Cardiac Systolic and Diastolic Dysfunction After a Cholesterol-Rich Diet

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Background—Although hypercholesterolemia is a well-established risk factor for coronary artery disease, little is known regarding its direct effects on cardiac function.

Methods and Results—We examined the effects of cholesterol feeding (0.5%) on cardiac function in rabbits. After 10 weeks, both systolic shortening and diastolic relaxation rates were impaired without any change in aortic pressure or ventricular hypertrophy. However, sarcoplasmic/endoplasmic reticulum Ca$^{2+}$-ATPase (SERCA)-2 mRNA levels were reduced within 4 days after initiation of cholesterol feeding. After this effect, SERCA-2 protein and SERCA-mediated Ca$^{2+}$ uptake into sarcoplasmic reticulum vesicles were impaired, and the ratio of MHC-$\beta$ to MHC-$\alpha$ mRNA increased 5-fold. Suppression of the SERCA-2 message correlated temporally with enrichment of the cardiac sarcolemma with cholesterol.

Conclusions—These data demonstrate that dietary hypercholesterolemia induces a “cholesterol cardiomyopathy” characterized by systolic and diastolic dysfunction. These alterations were independent of vascular disease and demonstrate a dietary link to cardiac dysfunction. (Circulation. 2004;109:97-102.)

Key Words: heart failure ▪ cardiomyopathy ▪ myosin ▪ sarcoplasmic reticulum ▪ hypercholesterolemia
ceeded by significant reductions in SERCA-2 mRNA levels after only 4 days of cholesterol feeding. These findings demonstrate, for the first time, a dietary link to the development of an intrinsic “cholesterol cardiomyopathy.”

Methods

Animals

Control and cholesterol-fed (“diet”) New Zealand White rabbits were used in this investigation. Control rabbits were maintained on standard calibrated chow, and diet rabbits were fed batch-matched standard chow supplemented with cholesterol (0.5%) for up to 10 weeks. All animals were handled in accordance with Accreditation of Animal Laboratory Animal Care guidelines.

Isolation of Myocytes

Calcium-tolerant myocytes were isolated and maintained in Krebs-Henseleit buffer containing 1 mmol/L CaCl₂ and 1% BSA at 35°C and gassed with 95% O₂/5% CO₂.

SERCA-2 Activity

Thapsigargin (TSG)-sensitive ⁴⁰Ca uptake was measured in microsomes prepared from LV myocardium. Fresh LV tissue was homogenized and centrifuged at 4000g for 5 minutes, and the postnuclear supernatant was collected for assay. ⁴⁰Ca⁺ (1 μCi) was added to Ca⁺⁺ uptake incubation medium (500 μL) containing ATP (2 mmol/L) and oxalate (10 mmol/L) with and without TSG (1 μmol/L) at 37°C and 200 μg protein and incubated at 37°C for 0, 10, 30, and 60 minutes followed by scintillation counting.

MHC Isoform Expression

After reverse transcription of mRNA isolated from fresh LV tissue, forward (5’-GCCAAGGTGTAAGGAGATGAA-3’) and reverse (5’-CTCTCCGTGGTCAGTT-CAG-3’) primers were used to amplify MHC-α and MHC-β cDNA (Accession No. S62056 and Z34886, respectively). After complete digestion of the polymerase chain reaction product with HindIII, fragments of the amplification product (lengths, 660 bp for α-MHC and 460+200 bp for β-MHC) were separated on 2% agarose gel, and the ratio of β- to α-MHC fragments was quantified by densitometry.

Membrane and Blood Cholesterol Measurements

Whole-cell homogenates from both control and diet animals were subjected to lipid extraction by standard methods. Free cholesterol was quantified by gas-liquid chromatography, and phospholipid mass was assessed with a phospholipid phosphorus assay.

Data Analysis

A total of 48 animals were used in this study (20 diet and 28 controls). For contractile data, ≥6 consecutive contractions were analyzed from a single cell, and 3 to 6 cells were averaged to provide an n=1 for each animal. Data are shown as the mean±SEM, and the data between groups were tested for significance with a nonpaired Student t test or ANOVA followed by a Bonferroni correction when appropriate. The null hypothesis was rejected at a value of P<0.05.

Results

Contractile Dysfunction Is Associated With Dietary Hypercholesterolemia (n=22 and 32 Cells From 8 Control [C] Versus 9 Diet [D] Animals)

Significant contractile alterations were found in the diet myocytes compared with the control group. Figure 1A illustrates typical tracings obtained from cells isolated from control and test animals. Although the magnitude of contraction was similar between the 2 groups (9.1±0.3% versus 9.3±0.3% resting cell length, C versus D), there was a 27% decrease (P<0.01) in the maximum rate of shortening (+dL/dt) and a 25% decrease (P<0.05) in the rate of relaxation (−dL/dt) (Figure 1B). The decrease in the rate of shortening was accompanied by a 60% increase in the time to peak (0.276±0.020 versus 0.453±0.18 seconds, C versus D; P<0.001). The decrease in the rate of relaxation was accompanied by a 34.8% increase in the time to relaxation (T½) in the diet group (0.290±0.012 versus 0.391±0.091 seconds, C versus D; P<0.01).

Only minor differences occur in action potentials (n=22 and 32 cells from 8 C versus 9 D animals) and calcium currents (n=22 and 32 cells from 8 C versus 9 D animals). Action potential durations were slightly shorter (3% to 8%) in the cells from the diet animals at the 25%, 50%, and 75% repolarization points but were not statistically different between the 2 groups (data not shown). There was, however, a small (2.6-mV) but significant difference in the resting membrane potential (−76.1±0.4 versus −73.5±0.3 mV; P<0.05, C versus D) and action potential amplitude (5.0 mV)
For calcium currents, the peak current density (normalized for capacitive surface area) was smaller in the diet group, but these differences were not statistically significant at any voltage except \(-10\) mV (\(P<0.05\)) (data not shown).

SERCA-2 mRNA, Protein, and Activity Are Suppressed by Dietary Hypercholesterolemia

Because the reduction in systolic and diastolic function in the diet group was not accompanied by remarkable alterations in membrane calcium currents, we measured SERCA-2 mRNA and protein levels in the control and diet cells. A decrease in SERCA-2 expression of 17\% approached significance (\(P=0.054\)) 4 days after the initiation of cholesterol feeding when the blood cholesterol levels were \(\approx 295\pm 31\) mg/dL and became significant at 8 days (21.5\%), decreasing further to 31\% by 10 weeks (\(P<0.05\)) (Figure 2A). Unlike mRNA levels, SERCA-2 protein levels remained unchanged through 16 days but were significantly reduced (40\%; \(P<0.05\)) by day 70 (Figure 2B). In addition, at day 70, SERCA-2 activity (TSG-sensitive \(^{45}\)Ca\(^{2+}\) uptake) was reduced on average by 50\% (\(P<0.05\) to 0.01) (Figure 2C).

MHC-\(\alpha\) and MHC-\(\beta\) mRNA Expression Levels Are Altered by Dietary Hypercholesterolemia

Because of the close similarity of the MHC-\(\alpha\) and MHC-\(\beta\) isoforms (molecular mass difference <0.2\%), separation of these proteins by Western blot has been problematic, especially in rabbits.\(^{14}\) Accordingly, we used a novel strategy in which primers for the MHC mRNA were designed that flanked a sequence difference between the \(\alpha\) and \(\beta\) isoforms that contained a recognition site for \(\text{HincII}\) on MHC-\(\beta\). After

(124.9±1.0 versus 119.9±0.8 mV; \(P<0.05\), C versus D). For calcium currents, the peak current density (normalized for capacitive surface area) was smaller in the diet group, but these differences were not statistically significant at any voltage except \(-10\) mV (\(P<0.05\)) (data not shown).

Figures 1 and 2.
Figure 3. A, Reverse transcription–polymerase chain reaction of MHC transcripts from normal and diet LV tissue before (top) and after (middle) restriction digestion with HincII. Decreased expression of MHC-α and increased expression of MHC-β transcripts are seen in diet tissue compared with control (N) tissue for each of 3 animals. B, Summarizing expression levels for MHC-α and MHC-β reveals a 5-fold increase in ratio of MHC-β to MHC-α.

Cardiac Sarcolemma Enriches With Cholesterol as Serum Cholesterol Levels Increase

Analysis of purified cardiac sarcolemmal membranes isolated from fresh LV tissue obtained from control and cholesterol-fed (70 days) animals revealed a significant increase (1.74-fold; P<0.05) in unesterified (free) cholesterol content, expressed as the FC/PL mole ratio (0.270±0.041 versus 0.470±0.037 FC/PL, C versus D; n=3 to 6). The total membrane phospholipid content did not differ between the 2 groups (333.1±62.0 versus 319.6±67.9 μg, C versus D). In addition, membrane lipid analyses of LV homogenates at days 0, 4, 8, 12, 16, and 70 days demonstrated a progressive rise in membrane cholesterol content between days 0 and 16 in the diet animals that paralleled the increase in blood cholesterol levels (Figure 4). The FC/PL molar ratio in tissue extracts accurately reflects plasma membrane cholesterol content.12,13,15

Myocardial Hypertrophy Does Not Occur With Dietary Hypercholesterolemia (n=17 and 13 Animals, C Versus D)

The heart weight (8.7±0.2 versus 9.1±0.4 g, C versus D), body weight (3.3±0.05 versus 3.5±0.14 kg, C versus D), and heart weight–to–body weight ratios (2.62±0.07 versus 2.57±0.08 mg/kg, C versus D) were similar in the 2 groups. At the cellular level, the capacitive surface area, an index of cell size, was also similar in the 2 groups (81.9±7.5 versus 81.2±7.7 μF, C versus D). These data are consistent with the conclusion that myocardial hypertrophy does not occur in this model.

Hypercholesterolemia Does Not Alter Aortic Pressure (n=17 and 13 Animals, C Versus D)

The mean arterial pressure, systolic and diastolic pressures, pulse pressure, and heart rate were also not altered in the diet group.

Discussion

In this report, we describe observations demonstrating that diet-induced hypercholesterolemia impairs systolic and diastolic function. Although the absolute magnitude of cell shortening was not affected, the rate of contraction and rate of relaxation were significantly suppressed. The contractile changes associated with cholesterol feeding are similar to those seen in models of myocardial hypertrophy16 but without the accompanying hypertrophy or hemodynamic overloading. In previous studies, we found that dietary hypercholesterolemia altered contractile function in arterial smooth muscle cells (SMCs), which was mediated by cholesterol enrichment of the SMC plasma membrane and alterations in membrane calcium permeability.13,17 Accordingly, we examined membrane lipid composition and calcium currents in LV myocytes. Like the SMCs, we found that the cardiac sarcolemmal membranes became enriched with cholesterol during the development of serum hypercholesterolemia. Specifically, the membrane FC/PL molar ratio increased 74% over the course of 10 weeks on the high-fat diet. Estimates of membrane cholesterol content at shorter time points indicated that membrane cholesterol content virtually paralleled blood cholesterol levels (Figure 4), elevating after only 4 days of cholesterol feeding when blood cholesterol levels were ~300 mg/dL. In SMCs12,13 in vivo and macrophages in culture,18 as well as in synthetic membranes, similar degrees of cholesterol enrichment result in a marked increase (up to 20%) in...
membrane bilayer width. We have suggested that this swelling effect of excess cholesterol on the cell membrane is linked to a variety of alterations in membrane protein activity, including reduced adenylate cyclase, Na/K-ATPase, and alkaline phosphatase activities, and may underlie the changes we observed in the present study. In addition, cholesterol enrichment may alter membrane caveolae and/or caveolin activity, as shown in other cell systems. Because caveolae are cholesterol-rich membrane microplatforms heavily involved in cell signaling, this possibility deserves further attention. The alterations in membrane lipids were accompanied by only minor electrophysiological alterations that were not altered sufficiently by cholesterol feeding to explain the functional alterations observed. This is in contrast to SMCs, and the basis for the differences between cardiac and SMC responses to cholesterol enrichment are not clear.

In the absence of altered membrane calcium currents, we hypothesized that the decreased rate of contraction and relaxation in cardiac myocytes of the diet group resulted from an alteration in intracellular calcium handling. We found that SERCA-2 mRNA levels were reduced by 17% after only 4 days of cholesterol feeding and continued to decline to 31% by day 70 (Figure 2A). Hence, the effect of hypercholesterolemia on SERCA-2 expression was surprisingly rapid. Interestingly, the SERCA-2 mRNA level was reciprocally related to the increase in membrane and blood cholesterol levels over the course of the cholesterol feeding period (Figure 4), suggesting a role for increased membrane cholesterol content in mediating the decrease in SERCA-2 message. SERCA-2 protein levels, however, were unchanged through 16 days on diet but fell by day 70, consistent with the explanation that SERCA-2 protein half-life is longer than that of SERCA-2 message. The reductions in SERCA-2 mRNA and protein content were accompanied by a 50% decrease in SERCA-2 activity. Thus, these alterations could account for the altered contractile function observed in the diet cells. In this scenario, sarcoplasmic reticulum (SR) calcium uptake would be reduced, leading to reduced relaxation rate and reduced SR calcium content and therefore reduced SR calcium release and decreased shortening velocity. There is a growing body of evidence implicating alterations in SERCA-2 expression in heart failure. In addition, transgenic mice overexpressing SERCA-2 show decreased mortality and preserved myocyte function even in the presence of hemodynamic overload. It has been suggested that downregulation of SERCA-2 activity is accompanied by a switch from the α to the β isofrom of MHC, a switch that would be expected to impair contractile mechanics. Consistent with this notion, we demonstrate an isoform shift away from the α- and toward the β-MHC isofrom that accompanied suppressed SERCA-2 mRNA, protein, and activity and the appearance of myocyte dysfunction induced by the cholesterol-rich diet. Accordingly, the alterations in contractile function observed may have resulted from the combined effects of altered calcium handling and altered MHC expression. In this regard, it is noteworthy that these alterations in cardiac function associated with changes in SERCA-2 expression, activity and MHC isoforms are similar to myocyte alterations associated with aging.
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