

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Hypercholesterolemia Effect on Potassium Channels

Anna N. Bukiya and Avia Rosenhouse-Dantsker

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/59761>

1. Introduction

Cholesterol is a major lipid component of the plasma membrane in mammalian cells constituting up to 45 mol % with respect to other lipids [1, 2]. Yet, even a limited increase in blood and/or tissue cholesterol of up to 2-3 fold above the physiological level is cytotoxic [1-3] and is associated with the development of cardiovascular disease [4-6]. The underlying source for the effect of cholesterol on cellular functions is its ability to alter the function of multiple membrane proteins including ion channels (see, for example, reviews [7-9]).

In recent years, high cholesterol diet has been shown to affect the function of multiple ion channels. In this chapter we focus on the effect of dietary-induced increase in blood and tissue cholesterol levels on potassium channels. Potassium channels are among the largest and most complex types of ion channels. They are widely expressed in human tissues and are involved in many aspects of cell function including membrane excitability, regulation of heart rate, neuronal signaling, vascular tone, insulin release and salt flow across epithelia (see, for example, reviews [10-17]). In addition, they also play a critical role in the protection of neurons and muscle under metabolic stress. As a result, mutations in potassium channels lead to a wide range of disease in the brain (epilepsy, episodic ataxia), ear (deafness), heart (arrhythmia), muscle (myokymia, periodic paralysis), kidney (hypertension), pancreas (hyperinsulinemic hypoglycemia, neonatal diabetes). Therefore, the effect of hypercholesterolemia on potassium channel function has important pathophysiological implications.

The most common effect of a high-cholesterol diet on ion channels in general and potassium channels in particular is a decrease in channel activity. Yet, the activity of some channels is increased following a high cholesterol diet. For example, hypercholesterolemia suppressed the function of the Kir2 subfamily of inwardly rectifying potassium (Kir) channels in different cell types by ~2 fold [18-19]. However, atrial G-protein gated inwardly rectifying potassium channels (GIRK or Kir3) that underlie K_{ACh} currents in the heart are enhanced by a high-

cholesterol diet [19]. Several types of voltage gated (K_v) channels were sensitive to changes in the level of cellular membrane and dietary cholesterol [20-26]. The majority of the reports described suppression of channel function by high-cholesterol diet. Moreover, large conductance calcium-activated potassium (BK) channels were often suppressed following a high-cholesterol diet [27-31].

In this chapter, we will describe the implications of high-cholesterol dietary intake on members of three major families of potassium channels: voltage gated potassium (K_v) channels, calcium-activated potassium (K_{Ca}) channels of large conductance (BK) and inwardly rectifying potassium (K_{ir}) channels. We will demonstrate that not only does high-cholesterol diet increase the levels of blood cholesterol but it also increases the level of cholesterol in tissues in which these types of channels are expressed. We will show that this cholesterol accumulation *in-vivo* is reflected in the function of potassium channels. Lastly, we will discuss the importance of the observed effects to organ function.

2. High-cholesterol diet model

Cholesterol-rich diet that is characteristic of Western societies critically controls blood lipid levels in several species, including humans [32-33]. Regression models have been reported for serum total cholesterol, triacylglycerol, and low-density-, high-density-, and very-low-density-lipoprotein cholesterol. In particular, correlations between increased levels of dietary cholesterol and these plasma lipids and lipoproteins were found to be 0.74, 0.65, 0.41, 0.14, and 0.34, respectively [32]. It has been predicted that compliance with the dietary recommendation to consume <300 mg cholesterol per day (with 30% of energy from fat, < 10% from saturated fat) will reduce plasma total and low-density-lipoprotein-cholesterol (LDL) concentrations by approximately 5% compared with amounts associated with the average American diet [32]. Restriction of dietary cholesterol intake represents a widely used preventative measure against numerous pathological conditions since increased total cholesterol and LDL levels are well-recognized risk factors for several largely prevalent pathologies, including stroke [34-37], coronary heart disease [38-39], vascular dementia [40], and atherosclerosis [41]. Therefore, it is not surprising that a cholesterol-rich diet has been recreated in a research laboratory setting to study the deleterious effects of cholesterol-rich food intake.

High-cholesterol diet-induced hypercholesterolemia is widely used for studies on monkeys [42], hamsters [43], guinea pigs [44], rabbits [20, 27, 29, 45, 46], rats [19, 47-49] and mice [50]. The dietary-induced hypercholesterolemia model has several advantages. First, it mimics the high-cholesterol food intake that is characteristic to the US population, and which impacts cholesterol levels in the blood of human individuals [32-33]. Second, it does not require alteration of the genetic background of the animal. These advantages make high-cholesterol diet a useful tool to manipulate cholesterol levels in species in which genetic alterations to achieve hypercholesterolemia are challenging. For example, diet-induced changes in blood cholesterol level have been detected in primates besides humans, such as baboons *Papio spp* (reviewed by [51]) and Japanese monkeys *Macaca fuscata* [42].

It should be noted that earlier studies have documented the existence of “hyper-” and “hyporesponders” to a cholesterol-rich diet in the human population. Trials with the same subjects demonstrated that the human population includes people with a consistently low or high response to increased dietary intake of cholesterol (reviewed by [52]). Similarly to humans, other primates also vary in their blood lipid responses to dietary lipid composition. Selective breeding of primates based on their individual responses to the composition of the diet resulted in lines that are characterized by low versus high responses to changes in dietary lipids. Thus, similarly to humans, changes in lipoprotein patterns in response to dietary cholesterol seem to be heritable in primates (reviewed by [51]). Further studies have shown that the differential response to cholesterol consumption in smaller laboratory animals also results in inbred strains of rabbits, rats, and mice that differ in their sensitivity to high-cholesterol diet. Their responsiveness to high-cholesterol diet is largely influenced by the genetic background [52]. Compared to humans, changes in blood cholesterol and LDL levels induced by high-cholesterol diet in lab animals are robust and of high magnitude. Therefore, a high-cholesterol diet represents a useful and practical tool to induce an increase in blood cholesterol levels that ultimately leads to hypercholesterolemia in animal models.

In a typical protocol for a high-cholesterol diet in animal models, the animal would be fed (*ad libitum* or via gavage) cholesterol-rich food. The ability of a high-cholesterol diet to increase blood cholesterol and LDL levels is well documented in various animal models. For instance, consumption of food containing 2% cholesterol for 20 months leads to up to a 2-fold increase in total cholesterol and LDL levels in the blood of a macaque [42]. An even more drastic increase was documented multiple times in rabbits: feeding animals with dietary cholesterol supplementation in the amount of 1 g per day for 4 weeks resulted in ~20-fold increase in total serum cholesterol concentration in rabbits [29], 1% cholesterol food for 8 weeks resulted in a nearly 30-fold increase in total serum cholesterol level [20], and 0.5% cholesterol for 12 weeks raised blood cholesterol level by nearly 33-fold [45]. Another study reported a 10-fold increase in plasma cholesterol concentration in rabbits fed by 0.5% cholesterol food for 16 weeks [27]. Mice and rats usually respond with a lower magnitude of increase in blood cholesterol and LDL levels in response to diet, yet these changes are still easily detected. In mice, consumption of 1,000 mg/kg cholesterol per day for 8 weeks increased blood cholesterol and LDL levels by 2-3 times [50]. Significant increase in blood cholesterol and LDL levels have been documented following feeding with 4% cholesterol food for 4 weeks in Sprague-Dawley rats [48], 2% cholesterol diet for 20-24 weeks in the same strain [19], and 5% cholesterol diet for 3 weeks led to a significant increase in the plasma cholesterol level in Wistar rats [47].

In our rat model of a high-cholesterol diet, male Sprague-Dawley rats (50 g) were subjected to an *ad libitum* high-cholesterol diet (2% cholesterol). The total cholesterol plasma level remained unchanged for 11-12 weeks of diet, but significantly increased during weeks 18-23 and was increased even further during weeks 27-28 of the high-cholesterol diet compared to the control group (Figure 1A). After receiving a high-cholesterol diet for 11-12 weeks, the rats displayed a significant increase in the LDL plasma level, with the increase becoming more pronounced during weeks 18-23 on a high-cholesterol diet and reaching a nearly 5-fold increase during weeks 27-28 on a high-cholesterol diet when compared to the control group (Figure 1B). High-

density-lipoproteins (HDL), however, seemed to be less sensitive to high-cholesterol diet intake, as HDL level increased significantly only during weeks 27-28 on a high-cholesterol diet (Figure 1C). Triglycerides followed the pattern of response of cholesterol and LDL: triglyceride level increased significantly during weeks 18-23 and became even higher during weeks 27-28 of the high-cholesterol diet (Figure 1D). Overall, changes in the blood lipid profile correlated with the duration of the high-cholesterol diet. Therefore, a high-cholesterol diet constitutes a valuable tool when the degree of change in the blood lipid profile needs to be controlled tightly.

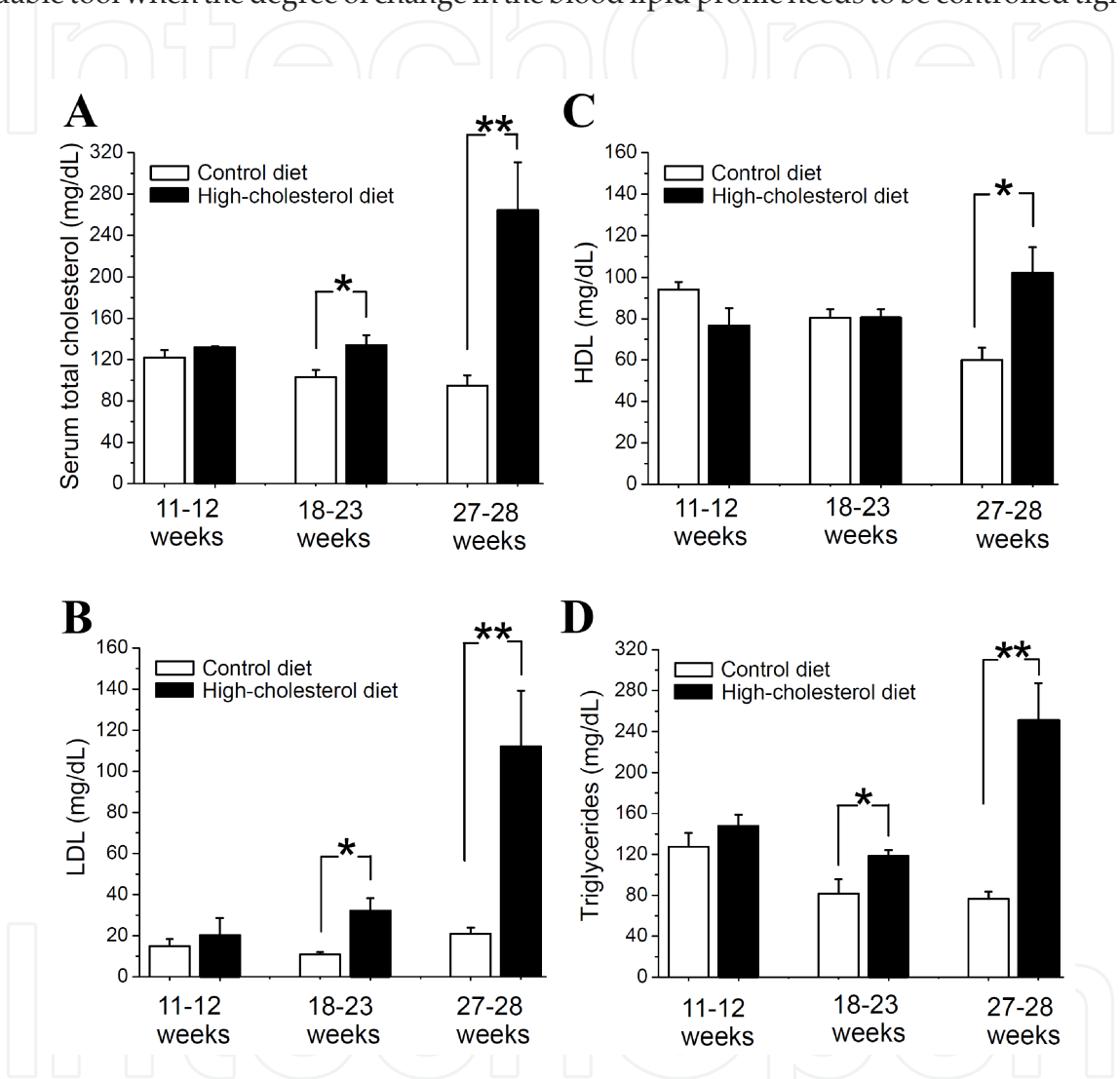


Figure 1. Blood lipid profile in Sprague-Dawley rats fed high-cholesterol diet. (A) Serum total cholesterol. (B) Low-density-lipoprotein-cholesterol. (C) High-density-lipoprotein. (D) Triglycerides. Here and in all figures: significantly different from control is indicated by an asterisk (*, $p < 0.05$; **, $p < 0.01$).

3. Effect of high-cholesterol diet on potassium channels.

In view of the advantages of a high cholesterol diet described above and its ability to adequately represent the characteristics of high cholesterol food intake in the US, it is widely used

for studies on ion channel function during dyslipidemia and hypercholesterolemia. For instance, dietary-induced hypercholesterolemia was shown to up-regulate the function of L-type Ca^{2+} -channels in detrusor smooth muscle [48], transient receptor potential channels 5 and 6 (TRPC5 and TRPC6) in aortic endothelial cells [53], cardiac G protein gated inwardly rectifying potassium channels [19], and epithelial Na^+ channels (eNaC) [54].

In this chapter, we will focus on high-cholesterol diet-driven changes in the function of potassium channels. In particular, we will discuss the effect of an increase in the dietary intake of cholesterol on voltage gated (K_v) channels, calcium activated potassium channels (K_{Ca}) and inwardly rectifying potassium channels (Kir).

Voltage-gated potassium (K_v) channels. Several reports describing the effect of a high-cholesterol diet on potassium channel function came from studies on voltage-gated potassium channels (K_v) [21]. These channels are transmembrane proteins that are located on the plasma membrane and sensitive to changes in the transmembrane potential [55]. Upon membrane depolarization, voltage-gated potassium channels conduct outward potassium currents as these channels exhibit the highest selectivity for K^+ ions compared to other monovalent cations. Activation of K_v channels usually results in decreased depolarization and the return of the plasma membrane potential to the resting level. Vertebrate K_v channels are tetramers of four pore-forming subunits, each contributing to the wall of the K^+ conducting pore. Each pore-forming subunit is composed of six transmembrane α -helices with intracellular N- and C-termini (Figure 2A). Helices S1-S4 contribute to voltage-sensing and S5-S6 form the pore region. Voltage-gated potassium channels play a key role in cellular excitability including vascular smooth muscle [55]. The effect of a high-cholesterol diet on K_v channel function has been extensively studied in the cardiovascular system.

High-cholesterol diet failed to modulate K_v channel function after a brief placement of an Ossabaw miniature swine model on a high-fat/high-cholesterol/high-fructose diet [26]. The animals were fed by the diet for 9 weeks. The authors considered the duration of diet administration to be relatively short. As a result, only an early stage of a complex metabolic syndrome developed. The diet caused ~4-fold increase in blood cholesterol level in the group on diet compared to the control group on standard chow. Coronary arterioles from both groups were isolated and pressurized to 60 cmH_2O for *in-vitro* pharmacological studies. Those include the assessment of the K_v channel contribution to the arteriolar response to the vasodilator 2-chloroadenosine. Pharmacological blockade of K_v channels by 4-aminopyridine reduced the arteriolar sensitivity to 2-chloroadenosine in both control and early stage metabolic syndrome groups. This result suggests that K_v channel regulation of the arteriolar diameter was still preserved at the early stage of the modeled metabolic syndrome. In contrast, blockade of Kir6 (K_{ATP}) channels (see below) with glibenclamide reduced the arteriolar sensitivity to 2-chloroadenosine in the control group only. Therefore, the involvement of K_v channels in the arteriolar responses to 2-chloroadenosine is resistant to the described diet.

A more complex scenario was observed in work by Heaps *et al* on a Yucatan miniature swine model that was placed on high-fat/high-cholesterol diet for 20 weeks [22-23]. Already after 4 weeks, blood cholesterol and LDL levels were increased on the average by 6 times when compared to the pre-diet and to the group on control diet levels. At the end of a 20 week-long

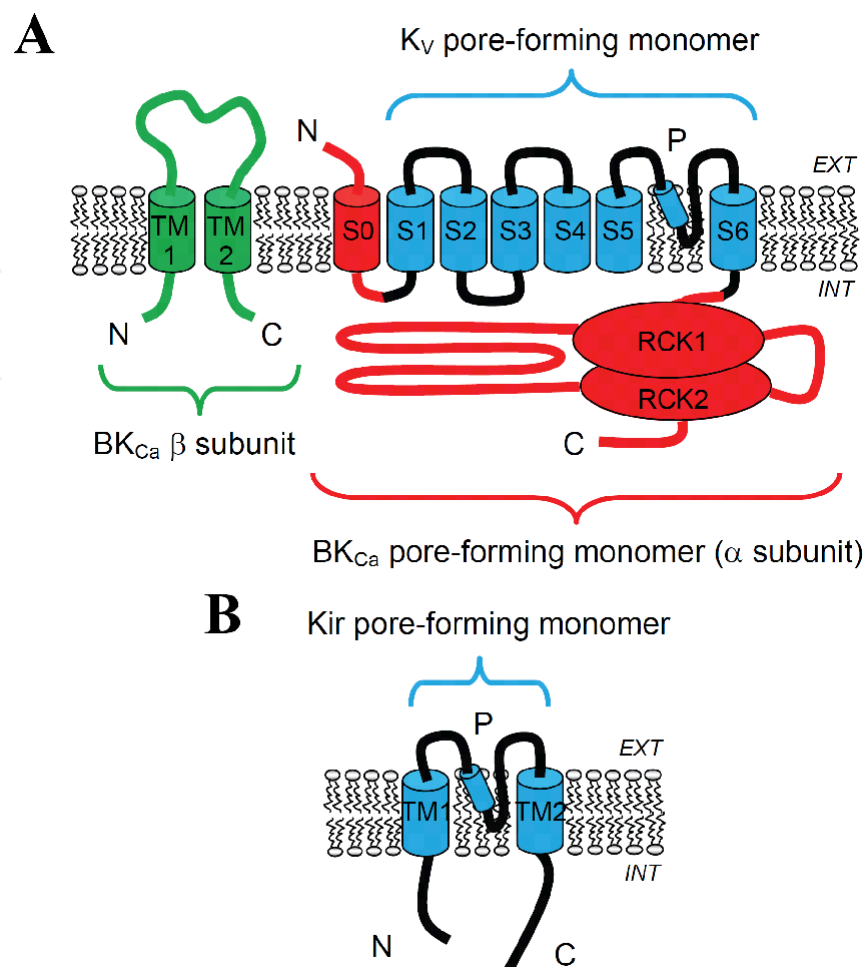


Figure 2. Schematic structures of K_v, K_{Ca}, and Kir channels. (A) Schematic structure of voltage-gated and voltage-/calcium gated potassium channel subunits. TM: transmembrane domain of β subunit; S: transmembrane domain of α subunit; RCK: regulator of conductance of potassium; EXT: extracellular media; INT: intracellular media. (B) Schematic structure of inwardly rectifying potassium channel subunit. TM1 is the outer transmembrane helix and TM2 is the inner transmembrane helix.

diet, *coronary vascular reactivity* was assessed. Coronary microvessels were removed, cannulated, pressurized at 40 mmHg and the luminal diameter was monitored. In this experimental setting, 4-aminopyridine attenuated adenosine-induced dilation of coronary arterioles in the control group, but did not affect adenosine-induced arteriole dilation in the group subjected to high-fat/high-cholesterol diet. Therefore, adenosine activation of K_v channels is attenuated during hypercholesterolemia. In contrast, Bender *et al* did not detect diet-induced changes in K_v channel contribution to the 2-chloroadenosine-induced dilation of swine coronary arterioles [26]. This controversy may be attributed to several differences between the two studies. For instance, in the study by Bender *et al.*, animals were subjected to a high-fructose diet in addition to high-fat/high-cholesterol food. Second, Bender *et al* were studying the consequences of brief (only 9 weeks) dieting as opposed to Heaps *et al* who placed the animals on a 20 weeks diet. It is therefore likely that a longer duration of diet is needed for K_v channels to lose their sensitivity to adenosine application. Loss of K_v channel contribution to adenosine-mediated

vasodilation was studied further at the level of coronary arteriole smooth muscle K_V currents. Outward K^+ currents were recorded in whole-cell configuration from freshly isolated arteriole myocytes. Noteworthy, the intracellular calcium was chelated to eliminate the calcium-dependent component from the whole-cell K^+ currents. K_V currents from myocytes in the high-cholesterol diet group were significantly smaller compared to the control [22-23]. Therefore, reduction in K^+ currents may underlie the attenuation of the K_V component in adenosine-induced arteriole dilations. Treatment of membrane patches with different concentrations of the non-selective potassium channel blocker tetraethylammonium (TEA) revealed that high-cholesterol diet primarily altered K_V channel isoforms with high sensitivity to TEA. RT-PCR experiments determined that $K_V3.1$ and $K_V3.3$ channel isoforms were expressed in coronary arterioles. However, expression levels of $K_V3.1$ and $K_V3.3$ were not changed by the diet. In contrast, it was shown that arteriole dilation caused by the receptor-independent activator of adenylyl cyclase forskolin was abolished in the high-fat/high-cholesterol diet group. Furthermore, the K_V channel blocker 4-aminopyridine and the non-selective blocker of potassium channels TEA significantly attenuated forskolin-mediated vasodilation in control, but not in the high-fat/high-cholesterol diet group. Taken together, these data suggest that hypercholesterolemia-mediated ablation of adenosine-induced vasodilation of coronary arterioles could be attributed to the impairment of the adenylyl cyclase pathway coupled to highly TEA-sensitive K_V channel isoforms.

These data showing a reduced K_V component in the whole-cell outward potassium current are consistent with another report that focused on potassium currents in swine coronary artery smooth muscle cells [25]. The animals were placed on a high-fat diet for 20 weeks. The diet significantly increased the total blood serum cholesterol level and triglycerides. Remarkably, the increase in both blood lipid components was higher in female swines. 4-aminopyridine-sensitive component of the whole-cell outward potassium current recorded from the isolated coronary artery smooth muscle cells was significantly diminished in the high-cholesterol diet group of male swines. In females, however, no significant reduction in K_V 4-aminopyridine-sensitive (K_V component) was detected [25]. This report suggests that the effect of high-cholesterol diet on the function of K_V channels may be gender-specific.

Loss of K_V channel current and its contribution to vasodilatory responses has not only been documented in coronary arteries, but also in the *middle cerebral artery*. In the latter work, New Zealand rabbits were placed on chow supplemented with 1% cholesterol for 8 weeks [20]. The total blood serum cholesterol increased close to 30 times at the end of the diet. In the vasodilatory response study, the middle cerebral arteries were dissected, mounted and pre-contracted with a high- K^+ (50 mM) physiologic saline solution. In the pre-contracted arteries isolated from control animals, acetylcholine produced artery relaxation. In the arteries from the high-cholesterol diet group, similar concentrations of acetylcholine induced less relaxation. In the control group, neither TBA, an inhibitor of K_{Ca} channels, nor glibenclamide, an inhibitor of K_{ATP} channels, significantly affected the concentration-response to acetylcholine, whereas 4-aminopyridine, a blocker of K_V channels, strongly inhibited this relaxation. In the high-cholesterol diet group, TBA, glibenclamide or 4-aminopyridine did not significantly affect the response to acetylcholine [20]. Loss of 4-aminopyridine effect on acetylcholine-induced

cerebral artery dilation suggests that either the function of K_v channels or their sensitivity to acetylcholine-activated pathway is diminished.

Apart from the vascular system, the effect of a high-cholesterol diet on K_v channels was studied in the *heart* itself [24]. Rabbits were subjected to a high-cholesterol diet (1.5% cholesterol) for 8 weeks. The diet resulted in an approximately 14 times increase in blood serum cholesterol level. RT-PCR studies were used to determine the impact of the high-cholesterol diet on the left ventricular mRNA expression level of several potassium channels that play a key role in the contractility of the heart. High-cholesterol diet significantly decreased the expression of $K_v4.2$, but did not alter the level of $K_v4.3$. Notably, a significant decrease and a significant increase in mRNA levels were detected for $K_v11.1$ (ERG1) and $K_v7.1$ (K_{VLQT1}) channels, respectively [24]. These data document that a high-cholesterol diet differentially affects the expression of voltage-gated potassium channels, with decrease, increase and “no-effect” being detected for this group of channels. The authors suggested that the arrhythmogenic nature of the hypercholesterolemia may be mediated by changed expression levels of the examined channels.

Calcium-activated potassium (K_{Ca}) channels. The effect of a high-cholesterol diet on calcium-sensitive potassium channels (K_{Ca}) is well described for calcium- and voltage-gated potassium channels of large conductance (BK). Fully functional BK channels result from the tetrameric association of 125-140 kDa polypeptides termed α , slo or slo1 subunits. Slo1 subunits share significant homology with K_v channels of the six transmembrane domain (TM6) family, with the S1-S4 regions contributing to voltage-sensing and the S5-S6 helices forming the activation gate-pore region (Figure 2A) [12, 56-57]. Unlike purely voltage-gated K_v channels, in addition to the S1-S6 core, BK slo1 subunits contain an additional transmembrane helix S0 leading to an extracellular N-terminus [58] and a large cytosolic tail domain (CTD) [59-61]. The CTD includes two domains that Regulate the Conductance of K^+ (RCK). RCKs contain sites for sensing intracellular Ca^{2+} levels and allow BK channels to increase channel activity in response to an increase in intracellular Ca^{2+} physiological levels [59-60]. In most tissues, BK channels usually result from the association of slo1 proteins with auxiliary β subunits. Four types of BK β subunits, $\beta1$ -4, have been identified. BK β subunits share an overall topology of two TM segments joined by a large extracellular loop, plus two short intracellular N and C termini [62]. Expression of β subunits is tissue-specific, with the $\beta1$ subunit being prevalent in smooth muscle, including vasculature [62-63]. The functional role of BK channels is similar to K_v channels: upon membrane depolarization BK channels generate outward potassium currents to oppose depolarization and favor the return of the transmembrane voltage to its resting level.

Several animal models have been used to document that plasmalemma BK channels are sensitive to dietary-induced hypercholesterolemia [64]. Considering the ample evidence linking hypercholesterolemia to cardiovascular disease, and the key role of BK channels in regulating vascular tone [65], most of the studies describe the effect of a high-cholesterol diet on BK channel function in the vascular system. One of the early studies addressed changes in endothelium-dependent and independent components *in-vitro* relaxation of carotid artery rings obtained from rabbits following administration of a 12 week-long high-cholesterol diet (0.5% cholesterol). Evaluation of isometric tension in carotid artery rings was performed in the

presence of nitric oxide (NO), sodium nitroprusside and 8-bromoguanosine 3', 5'-cyclic monophosphate (8-Brc-GMP). The responses to all three chemicals remained unaltered by dietary-induced hypercholesterolemia [45]. After application of a BK channel blocker, NO-mediated artery relaxation was significantly reduced in the hypercholesterolemic group. These findings could imply that BK channel contribution to the regulation of arterial tension during hypercholesterolemia is enhanced. It has been repeatedly suggested that dietary-induced hypercholesterolemia may lead to a compensatory increase in BK channel activity [45, 66]. Considering that smooth muscle BK channels are critical regulators of the arterial diameter, the compensatory increase in BK channel function during the course of high-cholesterol diet could take place in the arterial smooth muscle [45, 66]. Alternatively, BK channel activity could be reduced by hypercholesterolemia, in which case BK channels would be more available for activation [64], and would contribute more to an NO-induced decrease in vascular tension. However, the latter scenario is unlikely as an increase in vascular smooth muscle BK channel activity has been reported in cell-attached recordings from the area affected by the atherosclerotic plaque formation [67]. This difference is lost when BK channel function is evaluated in cell-free patches excised from the cells [67]. Thus, increased smooth muscle BK channel activity associated with atherosclerotic plaque likely requires the involvement of the intracellular organelles and/or freely diffusible cytosolic signals.

Hypercholesterolemia-driven increase in arterial K_{Ca} channel function compared to control chow was also reported in diabetic pigs receiving high-fat/high-cholesterol (2%) diet [21]. Patch-clamp recording of whole-cell outward potassium currents revealed increased density of the K_{Ca} -component in the high-cholesterol diet group. Western blot failed to detect a significant increase in the amount of the K_{Ca} pore-forming protein. In addition, intracellular calcium concentration did not differ in control versus high-cholesterol diet groups. The data indicated that diabetic hypercholesterolemia leads to an increased functional coupling between K_{Ca} and intracellular calcium release.

In contrast to the above *in-vitro* studies, *in-vivo* work conducted on hypercholesterolemic rabbits showed a reduction in NO-induced vasodilation as determined by monitoring the hindlimb vascular conductance in response to acetylcholine and bradykinin [27]. In this set of experiments, rabbits were fed high-cholesterol (0.5% cholesterol) diet for 16 weeks. The NO-independent vasodilation in response to acetylcholine and bradykinin, however, was larger in animals on high-cholesterol diet. Development of this NO-independent component of vasodilation was blocked by either TEA or by a mixture of the K_{Ca} channel blocker charybdoxin and the small conductance K_{Ca} channels blocker apamin. Thus, the authors concluded that hypercholesterolemia impaired K_{Ca} channel-mediated vasodilation [27].

Another study using hypercholesterolemic rabbits to test acetylcholine-induced vasorelaxation focused on renal artery. Rabbits were subjected to a high-cholesterol diet (0.5% cholesterol) for 5 weeks. This diet resulted in an over 50 fold increase in the total blood cholesterol and an almost 40-fold increase in LDL-cholesterol [28]. Contrary to the findings in the hindlimb circulation, acetylcholine-induced dilation of phenylephrine pre-constricted renal arteries was not changed by the high-cholesterol diet. However, the NO-independent (N(G)-nitro-L-arginine-resistant) component of this relaxation was significantly enhanced in arteries from

hypercholesterolemic animals. This component totally vanished after endothelial removal in both control and hypercholesterolemic groups, yet was only reduced significantly in the hypercholesterolemic group when an artery with endothelium was incubated in BK and the intermediate conductance K_{Ca} channel blocker charybdotoxin [28].

Studies on rat cerebral arteries yielded results that are in agreement with the conclusions obtained in the hindlimb of rabbits. Specifically, rat middle cerebral arteries obtained from a Sprague-Dawley strain on control versus high-cholesterol (2% cholesterol supplement for either 10 or 18-23 weeks) were dissected, de-endothelialized, cannulated and pressurized at 60 mm Hg. A blood lipid profile revealed a significant increase in the total serum cholesterol, LDL, and triglyceride levels only at 18-23 weeks of diet (Figure 1). The arterial responses to a depolarizing solution containing 60 mM KCl were similar in control and in all hypercholesterolemic arteries. However, treatment of arteries from either one of the high-cholesterol diet groups with the selective BK channel blocker paxilline resulted in vasoconstriction that was significantly smaller compare to the control group (Figure 3A). BK channel function seemed to be altered rather selectively: arterial diameter responses to the K_v channel blocker 4-aminopyridine were similar in control versus hypercholesterolemic animals (Figure 3A). First, these results demonstrated that the general contractile capability of the artery (as tested by a high KCl-containing solution) was largely preserved during the high-cholesterol diet. Second, endothelium-independent vasodilation that is mediated by the activity of smooth muscle BK channels was diminished during hypercholesterolemia [30, 31]. Moreover, the fact that reduced sensitivity to paxilline was observed after 10 weeks on a high-cholesterol diet, well before the changes in the blood lipid profile took effect (Figure 1) suggested that BK channels were highly sensitive to dietary cholesterol levels, independent of the increase in blood cholesterol.

Further experimentation took place in an effort to unveil molecular mechanisms that enable the sensitivity of BK channels to dietary cholesterol. First, it was shown that cholesterol accumulation in the wall of de-endothelialized cerebral arteries of hypercholesterolemic rats followed the pattern of increase in blood cholesterol level. In particular, the cholesterol level in cerebral artery tissue was only increased significantly during weeks 18-23 on diet, but not earlier (Figure 3B) [31]. Therefore, the direct accumulation of cholesterol in the vicinity of the BK channel might not be the sole reason for depressed BK channel sensitivity to paxilline during a high-cholesterol intake. The reduction in paxilline-induced cerebral artery constriction by hypercholesterolemia might result from a decreased number of BK channels in arterial smooth muscle. In particular, hypercholesterolemia may down-regulate accessory, smooth muscle-type β subunit ($\beta 1$) (Figure 2A). Indeed, cerebral arteries of $\beta 1$ (*KCNMB1*) knock-out mice were reported to be insensitive to selective BK channel block by the peptide blocker iberiotoxin [68]. Moreover, hypercholesterolemia-induced changes in BK $\beta 1$ subunit level have been studied in circular smooth muscle strips from the sphincter of Oddi in rabbits fed by high-cholesterol food (1 g cholesterol per day) for 4 weeks [29]. Immunohistochemical and Western blot protein analysis using a BK $\beta 1$ subunit-specific polyclonal antibody showed a decreased level of the BK $\beta 1$ protein in the cholesterol-fed group. Thus, in the sphincter of Oddi high-cholesterol diet down-regulated BK $\beta 1$ subunits [29]. However, in rat cerebral artery myocytes

high-cholesterol diet did not down-regulate, but actually significantly increased the fluorescent signal associated with selective labeling of BK $\beta 1$ subunits (Figure 3C, left panel). Since $\beta 1$ subunits themselves do not form functional channels, diminished responses of the artery to paxilline may be indicative of the reduction in the amount of the BK pore-forming (α) subunit protein. However, no significant differences between the BK α subunit-associated fluorescence signal in cerebral artery myocytes from rats on control versus high-cholesterol diet were detected (Figure 3C, right panel). Therefore, ablated paxilline-induced cerebral artery constriction observed in pressurized cerebral arteries from hypercholesterolemic rats could not be attributed to down-regulation of BK protein surface presence on myocyte membranes. Alternatively, ablated paxilline-induced cerebral artery constriction may arise from diminished functional properties of the BK channel itself. These may include but are not limited to the coupling (both, physical association and functional communication) between the BK pore-forming α subunit and the accessory $\beta 1$ subunits, changes in the gating pattern of the channel making the protein less responsive to paxilline binding. Alternatively, reduction in paxilline-induced cerebral artery constriction may not be a reflection of decreased BK channel function but rather be explained by changes in the affinity of the channel to paxilline. The latter possibility needs to be further studied as several BK channel blockers other than paxilline block BK channel with different efficacy depending on the presence of the accessory $\beta 1$ and $\beta 4$ subunits [69]. Theoretically, an increased amount of the BK $\beta 1$ protein by high-cholesterol diet may preclude paxilline from reaching its site in the BK α subunit and therefore, may result in a decreased paxilline effect. The most unequivocal evidence of the effect of a high-cholesterol diet on BK channel function must come from direct electrophysiological evaluation of BK voltage- and calcium-dependent gating following a high-cholesterol diet. The intrinsic mechanism(s) that underlie(s) alteration in BK channel function during a high-cholesterol diet is currently under investigation.

Remarkably, dietary cholesterol does not only alter the paxilline-sensitivity of the channel but also protects against ethanol-induced BK channel-mediated constriction of cerebral arteries. It was shown that BK channels represent a major target for ethanol in the cerebral vessels. Upon ethanol application, BK channel function is diminished, and cerebral artery constriction is observed [70]. The protective role of a high-cholesterol diet against alcohol-induced constriction of cerebral arteries was demonstrated *in-vivo* using a closed cranial window on anesthetized rats receiving conventional chow versus high-cholesterol (2% cholesterol) diet for 18-23 weeks [31]. Control or ethanol-containing solutions were infused into the cerebral circulation via a catheter in the carotid artery of the rat, and the diameter of the pial arterioles was determined. Infusion of 50 mM ethanol (e.g. the amount of ethanol detected in the blood during moderate-to-heavy alcohol intake in humans) into the cerebral circulation of control rats rendered a significant, 20% decrease in the pial arteriole diameter (Figure 4A). In contrast to the control group, infusion of 50 mM ethanol into the cerebral circulation of rats on a high-cholesterol diet resulted in no more than a 10% decrease in the pial arteriole diameter. To rule out the contribution of circulating factors in the observed protective effect of a high-cholesterol diet, the experiment was repeated using isolated cerebral arteries that were pressurized *in-vitro* at 60 mmHg. As observed with pial arterioles *in-vivo*, application of 50 mM ethanol to pressurized middle cerebral arteries from control rats resulted in up to 12% decrease in the

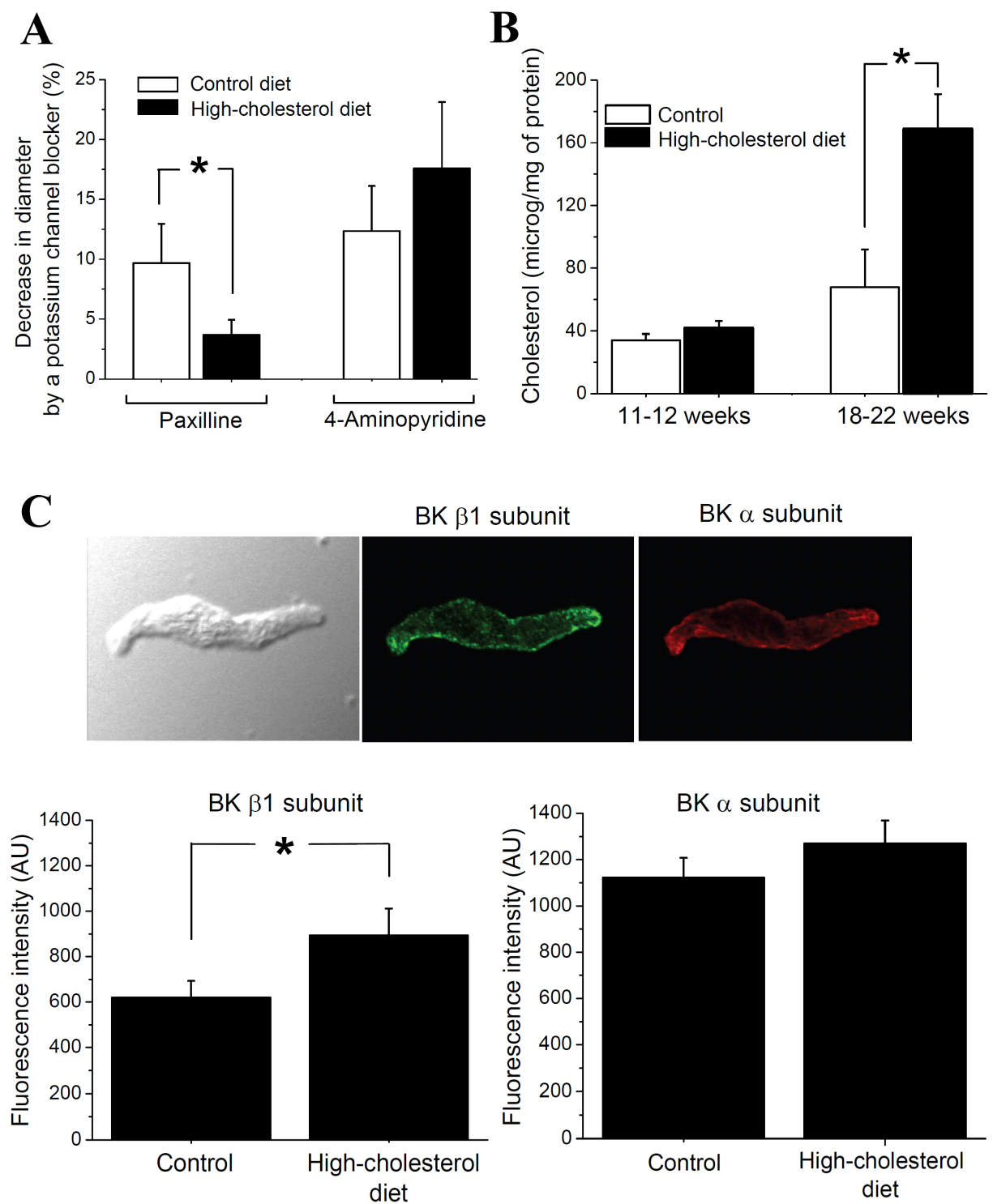


Figure 3. Effect of high-cholesterol diet on rat cerebral artery BK channel function. (A) Average data showing a decrease in arterial diameter by the selective BK channel blocker paxilline. (B) Cholesterol level in de-endothelialized cerebral arteries from rats fed control versus high-cholesterol diet. (C) Representative confocal microscope images showing an isolated cerebral artery myocyte and its fluorescence labeling of BK $\beta 1$ and α subunits. (D) Averaged fluorescence intensity associated with BK $\beta 1$ subunit. (E) Averaged fluorescence intensity associated with BK α subunit. (With modifications from [94]).

arterial diameter. In contrast, application of 50 mM ethanol to pressurized middle cerebral arteries from rats on a high-cholesterol diet resulted in only a 6-7% decrease in the arterial diameter (Figure 4B). Remarkably, the protective effect of a high-cholesterol diet against ethanol-induced constriction of cerebral arteries was similar in arteries with intact endothelium and in de-endothelialized vessels. Accordingly, dietary cholesterol-driven protection did not require the presence of functional endothelium and/or endothelium-derived vasoactive factors. Moreover, accumulation of cholesterol within the arterial wall was shown to play a major role in the observed protection by high-cholesterol diet against ethanol-induced constriction of cerebral arteries. In particular, after removal of the cholesterol that was accumulated in the cerebral artery tissue in the course of a high-cholesterol diet, ethanol-induced constriction was restored and reached the control value (Figure 4C) [31]. The molecular mechanisms by which membrane cholesterol affects the function of a major ethanol target in the artery, namely the BK channel, are still under investigation and are discussed in great detail elsewhere [71].

Inwardly rectifying potassium (Kir) channels. The effect of high-cholesterol diet on Kir channel function has been demonstrated for several members of the Kir family. Kir channels regulate important functions including membrane excitability, heart rate, vascular tone, insulin release and salt flow across epithelia (see, for example, reviews [10, 11, 13]). Structurally, they are comprised of four homo- or heteromeric subunits, each with two membrane spanning helices and intracellular N- and C- termini (Figure 2B). Fifteen Kir channels have been identified and classified in seven subfamilies (Kir1–7) [72]. Among these, it has been shown that a high-cholesterol diet affects the function of Kir2 channels, Kir3.1/Kir3.4 (K_{ACh}) channels and Kir6 (K_{ATP}) channels.

The effect of a high-cholesterol diet on Kir2 channels has been determined in different animal models and cell types. In earlier studies of Kir2 channels expressed in endothelial cells, hypercholesterolemia was induced by administering an atherogenic diet (0.5% cholesterol, 10% lard, and 1.5% sodium cholate) to castrated male Yorkshire pigs [18]. The properties of endothelial Kir2 channels and the values of the membrane potentials were compared in porcine aortic endothelial cells freshly isolated from the pig aortas. Cells isolated from hypercholesterolemic animals had significantly lower Kir currents than those isolated from control cells. Moreover, the membrane potential in hypercholesterolemic pigs was significantly more depolarized compared with that in control animals. More recently, the effect of a high-cholesterol diet on Kir2 channels expressed in cardiac myocytes was determined using a rat model [19]. In these experiments a group of 25-day-old male Sprague-Dawley rats was placed on a high-cholesterol diet (2% cholesterol in standard rodent food). Another group of the same age was fed an isocaloric, cholesterol-free diet from the same supplier. The rats were sacrificed as atrial tissue donors after 20-24 weeks on control or high-cholesterol diet. Notably, as a result of the high-cholesterol diet, in addition to an approximately 2.5-fold increase in serum LDL levels (see Figure 1), there was also an approximately 1.8-fold increase in cholesterol levels in the atrial tissue itself (see Figure 5A). This increase in cholesterol levels in the atrial myocytes following the high cholesterol diet resulted in an approximately 60% decrease in Kir2 currents in atrial cardiomyocytes [19].

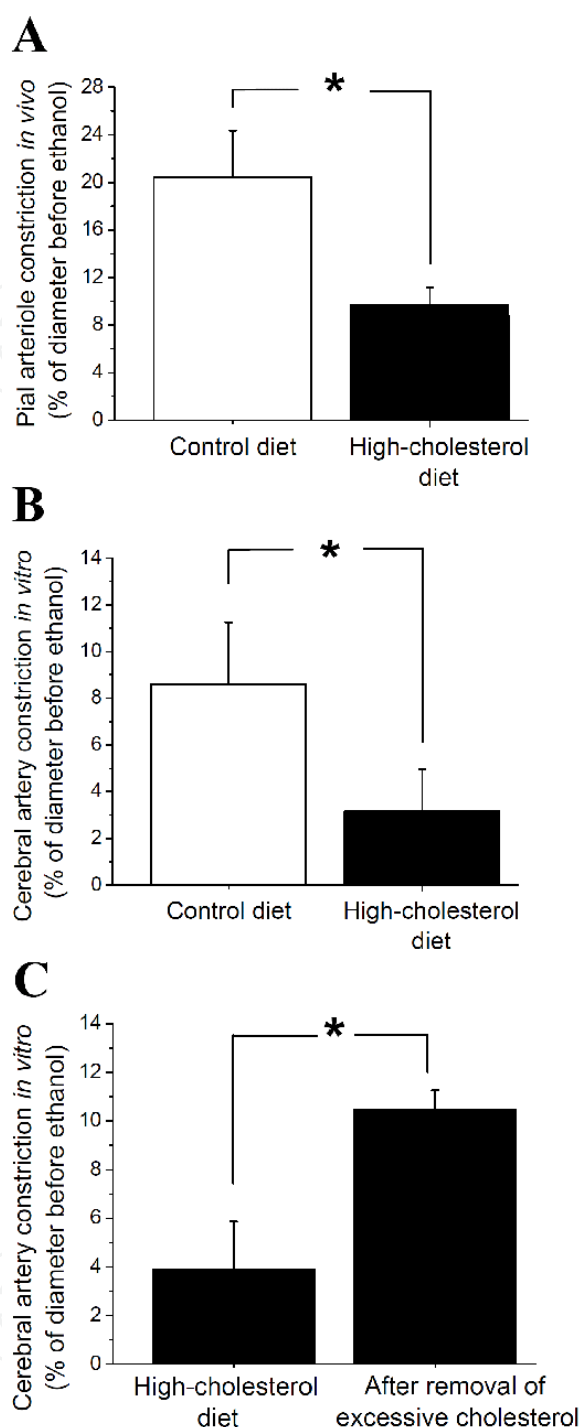


Figure 4. Cholesterol control of ethanol-induced constriction of cerebral artery. (A) Averaged data showing constriction of pial arterioles *in-vivo* in rats fed control versus high-cholesterol diet. (B) Ethanol-induced cerebral artery constriction in pressurized cerebral arteries *in-vitro* obtained from rats on control versus high-cholesterol diet. (C) Averaged data showing ethanol-induced cerebral artery constriction *in-vitro* in arteries from rats on a high-cholesterol diet before and after removal of excessive cholesterol using the cholesterol carrier methyl- β -cyclodextrin. (From [94]).

In addition to Kir2 channels, atrial myocytes also express Kir3 channels. In particular, atrial K_{ACh} channels are heterotetrameric proteins that consist of Kir3.1 and Kir3.4 [73]. Recent studies

[19] demonstrated that unexpectedly rats that were on a high-cholesterol diet for 18-22 weeks exhibited up to 2-3 fold increase in K_{ACh} currents that were sensitive to the selective $I_{K, ACh}$ -blocker tertiapin (Figure 5B-5E). The summary data in Figure 5D-E show that the high-cholesterol diet affected both inward and outward currents in a similar manner. Thus, while the effect was more visible for the larger inward currents, the physiologically relevant smaller outward currents were also significantly affected by cholesterol. This result was surprising because an increase in channel function following an increase in cholesterol levels (as shown in Figure 5A) is rare. These data suggest that an increase in cholesterol levels in atrial myocytes may underlie the increase in K_{ACh} currents in hypercholesterolemic rats.

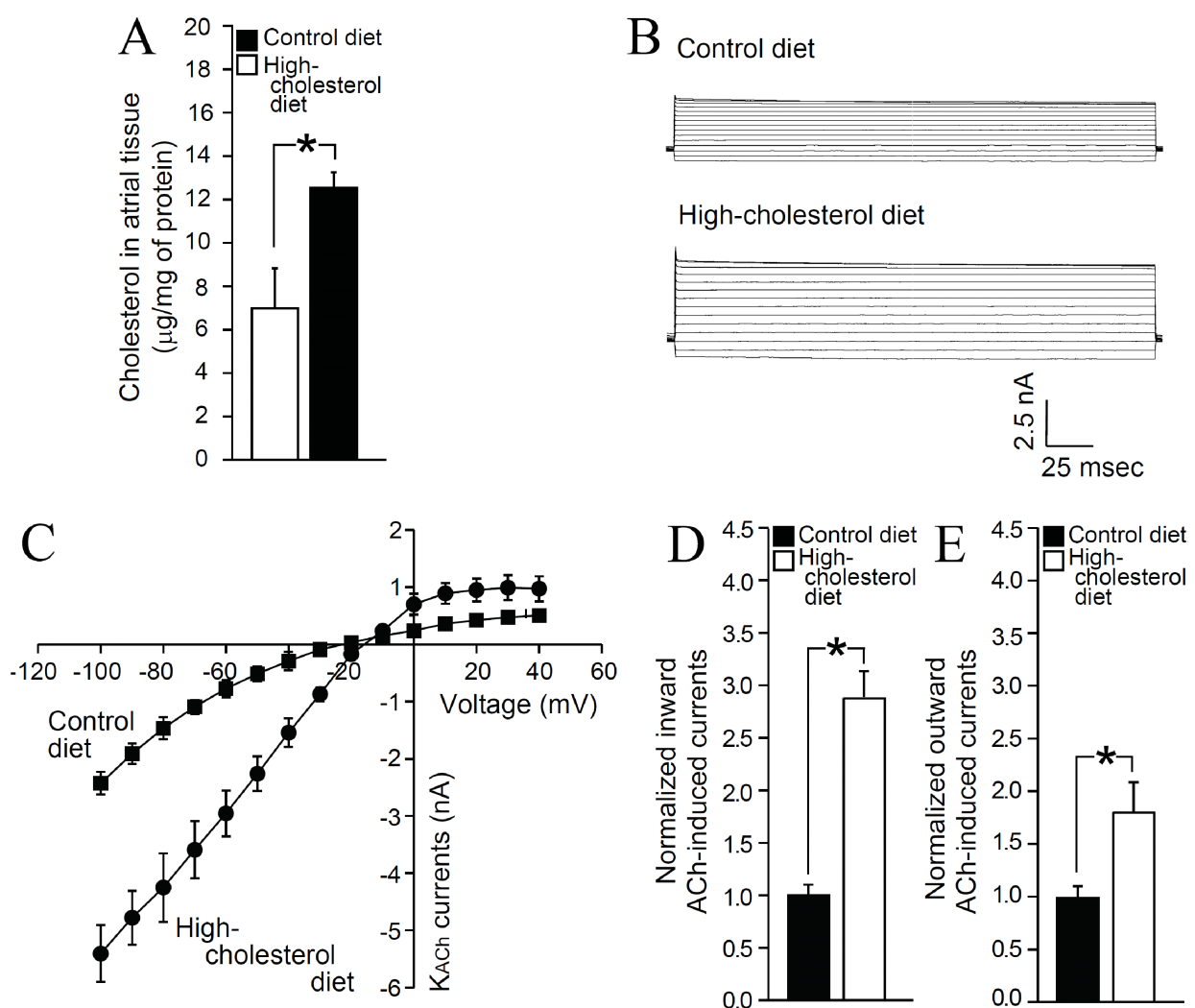


Figure 5. ACh-induced K_{ACh} currents in atrial cardiomyocytes are enhanced in hypercholesterolemic rats. (A) Cholesterol levels in atrial tissue of Sprague-Dawley rats fed a high-cholesterol diet compared to control. Tertiapin-sensitive currents (B) and I-V relationships (C) of ACh-induced current densities for atrial myocytes from control and hypercholesterolemic rats. Summary data: (D) inward ACh-induced current densities at -80 mV; (E) outward ACh-induced current densities at +40 mV. ((B)-(E) From [19]).

Several studies were also carried out to determine the effect of high-cholesterol diet on Kir6 channels. ATP-sensitive K^+ (K_{ATP}) channels are expressed in the sarcolemma of cardiomyocytes [74] and in the mitochondrial inner membrane [75]. Structurally, K_{ATP} channels are comprised of a pore forming Kir channel (Kir6.1 or Kir6.2) and an ATP-binding regulatory subunit, the sulfonylurea receptor (SUR1, SUR2A, or SUR2B).

Activation of K_{ATP} channels mediates coronary vasodilation during decreases in perfusion pressure within the autoregulatory range [76] and dilation of collateral and noncollateral vessels during ischemia [77]. However, dilation in response to the K_{ATP} channel activator aprikalim was not altered in monkeys following an atherogenic diet and reduction in dietary cholesterol [78].

In contrast, acidosis-induced coronary arteriolar dilation was impaired in hypercholesterolemic rabbits. When myocardial ischemia takes place [79], the interstitial pH of the heart rapidly decreases followed by an immediate decrease in coronary resistance by microvascular dilation [80]. It was shown that acidosis-induced coronary arteriolar dilation is mediated via the activation of pertussis toxin-sensitive G protein and consequent opening of the K_{ATP} channel [81-82]. Since hypercholesterolemia produces structural and functional abnormalities in blood vessels [83], its impact on coronary microvascular response to acidosis was investigated [84]. Coronary arterioles isolated from rabbit hearts were cannulated to micropipettes in a vessel chamber and microvascular responses were observed. The effect of the K_{ATP} channel blocker glibenclamide on the acidosis-induced microvascular responses was examined. Coronary arterioles significantly dilated as the pH was reduced and the dilation was significantly inhibited by glibenclamide. In another set of experiments, rabbits were randomly assigned to normal chow or high-cholesterol diet. After 8 weeks, the responses of isolated coronary arterioles to acidosis were examined in the two groups. Acidosis-induced dilation in the high-cholesterol group was significantly attenuated compared to the control group. These data suggest that K_{ATP} channels play an important role in the acidosis-induced dilation of rabbit coronary arterioles and that dilation of coronary arterioles is impaired in hypercholesterolemia. Notably, the impairment occurs upstream of K_{ATP} channel opening.

K_{ATP} channels play a key role in endogenous cardioprotective mechanisms [85-88]. Specifically, during cardiac ischemia, the levels of intracellular ATP may decrease. This would result in the opening of K_{ATP} channels that operate as molecular biosensors for coupling cellular energy metabolism and excitability [89]. The opening of K_{ATP} channels leads to increased influx of K^+ , which then leads to shortening of the action potential duration and to reduction of the Ca^{2+} overload that occurs during ischemia-reperfusion induced injury [90-92]. Since hyperlipidemia has been shown to interfere with cardioprotective mechanisms, studies were carried out to investigate the interaction of hyperlipidemia with cardioprotection induced by pharmacological activators of K_{ATP} channels [93]. Hearts isolated from rats fed a 2% cholesterol-enriched or normal diet for 8 weeks were subjected to 30 min of global ischemia and 120 min of reperfusion in the presence or absence of K_{ATP} modulators. In normal diet-fed rats, activation of K_{ATP} channels either by the nonselective K_{ATP} activator cromakalim or the selective mitochondrial K_{ATP} channel opener diazoxide significantly decreased infarct size compared with vehicle-treated control rats.

Moreover, the cardioprotective effect was abolished by blocking the channels using the nonselective K_{ATP} blocker glibenclamide or the selective mitochondrial K_{ATP} channel blocker 5-hydroxydecanoate. In contrast, in cholesterol-fed rats, the cardioprotective effect was not observed following administration of K_{ATP} channel activators, demonstrating that cardioprotection by K_{ATP} channel activators is impaired in cholesterol-enriched diet-induced hyperlipidemia. Notably, whereas protein levels of Kir6.1 and Kir6.2 remained unchanged, cardiac expression of Kir6.1 was significantly downregulated in cholesterol-fed rats.

Together, these data demonstrate a wide range of effects of a high-cholesterol diet on the function of inwardly rectifying potassium channels and on their physiological implications. Whereas the function of Kir2 and Kir6 channels was suppressed in several cases following a high-cholesterol diet, atrial Kir3 channels were enhanced. Moreover, in the case of Kir6 channels, whereas K_{ATP} -mediated coronary vasodilation was not altered in atherosclerotic monkeys, a high-cholesterol diet resulted in impaired cardioprotection by K_{ATP} channel activators in rats and impaired K_{ATP} -mediated acidosis-induced coronary arteriolar dilation in rabbits.

4. Conclusive remarks

Different types of potassium channels have been shown to be affected by high-cholesterol diet in a variety of species. The modulation of potassium channel activity by high-cholesterol diet results in alterations of organ function *in-vitro* and *in-vivo*. Notably, the effect of high-cholesterol diet on potassium channels varies and may result in decreased or increased channel function. In a few cases, potassium channels were found to be insensitive to dietary cholesterol manipulation. Among the potassium channels that were affected by a high-cholesterol diet are included several voltage-gated potassium channels (K_V), calcium-activated potassium (K_{Ca}) channels, and inwardly rectifying potassium (Kir) channels. Among the channels studied, only the currents of the heterotetrameric Kir3.1/Kir3.4 (K_{Ach}) channels were consistently enhanced by high-cholesterol dietary intake. The structural and molecular bases for the diverse effect of high-cholesterol diet on potassium channels remain largely unknown. Thus, considering the critical role of potassium channels in physiology and pathology, an important aspect of future studies will be to elucidate the intrinsic mechanisms leading to dietary cholesterol modulation of channel function.

Acknowledgements

This work was supported by a Scientist Development Grant 11SDG5190025 from the American Heart Association (to A.R.-D.), the Alcoholic Beverage Medical Research Foundation grant for New Investigator (A.B.), and NIH Support Opportunity for Addiction Research for New Investigators R03 AA020184 (A.B.)

Author details

Anna N. Bukiya³ and Avia Rosenhouse-Dantsker^{1,2*}

*Address all correspondence to: dantsker@uic.edu

1 Department of Chemistry, University of Illinois at Chicago, Chicago, IL, USA

2 Departments of Medicine and Pharmacology, University of Illinois at Chicago, Chicago, IL, USA

3 Department of Pharmacology, The University of Tennessee Health Science Center, Memphis, TN, USA

References

- [1] Yeagle PL. Cholesterol and the cell membrane. *BiochimBiophysActa* 1985;822 267-287.
- [2] Yeagle PL. Modulation of membrane function by cholesterol. *Biochimie* 1991;73 1303-10.
- [3] Kellner-Weibel G, Geng YJ, Rothblat GH. Cytotoxic cholesterol is generated by the hydrolysis of cytoplasmic cholesteryl ester and transported to the plasma membrane. *Atherosclerosis* 1999;146(2) 309-319.
- [4] Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med*. 1999;340(2) 115-126.
- [5] Kruth HS. Lipoprotein cholesterol and atherosclerosis. *Current Molecular Medicine* 2001;1(6) 633-653.
- [6] Steinberg D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat Med*. 2002;8(11) 1211-1217.
- [7] Maguy A, Hebert TE, Nattel S. Involvement of lipid rafts and caveolae in cardiac ion channel function. *Cardiovasc Res*. 2006; 69 798-807.
- [8] Levitan I. Cholesterol and Kir channels. *IUBMB Life* 2009;61 781-90.
- [9] Rosenhouse-Dantsker A, Mehta D, Levitan I. Regulation of ion channels by membrane lipids. *ComprPhysiol*. 2012;2 31-68.
- [10] Nichols C, Lopatin A. Inward rectifier potassium channels. *Annu Rev Physiol*. 1997;59 268-277.
- [11] Bichet D, Haass FA, Jan LY. Merging functional studies with structures of inward-rectifier K(+) channels. *Nat Rev Neurosci*. 2003;4 957-967.

- [12] Salkoff L, Butler A, Ferreira G, Santi C, Wei A. High-conductance potassium channels of the SLO family. *Nat Rev Neurosci* 2006;7(12) 921-931.
- [13] Hibino H, Inanobe A, Furutani K, Murakami S, Findlay I, Kurachi Y. Inwardly rectifying potassium channels: their structure, function, and physiological roles. *Physiol Rev*. 2010;90 291-366.
- [14] Castle NA. Pharmacological modulation of voltage-gated potassium channels as a therapeutic strategy. *Expert Opin Ther Pat*. 2010;20(11) 1471-503.
- [15] González C1, Baez-Nieto D, Valencia I, Oyarzún I, Rojas P, Naranjo D, Latorre R. K⁺ channels: function-structural overview. *Compr Physiol*. 2012;2(3) 2087-149.
- [16] Jan LY, Jan YN. Voltage-gated potassium channels and the diversity of electrical signalling. *J Physiol*. 2012;590 2591-9.
- [17] Ge L, Hoa NT, Wilson Z, Arismendi-Morillo G, Kong XT, Tajhya RB, Beeton C, Jadus MR. Big Potassium (BK) ion channels in biology, disease and possible targets for cancer immunotherapy. *Int Immunopharmacol*. 2014;22 427-443.
- [18] Fang Y, Mohler ER 3rd, Hsieh E, Osman H, Hashemi SM, Davies PF, Rothblat GH, Wilensky RL, Levitan I. Hypercholesterolemia suppresses inwardly rectifying K⁺ channels in aortic endothelium in vitro and in vivo. *Circ Res*. 2006;98 1064-1071.
- [19] Deng W, Bukiya AN, Rodríguez-Menchaca AA, Zhang Z, Baumgarten CM, Logothetis DE, Levitan I, Rosenhouse-Dantsker A. Hypercholesterolemia induces up-regulation of KACH cardiac currents via a mechanism independent of phosphatidylinositol 4, 5-bisphosphate and Gβγ. *J Biol Chem*. 2012;287(7) 4925-35.
- [20] Ghanam K, Javellaud J, Ea-Kim L, Oudart N. Effects of treatment with 17β-estradiol on the hypercholesterolemic rabbit middle cerebral artery. *Maturitas*. 2000;34(3) 249-60.
- [21] Mokolke EA, Hu Q, Song M, Toro L, Reddy HK, Sturek M. Altered functional coupling of coronary K⁺ channels in diabetic dyslipidemic pigs is prevented by exercise. *J Appl Physiol*. 2003;95(3) 1179-93.
- [22] Heaps CL, Tharp DL, Bowles DK. Hypercholesterolemia abolishes voltage-dependent K⁺ channel contribution to adenosine-mediated relaxation in porcine coronary arterioles. *Am J Physiol Heart Circ Physiol*. 2005;288(2) H568-76.
- [23] Heaps CL, Jeffery EC, Laine GA, Price EM, Bowles DK. Effects of exercise training and hypercholesterolemia on adenosine activation of voltage-dependent K⁺ channels in coronary arterioles. *J Appl Physiol*. 2008;105(6) 1761-71.
- [24] Varga A, Bagossi P, Tözsér J, Peitl B, Szilvássy Z. Effect of experimental hypercholesterolaemia on K⁺ channel alpha-subunit mRNA levels in rabbit hearts. *Eur J Pharmacol*. 2007;562(1-2) 130-1.

- [25] Yang Y, Jones AW, Thomas TR, Rubin LJ. Influence of sex, high-fat diet, and exercise training on potassium currents of swine coronary smooth muscle. *Am J Physiol Heart Circ Physiol*. 2007;293(3) H1553-1563.
- [26] Bender SB, Tune JD, Borbouse L, Long X, Sturek M, Laughlin MH. Altered mechanism of adenosine-induced coronary arteriolar dilation in early-stage metabolic syndrome. *Exp Biol Med (Maywood)*. 2009;234(6) 683-92.
- [27] Jeremy RW, McCarron H. Effect of hypercholesterolemia on Ca(2+)-dependent K(+) channel-mediated vasodilatation in vivo. *Am J Physiol Heart Circ Physiol* 2000;279(4) H1600-H1608.
- [28] Taniguchi J, Honda H, Shibusawa Y, Iwata T, Notoya Y. Alteration in endothelial function and modulation by treatment with pioglitazone in rabbit renal artery from short-term hypercholesterolemia. *Vascul Pharmacol*. 2005;43(1) 47-55.
- [29] Du P, Cui GB, Wang YR, Zhang XY, Ma KJ, Wei JG. Down regulated expression of the beta1 subunit of the big-conductance Ca²⁺ sensitive K⁺ channel in sphincter of Oddi cells from rabbits fed with a high cholesterol diet. *Acta Biochim Biophys Sin (Shanghai)*. 2006;38(12) 893-9.
- [30] Bukiya A, Dopico A. Endothelium-independent, smooth muscle BK channel-mediated dilation of resistance-size cerebral arteries is significantly impaired in rats on a high-cholesterol diet. *Arterioscler Thromb Vasc Biol*. 2008;28 e107.
- [31] Bukiya AN, Vaithianathan T, Kuntamallappanavar G, Asuncion-Chin M, Dopico AM. Smooth muscle cholesterol enables BK $\beta 1$ subunit-mediated channel inhibition and subsequent vasoconstriction evoked by alcohol. *Arterioscler Thromb Vasc Biol*. 2011;31(11) 2410-23.
- [32] Howell WH, McNamara DJ, Tosca MA, Smith BT, Gaines JA Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis. *Am J Clin Nutr*. 1997;65 1747-1764.
- [33] Asemi Z, Tabassi Z, Samimi M, Fahiminejad T, Esmailzadeh A. Favourable effects of the Dietary Approaches to Stop Hypertension diet on glucose tolerance and lipid profiles in gestational diabetes: a randomised clinical trial. *Br J Nutr* 2013;109 2024-2030.
- [34] Hadjiev DI, Mineva PP, Vukov MI. Multiple modifiable risk factors for first ischemic stroke: a population-based epidemiological study. *Eur J Neurol*. 2003;10(5) 577-82.
- [35] Ivan A, Azoicăi D, Stefanache F, Manole A, Hodorog D, Trifan M, Cuciureanu DI, Matei M. [Prospective epidemiologic and clinical observations on the prevalence of risk factors for stroke, performed on 374 inpatients, during the period of June 2001-June 2004]. *Rev Med Chir Soc Med Nat Iasi*. 2004;108(3) 561-5.

- [36] Weikert C, Berger K, Heidemann C, Bergmann MM, Hoffmann K, Klipstein-Grobusch K, Boeing H. Joint effects of risk factors for stroke and transient ischemic attack in a German population: the EPIC Potsdam Study. *J Neurol*. 2007;254(3) 315-21.
- [37] Castilla Guerra L, del Carmen Fernández Moreno M, López Chozas JM, Jiménez Hernández MD. Statins in stroke prevention: what an internist should know. *Eur J Intern Med*. 2008;19(1) 8-14.
- [38] Cui R, Iso H, Toyoshima H, Date C, Yamamoto A, Kikuchi S, Kondo T, Watanabe Y, Koizumi A, Inaba Y, Tamakoshi A; JACC Study Group. Serum total cholesterol levels and risk of mortality from stroke and coronary heart disease in Japanese: the JACC study. *Atherosclerosis* 2007;194(2) 415-20.
- [39] Uchiyama S, Shibata Y, Hirabayashi T, Mihara B, Hamashige N, Kitagawa K, Goto S, Origasa H, Shimada K, Kobayashi H, Isozaki M, Ikeda Y; J-TRACE Investigators. Risk factor profiles of stroke, myocardial infarction, and atrial fibrillation: a Japanese Multicenter Cooperative Registry. *J Stroke Cerebrovasc Dis*. 2010;19(3) 190-7.
- [40] Stella F, Banzato CE, Gasparetto Sé EV, Scudeler JL, Pacheco JL, Kajita RT. Risk factors for vascular dementia in elderly psychiatric outpatients with preserved cognitive functions. *J Neurol Sci*. 2007;257(1-2) 247-9.
- [41] Babiak J, Rudel LL. Lipoproteins and atherosclerosis. *Baillieres Clin Endocrinol Metab*. 1987;1(3) 515-50.
- [42] Ayajiki K, Fujioka H, Torii R, Toda N, Okamura T. Endothelial and neuronal functions in cerebral and temporal arteries from monkeys fed a high-cholesterol diet. *J Cardiovasc Pharmacol*. 2002;40(3) 456-66.
- [43] Chan YC1, Leung FP, Tian XY, Yung LM, Lau CW, Chen ZY, Yao X, Laher I, Huang Y. Raloxifene improves vascular reactivity in pressurized septal coronary arteries of ovariectomized hamsters fed cholesterol diet. *Pharmacol Res*. 2012;65(2) 182-8.
- [44] Amran AA, Zaiton Z, Faizah O, Morat P. Effects of *Garcinia atroviridis* on serum profiles and atherosclerotic lesions in the aorta of guinea pigs fed a high cholesterol diet. *Singapore Med J*. 2009;50(3) 295-9.
- [45] Najibi S, Cohen RA. Enhanced role of K⁺ channels in relaxations of hypercholesterolemic rabbit carotid artery to NO. *Am J Physiol*. 1995;269(3 Pt 2) H805-811.
- [46] Irie H, Koshiba H, Koyama M, Asakura E, Shibata H, Kimura K, Naito K, Yamauchi T, Yada K, Hanamura T, Hanada S, Abe Si, Nakamura N. Effects of recombinant human macrophage colony-stimulating factor on atherosclerotic lesions established in the aorta of high cholesterol-fed rabbits. *J Biochem*. 2001;129(5) 717-24.
- [47] Liu RY, Gu R, Qi XL, Zhang T, Zhao Y, He Y, Pei JJ, Guan ZZ. Decreased nicotinic receptors and cognitive deficit in rats intracerebroventricularly injected with beta-amyloid peptide(1-42) and fed a high-cholesterol diet. *J Neurosci Res*. 2008;86(1) 183-93.

- [48] Balkanci ZD, Pehlivanoglu B, Bayrak S, Karabulut I, Karaismailoglu S, Erdem A. The effect of hypercholesterolemia on carbachol-induced contractions of the detrusor smooth muscle in rats: increased role of L-type Ca^{2+} channels. *NaunynSchmiedeberts Arch Pharmacol.* 2012;385(11) 1141-8.
- [49] El-Sayyad HI, Khalifa SA, Fouda YA, Yonis AS Effects of diabetes and/or hypercholesterolemia on skin development of rat fetuses. *Nutrition* 2012;28 698–706.
- [50] Yeap SK, Beh BK, Kong J, Ho WY, MohdYusof H, Mohamad NE, Hussin AB, Jagannath IB, Alitheen NB, Jamaluddin A, Long K. In Vivo Hypocholesterolemic Effect of MARDI Fermented Red Yeast Rice Water Extract in High Cholesterol Diet Fed Mice. *Evid Based Complement Alternat Med.* 2014;2014 707829.
- [51] Kushwaha RS, McGill HC Jr. Diet, plasma lipoproteins and experimental atherosclerosis in baboons (*Papio sp.*). *Hum Reprod Update.* 1998;4(4) 420-9.
- [52] Beynen AC, Katan MB, Van Zutphen LF. Hypo- and hyperresponders: individual differences in the response of serum cholesterol concentration to changes in diet. *Adv Lipid Res.* 1987;22 115-71.
- [53] Rosenbaum MA, Chaudhuri P, Graham LM. Hypercholesterolemia inhibits re-endothelialization of arterial injuries by TRPC channel activation. *J Vasc Surg.* 2014;pii S0741-5214(14)00834-9.
- [54] Awayda MS, Awayda KL, Pochynyuk O, Bugaj V, Stockand JD, Ortiz RM. Acute cholesterol-induced anti-natriuretic effects: role of epithelial Na^{+} channel activity, protein levels, and processing. *J Biol Chem.* 2011;286 1683-95.
- [55] Hille B. Ion channels of excitable membranes. 3rd Edition. Sinauer Associates, Inc; 2001.
- [56] Latorre R, Brauchi S. Large conductance Ca^{2+} -activated K^{+} (BK) channel: activation by Ca^{2+} and voltage. *Biol Res.* 2006;39(3) 385-401.
- [57] Lee US, Cui J. BK channel activation: structural and functional insights. *Trends Neurosci.* 2010;33(9) 415-423.
- [58] Wallner M, Meera P, Toro L. Determinant for beta-subunit regulation in high-conductance voltage-activated and Ca^{2+} -sensitive K^{+} channels: an additional transmembrane region at the N terminus. *ProcNatlAcadSci U S A.* 1996;93(25) 14922-14927.
- [59] Wei A, Solaro C, Lingle C, Salkoff L. Calcium sensitivity of BK-type K_{Ca} channels determined by a separable domain. *Neuron* 1994;13(3) 671-681.
- [60] Cox DH. The BKCa channel's Ca^{2+} -binding sites, multiple sites, multiple ions. *J Gen Physiol* 2005;125(3) 253-255.
- [61] Yuan P, Leonetti MD, Pico AR, Hsiung Y, MacKinnon R. Structure of the human BK channel Ca^{2+} -activation apparatus at 3.0 Å resolution. *Science* 2010;329(5988) 182-186.

- [62] Orio P, Rojas P, Ferreira G, Latorre R. New disguises for an old channel: MaxiK channel beta-subunits. *News Physiol Sci*. 2002;17 156-161.
- [63] Brenner R, Jegla TJ, Wickenden A, Liu Y, Aldrich RW. Cloning and functional characterization of novel large conductance calcium-activated potassium channel beta subunits, hKCNMB3 and hKCNMB4. *J Biol Chem*. 2000;275(9) 6453-6461.
- [64] Sobey CG. Potassium channel function in vascular disease. *ArteriosclerThrombVasc Biol*. 2001;21(1) 28-38.
- [65] Brayden JE, Nelson MT. Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science* 1992;256(5056) 532-535.
- [66] Najibi S, Cowan CL, Palacino JJ, Cohen RA. Enhanced role of potassium channels in relaxations to acetylcholine in hypercholesterolemic rabbit carotid artery. *Am J Physiol*. 1994;266(5 Pt 2) H2061-H2067.
- [67] Wiecha J, Schläger B, Voisard R, Hannekum A, Mattfeldt T, Hombach V. Ca^{2+} -activated K^+ channels in human smooth muscle cells of coronary atherosclerotic plaques and coronary media segments. *Basic Res Cardiol*. 1997;92(4) 233-239.
- [68] Brenner R, Pérez GJ, Bonev AD, Eckman DM, Kosek JC, Wiler SW, Patterson AJ, Nelson MT, Aldrich RW. Vasoregulation by the beta1 subunit of the calcium-activated potassium channel. *Nature*. 2000;407(6806) 870-6.
- [69] Meera P, Wallner M, Toro L. A neuronal beta subunit (KCNMB4) makes the large conductance, voltage- and Ca^{2+} -activated K^+ channel resistant to charybdotoxin and iberiotoxin. *ProcNatlAcadSci U S A*. 2000;97(10) 5562-7.
- [70] Liu P, Xi Q, Ahmed A, Jaggar JH, Dopico AM. Essential role for smooth muscle BK channels in alcohol-induced cerebrovascular constriction. *ProcNatlAcadSci U S A*. 2004;101(52) 18217-22.
- [71] Dopico AM, Bukiya AN, Singh AK. Large conductance, calcium- and voltage-gated potassium (BK) channels: regulation by cholesterol. *PharmacolTher*. 2012;135(2) 133-50.
- [72] Reimann F, Ashcroft FM. Inwardly rectifying potassium channels. *CurrOpin Cell Biol*. 1999;11 503-8.
- [73] Krapivinsky G, Gordon EA, Wickman K, Velimirović B, Krapivinsky L, Clapham DE. The G protein-gated atrial K^+ channel IKACH is a heteromultimer of two inwardly rectifying K^+ channel proteins. *Nature* 1995;374 135-141.
- [74] Noma A. ATP-regulated K^+ channels in cardiac muscle. *Nature* 1983;305 147-148.
- [75] Inoue I, Nagase H, Kishi K, Higuti T. ATP-sensitive K^+ channel in the mitochondrial inner membrane. *Nature* 1991;352 244-247.

- [76] Komaru T, Lamping KG, Eastham CL, Dellsperger KC. Role of ATP-sensitive potassium channels in coronary microvascular autoregulatory responses. *Circ Res.* 1991;69 1146-1151.
- [77] Lamping KG, Bloom EN, Harrison DG. Regulation of native collateral vessel dilation following coronary occlusion in the dog. *Am J Physiol.* 1994;266 H769-H778.
- [78] Lamping KG, Piegors DJ, Benzuly KH, Armstrong ML, Heistad DD. Enhanced coronary vasoconstrictive response to serotonin subsides after removal of dietary cholesterol in atherosclerotic monkeys. *ArteriosclerThromb.* 1994;14(6) 951-7.
- [79] Momomura S, Ingwall JS, Paker JA, Sahagian P, Ferguson JJ, Grossman W. The relationships of high energy phosphates, tissue pH, and regional blood flow to diastolic distensibility in the ischemic dog myocardium. *Circ Res.* 1985;57 822-835.
- [80] Lee JC, Halloran KH, Taylor JF, Downing SE. Coronary flow and myocardial metabolism in newborn lambs: effects of hypoxia and acidemia. *Am J Physiol.* 1973;224 1381-1387.
- [81] Ishizaka H, Kuo L. Acidosis induced coronary arteriolar dilation is mediated by ATP-sensitive potassium channels in vascular smooth muscle. *Circ Res.* 1996; 78 50-57.
- [82] Ishizaka H, Gudi SR, Frangos JA, Kuo L. Coronary arteriolar dilation to acidosis. Role of ATP-sensitive potassium channels and pertussis toxin-sensitive G proteins. *Circulation* 1999;99 558-563.
- [83] Vanhoutte PM. Endothelial dysfunction and atherosclerosis. *Eur Heart J.* 1997;18(suppl) E19-E29.
- [84] Shioiri H, Komaru T, Sato K, Takahashi K, Takeda S, Kanatsuka H, Watanabe J, Shirato K. Impact of hypercholesterolemia on acidosis-induced coronary microvascular dilation. *Basic Res Cardiol.* 2003;98 76-83.
- [85] Kevelaitis E, Oubenaissa A, Peynet J, Mouas C, Menasche P. Preconditioning by mitochondrial ATP-sensitive potassium channel openers: an effective approach for improving the preservation of heart transplants. *Circulation* 1999;100(19 Suppl) II345-II350.
- [86] Ferdinandy P, Schulz R, Baxter GF. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. *Pharmacol Rev.* 2007;59 418-458.
- [87] Wang L, Cherednichenko G, Hernandez L, Halow J, Camacho SA, Figueredo V, Schaefer S. Preconditioning limits mitochondrial Ca^{2+} during ischemia in rat hearts: role of KATP channels. *Am J Physiol HeartCirc Physiol.* 2001;280 H2321-H2328.

- [88] Wang Y, Kudo M, Xu M, Ayub A, Ashraf M. Mitochondrial KATP channel as an end effector of cardioprotection during late preconditioning: triggering role of nitric oxide. *J Mol Cell Cardiol.* 2001;33 2037–2046.
- [89] Zingman LV, Alekseev AE, Hodgson-Zingman DM, Terzic A. ATPsensitive potassium channels: metabolic sensing and cardioprotection. *J Appl Physiol.* 2007;103 1888–1893.
- [90] Loussouarn G, Pike LJ, Ashcroft FM, Makhina EN, Nichols CG. Dynamic sensitivity of ATP-sensitive K⁺ channels to ATP. *J Biol Chem.* 2001;276 29098–29103.
- [91] Puskas LG, Nagy ZB, Giricz Z, Onody A, Csonka C, Kitajka K, Hackler L Jr, Zvara A, Ferdinandy P. Cholesterol diet-induced hyperlipidemia influences gene expression pattern of rat hearts: a DNA microarray study. *FEBS Lett.* 2004;562 99-104.
- [92] Olson TM, Terzic A. Human KATP channelopathies: diseases of metabolic homeostasis. *Pflügers Arch.* 2010;460 295–306.
- [93] Csonka C1, Kupai K, Bencsik P, Görbe A, Pálóczi J, Zvara A, Puskás LG, Csont T, Ferdinandy P. Cholesterol-enriched diet inhibits cardioprotection by ATP-sensitive K⁺ channel activators cromakalim and diazoxide. *Am J Physiol Heart Circ Physiol.* 2014;306(3) H405-13.
- [94] Bukiya A, Dopico AM, Leffler CW, Fedinec A. Dietary cholesterol protects against alcohol-induced cerebral artery constriction. *Alcohol ClinExp Res.* 2014 ;38(5) 1216-26.

