ウェブサイト公開用

（様式 10）
（海外特別研究員事業）

令和 2 年 3 月 20 日

海外特別研究員最終報告書

独立行政法人日本学術振興会 理事長 殿

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（氏名は必ず自署すること）

海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地： Duke-NUS Medical School （国名： シンガポール 国）

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
糖尿病性腎症の病勢に関わる遺伝子と分子経路の究明および治療への展開

3. 派遣期間：平成 31 年 4 月 1 日 ～ 令和 2 年 3 月 20 日

4. 受入機関名及び部局名
Cardiovascular and Metabolic Disorders Program, Duke-NUS Medical School

5. 所期の目的の遂行状況及び成果…書式任意 書式任意（A4 判相当 3 ページ以上、英語で記入も可）
（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

<Background>

Diabetic nephropathy (DN) is a leading cause of end-stage kidney disease (ESKD) in the developed world. Along with hyperglycemia, activation of the renin-angiotensin system (RAS) is a major contributor to the pathogenesis of DN. This has been most clearly demonstrated by clinical trials showing that RAS blockade slows the progression of kidney damage and reduces risk for ESKD in DN. However, the precise mechanisms of kidney protection by RAS blockade in DN are not clear. Here, we examined whether metabolic effects of RAS blockade might contribute to renoprotective effects since previous studies suggest a link between dysregulated mitochondrial metabolism and kidney injury. To investigate this issue, we used metabolomic profiling to test the effects of RAS blockade on kidney metabolism in a mouse model exhibiting cardinal characteristics of human DN.

<Methods>

We utilized a mouse model of DN combining the *Akita* mutation of the insulin 2 gene with a single-copy renin transgene (*ReninTG*) driven by the albumin promoter. We have previously shown that combining severe type I diabetes with low level activation of the RAS in *Akita-ReninTG* mice on a susceptible 129 strain background (129AR mice) produces major clinical features of human DN including hypertension, high-grade albuminuria, and glomerulosclerosis. By contrast, *Akita-ReninTG* mice on an inbred C57BL/6 background (B6AR mice) do not develop significant kidney disease (**Figure 1**). For the current study, 12-week old male 129AR and B6AR mice were treated with vehicle or the angiotensin receptor blocker (ARB) losartan 10 mg/kg/day in drinking water for 12 weeks. 24-hour urine samples were collected in metabolic cages every 6 weeks and urine albumin levels were measured by EIA. At the end of the 12-week treatment period, kidneys were harvested and metabolic profiling of kidney tissue was performed by liquid chromatography-mass spectrometry using our standardized targeted metabolomics platform. Kidneys from age-matched parental 129 and C57BL/6 wild-type (WT) mice were analyzed as controls.

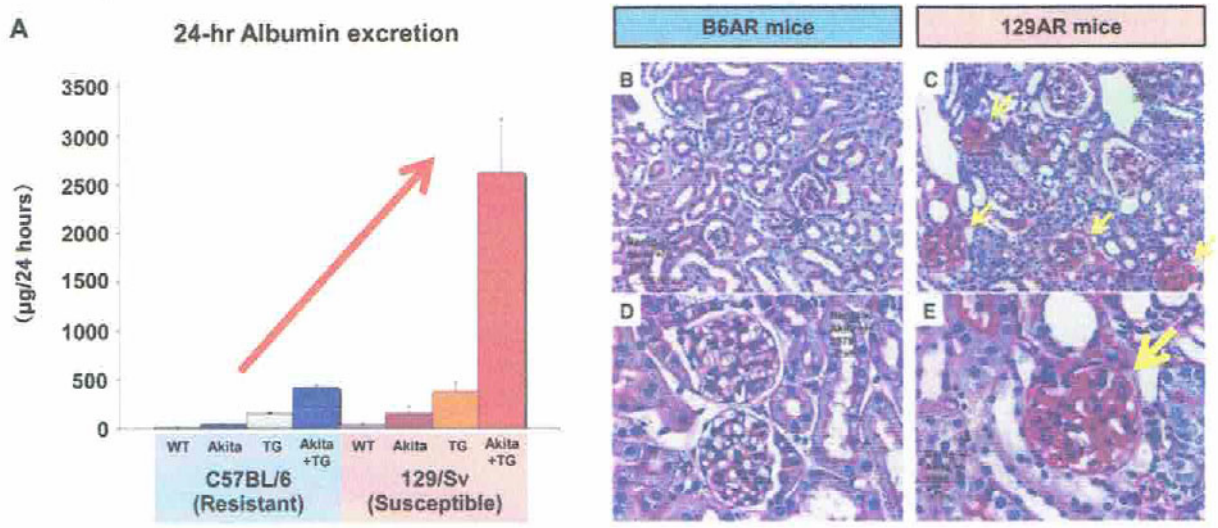

Figure 1. Mouse model of DN. (A) Urinary albumin excretion is markedly elevated by >20x-fold in 129AR mice. (B-E) Photomicrographs of PAS stained kidney sections. (B) and (D) are low- and high-powered photomicrographs of kidney cortex from a control showing minimal abnormalities. (C) and (E) depict kidney cortex from a 129AR mouse where there is marked mesangial expansion with nodular glomerulosclerosis and accumulation of inflammatory cells in the renal interstitium. DN, diabetic nephropathy; 129AR, 129/Sv-Akita-ReninTG mice; PAS, periodic acid-schiff.

<Results>

ARB treatment reduces albuminuria in mice with DN: Before treatment, 12-week old 129AR mice had significant albuminuria (833±112 µg/day). Over the next 12 weeks, there was a progressive increase of

albuminuria in the vehicle group to 1480±562 μg/day that was dramatically attenuated in the ARB group (193±42 μg/day; p=0.045). By contrast, B6AR mice had minimal levels of albuminuria at all time points (**Figure 2**).

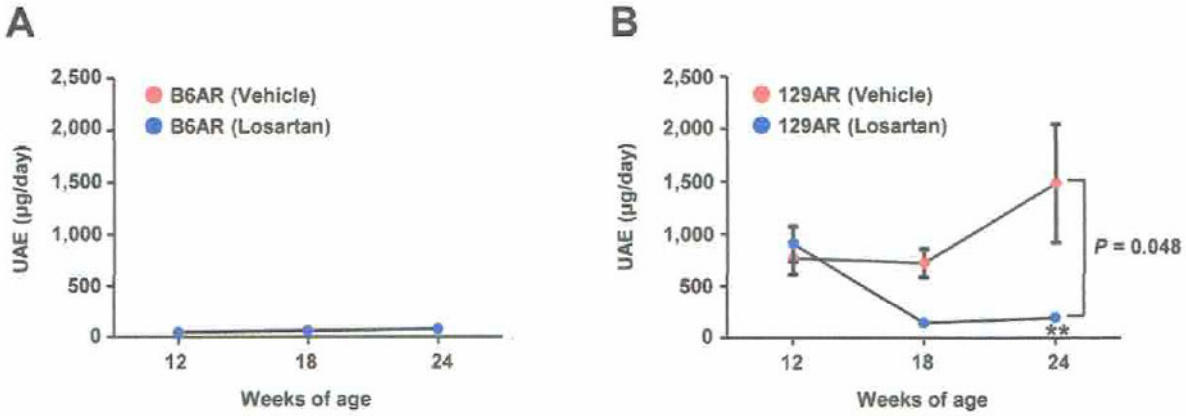

Figure 2. Effects of ARB treatment on UAE and renal pathological changes in B6AR and 129AR mice. (A) B6AR mice had minimal albuminuria at all time points and ARB had no obvious effect. (B) 129AR mice had high-grade albuminuria before the treatment and ARB significantly reduced albuminuria levels. (A, B) Data were analyzed by two-way repeated measures ANOVA with Bonfferoni post hoc test. ** $P < 0.01$, vs vehicle group. ARB, angiotensin receptor blocker; UAE, urinary albumin excretion; B6AR, C57BL/6-Akita-ReninTG mice; 129AR, 129/Sv-Akita-ReninTG mice.

ARB treatment normalizes levels of C2 Acyl-carnitine: Levels of most even-chain acyl-carnitine, which include products of mitochondrial fatty acid β-oxidation, were significantly reduced in kidneys from vehicle-treated 129AR mice and were largely unaffected by ARB treatment. The exception to this was C2-carnitine, acetyl-carnitine generated from acetyl CoA-the final common metabolite of mitochondrial fuel oxidation. Levels of C2-carnitine were substantially reduced in vehicle-treated 129AR mice and ARB increased C2-carnitine to levels approaching those seen in WT controls. Reduced levels of even-chain acyl-carnitine were not observed in kidneys of B6AR mice (**Figure 3**).

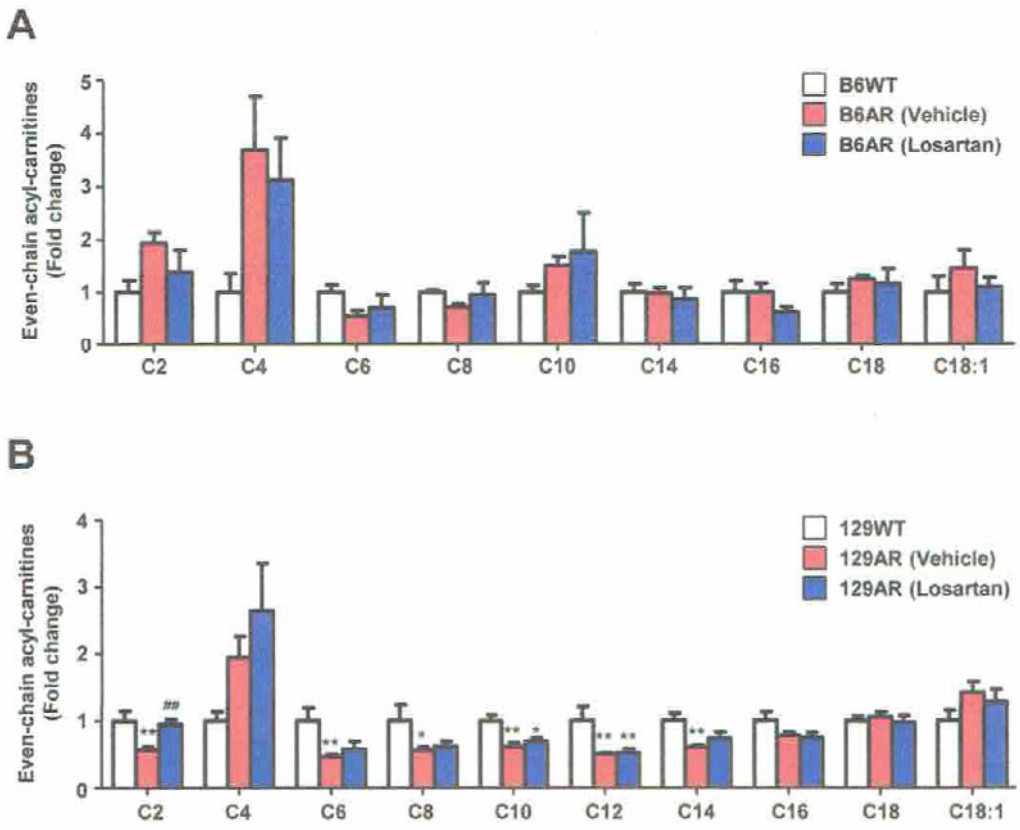

Figure 3. Renal even-chain acyl-carnitine profiles in B6AR and 129AR mice. (A) B6AR mice had no statistical difference in renal even-chain acyl-carnitine levels between all experimental groups. (B) Renal even-chain acyl-carnitine levels were broadly reduced in 129AR mice compared to WT mice which were almost not affected by ARB. However, C2-carnitine was completely restored by ARB. (A and B) Data were analyzed by one-way ANOVA with Tukey post-test. * $P < 0.05$, ** $P < 0.01$, vs WT group. ## $P < 0.01$, vs Vehicle group. B6AR, C57BL/6-Akita-ReninTG mice; 129AR, 129/Sv-Akita-ReninTG mice; WT, wild type; ARB, angiotensin receptor blocker.

Improvement in free fatty acid oxidation with RAS blockade: To further explore effects of DN on fatty acid oxidation by the kidney, we examined renal expression of fatty acid metabolism related genes. Compared to 129 WT controls, mRNA levels of key genes involved in mitochondrial fatty acid oxidation (CPT1 and CPT2) and key regulators of mitochondrial metabolism (PPAR-α and PGC-1α) are increased ≈2.5-fold in the vehicle-treated 129AR mice. ARB treatment restored mRNA levels for these genes to levels that were similar to WT (**Figure 4**).

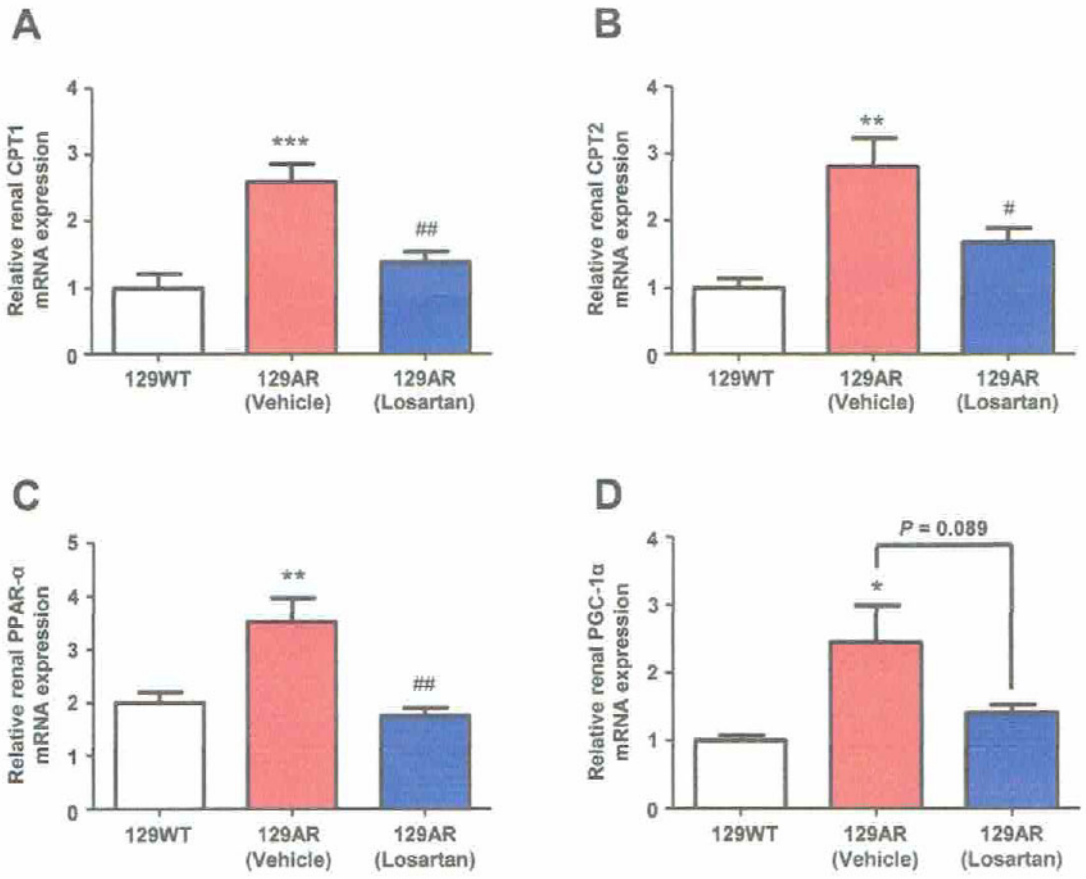

Figure 4. Renal mRNA expression of FAO-related genes in 129AR mice. (A) Renal mRNA levels of CPT1 were increased in 129AR mice and significantly restored by ARB. (B) Renal mRNA levels of CPT2 were increased in 129AR mice and significantly restored by ARB. (C) Renal mRNA levels of PPAR-α were increased in 129AR mice and significantly restored by ARB. (D) Renal mRNA levels of PGC-1α were increased in 129AR mice and tended to be restored by ARB. (A, B, C and D) Data were analyzed by one-way ANOVA with Tukey post-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, vs WT group. # $P < 0.05$, ## $P < 0.01$, vs Vehicle group. FAO, fatty acid oxidation; 129AR, 129/Sv-Akita-ReninTG mice; CPT, palmitoyltransferase; ARB, angiotensin receptor blocker; PPAR-α, peroxisome proliferator-activated receptor-alpha; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; WT, wild type.

< Conclusions>

Our study demonstrates substantial alterations of kidney mitochondrial fuel metabolism in a mouse model of DN. Notably, these abnormalities were only seen in *Akita-ReninTG* mice on the susceptible 129 background that develop features of human DN, but not on the C57BL/6 background that is resistant to the development of DN. We found broad reductions in even-chain acyl-carnitines, possibly reflecting impaired β-oxidation of mitochondrial fatty acids. The dramatic reduction in acetyl-carnitine generated from acetyl CoA, the final common metabolite of mitochondrial fuel oxidation, was almost completely reversed with ARB treatment. Likewise, changes in renal expression of fatty acid metabolism related genes seen in mice with DN were normalized with ARB treatment, suggesting a causal role for RAS activation in driving these abnormalities. Taken together, our findings suggest a critical role for RAS activation to promote renal metabolic abnormalities in DN, and these changes were only observed on a susceptible genetic background where they were associated with the development of kidney disease. As previous studies have linked impaired free fatty acid oxidation to chronic kidney injury, the reversal of these metabolic abnormalities by RAS blockade may contribute to renal protective actions in DN.

<Perspectives>

Results of our study suggest that, similar to the pathophysiology of other kidney diseases, impaired renal fatty acid metabolism is associated with the progression of DN. The improvement of impaired renal fatty acid metabolism may be one of the mechanisms for the renoprotective effect of RAS blockade in DN. Therefore, interventions for renal energy metabolism, especially fatty acid metabolism, would be novel therapeutic candidates to treat DN.