**Supplementary Table 1.** Mechanisms Associated with Metformin and Lipogenesis Reduction: Evidence from *In Vitro* Reports

| Author (year) | Cell types | Model | Metformin dose | Time (min) | Effects | Interpretations |
|---|---|---|---|---|---|---|
| Fullerton *et al.* (2013)[1] | C57bl/6 mice primary hepatocytes | Palmitate (0.5 mmol)/ 18 hours | 0.5 mmol | 1,080 | ↓ Lipogenesis | Metformin decreased *de novo* hepatic lipogenesis. |
| Song *et al.* (2015)[2] | HepG2 cell | Oleic acid (1 mmol) and glucose (30 mmol)/8 hours | 0.5 mmol<br>0.1, 0.5, 1.0 mmol | 120<br>120 | ↓ Lipid accumulation<br>↑ SIRT1 activity and expression<br>↑ Autophagy<br>↑ P-ACC | Metformin restored SIRT1-mediated autophagy. |
| | C57bl/6 mice primary hepatocytes | Oleic acid (2 mmol) and glucose (30 mmol)/8 hours | 0.5 mmol<br>0.1, 0.5, 1.0 mmol | 120<br>120 | ↓ Lipid accumulation<br>↑ SIRT1 activity and expression<br>↑ Autophagy<br>↑ P-ACC | |
| Ford *et al.* (2015)[3] | C57bl/6 mice primary hepatocytes | Mice fed with HFD | Various doses<br>0.1 mmol | 240<br>120 | ↓ Lipogenesis (dose-dependent)<br>↔ Lipogenesis<br>↔ P-ACC<br>↔ Fatty acid oxidation | Metformin treatment resulted in dose-dependent lipogenesis inhibition. |
| | Human primary hepatocytes | Palmitate (200 μmol), L-carnitine (500 μmol) | Various doses<br>0.1 mmol | 60<br>120 | ↓ Lipogenesis (dose-dependent)<br>↓ Lipogenesis<br>↔ P-ACC/ACC | |
| Huang *et al.* (2018)[4] | C57bl/6 mice primary hepatocytes | | 0.5, 1, 2, 5 mmol | 180–360 | ↓ Lipogenic rates (1, 2, 5 mmol)<br>↓ ROCK1 activity (2, 5 mmol)<br>↑ AMPK activity (2, 5 mmol)<br>↓ FAS, SCD1, ACC, SREBP-1c (dose not specified) | Metformin suppressed ROCK1 activity, resulting in AMPK activation and decreased lipogenic rates. |
| | L-ROCK1$^{-/-}$ primary hepatocytes | | | 360 | ↔ AMPK activity<br>↔ FAS, SCD1, ACC, SREBP-1c (dose not specified) | |
| Li *et al.* (2019)[5] | AML12 mouse hepatocytes | MCD medium/ 24 hours | 5, 10, 20, mmol<br><br>10, 20 mmol<br>20 mmol | 120 | ↓ STAT3 protein and mRNA expression (dose-dependent)<br>↑ Autophagy (dose-dependent)<br>↓ IL-1β, IL-6, TNF-α | Metformin inhibited STAT3 mRNA and protein expression, resulting in autophagy restoration and alleviation of inflammation. |
| Geng *et al.* (2019)[6] | Male Wistar rats hepatocytes | Palmitate (1.0 mmol)/ 12–16 hours | 1.0 mmol | 30 | ↔ Intracellular TG<br>↓ Necrotic cell death<br>↔ Mitochondrial respiratory chain complex I | Metformin protected against palmitate induced necrotic and apoptotic cell death and decreased oxidative stress. |
| | HepG2 cells | Palmitate (0.5 mmol)/ 12–16 hours | 1.0 mmol | 30 | ↔ Intracellular TG<br>↓ Necrotic cell death<br>↓ Apoptotic cell death<br>↓ ROS generation<br>↑ SOD2 mRNA expression<br>↑ Mitochondrial respiratory chain complex I | |

ACC, acetyl-CoA carboxylase; AML12, alpha mouse liver 12 hepatocyte; ER, endoplasmic reticulum; FAS, fatty acid synthase; HFD, high fat diet; IL, interleukin; L-ROCK1$^{-/-}$, liver-specific ROCK-1 deficient mice; MCD, methionine-and choline-deficient diet; P-ACC, phosphorylation of acetyl-CoA carboxylase; ROCK1, Rho-kinase 1; ROS, reactive oxygen species; SCD1, stearoyl-CoA desaturase-1; SIRT1, sirtuin 1; SOD2, superoxide dismutase2; SREBP-1c, Sterol regulatory element-binding protein 1; STAT3, signal transducer and activator of transcription 3; TG, triglyceride content; TNF-α, tumor necrosis factor α; ↓, significant decrease; ↑, significant increase; ↔, no significant change.

**Supplementary Table 2.** Other Proposed Mechanisms of Metformin: Evidence from *In Vivo* Reports

| Reference | Model (age) | Method | Metformin (dose/route/duration) | Effects of metformin | Interpretations |
|---|---|---|---|---|---|
| Karavia *et al.* (2015)[7] | Male C57bl/6j mice (10–12 weeks old) | - Western diet<br>- *Apoa1*$^{-/-}$ | - 300 mg/kg/day/PO/18 weeks<br>- 300 mg/kg/day/PO/18 weeks | ↓ Hepatic TG<br>↓ Histologic steatosis<br>↔ Hepatic TG<br>↔ Histologic steatosis | ApoA-I deficiency blunted the beneficial effect of metformin on hepatic lipid content. |
| Shin *et al.* (2017)[8] | Male OLETF and LETO rats (6 weeks old) | - OLETF rats | - 100 mg/kg/day/PO/12 weeks | ↓ Hepatic TG<br>↔ Hepatic TC<br>↓ Fecal endotoxin, MDA | Metformin reduced fecal endotoxin contents. |
| Brandt *et al.* (2019)[9] | Female C57b1/6j mice (6–8 weeks old) | - Fat-fructose-and cholesterol-rich diet | - 300 mg/kg/day/PO/4 days<br>- 300 mg/kg/day/PO/6 weeks | ↓ Hepatic TG<br>↓ Liver iNOS, 4NHE<br>↑ Occludin in prox. SB<br>↔ MMP13 in prox. SB<br>↔ Hepatic TG<br>↓ Liver iNOS, 4NHE<br>↔ Occludin in prox. SB<br>↓ MMP13 in prox. SB | Metformin treatment reversed the alteration of tight junction proteins in the proximal small intestine and the markers associated with bacterial endotoxins. |
| Stachowicz *et al.* (2012)[10] | Female C57BL/6j mice (8 weeks old) | - apoE$^{-/-}$ | - 10 mg/kg/day/PO/16 weeks | 3.2-fold ↑ GNMT | Metformin up-regulated GNMT. |
| Guo *et al.* (2018)[11] | Male C57bl/6j mice (3 weeks old) | - HFD | - 3 mg/kg/day/PO/5 weeks<br>- Starting after HFD for 12 weeks | ↓ Hepatic TG, TC<br>↓ SERPINA 12, INSIG1<br>↑ FABP2 | Metformin alters the gene expression related to diabetes, obesity and fatty liver phenotypes. |

4HNE, 4-hydroxynonenal; ApoA-1, apolipoprotein A-I; *Apoa1*$^{-/-}$, ApoA-I knock-out mice; apoE$^{-/-}$, apolipoprotein E knock-out mutation; GNMT, glycine N-methyltransferase; HFD, high fat diet; iNOS, inducible nitric oxide synthase; LETO, Long-Evans Tokushima Otsuka rats; MDA, malondialdehyde; MMP13, matrix-metalloproteinase 13; OLETF, Otsuka Long-Evans Tokushima Fatty rat; PO, per oral; prox. SB, proximal small bowel; TC, total cholesterol contents; TG, triglyceride content; WT, wild type; ↓, significant decrease; ↑, significant increase; ↔, no significant change.

## REFERENCES


1. Fullerton MD, Galic S, Marcinko K, et al. Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. Nat Med 2013;19:1649-1654.
2. Song YM, Lee YH, Kim JW, et al. Metformin alleviates hepatosteatosis by restoring SIRT1-mediated autophagy induction via an AMP-activated protein kinase-independent pathway. Autophagy 2015;11:46-59.
3. Ford RJ, Fullerton MD, Pinkosky SL, et al. Metformin and salicylate synergistically activate liver AMPK, inhibit lipogenesis and improve insulin sensitivity. Biochem J 2015;468:125-132.
4. Huang H, Lee SH, Sousa-Lima I, et al. Rho-kinase/AMPK axis regulates hepatic lipogenesis during overnutrition. J Clin Invest 2018;128:5335-5350.
5. Li YL, Li XQ, Wang YD, Shen C, Zhao CY. Metformin alleviates inflammatory response in non-alcoholic steatohepatitis by restraining signal transducer and activator of transcription 3-mediated autophagy inhibition in vitro and in vivo. Biochem Biophys Res Commun 2019;513:64-72.
6. Geng Y, Hernández Villanueva A, Oun A, et al. Protective effect of metformin against palmitate-induced hepatic cell death. Biochim Biophys Acta Mol Basis Dis 2020;1866:165621.
7. Karavia EA, Hatziri A, Kalogeropoulou C, et al. Deficiency in apolipoprotein A-I ablates the pharmacological effects of metformin on plasma glucose homeostasis and hepatic lipid deposition. Eur J Pharmacol 2015;766:76-85.
8. Shin NR, Bose S, Wang JH, et al. Flos Lonicera combined with metformin ameliorates hepatosteatosis and glucose intolerance in association with gut microbiota modulation. Front Microbiol 2017;8:2271.
9. Brandt A, Hernández-Arriaga A, Kehm R, et al. Metformin attenuates the onset of non-alcoholic fatty liver disease and affects intestinal microbiota and barrier in small intestine. Sci Rep 2019;9:6668.
10. Stachowicz A, Suski M, Olszanecki R, Madej J, Okoń K, Korbut R. Proteomic analysis of liver mitochondria of apolipoprotein E knockout mice treated with metformin. J Proteomics 2012;77:167-175.
11. Guo J, Zhou Y, Cheng Y, et al. Metformin-induced changes of the coding transcriptome and non-coding RNAs in the livers of non-alcoholic fatty liver disease mice. Cell Physiol Biochem 2018;45:1487-1505.