行政院國家科學委員會專題研究計畫　期中進度報告

高血脂與心臟神經再塑暨電生理特性改變之探討(1/2)

計畫類別：個別型計畫
計畫編號：NSC93-2314-B-002-204-
執行期間：93 年 08 月 01 日至 94 年 07 月 31 日
執行單位：國立臺灣大學醫學院內科

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報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 94 年 6 月 1 日

期中精簡報告:

計劃編號: 93-2314-B-002-204
計劃名稱: 高血脂與心臟神經再塑暨電生理特性改變之探討
(Cardiac Neural Remodeling and Electrophysiological Alterations in Hypercholesterolemia)

The broad and long-term objective of this research project is to test the hypothesis that the interaction between neural remodeling (nerve sprouting) and electrical remodeling underlies the mechanisms of cardiac arrhythmogenesis and sudden cardiac death in patients with hypercholesterolemia (HC). There are 4 Specific Aims in this project. We have some preliminary results in the Specific Aim 1-3 after one year work.

Specific Aim 1: *The aim is to perform optical mapping studies in hearts of HC rabbits, demonstrating the electrophysiological consequences of neural and electrical remodeling.*

Our previous works demonstrated that the HC group showed significant APD prolongation and increased APD dispersion after 8 weeks of feeding. The mean APD_{80} of the anterior wall at a PCL of 400 ms was significantly longer in the HC group than the control group (APD_{80}: 193±21 ms vs. 174±17 ms; $p<0.05$). The spatial heterogeneity of APD, represented as either the SD or difference of APD_{80} over the anterior wall, was also significantly greater in HC group compared to the control group (SD: 8.7±2.9 ms vs. 5.5±2.0 ms, $p<0.01$; difference: 44.7±16.9 ms vs. 25.1±7.3 ms, $p<0.01$). Meanwhile, the ERP was longer in the HC group than in the control group.

In present study, we found that the dynamic instability, restitution characteristics, seemed increased in HC group than control group. (Figure 1) However, the APD restitution (APDR) did show a spatial heterogeneity. We need to further measure the APDR characteristics at multiple sites to clarify that the increased dynamic instability resulted from either intrinsic restitution characteristics or spatial heterogeneity.

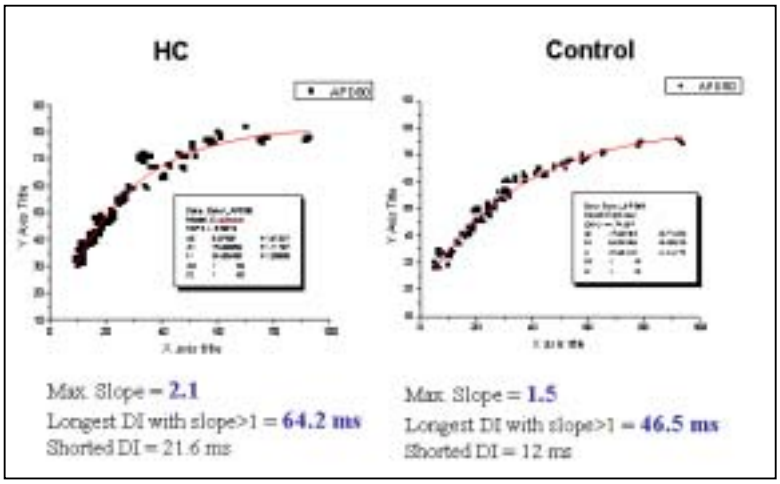

Figure 1: Increased dynamic instability in HC rabbits

We also demonstrated that the conduction velocity was significantly decreased (about 15% decrease) in HC rabbits as compared with control rabbits. (Figure 2)

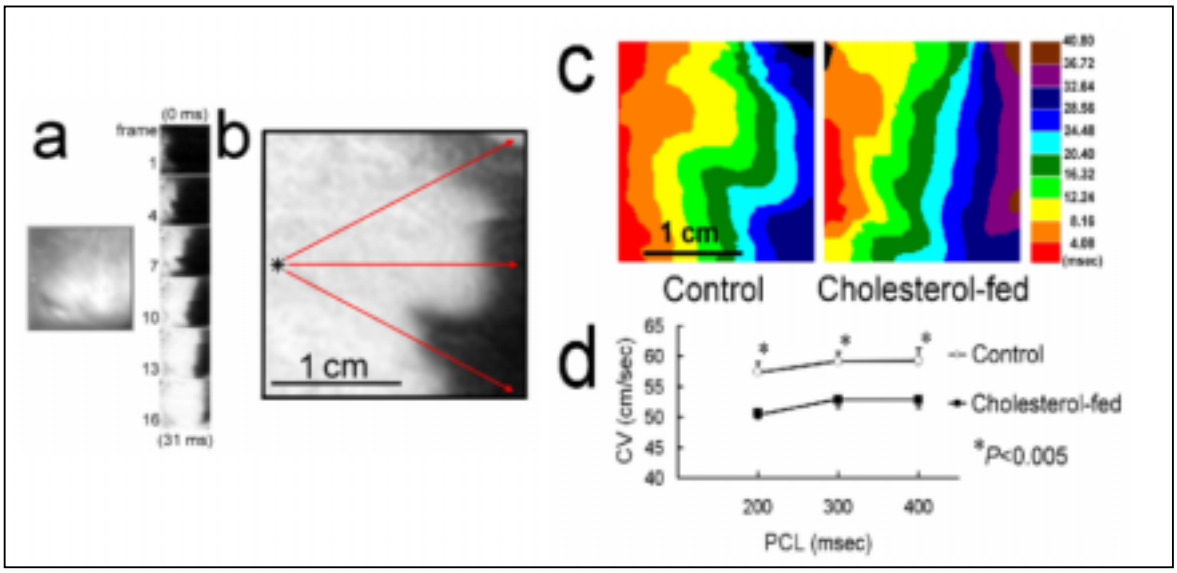

Figure 2: Decreased conduction velocity in HC rabbits

Due to increased in pre-existing APD dispersion, increased APDR slope and decreased conduction velocity, the ventricular fibrillation (VF) dynamic was more complex in HC rabbits (Figure 3) and the wavebreak number was significantly increased during VF in HC rabbit hearts (Figure 4).

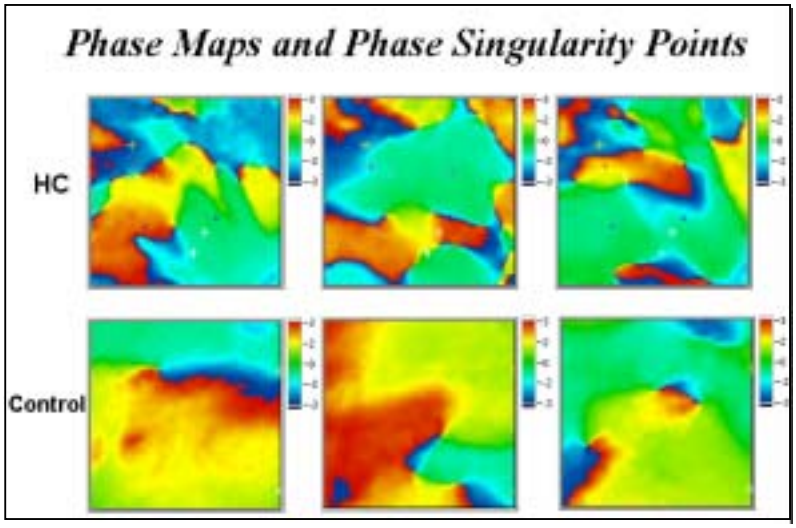

Figure 3: Increased VF dynamics in HC rabbits by phase map

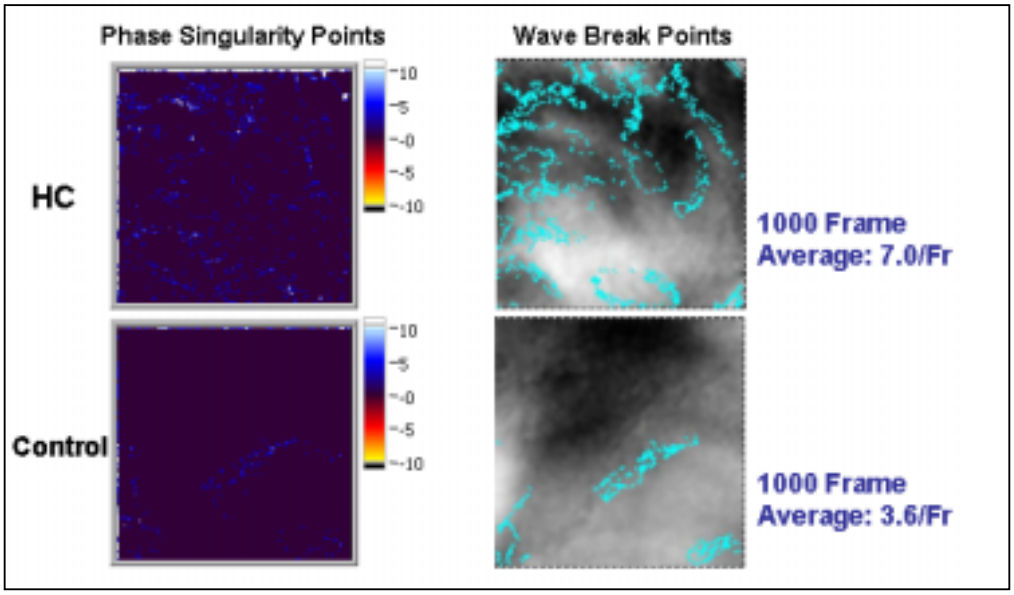

Figure 4: Increased phase singularity and wavebreak points during VF in HC rabbits

Specific Aim 2: *The aim is to test the hypothesis that HC results in both increased sympathetic nerve density and sympathetic activity. We performed ^{123}I-MIBG imaging in HC rabbits to access the sympathetic activity in vivo. The heart rate variability and baroreflex sensitivity were also measured during the feeding period.*

We found that the ^{123}I-MIBG myocardial uptake was increased in Rabbits with HC. Twelve rabbits (6 in control and 6 in HC) underwent MIBG scintigraphy. Figure 5 is the representative planar images of whole body distribution in hypercholesterol rabbits at 5 min, 30 min, 1 and 3 hr after injection of the ^{123}I-MIBG. At the initial period of this study (without starting the diet test), all the rabbits had almost the same uptake and washout ratios at the baseline period (Table 1). After 3-month lipid-enriched diet feeding, we performed the same imaging protocol with these rabbits. It showed that HC rabbit significantly had a higher uptake ratio than control group (3.39 ± 0.11 in HC group and 2.90 ± 0.10 in control group). The washout ratio of ^{123}I-MIBG from the myocardium in HC group was slightly higher than that in age-matched control rabbits (0.53 ± 0.02 in HC group and 0.46 ± 0.05 in control group). Thus, the sympathetic innervation was significantly increased in rabbit hearts with HC. But the sympathetic activity seemed only slightly elevated in anesthetized rabbits.

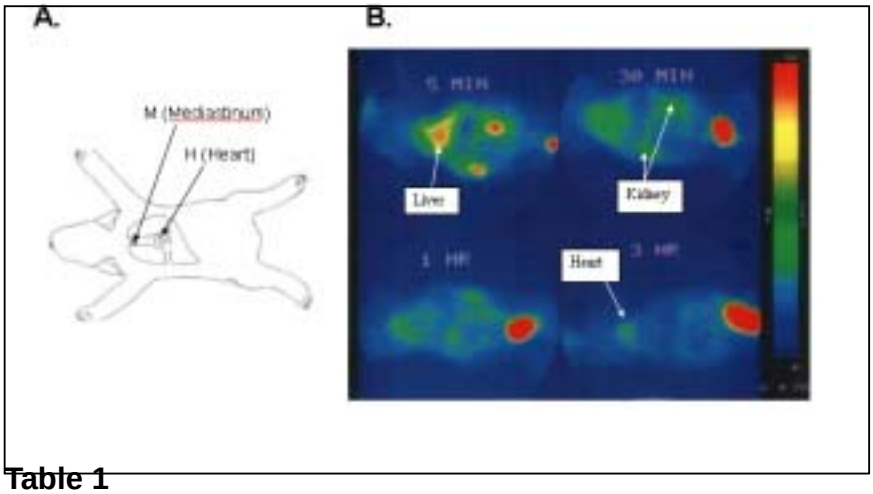

Figure 5: Representative example of MIBG imaging

Table 1

	Hypercholesterol Group		Control Group	
Study period	Initial	End	Initial	End
Washout ratio	0.47 ± 0.07[a]	0.53 ± 0.02	0.46 ± 0.09	0.46 ± 0.05
Uptake ratio	2.69 ± 0.11	3.39 ± 0.11[*,+]	2.77 ± 0.09	2.90 ± 0.10

For baroreflex sensitivity (BRS) and heart rate variability, Figure 6 shows an example of our measurement in anesthetized rabbits. We could get both time-domain and frequency-domain heart rate variability. Meanwhile, we used two methods to measure the BRS. The first one is to get spontaneous BRS by 15 min recording and the second one is to get “classical” BRS by phenylephrine injection. The correlation coefficiency between spontaneous and classical BRS is about **0.81** in our study.

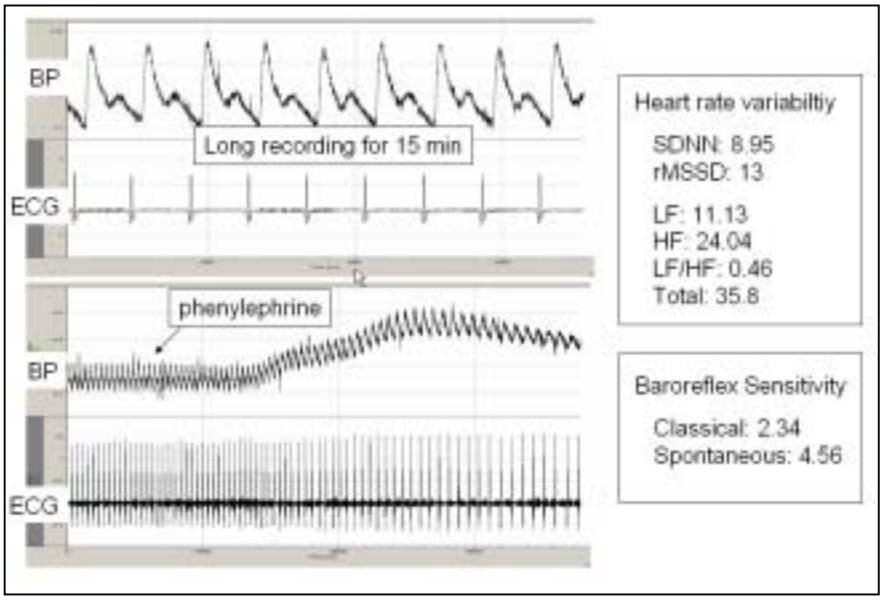

Figure 6: Measurement of BRS and HRV

However, repeated measurement of BRS in 2-wk interval (according to our protocol) resulted in severe vascular injury, which changed the vascular compliance and affected the results of BRS. Thus, we modified our protocol to repeated measurement of BRS in 4-wk interval. This protocol will be completed by July 31.

Specific Aim 3: *The aim is to test the hypothesis that pharmacological and dietary intervention can reduce/reverse the proarrhythmic neural and electrical remodeling induced by HC.*

Our previous work demonstrated that HC in rabbit induced cardiac nerve sprouting and sympathetic hyperinnervation that increased the vulnerability to ventricular arrhythmias. There is an excellent correlation between serum cholesterol concentration and the cardiac nerve density by dietary intervention only. To test the hypothesis that statin therapy can reduce cardiac nerve density in hypercholesterolemic rabbits, we treated 7 rabbits orally with simvastatin (20 mg/day). The cholesterol leve at 8 weeks were 873 ±111 mg/dl. The cholesterol levels of untreated rabbits are 1718 ± 345 mg/dL at 8 week. These data indicate that simvastatin therapy significantly reduces the serum cholesterol level by roughly 50%. ($p<0.001$) Figure 7 shows several examples of GAP 43 staining of HC rabbits with or without simvastatin treatment. We could see the GAP-43 positive nerve was much less abundant in statin-treated group than HC group. Figure 8 summarized the GAP-43 positive nerve density in both group. The density of GAP43-positive nerves in simvastatin treated hearts was significantly less than that in untreated rabbits. ($p<0.01$) Furthermore, the percentage of decreases in nerve density is approximately the same as the percentage of decreases in serum cholesterol level, which is consistent with the nerve density associated with that cholesterol level.
In addition to the effects on nerve sprouting and cholesterol, simvastatin also significantly reduced the incidences of spontaneous and inducible ventricular fibrillation in Langendorff-perfused hearts harvested from these rabbits. Our study showed that VF could be demonstrated in about 79% of HC rabbits. Despite dietary modification lowering the serum cholesterol level, VF still could be demonstrated in about 66% of HC rabbits with dietary modification. However, there was **NO** HC rabbits treated with simvastatin could induce VF. These preliminary results suggest that statin therapy results in an antiarrhythmic effect on HC rabbits. Further studies to clarify the difference between dietary modification and statin treatment and the effects of statin treatment on TH-positive nerve will be preceded in the coming year.

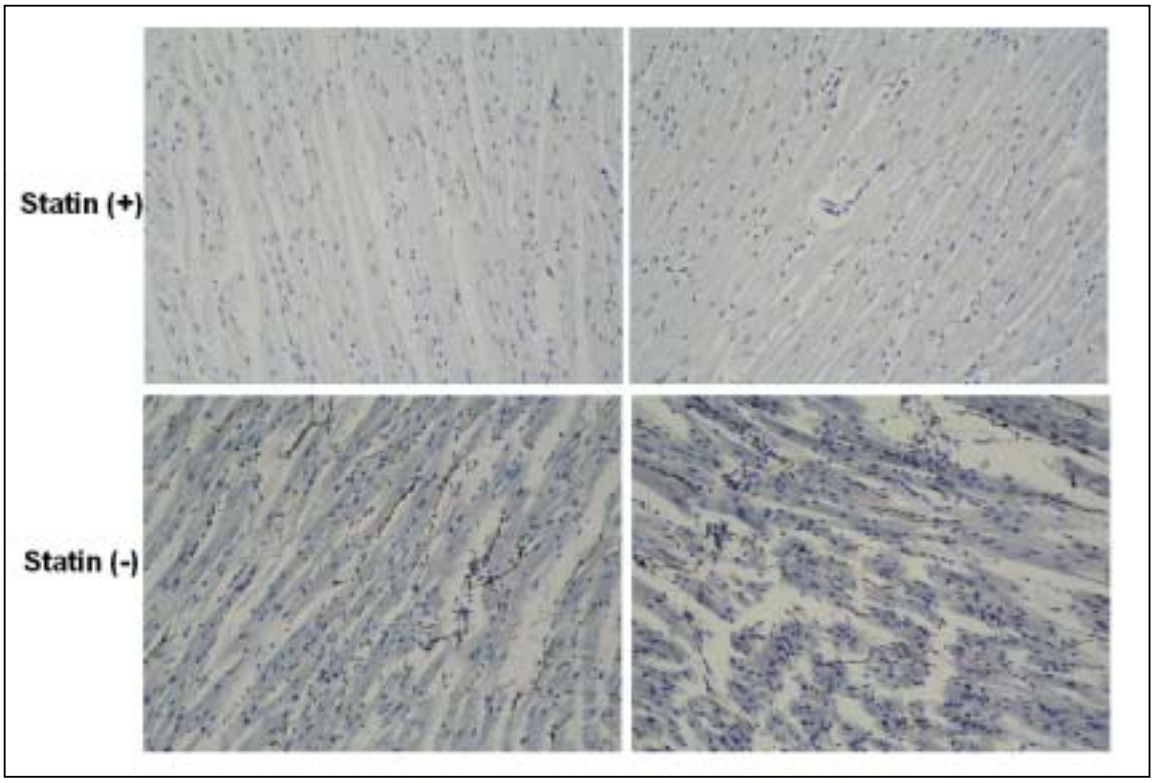

Figure 7: Examples of GAP-43 immunocytochemical stain in HC rabbits with or without simvastatin treatment

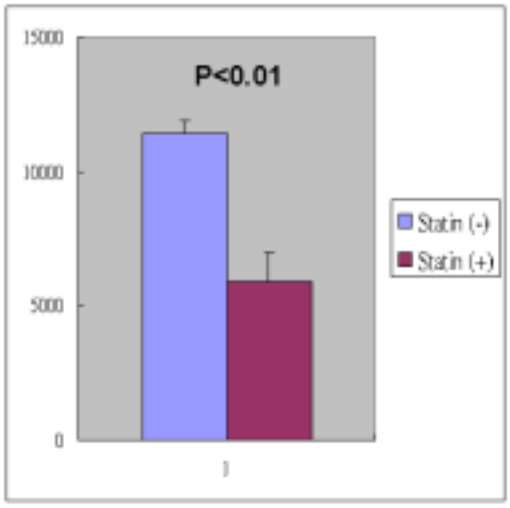

Figure 8: The density of GAP43-positive nerves in simvastatin treated hearts was significantly less than that in untreated rabbits.

In summary, we found the VF dynamics did more complex in HC rabbit hearts. It may result from increased APD dispersion and decreased conduction velocity in HC rabbit after neural and electrical remodeling. The importance of restitution characteristics for VF dynamics needs further data analysis to clarify. By I-131 MIBG imaging, we successfully demonstrated

that sympathetic hyperinnervation of HC rabbit hearts *"in vivo"*, which was compatible with our previous immonocytochemical studies. The function significance of sympathetic hyperinnervation on HRV and BRS needs further study to explore. In simvastain-treated HC rabbits, we found that the GAP-43 positive nerve (sprouting nerve) was significantly reduced after statin treatment, which may result from a parallel reduction of serum cholesterol level. The ventricular vulnerability to fibrillation was also significantly decreased after statin treatment. Its effects were more prominent than dietary modification alone. The underlying mechanisms responsible for the differences between dietary modification and statin treatment need further data analysis to clarify.