Online supplement to Dikkers et al.

"Differential Impact of Hepatic Deficiency and Total Body Inhibition of Microsomal Triglyceride Transfer Protein on Cholesterol Metabolism and Reverse Cholesterol Transport in Mice"

Supplemental Materials and Methods

VLDL production studies

VLDL production was essentially determined as described before (1). Mice were fasted for 4 hours, injected i.p. with poloxamer 407 (1000 mg/kg body weight) and blood samples were drawn at 0, 30, 60 and 180 minutes after injection. Plasma triglycerides were measured enzymatically (Diasys, Holzheim, Germany) and the triglyceride production rate was calculated as described (1).

Chylomicron production studies

Mice were fasted for 4 hours and then injected i.p. with poloxamer 407 (1000 mg/kg body weight). Immediately, 150 µl olive oil containing 2 µCi ^{3}[H]triolein was administered via gavage and a blood sample was drawn at 3 hours. Counts in plasma were assessed directly by liquid scintillation counting (Packard 1600CA Tri-carb, Packard, Meriden, CT). Plasma triglycerides were measured enzymatically (Diasys, Holzheim, Germany).

MTP activity measurements

MTP activity was measured in liver and proximal small intestine using a fluorigenic assay essentially as described before (2).

Western blotting.

Western blots for MTP were carried out as described before on total liver and proximal small intestine homogenates (3). The bicinchoninic acid assay (Pierce Biotechnology, Inc., Rockford, IL) was used to determine protein concentrations. 50 µg protein was resolved by SDS-PAGE electrophoresis and blotted onto nitrocellulose. MTP was visualized using a mouse MTP antibody (BD Biosciences, Franklin Lakes, NJ), followed by a HRP-conjugated secondary antibody. HRP was detected using chemiluminescence (ECL, GE Healthcare, Piscataway, NJ). Densitometry analysis of the bands was performed on Western blots using ImageJ software (National Institutes of Health, Bethesda, MD).

Supplemental figure legends

Supplemental figure I: Impact of hepatocyte-specific MTP deficiency (L-*Mttp*$^{-/-}$) and pharmacological MTP inhibition on MTP protein expression as well as MTP activity in liver and small intestine.

L-*Mttp*$^{-/-}$ and MTP inhibitor-treated mice were fasted for 4 hours before liver and proximal small intestine were excised and stored at -80°C until analysis. MTP activity

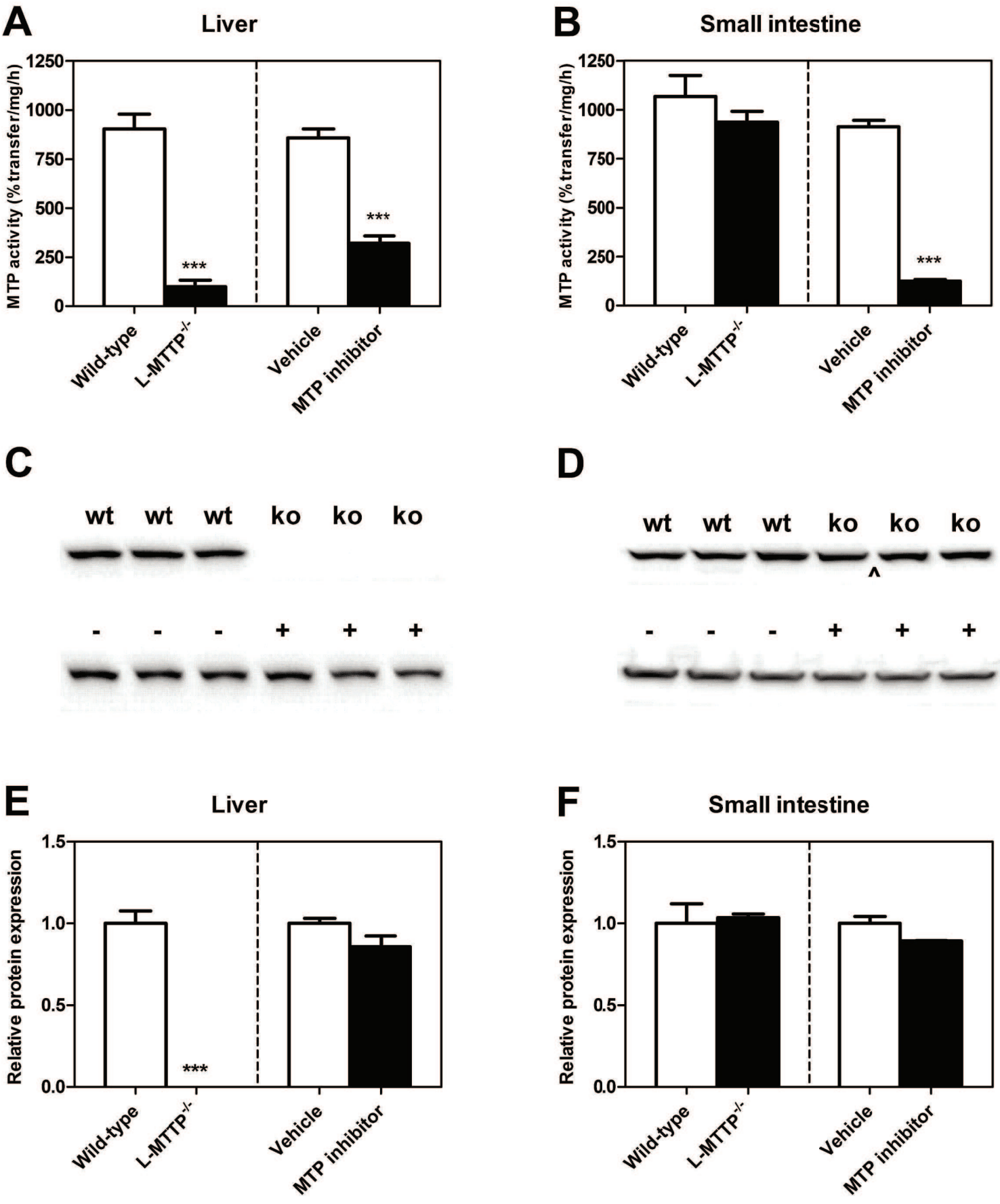
and protein expression were determined as detailed in Supplemental Materials and Methods. (A) MTP activity in liver. (B) MTP activity in small intestine. (C) MTP protein expression and (E) quantification in liver. (D) MTP protein expression and (F) quantification in small intestine. Data are presented as means ± SEM. At least n = 6 for each group (A and B), n = 3 for each group (C-F). For Western blot (C and D) wild-type control, L-*Mttp*$^{-/-}$, vehicle treated and pharmacologically treated mice are indicated as wt, ko, - and + respectively. ^ indicates were the marker was cut out. Statistically significant differences from the control group are indicated as ***p<0.001.

Supplemental figure II: Impact of pharmacological MTP inhibition on VLDL and chylomicron production.

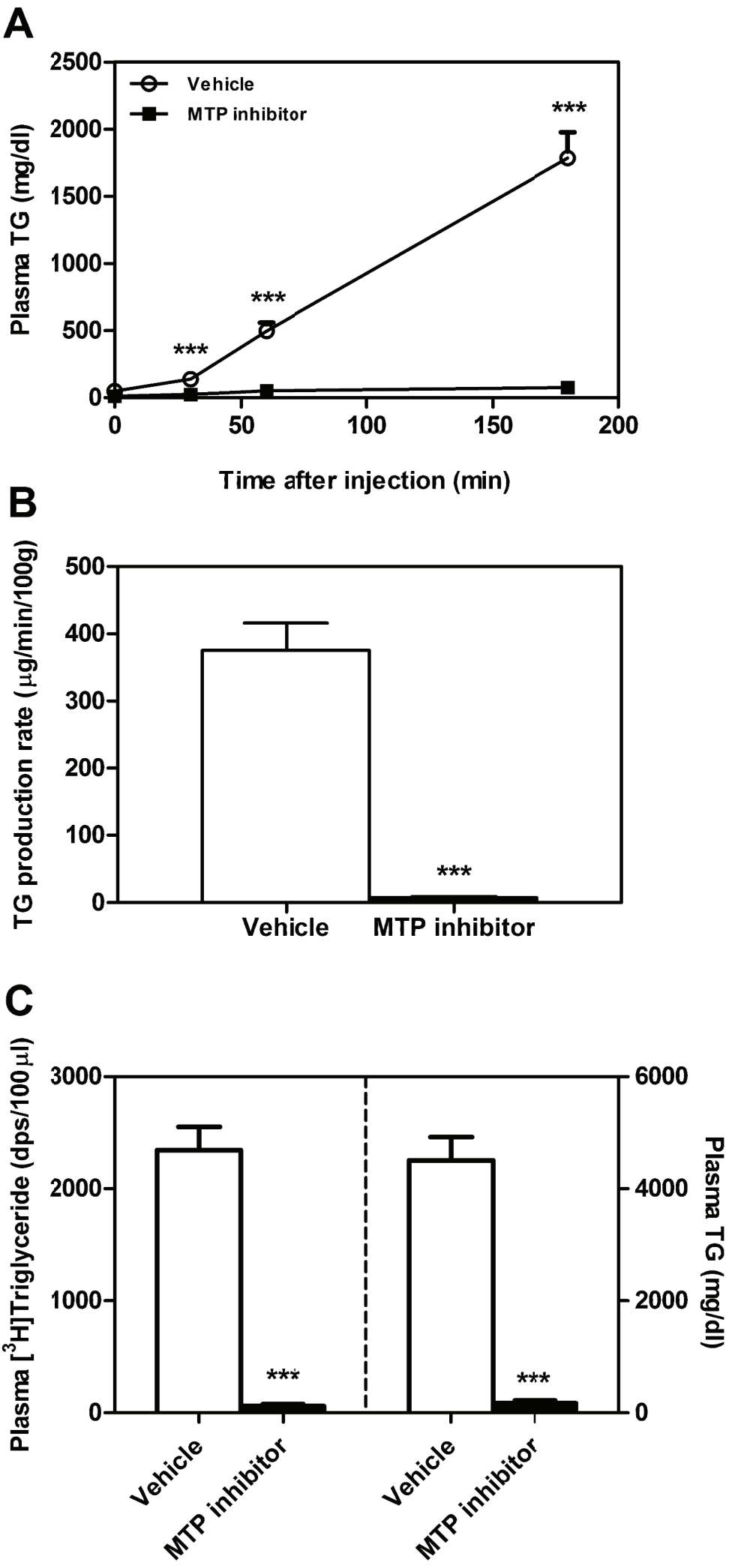
MTP inhibitor-treated mice were fasted for 4 hours and poloxamer 407 was injected i.p. (A) plasma triglyceride levels at 0, 30, 60 and 180 minutes after poloxamer 407 administration. (B) calculated triglyceride production rates. To determine chylomicron production mice were fasted for 4 hours, poloxamer 407 was injected i.p. and 150 µl olive oil containing 2 µCi ^{3}[H]triolein was administered via gavage. (C) Plasma ^{3}[H]triglyceride tracer recovery and plasma triglyceride concentrations 3 hours after gavage. Data are presented as means ± SEM. At least n = 6 for each group. Statistically significant differences from the control group are indicated as *p<0.05, ***p<0.001.

Supplemental references

1. Wiersma, H., N. Nijstad, T. Gautier, J. Iqbal, F. Kuipers, M. M. Hussain, and U. J. Tietge. 2009. Scavenger receptor BI (SR-BI) facilitates hepatic very low density lipoprotein (VLDL) production in mice. *J Lipid Res* **51**: 544-553.
2. Athar, H., J. Iqbal, X. C. Jiang, and M. M. Hussain. 2004. A simple, rapid, and sensitive fluorescence assay for microsomal triglyceride transfer protein. *J Lipid Res* **45**: 764-772.
3. Dikkers, A., J. F. de Boer, W. Annema, A. K. Groen, and U. J. Tietge. 2013. Scavenger receptor BI and ABCG5/G8 differentially impact biliary sterol secretion and reverse cholesterol transport in mice. *Hepatology* **58**: 293-303.



Supplemental figure I



Supplemental figure II