Mechanism of Cholesterol Transport Promotion by Hepatic *Cdkal1*-Deletion

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Mechanism of Cholesterol Transport Promotion by Hepatic *Cdkal1*-Deletion

Directed by Professor Sang-Hak Lee

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Ji Eun Lee

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ABSTRACT

Mechanism of Cholesterol Transport Promotion by Hepatic *Cdkal1*-Deletion

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(Directed by Professor Sang-Hak Lee)

Previous studies have shown that the effects of *Cdkal1* deletion on liver were observed only in male mice, however, in this study, we aimed to determine the effects of *Cdkal1* deletion on reverse cholesterol transport (RCT) and cholesterol metabolism in both male and female mice.

In this study, we investigated the effects of *Cdkal1* using a liver-specific *Cdkal1*-deficient mice model by RCT and Western blotting. Macrophages were treated with ^{3}H-labeled cholesterol and subsequently injected intraperitoneally to mice. After 48 h, plasma, liver, and feces were collected and radioactivity was measured using a liquid scintillation counter.

RCT analysis showed that radioactivity measured in the liver of female *Cdkal1*-deficient mice was 154% (p = 0.006) higher than that of wild type (WT) mice.

Additionally, the total sterol, cholesterol, and bile acid excretion in feces increased in *Cdkal1* KO mice.

Total sterols in *Cdkal1* KO mice were 57% ($p = 0.006$) higher in males and 200% ($p = 0.006$) in females, and cholesterol was 93% in females ($p = 0.006$) higher. Bile acids were 117% ($p = 0.04$) higher in males and 97% ($p = 0.006$) in females of *Cdkal1* KO mice.

Western blotting analysis of liver tissue revealed an increase in CYP7A1 compared to WT mice in males ($p = 0.01$) and females ($p = 0.10$) of *Cdkal1* mice and an increase in ABCG5 in males ($p = 0.004$) and females ($p = 0.006$) of *Cdkal1*1 mice. However, it was not changed in liver X receptor α (LXRα).

In conclusion, higher RCT in liver-specific *Cdkal1* KO mice was associated with activated liver cholesterol uptake, bile acid synthesis, and cholesterol excretion but not with LXR.

This suggests that *Cdkal1* may provide a potential treatment approach in atherosclerotic vessels.

Key words: cardiovascular disease, reverse cholesterol transport, liver, cholesterol metabolism

1. Introduction

Abnormal cholesterol levels have been identified as a major risk factor for the development of cardiovascular disease (CVD) [1]. CVD can occur when excessive cholesterol accumulates in blood vessels. [2] In particular, low-density lipoprotein cholesterol (LDL-C) has been proven to be an important risk factor for CVD because it causes atherosclerosis [3]. On the other hand, high-density lipoprotein cholesterol (HDL-C) acts as a lipoprotein that removes cholesterol from the body and prevents atherosclerosis [4]. In cholesterol metabolism, which plays an important role in cardiovascular health, reverse cholesterol transport (RCT) selectively absorbs cholesterol from HDL particles outside the hepatocytes by SR-B1, which accumulates cholesterol in peripheral tissues. It is then converted to bile acid or excreted through the intestines into bile [5]. In this process, cholesterol, phospholipids, and free cholesterol combine with ApoA1 to form HDL-C particles. In addition, LDLR removes LDL cholesterol in the blood through the receptor to prevent excessive cholesterol in the blood vessels [6]. It reduces cholesterol accumulation in macrophages through a series of processes, contributing to the prevention of atherosclerosis. Therefore, RCT is considered a key metabolic pathway in the prevention of CVD, and studying genes such as *Cdkal1* may play a key role in understanding atherosclerotic disease.

Mutations in the Cdk5 regulatory protein 1-like 1 (*Cdkal1*) gene, one of the risk genes for type 2 diabetes in various populations, are associated with increased risk of diabetes and abnormal insulin secretion. [7,8]. However, the specific role of CDKAL1 in atherosclerotic disease has not yet been elucidated.

In a previous study, we identified factors that affect increased cholesterol efflux capacity (CEC) and changes in RCT in the liver of *Cdkal1* KO male mice, as well as determined the factors and pathways affected by *Cdkal1*. Until now, the effect of CDKAL1 in atherosclerosis has been mainly studied in males, however, in this study, we also confirmed it in females. In this study, we aimed to compare the impacts of liver-specific *Cdkal1* KO on the basic mechanisms of cholesterol metabolism and bile acid metabolism in both male and female mice compared with those in wild-type (WT) controls. We used RCT and Western blotting to identify proteins and physiological pathways that may mediate the effect of this gene in *Cdkal1* mice.

Therefore, the aim of this study was to elucidate the biological effects of CDKAL1 in a liver-specific *Cdkal1*-deficient mice model, especially the effects on cholesterol metabolism following *Cdkal1* KO. We also aimed to evaluate additional relevant mechanisms for the altered cholesterol transport and potential differences in *Cdkal1* KO. Based on our results, we suggest future research directions for *Cdkal1* as a therapeutic agent for atherosclerosis.

II. MATERIALS AND METHODS

1. Animals

Liver specific *Cdkal1* deficient mice were generated by crossing *Cdkal1*$^{fl/fl}$ mice (donated by Professor Tomizawa, Kumamoto University, Japan) with *Alb-Cre* mice (The Jackson Laboratory, Bar Harbor, U.S.A.). Liver specific *Cdkal1* deficient mice were used at 8 weeks of age. Eight-week-old C57BL/6 mice were purchased from Orientbio (Seongnam, Korea). Mice were housed in a regulated environment with a 12-hour day and night cycle (dark from 8:00 PM to 8:00 AM) in individually ventilated cages at 22°C and had free access to standard food and water. All mice experiments were conducted after the approval of Yonsei University's Animal Experiment Ethics Committee (2023-0213).

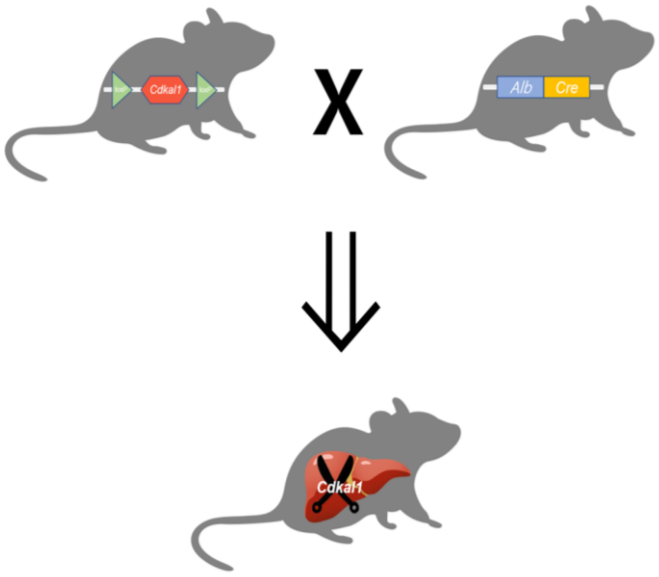


Figure 1. Creation of liver-specific *Cdkal1* deficit mice. Mice were produced through the locus of X-over P1 (loxP) system. To generate these liver-specific Cdkal1-deficient mice, *Alb-Cre* mice were bred with *Cdkal1*$^{fl/fl}$ mice [8].

2. Genotyping

To confirm whether the mice was liver-specifically *Cdkal1*-deficient, the toes of 10-day-old pups were cut and stored in a 1.5 mL tube. The tube containing the tissue was centrifuged with Dilution buffer (20 μL) and DNA release (0.5 μL). (F-140WH, Thermo Scientific™, Waltham, MA, USA). The reaction was conducted for 5 min at room temperature, followed by heat-block at 98 ℃ for 2 min. Subsequently, the reaction was cooled to room temperature and DNA extraction was conducted, and the supernatant was transferred to a new 1.5 mL tube.

Polymerase chain reaction(PCR) was conducted by adding DNA (1 μL), forward primer (1 μL), reverse primer (1 μL), and distilled water (DW) (17 μL) to a PCR kit tube to confirm the *Cre* band. Subsequently, to confirm the *Cdkal1* band, DNA (1 μL), forward primer (1 μL), reverse primer (1 μL), 5X PrimeSTAR GXL Buffer (2 μL), (deoxynucleoside triphosphate) (dNTP) mixture (0.8 μL), PrimeSTAR GXL DNA Polymerase (0.4 μL), and DW (3.8 μL) were added to a PCR tube. (Takara, R050A) After the completion of PCR, 100 bp DNA ladder (BIONEER, 3 μL) and PCR-synthesized genomic DNA (8.5 μL) were electrophoresed to confirm the bands. The band sizes of each gene are as follows. *Cdkal1* – wild type (300 bp), *fl/fl* (500 bp), heterozygous (300 & 500 bp), and *Cre* (100 bp).

3. Tissue preparation

Cleaned liver tissue was obtained by perfusing phosphate-buffered saline (PBS) into the left atrium of mice. The liver was excised from a portion of the largest lobe and placed in a 1.5 mL tube. The tissue was homogenized evenly using a homogenizer, 100 μL of lysis buffer was added, and brief centrifugation was performed. After placing on ice for 30 min to 60 min, the liver was centrifuged at 13,000 rpm, 4°C for 30 min to 60 min. The supernatant was moved into a new 1.5 mL tube to obtain protein and used for the next experiment.

4. Reverse cholesterol transport

J774 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, with 10% fetal bovine serum (FBS) and 1% antibiotics(Abs)) and treated with 5 μCi/mL of ^{3}H-cholesterol and 100 μg/mL acetylated low-density lipoprotein for 48 h. Cells were cultured at 37℃ with 5% CO_2. Approximately 1×10^7 cells, diluted in 500 μL of PBS or DMEM, were injected intraperitoneally into mice. Plasma was collected from the peritoneal vein after 48 h, and feces were collected once 48 h later and stored overnight at 4°C for the extraction of cholesterol and bile acids. Liver tissue was harvested after perfusion with PBS, and 100 mg of liver tissue was used for analysis. Lipid extraction from liver tissue was conducted based on the method of Bligh and Dyer. The radioactivity of plasma, liver, and stool samples was measured using a liquid scintillation counter (LSC). This study was certified by the Institutional Review Board of Yonsei University's Animal Experiment Ethics Committee (2023-0213).

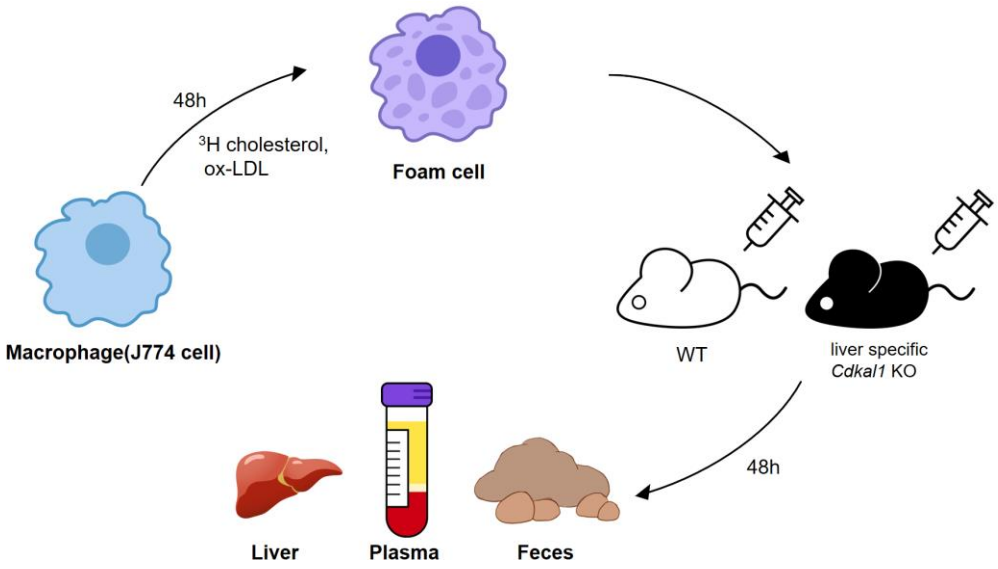


Figure 2. Reverse cholesterol transport (RCT) experiment.

5. Western blotting

Protein was quantified with the bicinchoninic acid (BCA) assay (Pierce™ BCA Protein Assay Kit, Thermo Scientific™, #23227). Equal amounts of total protein were run on a 10% sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gel and transferred to a polyvinylidene fluoride membrane (PVDF) using a Bio-Rad Western blotting system (Mini-Protean® Tetra Cell and Mini Trans-Blot module; Bio-Rad Laboratories, Hercules, CA, USA). The membrane was blocked with 5% skim milk in 0.1% Tween-20 (TBST) at room temperature for 1 hour. subsequently, it was washed thrice for 7 minutes each with Tris-buffered saline containing 0.1% Tween-20. Thereafter, the membranes were incubated with primary antibodies

(CDKAL1(Cat NO. ab169531; Abcam, Cambridge, UK), SR-B1(ab217318, Abcam), CYP7A1(A10615, ABclonal, Boston, USA), ABCG5(27722-1-AP, Proteintech, Illinois, USA), ABCG8(PA5-104397, Waltham, MA, USA), and LXRα(A2141, ABclonal)) overnight at 4°C. Afterwards, the membrane was reacted with a secondary antibody conjugated with horseradish peroxidase (HRP) for more than 1 hour (sc-2357, sc-516102, Santa Cruz Biotechnology). The membranes were washed four times, each for 10 minutes, with TBST. And visualized and images were acquired using Pierce™ ECL Western Blotting Substrate (Thermofisher, Waltham, MA, USA) and Amersham™ ImageQuant™ 800 (GE Healthcare). Protein expression was normalized to β-actin (sc-47778, Santa Cruz Biotechnology) and the total protein content of each sample, and the densities of protein expression were analyzed using Image J.

6. Statistical analysis

All graphs were created using GraphPad Prism 9 (GraphPad Software Ltd, La Jolla, CA, U.S.A.). Values were expressed as mean ± standard error of the mean. In particular, the mean values of RCT and western blot data were expressed as ± SD. Statistical analysis of all results was conducted using the Mann-Whitney U test, a nonparametric statistical method suitable for analyzing the differences between two groups in samples with relatively small sample sizes. In this study, a *P* value of less than 0.05 was considered statistically significant difference.

III. RESULTS

1. Mice Characteristics

Information on mice used in the experiment is summarized in Table 1. WT mice were used as the control group, no signal was observed in body weight between the control and other groups.

Table 1. Characteristics of mice

Group	Strain	Genotype	Number	Sex	Age (weeks)	Weight (g)
Control	C57BL/6	WT	6	Male	8	24.8±1.2
Control	C57BL/6	WT	6	Female	8	19.8±1.0
Cdkal1 KO	C57BL/6	*Cdkal1*$^{fl/fl}$ × *Alb-Cre*	6	Male	8	24.2±0.5
Cdkal1 KO	C57BL/6	*Cdkal1*$^{fl/fl}$ × *Alb-Cre*	6	Female	8	18.2±0.5

Mean ± standard deviation (SD). Six mice were used for RCT and western blot analysis.

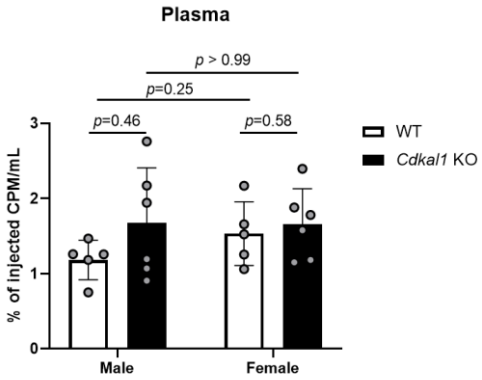
2. Effect of liver-specific *Cdkal1* deletion on RCT

In vivo RCT experiments were conducted in 8-week-old WT and *Cdkal1* KO mice, as described in the Materials and Methods section. When comparing WT and *Cdkal1* KO mice, the radioactivity of plasma was not different (Figure 4A).

However, the radioactivity of liver was higher only in *Cdkal1* KO mice, especially in females (1.53 ± 0.43 and 3.9 ± 3.5 CPM/g in female WT and *Cdkal1* KO mice, respectively; p=0.006; Fig 4B).

The radioactivity of total sterol in feces was higher in *Cdkal1* KO than in WT, and was higher in both males (0.73 ± 0.27 and 1.15 ± 0.24 CPM/g in male WT and male *Cdkal1* KO mice, respectively; *p*=0.006) and females (0.81 ± 0.3 and 2.43 ± 0.67 CPM/g in female WT and female *Cdkal1* KO mice, respectively; *p*=0.006). And then the radioactivity of cholesterol measured in feces was higher in the *Cdkal1* KO group, and the cholesterol activity in feces was similar in males (0.53 ± 0.39 and 0.87 ± 0.42 CPM/g in male WT and male *Cdkal1* KO mice, respectively; *p*=0.26), we confirmed that it was higher only in females (0.62 ± 0.34 and 1.19 ± 0.14 CPM/g in female WT and female *Cdkal1* KO mice, respectively; *p*=0.04). Additionally, the radioactivity of bile acid measured in feces was higher in *Cdkal1* KO mice than in WT mice, and was significantly higher in both males (0.20 ± 0.15 and 0.43 ± 0.12 CPM/g in male WT and male *Cdkal1* KO mice, respectively; *p*=0.04) and females (0.29 ± 0.07 and 0.57 ± 0.1 CPM/g in female WT and female *Cdkal1* KO mice, respectively; *p*=0.006) (Fig 4C).

A



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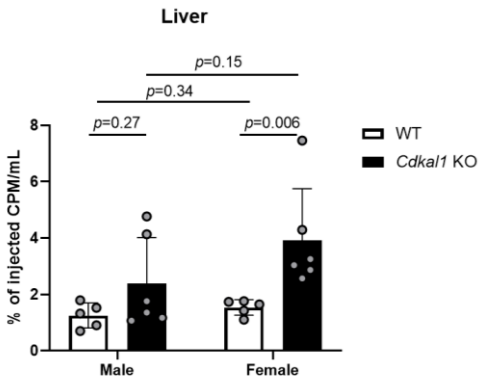


C

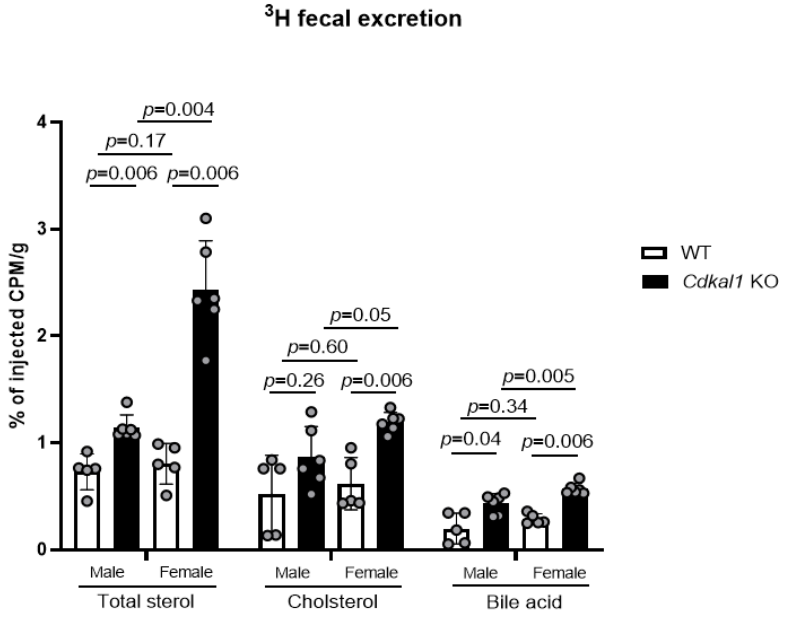


Figure 4 legend (continued)

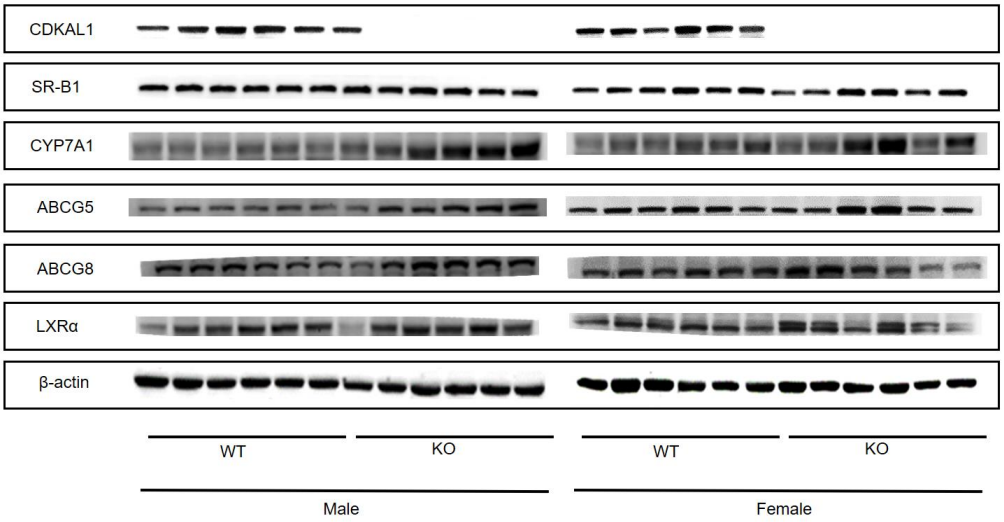
Figure 4. Promoted cholesterol transport in hepatic *Cdkal1*-deficient mice.

We measured the radioactivity of plasma, liver, and feces from WT and *Cdkal1*-deficient mice to determine the differences between individuals. Analysis was conducted on the last collected sample of feces after 48 h. No difference was observed in plasma activity. However, the radioactivity in the liver and total sterols and bile acids in feces was higher in both male and female *Cdkal1*-deficient mice. Moreover, the radioactivity observed in the cholesterol in feces was higher in female *Cdkal1*-deficient mice (permutation test; n = 5-6). CPM: counts per minute.

3. The impact of *Cdkal1* deficiency on bile acid synthesis and excretion

The expression of proteins related to cholesterol metabolism and excretion was confirmed in the liver tissues of WT and *Cdkal1* KO mice by western blotting. First, we confirmed whether the mice were liver-specific Cdkal1 deficient mice by examining the level of CDKAL1 protein. CDKAL1 protein was expressed in the livers of WT mice but not in those of *Cdkal1* KO mice ($P < 0.001$ for both males and females, respectively Next, we confirmed the expression of SR-B1 protein, which functions as an HDL receptor in the liver and plays a key role in RCT. It was significantly increased in male *Cdkal1* KO mice compared with WT mice, but no differences were found between individuals in females (0.56 ± 0.13 and 0.94 ± 0.15 AU in male WT and male *Cdkal1* KO mice, respectively; $p = 0.004$). Additionally, the expression of CYP7A1 protein, known to be involved in cholesterol metabolism, was significantly increased in male *Cdkal1* KO compared to male WT mice. (1.23 ± 0.28 and 1.55 ± 0.22 AU in male WT and male *Cdkal1* KO mice, respectively; $p = 0.01$) Moreover, ABCG5 expression, which plays an important role in bile acid metabolism related to cholesterol excretion, was higher in *Cdkal1* KO mice compared with that in WT mice in both male and female mice. (0.53 ± 0.21 and 0.77 ± 0.22 AU in male WT and male *Cdkal1* KO mice, respectively; $p = 0.004$) (0.34 ± 0.13 and 0.58 ± 0.13 AU in female WT and female *Cdkal1* KO mice, respectively; $p = 0.006$) No inter-individual differences were identified in LXRα and ABCG8. (Figure 5A and 5B)

A



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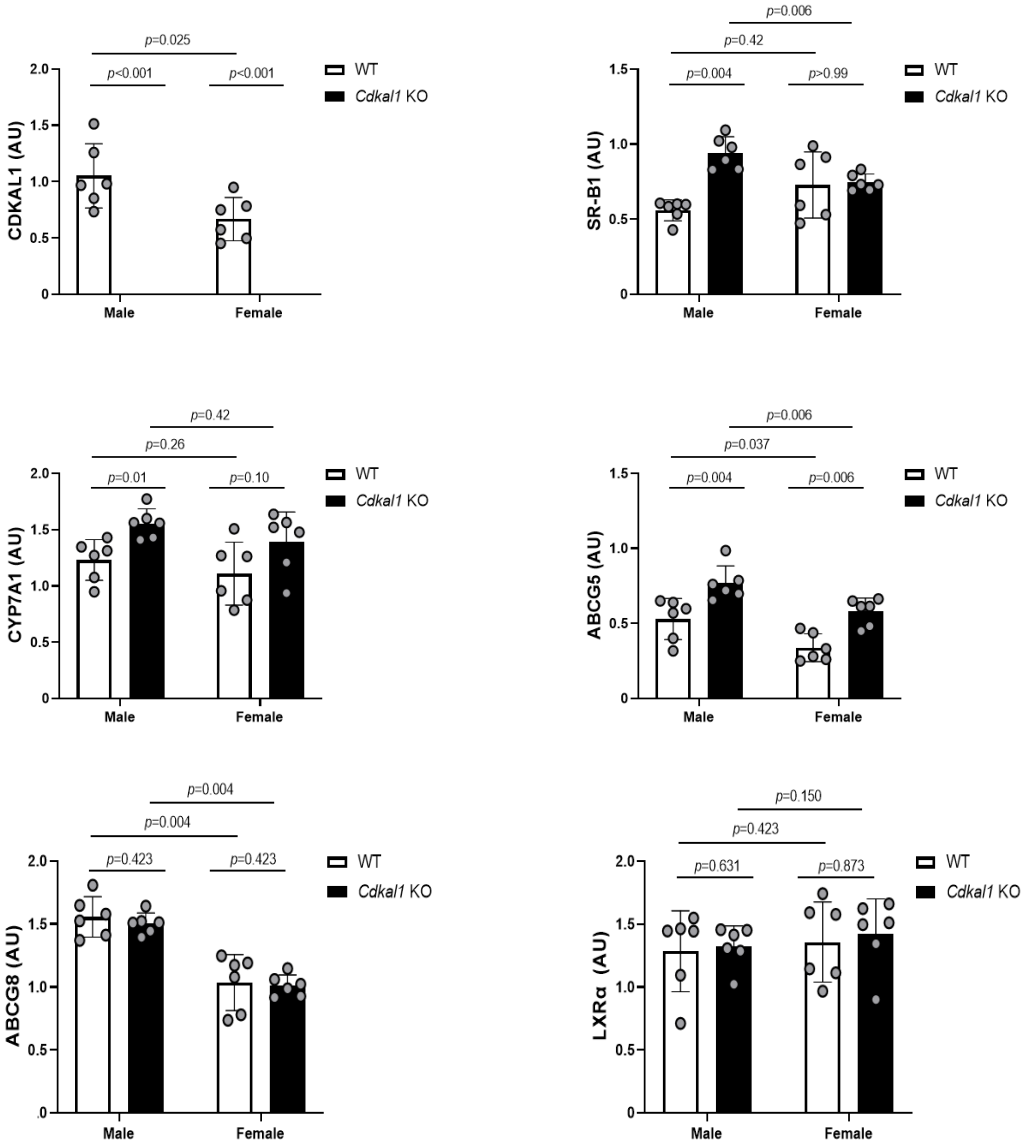

Figure 5 legend (continued)

Figure 5. Difference in molecules in the hepatic bile acid synthesis pathway.

Western blot analysis of CDKAL, SR-B1, CYP7A1, ABCG5/8 and LXRα in WT and *Cdkal1* KO mice. The expression of cholesterol metabolism-related proteins was confirmed in liver tissues of 8-week-old WT and *Cdkal1* KO mice. (n = 6 per group; experiments were repeated 3 thrice and averaged) (A) Characteristic image of western blotting in liver tissue. (B) Graph depicting the quantification of western blot images using the Image J program. Results are presented as the mean ± SD

IV. DISCUSSION

The main findings of this study are as follows: 1) in RCT, the radioactivity of the liver was higher in liver-specific *Cdkal1*-deficient mice, especially in females, than in WT females. 2) Fecal total sterol, cholesterol, and bile acid radioactivity in RCT were higher in *Cdkal1* KO mice than in WT mice. 3) SR-B1 and CYP7A1 were upregulated in hepatocytes of male *Alb-Cre*:*Cdkal1*$^{fl/fl}$ mice compared with those in WT, and ABCG5 was upregulated in both male and female *Cdkal1* KO mice. These effects were LXR-independent.

Our most important finding is that Cdkal1 deficiency promotes RCT. Previous studies have shown that *Cdkal1* KO mice promote RCT only in males compared with controls [9], but in this study we additionally confirmed this phenomenon in females. Additionally, in contrast to studies that focused on *Cdkal1* in insulin secretion or diabetes [10], we specifically identified the effect of the *Cdkal1* gene on cholesterol metabolism in RCT. Previous studies have also reported some differences in human cholesterol and HDL metabolism depending on gender [11], but the effects of *Cdkal1* KO have not been studied in mice based on gender. Our study will provide an opportunity to determine how *Cdkal1* KO affects cholesterol metabolism compared with that in the control group. These results will be the first step toward developing *Cdkal1* as a target for the prevention and treatment of CVDs, considering its important role in cholesterol metabolism.

In this study, we confirmed that SR-B1, CYP7A1 and ABCG5 protein levels increased in the liver of *Cdkal1*-deficient mice. CYP7A1 is expressed in the liver and is the first step and rate-determining enzyme of the classical pathway, wherein cholesterol is synthesized into bile acids, thereby controlling cholesterol and bile acid homeostasis [12]. CYP7A1 metabolizes cholesterol into 7α-

hydroxycholesterol, which then enters the bile acid biosynthesis pathway. In our study, the protein expression of CYP7A1 was increased in *Alb-Cre*:*Cdkal1*$^{fl/fl}$ mice. CYP7A1 promotes the process of cholesterol removal from the liver and helps to convert cholesterol into bile acids for excretion. AS this enzyme was increased in *Cdkal1* KO mice, cholesterol metabolism and bile acid production may have been enhanced.

Additionally, ABCG5/8 is a protein predominantly expressed in the liver and small intestine that forms sterol transporters and plays a role in suppressing the accumulation of dietary sterols [13,14]. ABCG5/8 forms a heterodimer, which effectively plays a role in the excretion of sterols from the small intestine into the intestinal lumen and secretion of cholesterol and plant sterols from the liver into bile. This inhibits the absorption of cholesterol and plant sterols [15]. In our study, the protein expression of ABCG5 increased in *Alb-Cre*: *Cdkal1*$^{fl/fl}$ mice. The increased expression of ABCG5 promotes the excretion of cholesterol and neutral sterols from the liver through bile, and reduces cholesterol accumulation in the body by decreasing intestinal cholesterol absorption [16].

In a previous study, SR-B1 was found to increase in *Cdkal1* KO mice, and in this study, *Cdkal1* deletion not only promoted cholesterol uptake into the liver through SR-B1 increase; but also promoted bile acid activity through the increase in CYP7A1; it also promoted cholesterol transport through the increase in ABCG5 increase. These two additional mechanisms were identified. Although these two molecules are upregulated in the liver, this is not attributed to LXR activation in response to cholesterol elevation in hepatocytes.

In SR-B1/ABCG5 double knockout mice (DKO), SR-B1 contributes to bile cholesterol secretion and macrophage-to-feces RCT independent of ABCG5/8, and

ABCG5/8 is known to secrete cholesterol under bile acid-stimulated conditions [17]. This suggests that SR-B1 and ABCG5/8 play independent roles in cholesterol metabolism, which is consistent with our findings on the RCT-promoting mechanism and cholesterol metabolism in *Cdkal1* deletion mice.

A limitation of this study is that although RCT was increased in *Cdkal1*-deficient mice compared with that in WT mice, the exact reason for this increase was not identified. Although proteins such as CYP7A1 and ABCG5 appear to be involved, it is still unclear through which pathways they promote RCT are still unclear. To confirm and address this limitation, future studies need to generate mice with knockout or KO of proteins such as CYP7A1 using CRISPR, adenoviral vectors, shRNA technology, or RNA interference to further determine their role in the RCT pathway.

Moreover, investigating the sex-specific effects of *Cdkal1* deficiency on these pathways will be an important aspect of future studies. In this study, we focused on WT and *Cdkal1* KO mice, and sex differences in *Cdkal1* KO have not yet been demonstrated. However, as sex may potentially influence cholesterol metabolism, further investigation of sex differences will provide meaningful results regarding the sex-specific effects of *Cdkal1* KO and underlying mechanisms. In conclusion, we elucidated an additional mechanism through which *Cdkal1* promotes cholesterol transport in hepatic *Cdkal1*-deficient mice, which may provide additional therapeutic avenues for *Cdkal1* in atherosclerotic diseases.

V. CONCLUSION

In this study, we confirmed that liver-specific *Cdkal1* deficiency affects RCT regardless of sex. Liver-specific *Cdkal1* deficiency affected total sterols, cholesterol, and bile acids in the liver of females and in feces of both sexes. In addition, we described the effects of *Cdkal1* deficiency on cholesterol metabolism by identifying several proteins. This study provides a new direction for research by confirming the results previously found only in male mice in female mice. That is, it provides new insights into *Cdkal1*.

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Abstract in Korean

간 *Cdkal1*-deletion에 의한 콜레스테롤 수송 촉진 메커니즘

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이지은

이전 연구에서는 수컷 마우스에서만 간 *Cdkal1* 결손에 대한 영향이 확인되었지만 이번 연구에서는 수컷과 암컷 모두에서 *Cdkal1* 결손에 대한 역 콜레스테롤 수송(RCT)와 콜레스테롤 대사에 미치는 영향을 확인하고자 했다.

이 연구에서는 간 특이적 *Cdkal1* 결손 마우스 모델을 사용하여 RCT와 Western blotting을 통해 *Cdkal1*의 영향을 조사했다. 대식세포를 ^{3}H 표지 콜레스테롤로 처리하고 마우스에 복강 내 투여했다. 48 시간 후

혈장, 간, 대변을 수집하고 액체 섬광 계수기를 이용하여 방사능을 측정했다.

RCT 분석 결과, 암컷 *Cdkal1* 결핍 마우스의 간에서 측정한 방사능은 WT 마우스보다 154%(p = 0.006) 더 높았다. 또한 대변에서 측정한 총 스테롤, 콜레스테롤, 담즙산 배설이 *Cdkal1* KO 마우스에서 증가했다. *Cdkal1* KO 마우스의 총 스테롤은 수컷에서는 57%(p = 0.006), 암컷에서 200%(p = 0.006) 더 높았으며 콜레스테롤은 암컷에서 93%(p= 0.006) 더 높았다. *Cdkal1* KO 마우스의 담즙산은 WT 마우스에 비해 수컷에서 117% p = 0.04), 암컷에서 97%(p = 0.006) 더 높았다.

간 조직에서 진행한 western blotting 분석 결과 WT 마우스에 비해 *Cdkal1* KO mice 에서 CYP7A1 이 수컷(p = 0.01)과 암컷(p = 0.10)에서 증가하였다. 그리고 ABCG5 단백질은 WT 에 비해 *Cdkal1* 마우스의 수컷(p = 0.004)과 암컷(p = 0.006)에서 증가한 것으로 나타났다. 하지만 LXRα 에서는 변화되지 않았다.

결론적으로, 간 *Cdkal1* 결핍 마우스는 특히 암컷에서 RCT 가 촉진되었다. 또한, *Cdkal1* 결핍 마우스의 간에서 SR-B1, CYP7A1 및 ABCG5가 상향 조절되었지만 LXRα 발현은 WT 마우스와 다르지 않았다.

이를 통해 *Cdkal1* 이 죽상경화성 혈관에서 잠재적인 치료 접근 방안을 제공할 수 있음을 시사한다.

핵심되는 말: 심혈관 질환, 콜레스테롤 역수송, 간, 콜레스테롤 대사