

Liver Selective Acetyl-CoA Carboxylase Inhibition by ND-654 and Related Analogs Inhibits Hepatic Fatty Acid Synthesis, Stimulates Hepatic Fatty Acid Oxidation, Reduces Hepatic Steatosis, and Modulates Dyslipidemia in Diet-Induced Obese Rats

Geraldine Harriman¹, Jeremy Greenwood², Sathesh Bhat², Liang Tong³, Ruiying Wang³, Debamita Paul³, Katherine Allen⁴, Asish Saha⁴, Neil Ruderman⁴, Rosana Kapeller¹, and H. James Harwood Jr.¹

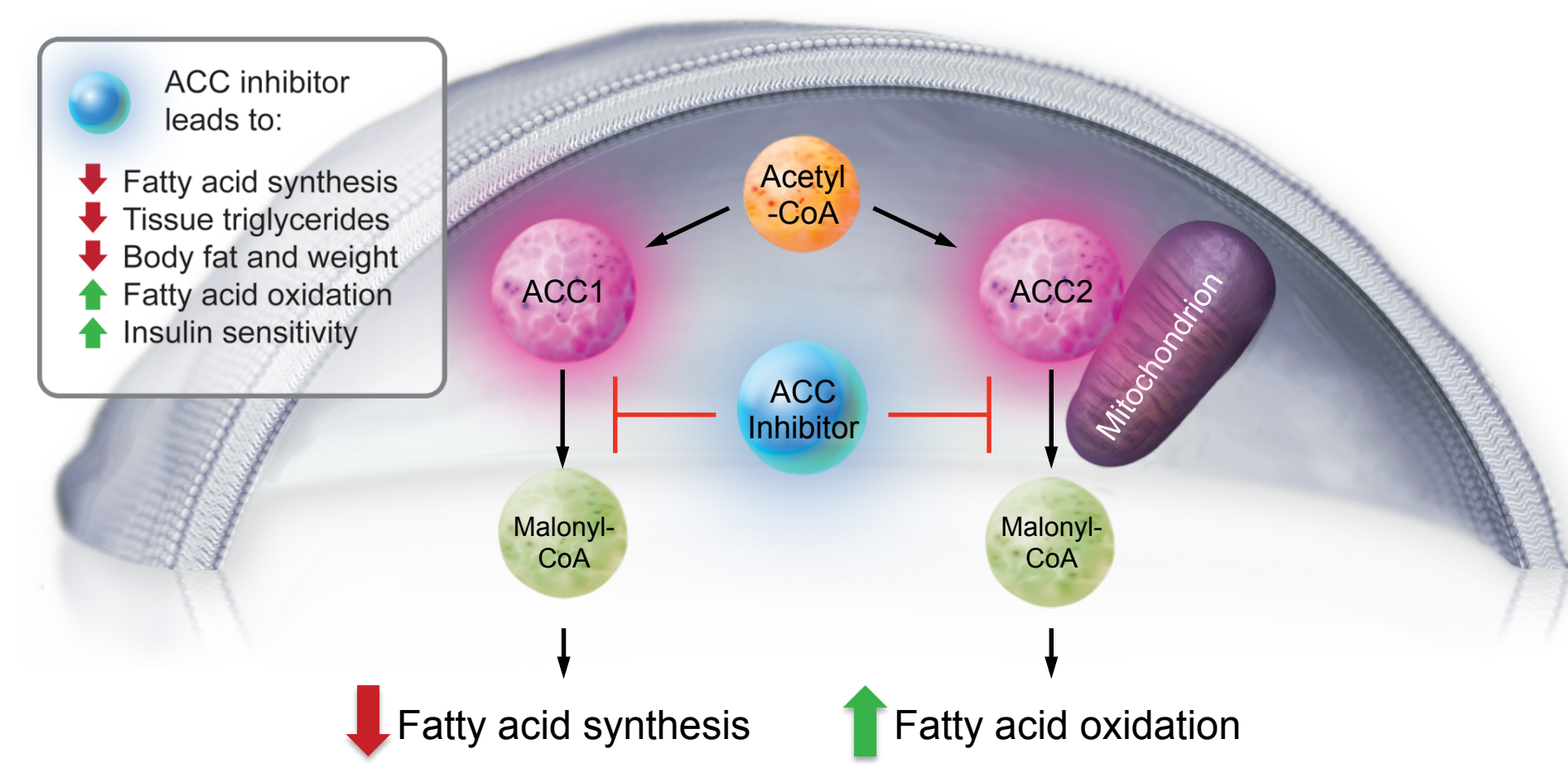
¹Nimbus Discovery, Cambridge, MA (USA), ²Schrodinger, New York, NY (USA), ³Columbia University New York NY (USA), ⁴Boston University, Boston, MA (USA)



ABSTRACT

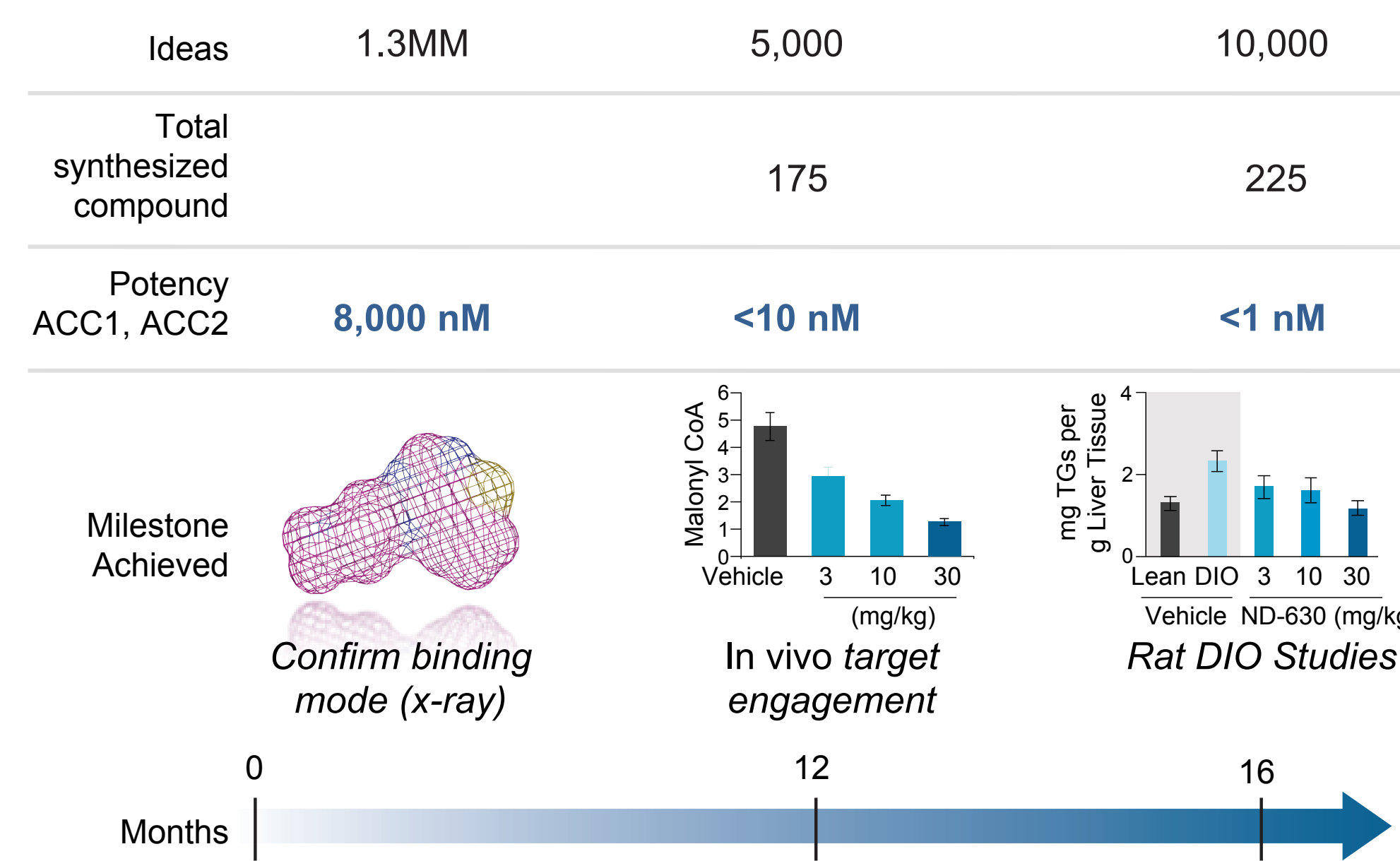
- Our efforts to discover hepatoselective ACC inhibitors have focused on interaction with the subunit dimerization site on the biotin carboxylase domain of the enzyme to which the phosphopeptide of AMP-activated protein kinase-phosphorylated ACC binds to prevent dimerization and to which the fungal metabolite Soraphen A interacts.
- Using state-of-the-art structure-based drug design and crystal structures of the human ACC2 biotin carboxylase domain, we have identified a unique series of hepatoselective allosteric inhibitors that bind to the dimerization site, inhibit the enzymatic activity of both ACC1 and ACC2, reduce FASyn and stimulate FAoxn in cultured cells, and exhibit acute and chronic *in vivo* efficacy.
- Herein we report on two of our ACC inhibitors, *liver selective* ND-630 (clinical candidate for NASH) and *liver specific* ND-654, experimental compound for HCC.

FIGURE 1: Acetyl CoA Carboxylase (ACC): Master Regulator of Fatty Acid Synthesis & Oxidation



- Beneficial effects on lipids, blood glucose, weight, potentially diabetes and CV risk
- Nimbus: **First** small molecule **allosteric** inhibitor successfully targeting BC domain

FIGURE 2: Nimbus Approach has Delivered ACC Inhibitors with Demonstrated *in Vivo* Efficacy in 16 Months



- A structure-based virtual screen of 1.3M lead-like molecules was performed utilizing Schrodinger's computational tools including WaterMap and Glide.
- In an iterative design fashion over the next 16 month period, the potency of this family of hits were improved 1000x utilizing the computational model focusing on the replacement or stabilization of high energy hydration sites.
- Simultaneous to the potency improvements, drug-like properties were optimized to deliver Development Candidate quality molecules.

FIGURE 3: Liver **Specific ND-654 has Favorable Drug-Like Properties, 2700:1 Liver to Muscle Exposure**

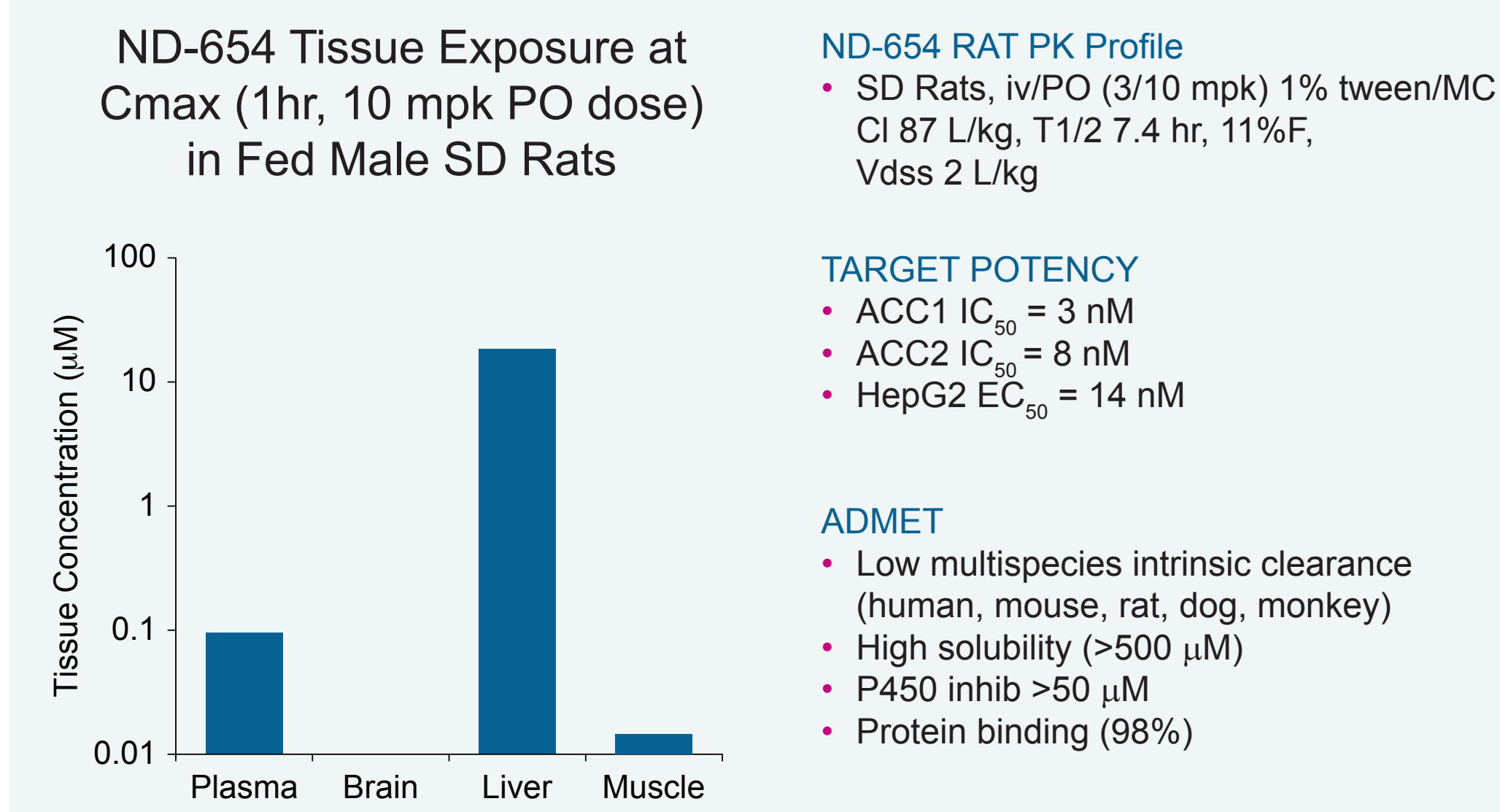
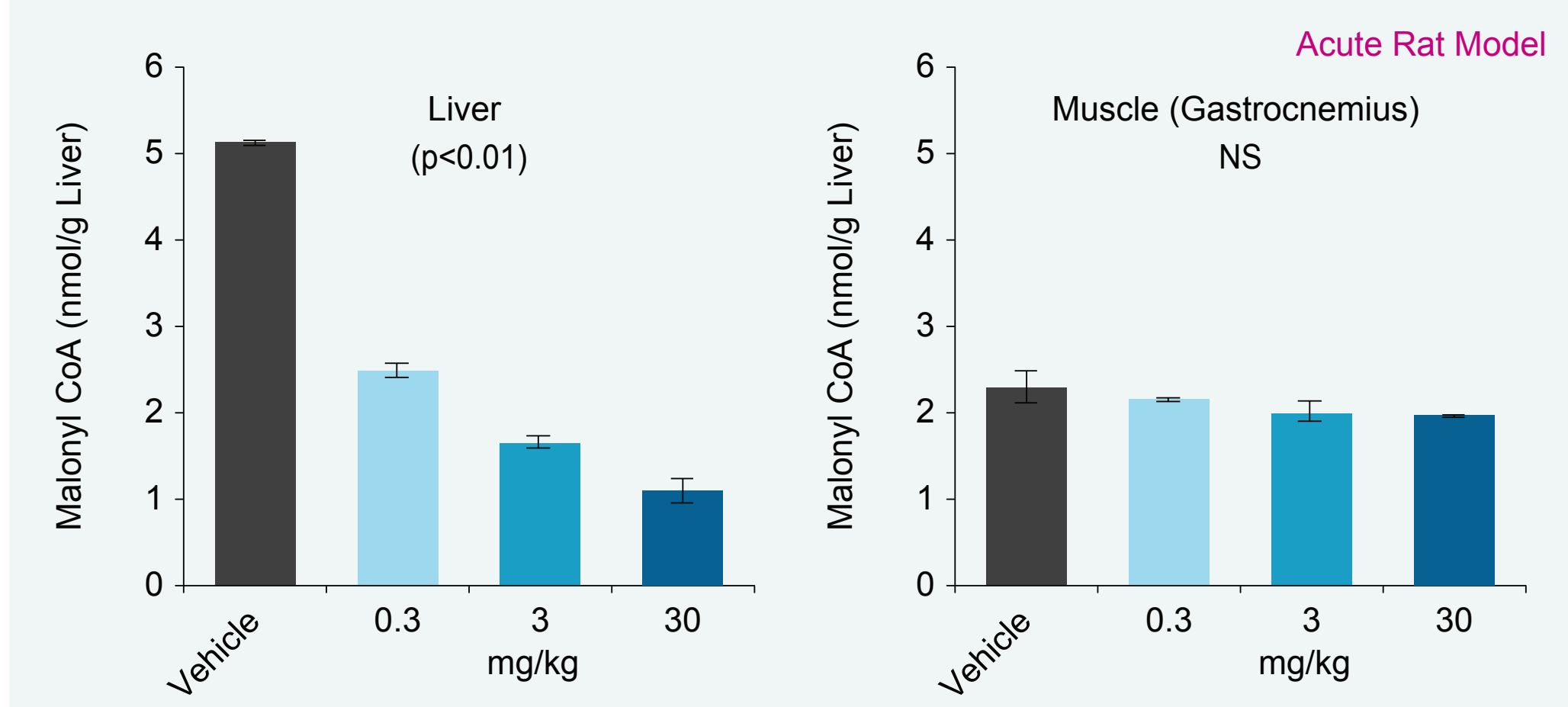


FIGURE 4: Proof-of-Mechanism: ND-654 Acutely Inhibits ACC Prevents Malonyl-CoA Production in Liver but Not Muscle



- Single oral dose of ND-654, measurements obtained after 1 hr.
- Liver has **higher ACC expression** and thus higher levels of malonyl-CoA
- **Efficacy in line with liver-specific biodistribution**; virtually no effect on muscle minimizes effects on non-target tissues

FIGURE 5: ND-654 *in Vivo* Proof of Concept: Target Engagement in the Liver and Muscle

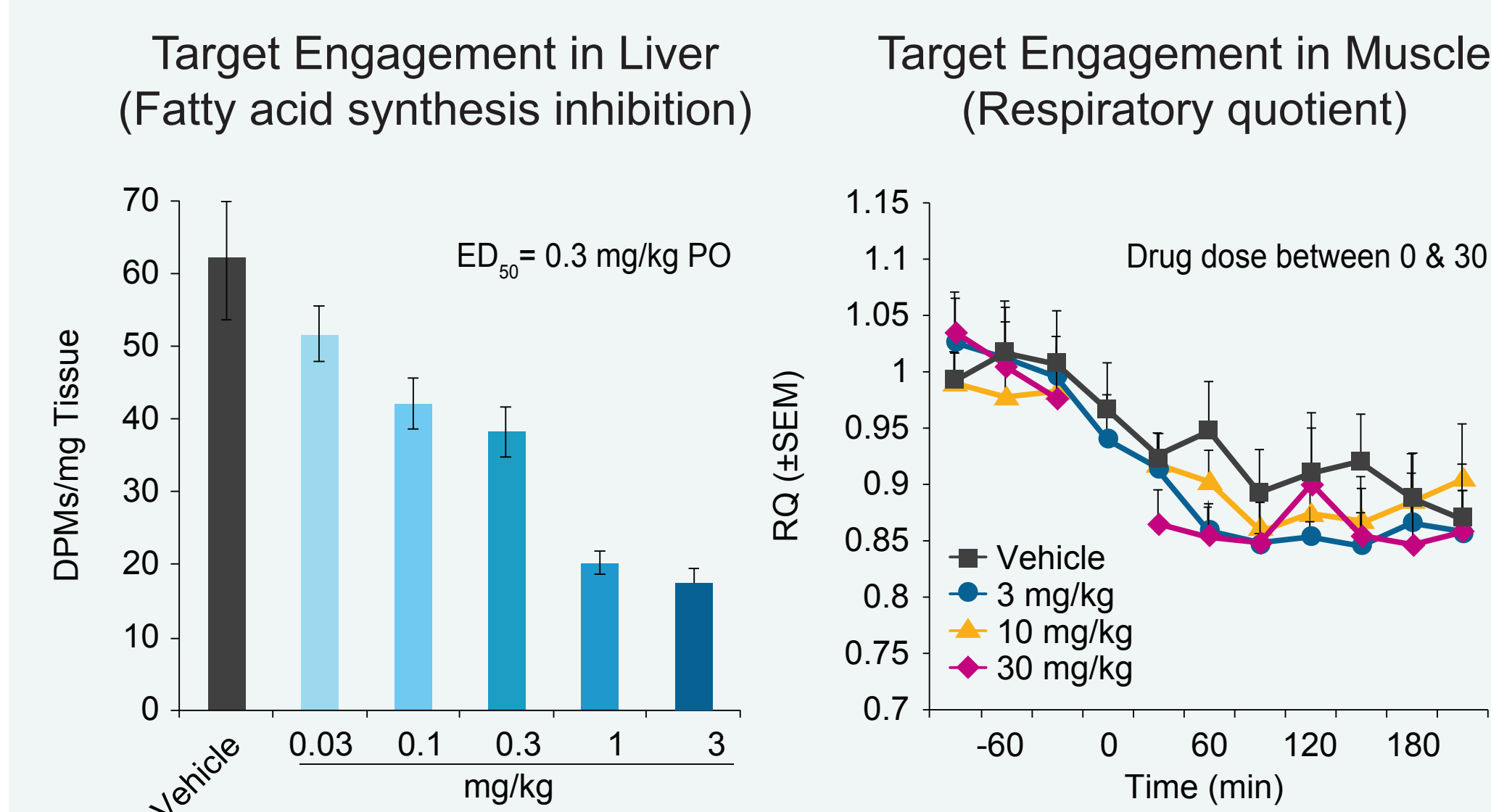


FIGURE 9: Consistent *in Vivo* Results for ND-630 Across Several Rat Models

Endpoints	14-day rat DIO study high fat diet with 4w diet run-in	28-day rat DIO study high sucrose diet with 4w diet run-in	ZDF rats with dosing from 7 weeks to 12 weeks of age
Body weight	↓	↓	-
Food intake	-	-	-
Hepatic triglycerides	↓	↓	↓
Hepatic cholesterol	↓	↓	↓
Glycemic Control (oGTT)	↑	↑	↑
HbA1c	nd	nd	↓
Plasma leptin	↓	↓	na
Plasma adiponectin	nd	↑	↑
Plasma triglycerides	-	↓	pd
Plasma free fatty acids	nd	↓	pd
Plasma cholesterol	-	↓	-
Plasma ketone bodies	nd	↑	↑
Plasma insulin	↓	-	-
Plasma glucose	-	-	-

*Animals decompensated more rapidly than anticipated so prevention of plasma glucose elevation could not be adequately demonstrated. nd = not determined; na = not applicable; pd = prevented decrease

FIGURE 6: Liver **Selective ND-630 has Favorable Drug-Like Properties, 100:1 Liver to Muscle Exposure**

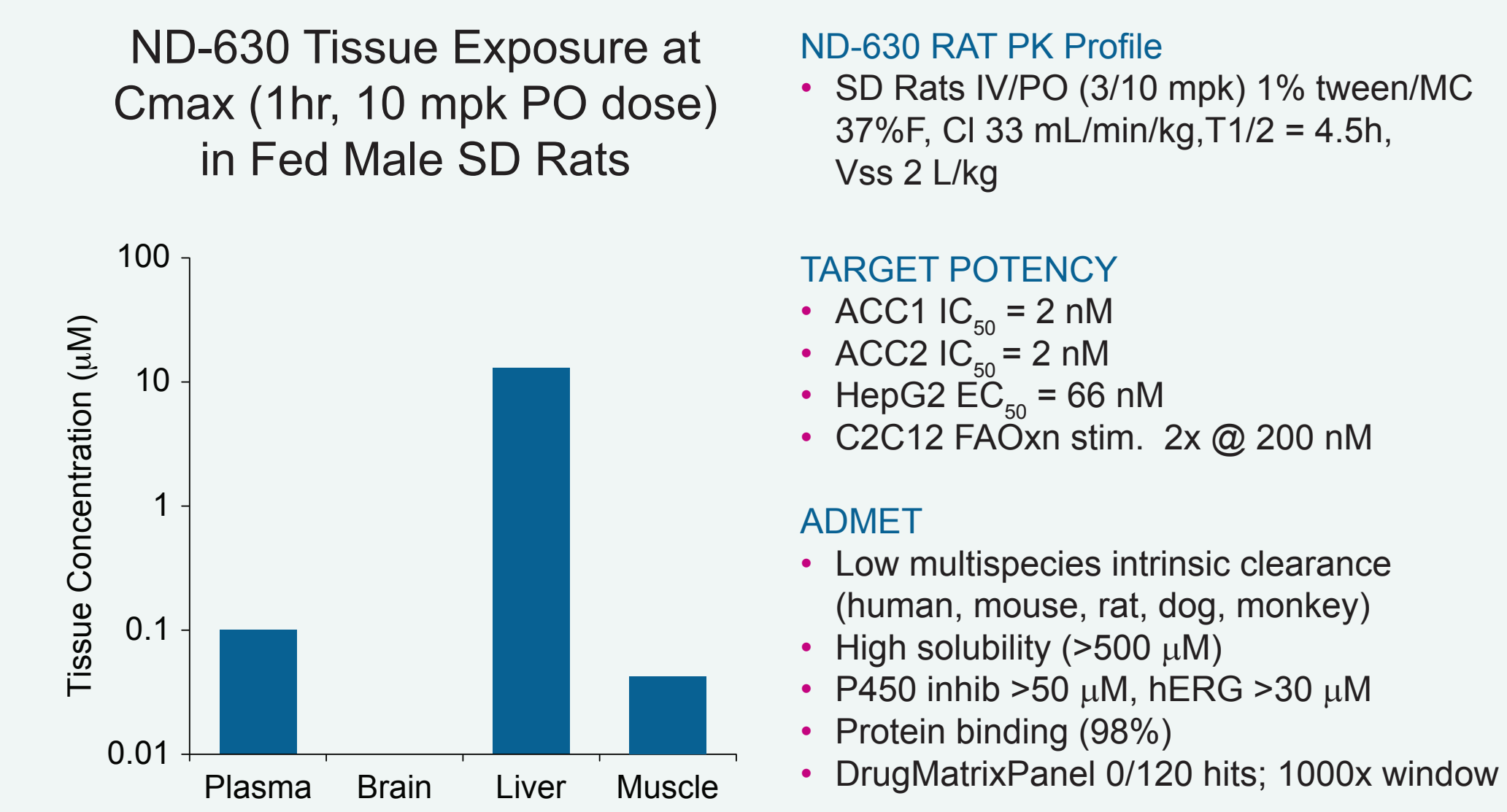
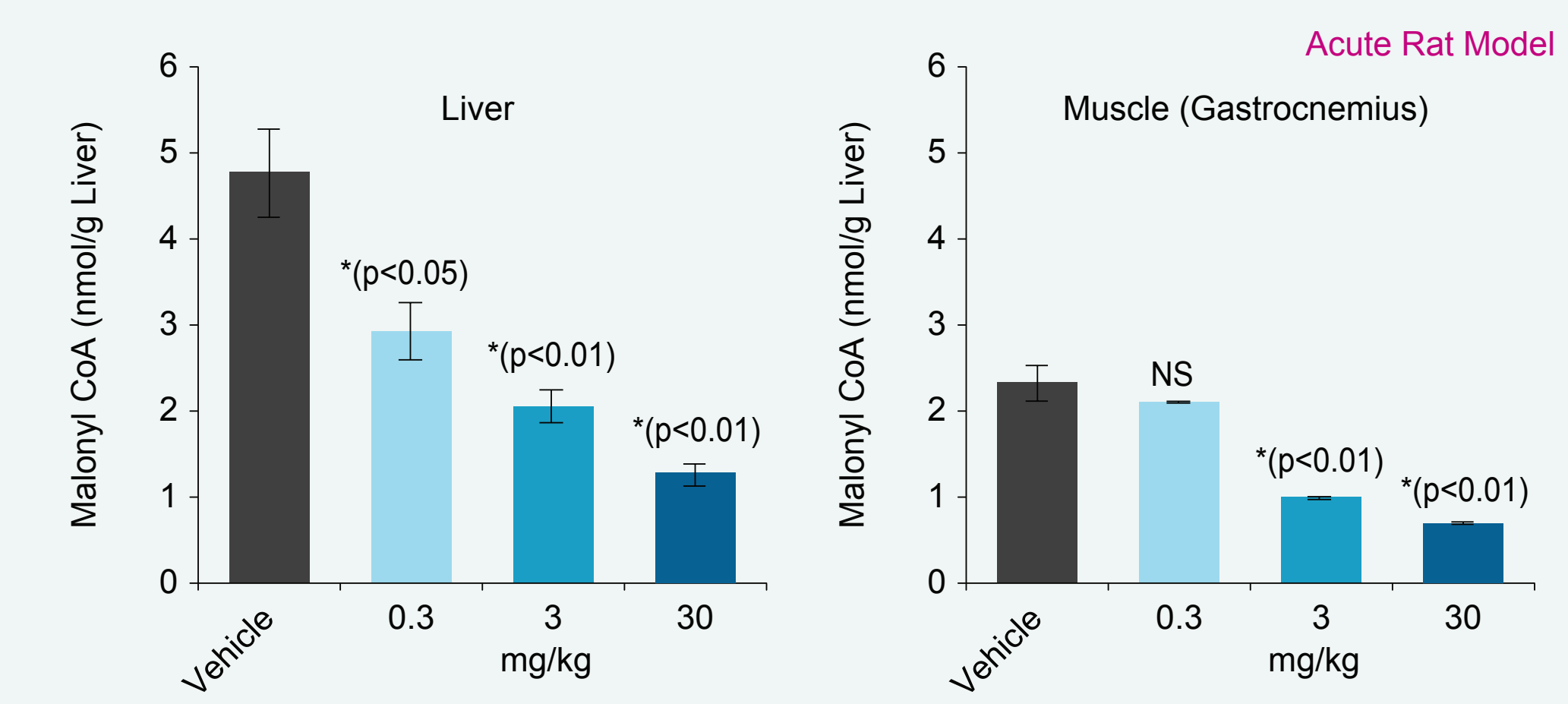


FIGURE 7: Proof-of-Mechanism: ND-630 Acutely Inhibits ACC Prevents Malonyl-CoA Production in Liver and Muscle



- Single oral dose of ND-630, measurements obtained after 1 hr.
- Liver has **higher ACC expression** and thus higher levels of malonyl-CoA
- Tissue expression of ACC in line with **liver-targeted ND-630 biodistribution**
- **Efficacy across both tissues**; perfectly aligned exposure for desired effect

FIGURE 8: ND-630 *in Vivo* Proof of Concept: Target Engagement in the Liver and Muscle

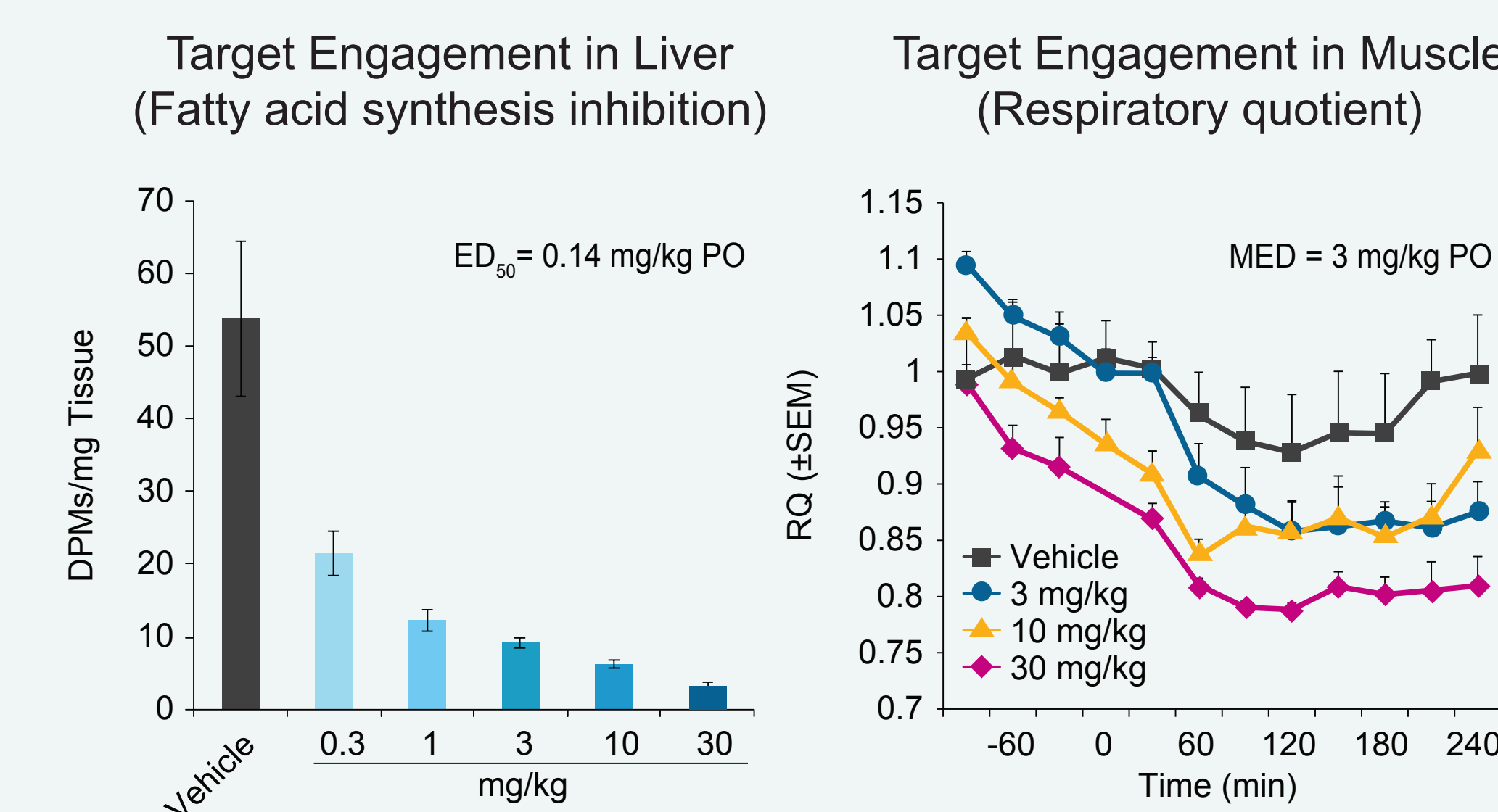


FIGURE 10: ND-630 Dosed High Sucrose Fed DIO Rats Show Improvement in Insulin Sensitivity in 28d Study

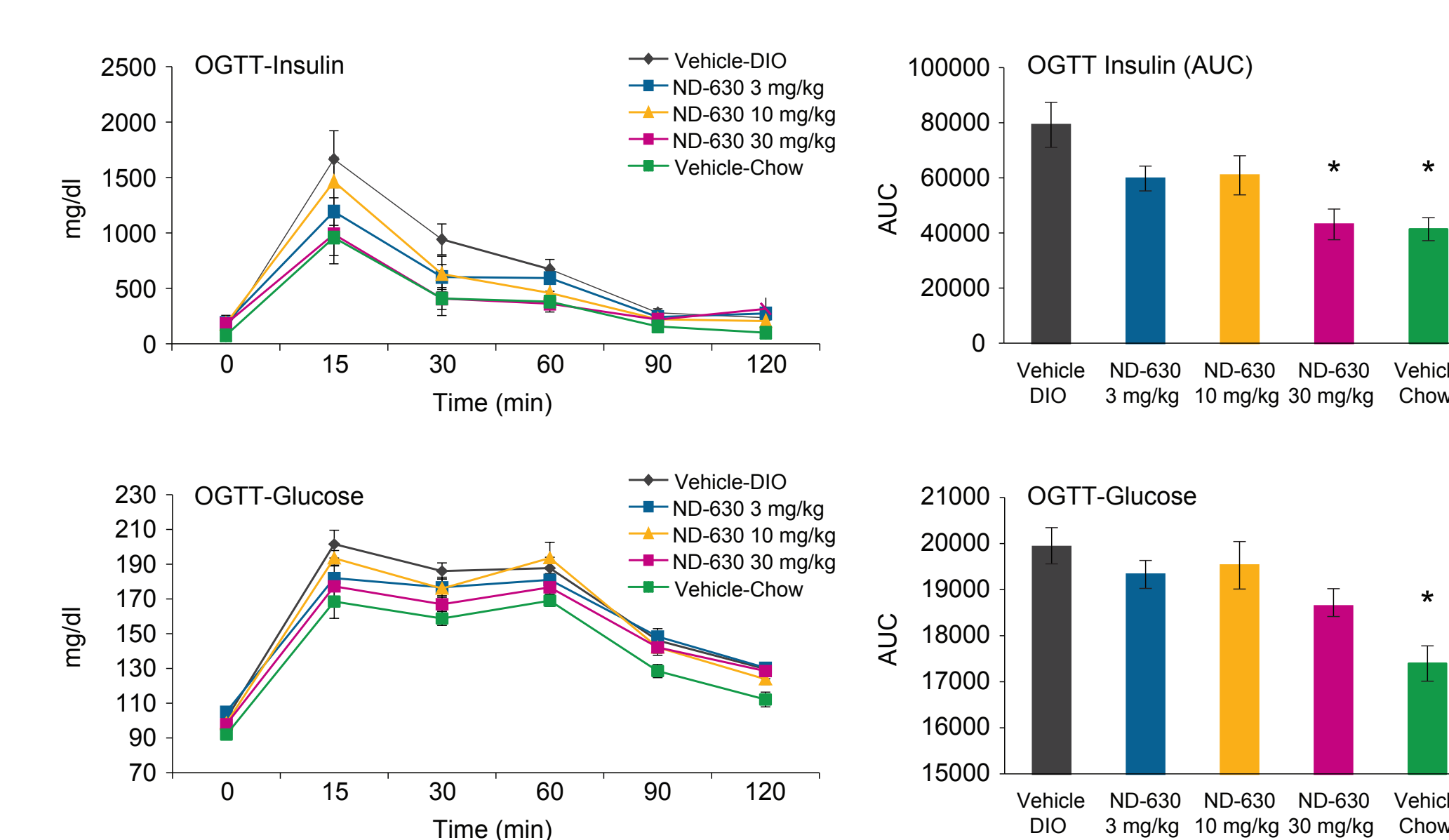


FIGURE 11: ND-630 Dosed High Sucrose Fed DIO Rats Show Improvement in Hepatic Cholesterol & Normalization of Hepatic Triglycerides in 28d Study

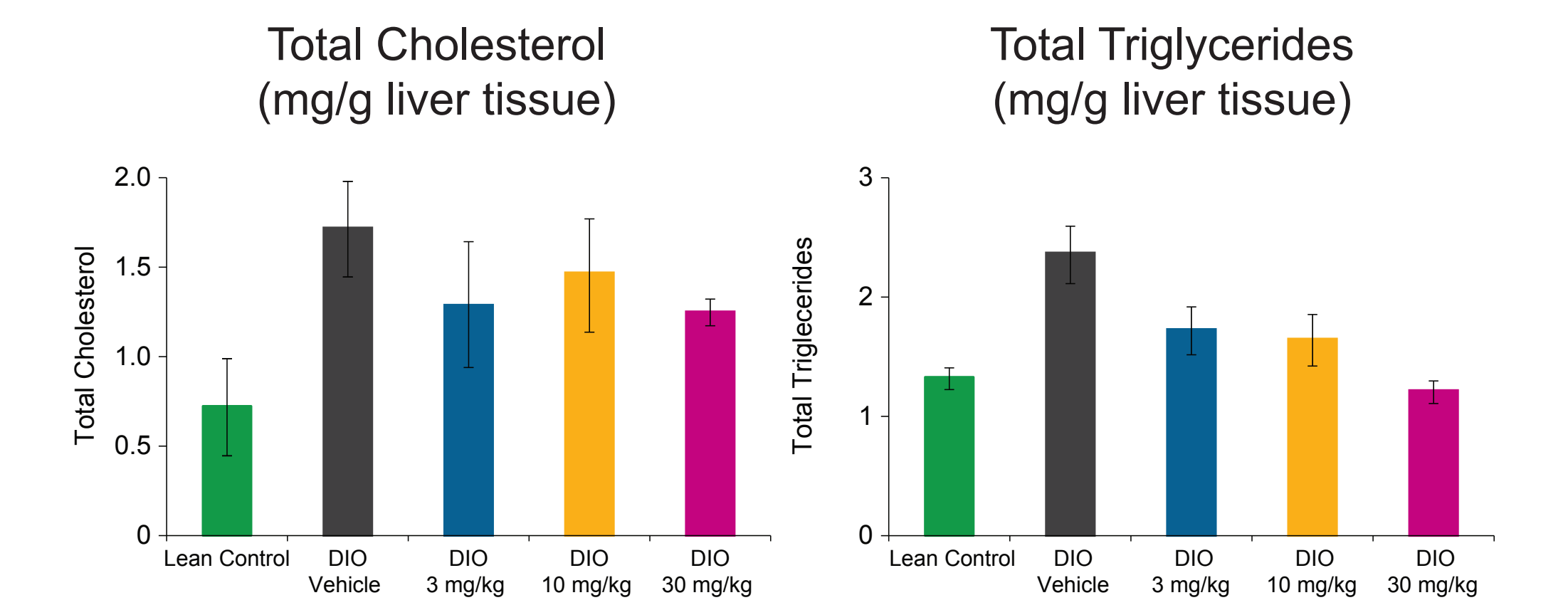


FIGURE 12: ND-630 Dosed High Sucrose Fed DIO Rats Show Dose Dependent Decrease of Plasma Triglycerides & FFAs

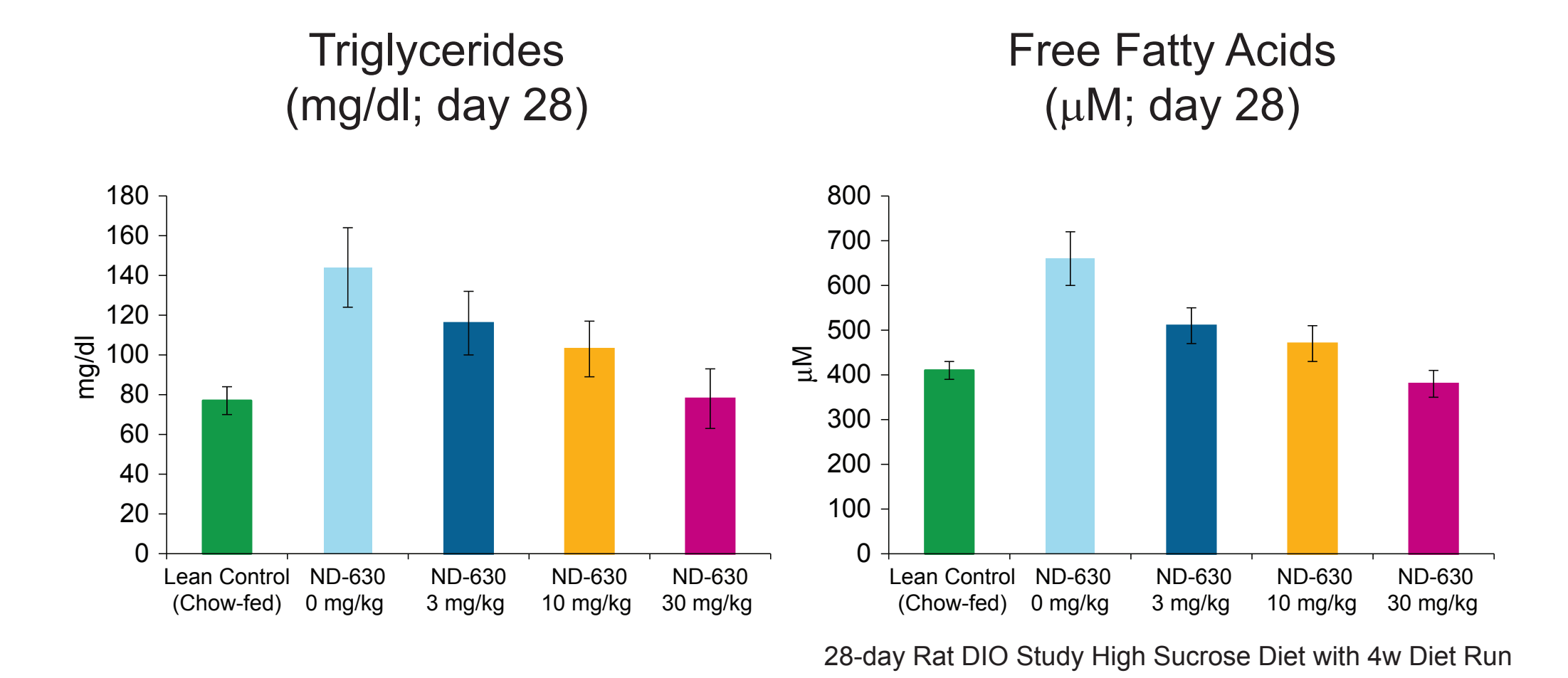


FIGURE 13: ND-630 Dosed High Sucrose Fed DIO Rats Show Decrease in Plasma Cholesterol

Dose	Plasma Cholesterol (mg/dl)				
	Prebleed*	Day 7*	Day 14*	Day 20**	Day 28**
0 mg/kg	100 ± 3	106 ± 3	110 ± 6	118 ± 5	107 ± 4
3 mg/kg	96 ± 2	94 ± 3	96 ± 3	101 ± 4	102 ± 4
10 mg/kg	96 ± 3	82 ± 3	87 ± 3	86 ± 4	91 ± 3
30 mg/kg	99 ± 3	78 ± 3	78 ± 2	74 ± 3	90 ± 3
Chow-fed Controls	109 ± 2	107 ± 3	107 ± 3	111 ± 6	103 ± 3

* Data are mean values for n=14 animals per group ± SEM

** Data are mean values for n=8 animals per group ± SEM

- ND-630 reduces both plasma cholesterol and plasma triglycerides

SUMMARY

- A unique series of allosteric inhibitors that bind to the BC domain of ACC were successfully optimized for excellent potency, drug-like properties and *in vivo* efficacy in 16 months

- Both ND-630 and ND-654 demonstrated desirable *in vitro* and *in vivo* efficacy in experimental models of metabolic disease

ONGOING STUDIES

- ND-630 is in pre-clinical development and **will enter the Clinic** in early 2015 for NASH and Diabetes
- ND-654 is being evaluated in a rat DEN model of **hepatocellular carcinoma (HCC)** and this data will be presented at AACR

