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# Empagliflozin moderately increases LDL-cholesterol levels through reduced LDL catabolism while it increases slightly reverse cholesterol transport in hamsters

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THE METABOLIC DISEASES EXPERTS

## OBJECTIVES

Chronic treatment with SGLT2 inhibitors may induce moderate increase in plasma LDL-cholesterol levels. This effect could result from heamo-concentration in association with glucosuria and osmotic diuresis or from a shift from carbohydrate to lipid metabolism with increased ketone bodies production. Here we investigated some potential mechanisms in hamster, a preclinical model with a human-like cholesterol metabolism.

## METHODS

6-week old Golden Syrian hamsters were made dyslipidemic with a 4-week high fat/high cholesterol diet with 10% fructose in drinking water.
After 2 weeks of diet, hamsters were randomized into two treatment groups according to their LDL-cholesterol and blood glucose levels.
Hamsters were then treated for 2 weeks orally, once daily with vehicle or empagliflozin at 30mg/kg, a dose inducing a 1200-fold increase in urine glucose excretion in this preclinical model.
At the end of treatment, a first set of hamsters was used to measure blood, plasma and liver biochemical parameters in fed or overnight fasting conditions. A second set of hamsters underwent radiotracer-based in vivo experiments to assess either intestinal cholesterol absorption, LDL-cholesterol metabolism or macrophage-to-feces reverse cholesterol transport.
Data are expressed as mean ± SEM. Student t-test or 2-way ANOVA+Bonferroni post test were used for statistics.

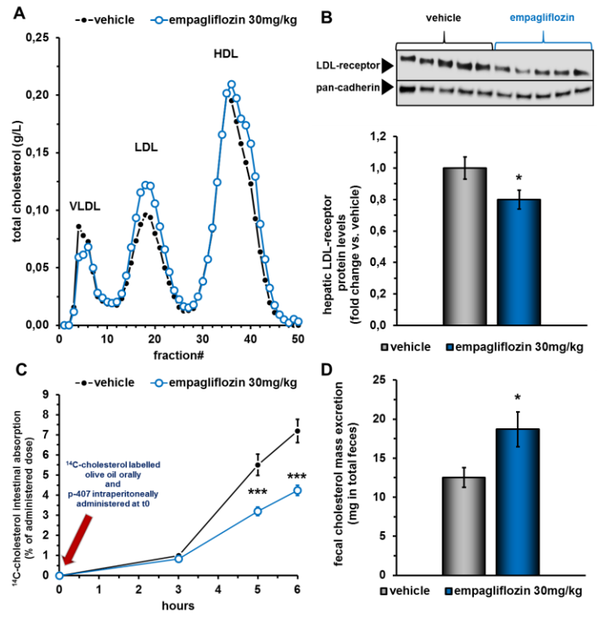
## RESULTS

### 1. Empagliflozin raises plasma LDL-cholesterol and hepatic cholesterol levels in overnight fasting, but not fed, conditions.

| | fed conditions | | overnight fasting conditions | |
|---|---|---|---|---|
| parameters | vehicle | empagliflozin 30mg/kg | vehicle | empagliflozin 30mg/kg |
| body weight (g) | 110 ± 2 | 114 ± 2 | 110 ± 2 | 111 ± 1 |
| hematocrit (%) | 49.8 ± 0.7 | 47.9 ± 0.6* | 48.3 ± 0.5 | 49.4 ± 0.6 |
| plasma total protein (g/L) | 81.2 ± 1.8 | 81.9 ± 1.8 | 79.6 ± 2.5 | 76.0 ± 1.0 |
| blood glucose (mg/dL) | 86.0 ± 5.5 | 88.6 ± 2.6 | 73.4 ± 4.0 | 59.9 ± 2.5* |
| Plasma total cholesterol (g/L) | 4.0 ± 0.2 | 4.0 ± 0.2 | 3.0 ± 0.1 | 2.9 ± 0.2 |
| Plasma LDL-cholesterol (g/L) | 1.8 ± 0.1 | 1.6 ± 0.1 | 1.2 ± 0.1 | 1.5 ± 0.1* |
| Plasma ketone bodies (µM) | 773 ± 76 | 909 ± 124 | 3094 ± 171 | 6685 ± 510*** |
| Liver weight (g) | 5.61 ± 0.13 | 6.04 ± 0.13* | 4.90 ± 0.13 | 4.75 ± 0.06 |
| Hepatic triglycerides (mg/g liver) | 15.1 ± 0.9 | 16.9 ± 0.1 | 16.6 ± 1.3 | 15.3 ± 0.7 |
| Hepatic cholesterol (mg/g liver) | 38.9 ± 0.8 | 40.2 ± 1.7 | 43.1 ± 1.9 | 47.7 ± 1.1* |
| Hepatic fatty acids (µmol/g liver) | 362 ± 9 | 352 ± 12 | 386 ± 11 | 418 ± 8* |
| Hepatic ketone bodies (µmol/g liver) | 12.4 ± 0.5 | 12.1 ± 0.5 | 14.7 ± 0.6 | 16.8 ± 0.8# |
| Hepatic pyruvate (µmol/g liver) | 6.2 ± 0.5 | 6.4 ± 0.3 | 6.7 ± 0.4 | 8.0 ± 0.3* |
| Hepatic glycogen (mg/g liver) | 39.1 ± 3.9 | 37.3 ± 2.2 | 4.31 ± 0.64 | 0.7 ± 0.4*** |
| Hepatic HMGCoA reductase activity (mU/mg protein) | 0.302 ± 0.034 | 0.357 ± 0.040 | 0.255 ± 0.019 | 0.334 ± 0.028* |

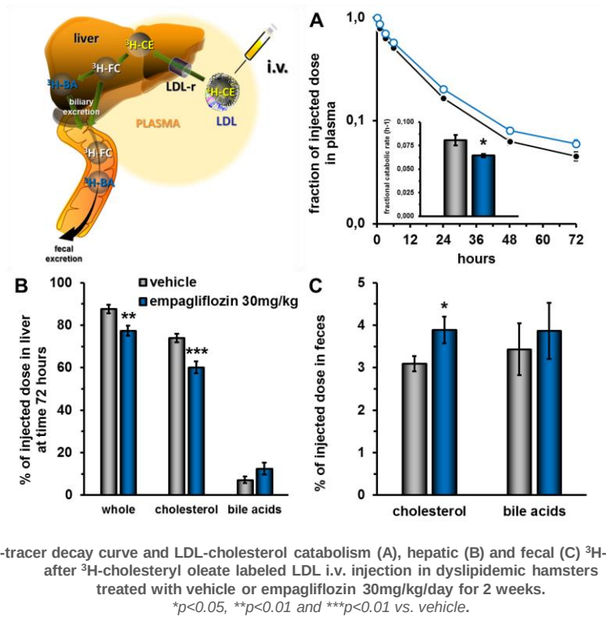
Body weight and biochemical parameters in fed or overnight fasted dyslipidemic hamsters treated with vehicle or empagliflozin 30mg/kg/day for 2 weeks.
**p<0.05 and ***p<0.01 vs. respective vehicle.*
*#p=0.054 vs. respective vehicle.*

### 2. Empagliflozin alters lipoprotein profile with concomitant reduction in hepatic LDL-receptor expression, lower intestinal cholesterol absorption and higher fecal cholesterol mass excretion.



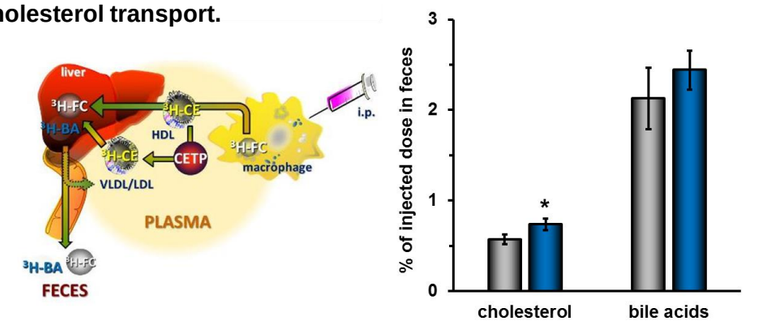

FPLC total cholesterol lipoprotein profile (A), hepatic LDL-receptor expression (B), intestinal cholesterol absorption (C) and fecal cholesterol mass excretion (D) in dyslipidemic hamsters treated with vehicle or empagliflozin 30mg/kg/day for 2 weeks.
**p<0.05 and ***p<0.01 vs. vehicle.*

### 3. Empagliflozin raises LDL-cholesterol levels through lower LDL-cholesterol catabolism, but increases LDL-derived cholesterol fecal excretion through lower intestinal cholesterol absorption.



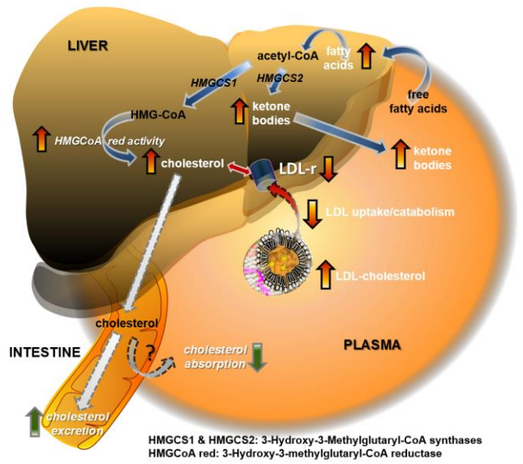

Plasma $^3$H-tracer decay curve and LDL-cholesterol catabolism (A), hepatic (B) and fecal (C) $^3$H-tracer recovery after $^3$H-cholesteryl oleate labeled LDL i.v. injection in dyslipidemic hamsters treated with vehicle or empagliflozin 30mg/kg/day for 2 weeks.
**p<0.05, **p<0.01 and ***p<0.01 vs. vehicle.*

### 4. Empagliflozin slightly promotes macrophage-to-feces reverse cholesterol transport.




$^3$H-tracer recovery in feces after $^3$H-cholesterol labeled/oxidized LDL loaded macrophages i.p. injection in dyslipidemic hamsters treated with vehicle or empagliflozin 30mg/kg/day for 2 weeks.
**p<0.05 vs. vehicle.*

### 5. Proposed mechanisms for the alteration of cholesterol metabolism by empagliflozin .




HMGCS1 & HMGCS2: 3-Hydroxy-3-Methylglutaryl-CoA synthases
HMGCoA red: 3-Hydroxy-3-methylglutaryl-CoA reductase

***SGLT2 inhibition switches from carbohydrate to fat oxidation and stimulates ketone bodies production and hepatic cholesterol synthesis in fasting conditions.***
***These metabolic alterations result in lower LDL-receptor expression and moderate increase in LDL-cholesterol levels .***
***A reduced intestinal cholesterol absorption and higher LDL-derived cholesterol fecal excretion were aslo observed.***

## CONCLUSION

- **Empagliflozin moderately increases fasting LDL-cholesterol levels and alters cholesterol metabolism at both hepatic (cholesterol synthesis) and intestinal (cholesterol absorption) levels.**
- **The increase in LDL-cholesterol levels is related to lower LDL-receptor expression and LDL-cholesterol catabolism.**
- **Despite the moderate increase in LDL-cholesterol levels, empagliflozin promotes two anti-atherogenic mechanisms by increasing both macrophage-to-feces reverse cholesterol transport and LDL-derived cholesterol fecal excretion,**

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