# Carnitine supplementation in long-chain acyl-CoA dehydrogenase knock-out mice is not detrimental for cardiac function

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## Introduction

Patients with long-chain fatty acid β-oxidation (FAO) disorders may present with cardiac abnormalities such as hypertrophic cardiomyopathy and arrhythmias. Elevation of long-chain acylcarnitine levels is a hallmark of long-chain FAO disorders, and is accompanied by low free carnitine levels. To normalize free carnitine levels, it has been proposed to supplement patients with carnitine. However, this approach could further enhance accumulation of long-chain acylcarnitines [1], which may exert a lipotoxic effect on the heart [2]. Using a non-invasive MR toolkit to assess left ventricular (LV) function and myocardial triglyceride (TG) levels, we investigated the effects of carnitine supplementation in long-chain acyl-CoA dehydrogenase knock-out (LCAD KO) mice and wild-type (WT) C57BL/6 controls.

## Methods

*Animals* - Male animals were housed with ad libitum access to water and a standard rodent diet. At 5 weeks of age, baseline MR data were acquired as described below. Subsequently, mice (LCAD KO+: $n$ = 7, WT+: $n$ = 10) received L-carnitine supplementation (50 mg $kg^{-1}$ $day^{-1}$) via their drinking water. Non-supplemented animals served as controls (LCAD KO: $n$ = 10, WT: $n$ = 8). The MR protocol was repeated at 7 and 9 weeks of age. Finally, heart tissue was collected for acylcarnitine profiling with tandem mass spectroscopy.

*MR protocol* - Anesthetized mice were positioned supine in a purpose-built cradle. ECG and respiration were used to monitor vital signs, and to enable cardiac triggering and respiratory gating. The cradle was entered into a horizontal bore 9.4 T MR system (Bruker Biospin), equipped with a quadrature $^1$H birdcage resonator (Ø 35 mm) for RF transmission and signal reception. Cardiac cine images were made for reference purposes and to assess LV function and morphology. Imaging parameters: FOV=30×30$mm^2$, matrix size=192×192, slice thickness=1mm, TE/TR=1.8ms/7ms, flip angle=15º, NA=6.

Localized $^1$H-MRS was performed in the diastolic interventricular septum using a respiratory gated and cardiac triggered PRESS sequence preceded by a respiratory gated and cardiac triggered CHESS water suppression module [3]. PRESS parameters: voxel size=1×2×2$mm^3$, TE/TR=9.1ms/~2s, water-suppressed spectra: NA=256, unsuppressed spectra: NA=32.

*Data analysis* - LV ejection fraction (EF) and mass (LVM) were determined from the cine MR images using the semiautomatic segmentation software CAAS MRV FARM (Pie Medical Imaging, Maastricht, The Netherlands). MR spectra were analyzed using AMARES in jMRUI. TG level was quantified relative to the unsuppressed water signal. Statistical significance of effects of genotype, aging, and carnitine supplementation on traits derived from the *in vivo* MR data was assessed using a linear mixed-effects model with mouse number ($n$ = 35) as random factor, and genotype, carnitine supplementation, and the time as fixed factors.

## Results

*In vivo MR* - Mouse body weight was similar for LCAD KO and WT mice at baseline, and was unaffected by carnitine supplementation or genotype. At 5 weeks of age, LVM was 22% higher ($P$ < 0.001) in LCAD KO mice compared to WT mice, indicating mild hypertrophy. In addition, EF was lower ($P$ < 0.05, Fig. 1) and myocardial TG levels were elevated ($P$ < 0.01, Fig. 1) in LCAD KO mice when compared to WT animals. Carnitine supplementation did not affect LVM or EF, but lowered myocardial TG levels, normalizing TG content in LCAD KO myocardium.

*Ex vivo tandem MS* - Myocardial free carnitine content was lower in LCDK KO mice ($P$ < 0.0001), and was elevated by carnitine supplementation. Compared with WT mice, C14:2- and C14:1-acylcarnitine levels were elevated in LCAD KO myocardium, whereas LCAD KO myocardial C16-C18 long-chain acylcarnitine levels were similar. Carnitine supplementation did not enhance myocardial accumulation of these acylcarnitines (Fig. 2).

## Conclusion

At a clinically relevant dose, carnitine supplementation lowers myocardial TG storage, and is not detrimental for cardiac function in mice deficient in long-chain FAO. We found no evidence for lipotoxic effects of carnitine supplementation.

## References

[1] Primassin, S. *et al*., 2008, *Pediatr Res*, 63:632-37. [2] Bonnet, D. *et al*., 1999, *Circulation*, 100:2248-53. [3] Bakermans, A.J. *et al*., 2011, *Circ Cardiov Imaging*, 4:558-65.

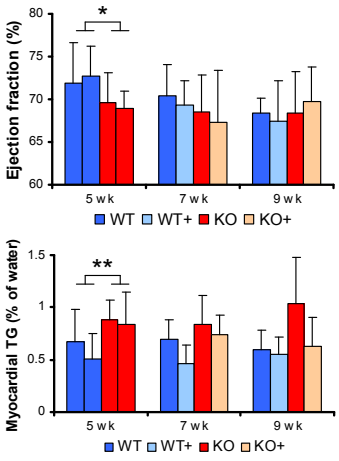


**Figure 1** Top: Decreased LV ejection fraction in LCAD KO mice at baseline. Bottom: Myocardial TG levels determined with *in vivo* with $^1$H-MRS. *, $P$ < 0.05; **, $P$ < 0.01.

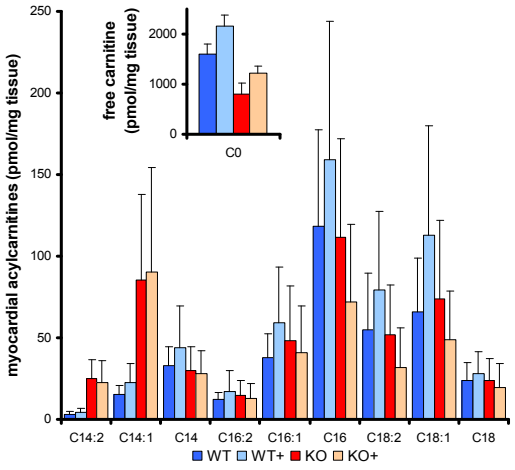


**Figure 2** Myocardial acylcarnitine profile. LCAD KO mice display low levels of free carnitine (C0), which are elevated by carnitine supplementation.