
- Simulation results consistent with measured data
  - Multiple simulations performed to capture range of liver TG accumulation with small (n=36)
  - SimCohorts used to capture variability in ROS-induced apoptosis
  - Simulated cleaved CK18 increases within range observed for FH patients
  - Lipotoxicity due to increased ROS via increased SFA



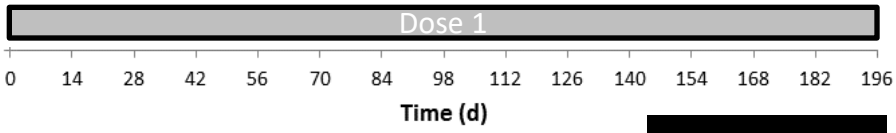
HUMANS



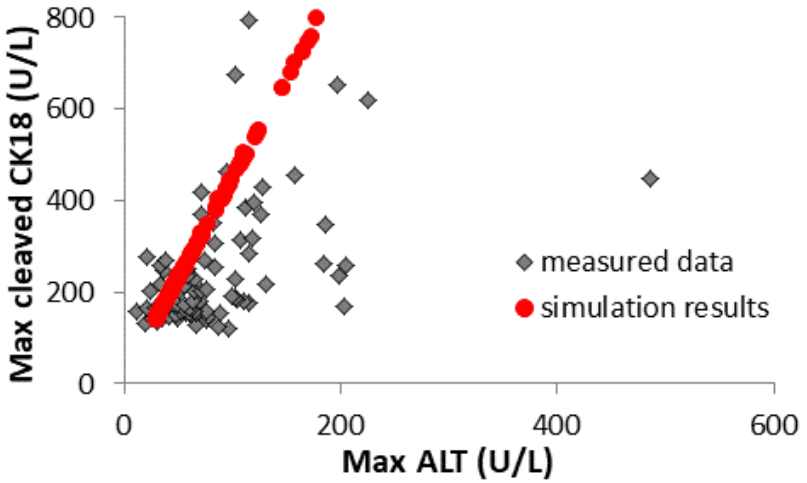
FDA briefing document
NDA 203568 2012
Stein 2013

Kynamro-Induced Increases In ALT Included in DILIsym

- Kynamro (Mipomersen) is an apoB100 ASO that is available to treat homozygous familial hypercholesterolemia (Stein 2013)
  - Safety concerns raised from clinical trial data
  - Example trial: consistent dosing over 26 weeks
- High fraction of patients had increased liver TG
  - Median increase was 5%--clinical steatosis
  - Liver TG increased in >60% of patients
- Cleaved CK18 reported to increase
  - Indicative of lipotoxicity-induced apoptosis
  - Correlation between steatosis and cleaved CK18
  - ALT increases also observed in a number of patients
- Simulation results consistent with measured data
  - Multiple simulations performed to capture range of liver TG accumulation with small (n=36)
  - SimCohorts used to capture variability in ROS-induced apoptosis
  - Simulated ALT and cleaved CK18 increases within range observed for FH patients
  - Lipotoxicity due to increased ROS via increased SFA
  - These simulation results help to optimize apoptosis, and secondary necrosis representation within DILIsym

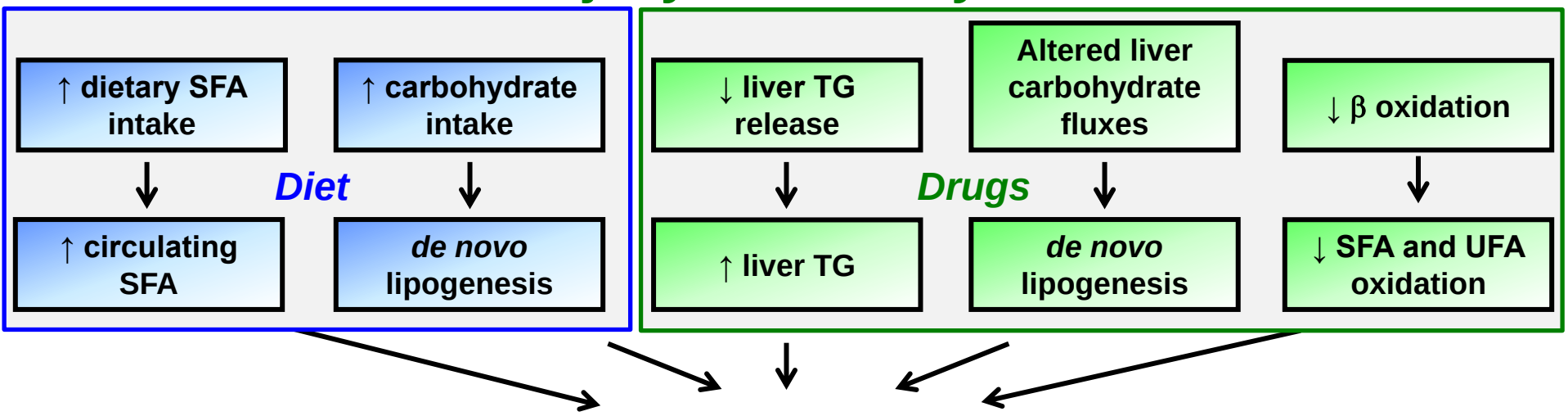


HUMANS

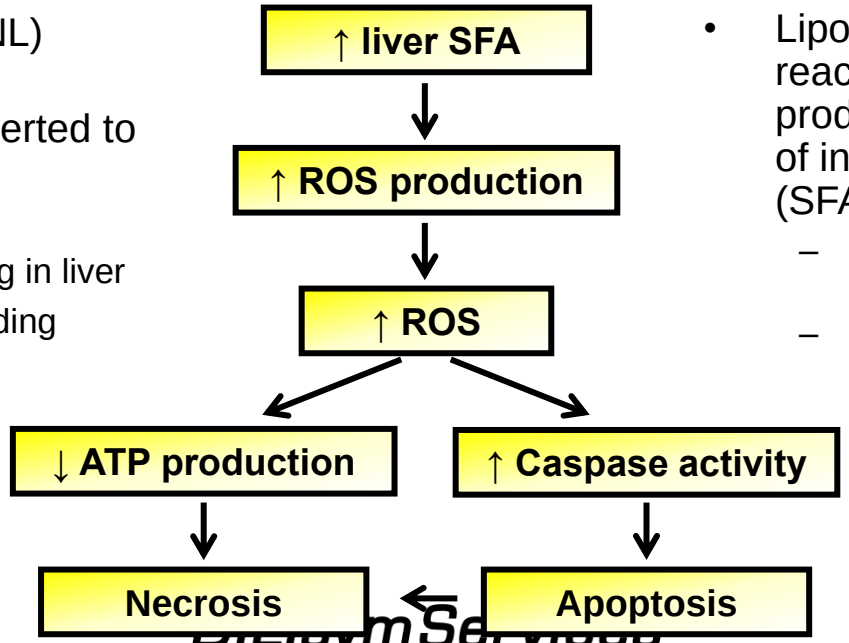


Stein 2012,
FDA briefing document
NDA 203568 2012

How Lipid Accumulation Is Connected to Injury in DILIsym



- *de novo* lipogenesis (DNL) provides pathway for carbohydrate to be converted to fatty acids
  - Drugs that manipulate carbohydrate trafficking in liver
  - Carbohydrate overfeeding

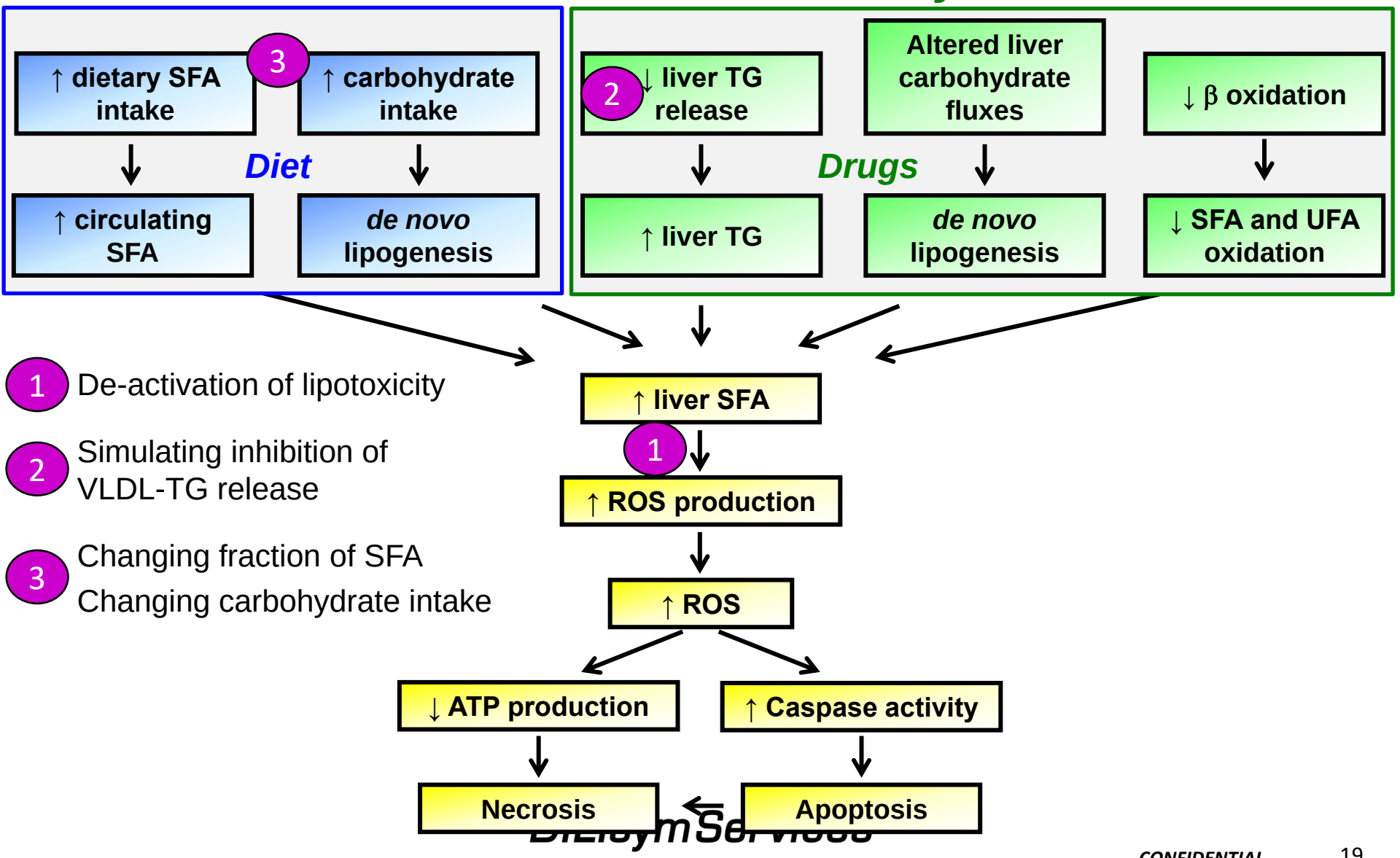


- Lipotoxicity due to increased reactive oxygen species (ROS) production as a consequence of increased saturated fatty acid (SFA) levels
  - SFA typically minority component
  - Can increase in certain situations and initiate lipotoxicity in liver

DILIsym Review Session Agenda

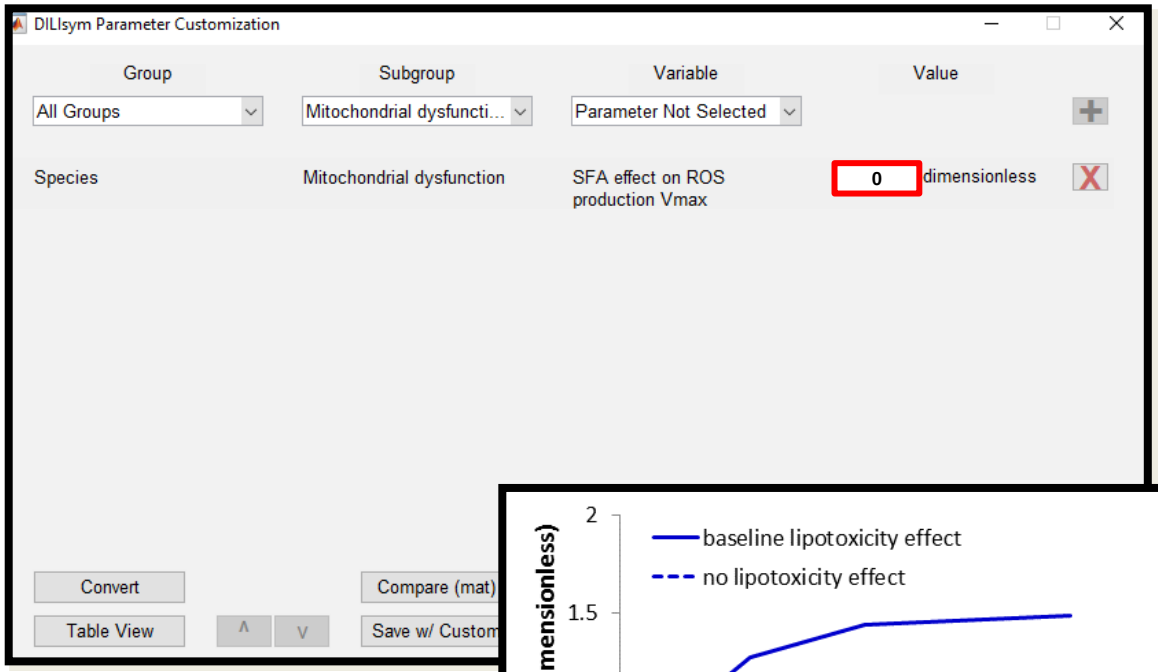
- Brief lipotoxicity overview
- Representation of lipotoxicity in DILIsym
- Adjusting DILIsym parameters to simulate lipotoxicity
- Predicted toxicity risk of elevated DNL due to lipotoxicity

Modifying Lipotoxicity Parameters in DILIsym



Parameters to Use to Ensure Lipotoxicity Is Activated in DILIsym

- Lipotoxicity is active with default human parameters in DILIsym
  - May want to perform simulations in absence of effect
  - Not active in dog, rat, mouse
- SFA effect on ROS production Vmax = 0 to deactivate lipotoxicity effect
  - Located in 'Species Parameters'
  - Located in 'mitochondrial dysfunction' parameter sub-set
  - Baseline value for humans is 0.0138
  - Set to 0 for dog, rat, mouse



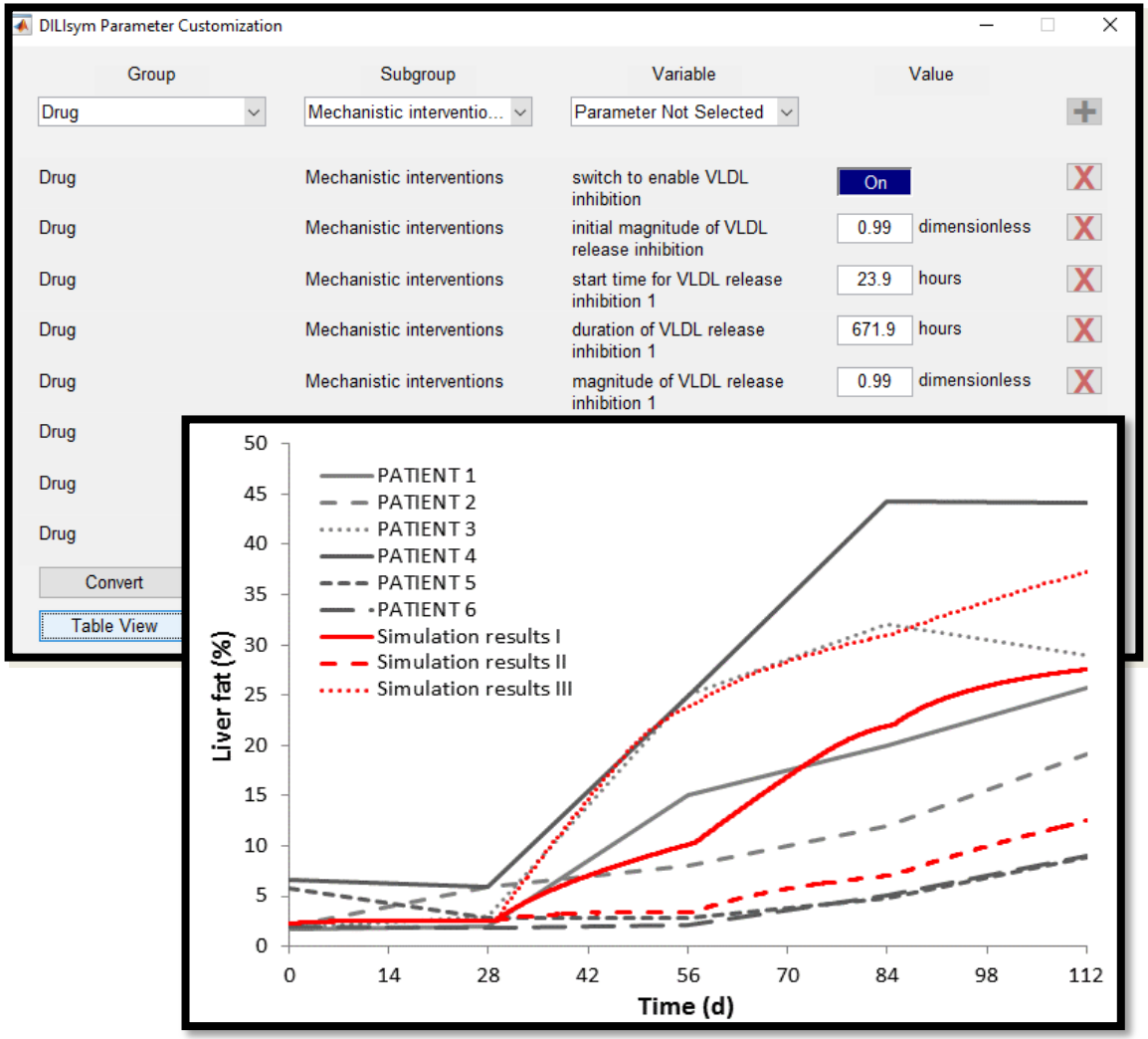
Simulation Results

DILIsym Services

A SIMULATIONS PLUS COMPANY

Simulating the Inhibition of VLDL-TG Release with DILIsym

- Can simulated reductions in VLDL-TG release with DILIsym
  - The details of the pharmacology are not represented
  - Magnitude of reduction can be set at specific time points
- Multiple parameters allow for simulating VLDL-TG inhibition
  - Located in 'Drug Parameters'
  - Located in 'mechanistic interventions' parameter sub-set
  - Switch to enable VLDL inhibition = 1
  - Magnitude of VLDL release inhibition = 1- inhibition
  - Start time for VLDL release inhibition = start time
  - Duration of VLDL release inhibition = period of inhibition
  - Can simulate 4 inhibition levels during a single simulation
- Data Comparison experiment provides use example
  - Cuchel_2007_TG_mid
  - Cuchel 2007



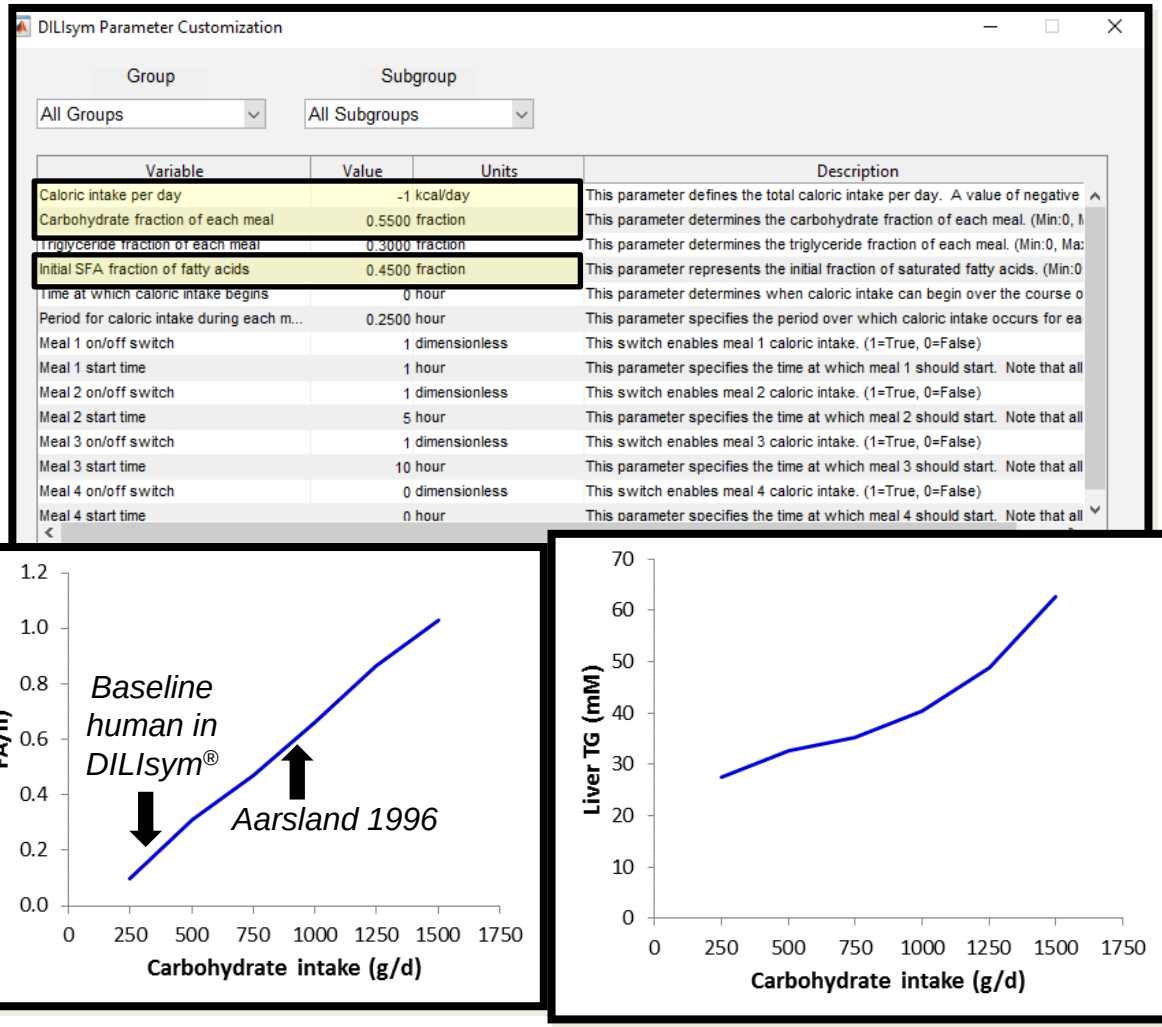
Clinical Data and Simulation Results

DILIsym Services

A SIMULATIONS PLUS COMPANY

Simulating Dietary Changes Relevant to Lipotoxicity in DILIsym

- Changes to diet can impact accumulation of liver lipids and subsequent lipotoxicity in DILIsym
  - Carbohydrate intake can increase DNL
  - SFA intake can alter sensitivity to lipotoxicity
  - DNL only active in humans in v4A
- Use 'Caloric Intake' parameter set to adjust dietary intake
  - 'Caloric intake per day' to adjust total calories
  - 'Carbohydrate fraction of each meal' to adjust the fraction of carbohydrate
  - 'Initial SFA fraction of fatty acids' to adjust the fraction of SFA
- DNL is dependent upon carbohydrate intake
  - Can cause significant increases in liver TG



Simulation Results

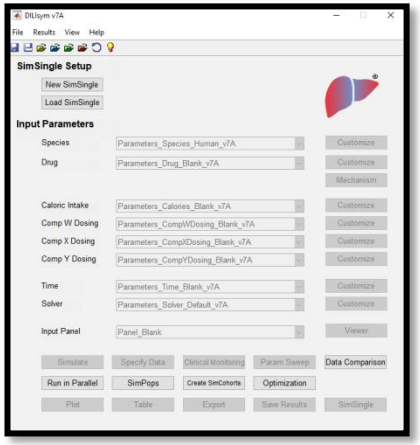
DILIsym Services
A SIMULATIONS PLUS COMPANY

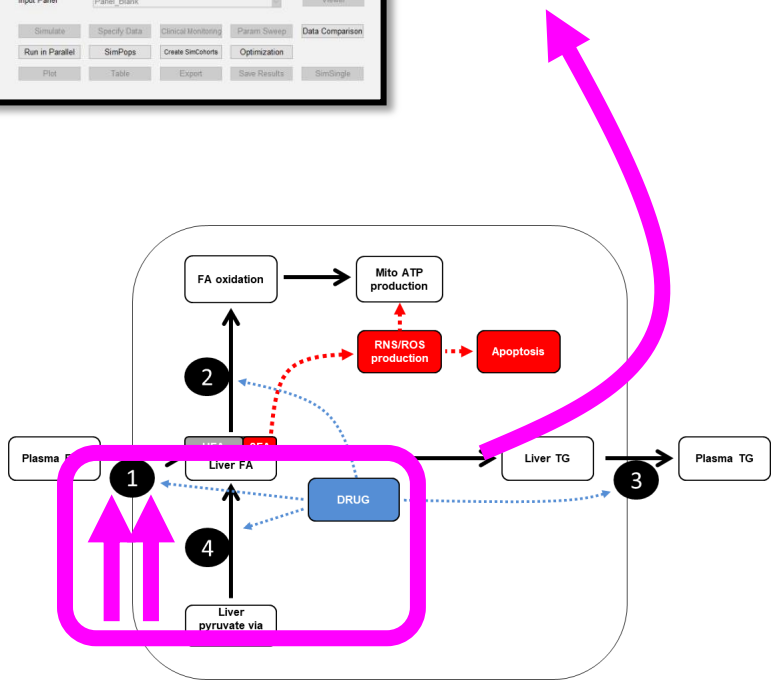
DILIsym Review Session Agenda

- Brief lipotoxicity overview
- Representation of lipotoxicity in DILIsym
- Adjusting DILIsym parameters to simulate lipotoxicity
- Predicted toxicity risk of elevated DNL due to lipotoxicity

Predicting the Impact of DNL on Liver Lipids and Lipotoxicity with DILIsym

- Performed simulations with DILIsym to predict the hepatotoxic risk due to *de novo* lipogenesis (DNL)
  - Potential risk for some drugs developed to treat metabolic diseases
- Simulated 7x or 3.5x increase in DNL over 12 weeks
  - 7x is apparent maximal DNL rate
  - DNL stimulus provided by continuous overfeeding
    - Comparable to alterations in carbohydrate fluxes with some metabolic disease compounds
  - Maintained plasma FFA at basal values
  - N=36 SimCohorts (Human_ROS_apop_mito_BA_v4A_1_RS36)





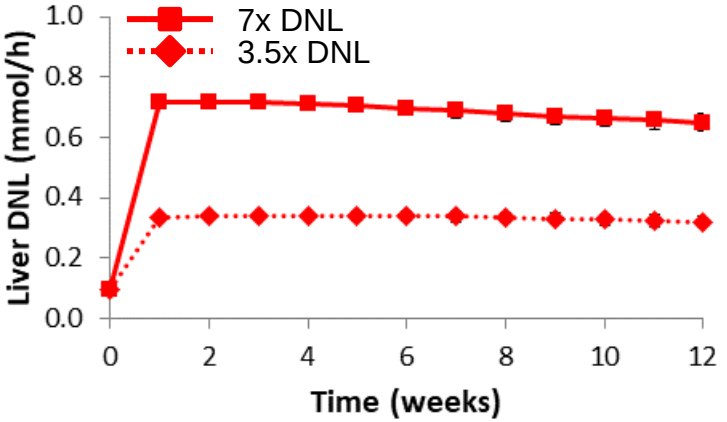
DILIsym Services

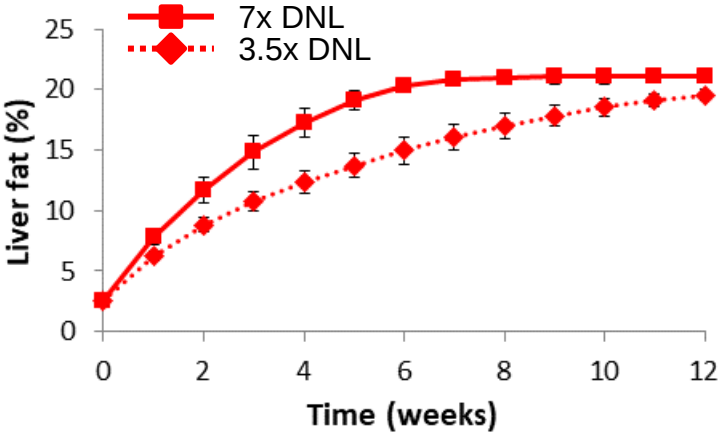
S+ A SIMULATIONS PLUS COMPANY

Substantial Increases in DNL Predicted to Elicit Increases in Liver Fat

HUMANS

- Simulated 7x or 3.5x Increase in DNL for 12 weeks
  - N=36 SimCohorts
- Substantial predicted increases in liver fat
  - All simulated patients were predicted to develop steatosis with increased DNL
  - Delayed presentation with lower DNL stimulus
- Liver triglyceride levels regulated by inputs from both DNL and uptake of plasma FFA
  - No change in FFA in these simulations

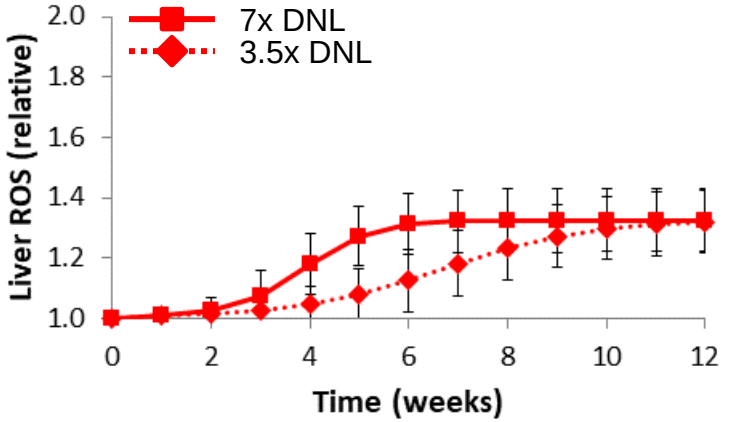


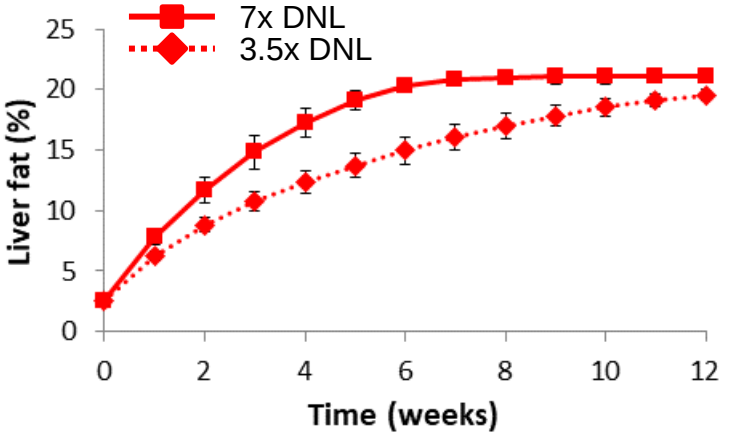


Increased Liver Fat due to DNL Stimulus Drives Liver ROS Production

HUMANS

- Simulated 7x or 3.5x Increase in DNL for 12 weeks
  - N=36 SimCohorts
- Substantial predicted increases in liver fat
  - All simulated patients were predicted to develop steatosis with increased DNL
  - Delayed presentation with lower DNL stimulus
- Oxidative stress developed in all simulated patients
  - Lipotoxicity
  - Increased liver saturated fatty acids (*not shown*) motivated ROS production
  - Delayed presentation with lower DNL stimulus





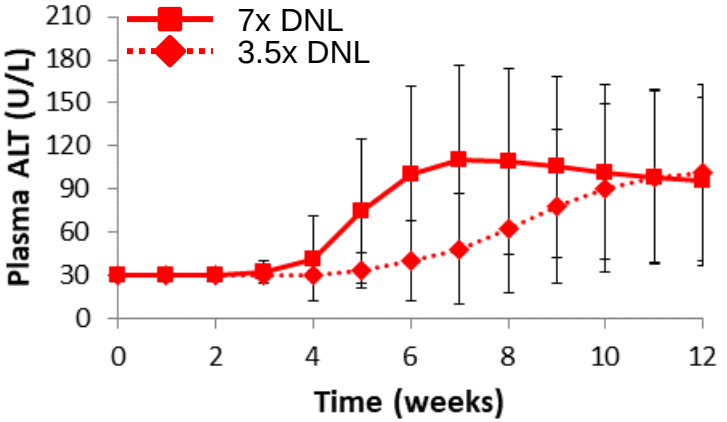
Simulation Results

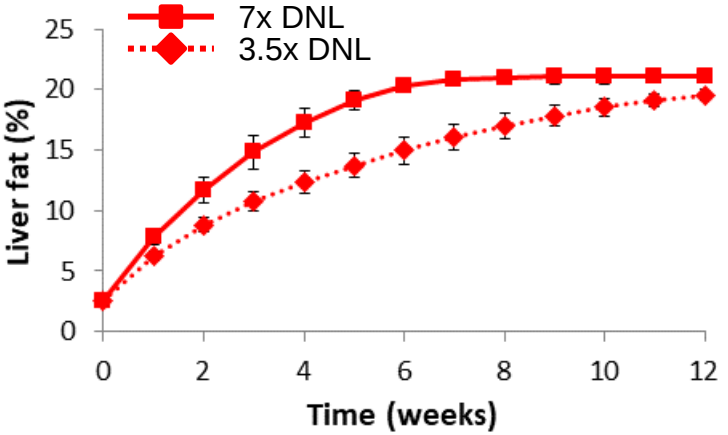
DILIsym Services
A SIMULATIONS PLUS COMPANY

Predicted ALT Increases Due To Increased DNL, Liver Fat, and Lipotoxicity

HUMANS

- Simulated 7x or 3.5x Increase in DNL for 12 weeks
  - N=36 SimCohorts
- Substantial predicted increases in liver fat
  - All simulated patients were predicted to develop steatosis with increased DNL
  - Delayed presentation with lower DNL stimulus
- Plasma ALT predicted to increase in all simulated patients
  - Due to liver lipotoxicity
  - Variability due to diversity in response to liver ROS within SimCohorts
  - Delayed presentation with lower DNL stimulus



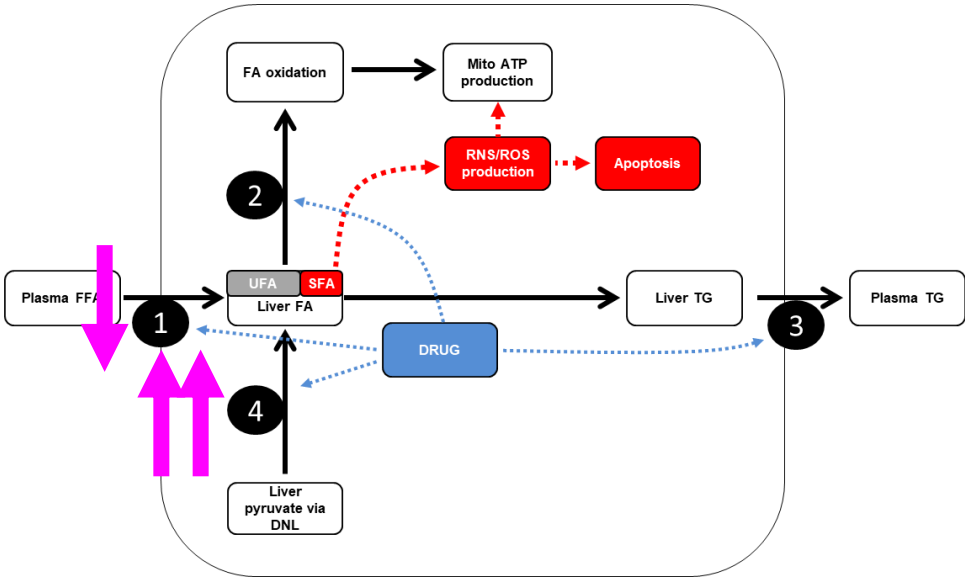


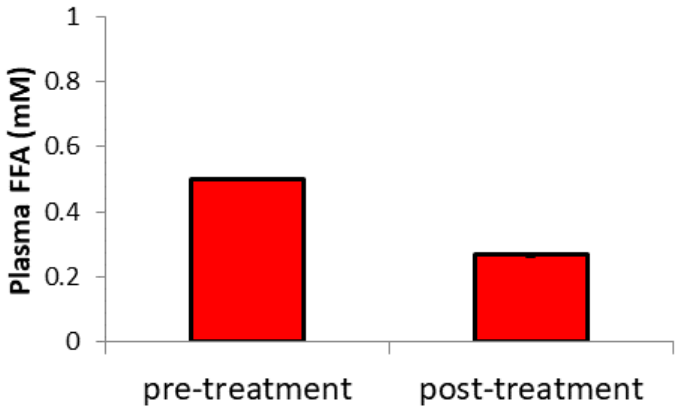
Simulation Results

DILIsymServices
A SIMULATIONS PLUS COMPANY

Predicting the Impact of DNL on Liver Lipids and Lipotoxicity with DILIsym

- Performed simulations with DILIsym to predict the hepatotoxic risk due to *de novo* lipogenesis (DNL)
  - Potential risk for some drugs developed to treat metabolic diseases
- Simulated 7x increase in DNL over 12 weeks
  - Apparent maximal DNL rate
  - DNL stimulus provided by continuous overfeeding
    - Comparable to alterations in carbohydrate fluxes with some metabolic disease compounds
  - **Plasma FFA reduced**
    - Comparable to post-prandial reductions
  - N=36 SimCohorts (Human_ROS_apop_mito_BA_v4A_1_RS36)

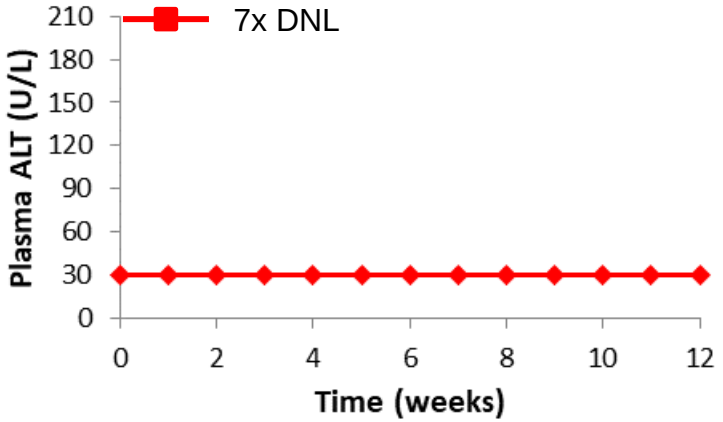


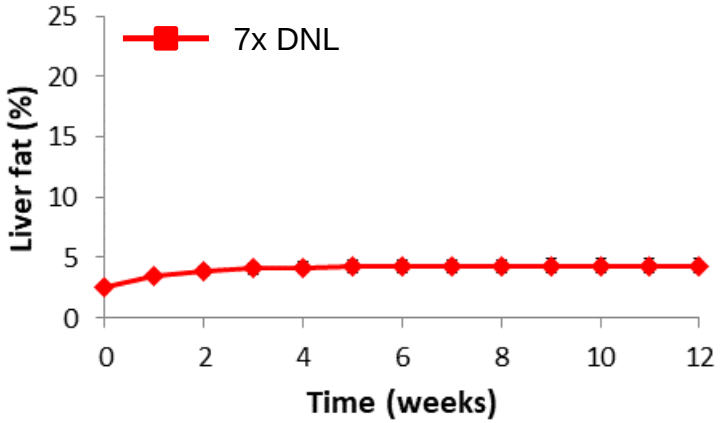


Minimal Increases in Liver Fat and No Lipotoxicity Predicted When FFA Reduced Along with Increased DNL

HUMANS

- Simulated 7x Increase in DNL for 12 weeks
  - Also reduced plasma FFA 50%
  - N=36 SimCohorts
- Minimal predicted increases in liver fat
  - All simulated patients were predicted to develop steatosis
- Liver triglyceride levels regulated by inputs from both DNL and uptake of plasma FFA
  - Reductions in plasma FFA largely offset increased input from DNL
  - Interpatient variability in impact of DNL vs. FFA in metabolic disease patients
- No increases in plasma ALT predicted
  - Liver lipid levels not elevated enough to elicit liver lipotoxicity





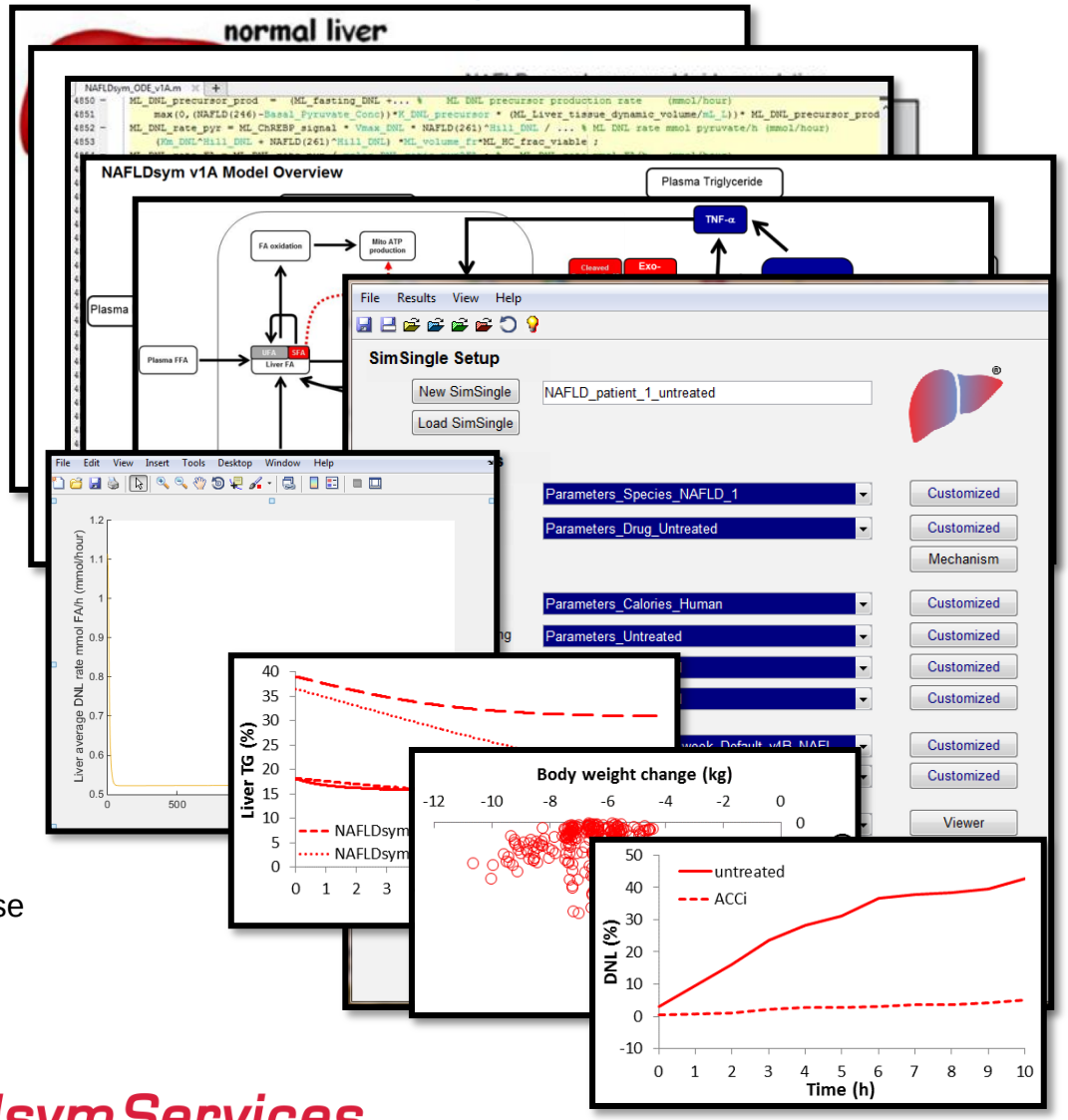
Summary

- DILIsym includes representation of lipotoxicity, enabling prediction of hepatotoxic risk for compounds that alter carbohydrate or lipid metabolic fluxes

- Predicted lipotoxicity due to increased DNL depends on associated changes in plasma FFA

NAFLDsym Is Designed to Support Drug Development with Efficacy Predictions

- NAFLD incidence is growing worldwide with few treatment options
  - Substantial opportunity to improve health for many patients by developing treatments
- NAFLDsym is a QSP model of NAFLD and NASH
  - v1A focuses on key pathways that contribute to steatosis and lipotoxicity; currently in use
  - Currently developing v2A, which will include inflammation and fibrosis sub-models; available Q4 2018
  - Includes >300 diverse simulated patients in SimPops™
  - NAFLDsym utilizes many key aspects of DILIsym®
- NAFLDsym can be used to support NAFLD drug development
  - Combines PK, PD, pathophysiology to predict efficacy of novel treatments
  - Flexible framework facilitates addition of new targets as needed
  - Can be used to optimize clinical trial protocols and identify key hypotheses related to mechanistic underpinnings of predicted response to treatment
- NAFLDsym has been used in collaborative research agreement with Pfizer, Gilead and other companies to inform clinical programs



DILIsym Services

A SIMULATIONS PLUS COMPANY

Upcoming DILIsym Review Sessions

Join us for:

- **Review Session 25: “SimPops Design and Construction.” August 30, 2018**