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Hypercholesterolemia Impairs Exercise Capacity in Mice

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Abstract

Objective—We previously reported an attenuation of both exercise hyperemia and measures of aerobic capacity in hypercholesterolemic mice. In this study we expanded upon the previous findings by examining the temporal and quantitative relationship of hypercholesterolemia to aerobic and anaerobic capacity and by exploring several potential mechanisms of dysfunction.

Methods—Eight-week old wild type (n=123) and apoE knockout (n=79) C57BL/6J mice were divided into groups with distinct cholesterol levels by feeding regular or high fat diets. At various ages the mice underwent treadmill ergospirometry. To explore mechanisms, aortic ring vasodilator function and nitrate (NO_x) activity, urinary excretion of NO_x, running muscle microvascular density and citrate synthase activity, as well as myocardial mass and histologic evidence of ischemia were measured.

Results—At 8 weeks of age, all mice had similar measures of exercise capacity. All indices of aerobic exercise capacity progressively declined at 12 and 20 weeks of age in the hypercholesterolemic mice as cholesterol levels increased while indices of anaerobic capacity remained unaffected. Across the 4 cholesterol groups, the degree of aerobic dysfunction was related to serum cholesterol levels; a relationship that was maintained after correcting for confounding factors. Associated with the deterioration in exercise capacity was a decline in measures of nitric oxide-mediated vascular function while there was no evidence of aberrations in functional or oxidative capacities or in other components of transport capacity.

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Conclusion—Aerobic exercise dysfunction is observed in murine models of genetic and diet-induced hypercholesterolemia and is associated with a reduction in vascular nitric oxide production.

Keywords

Endothelial function; nitric oxide; oxygen consumption; cholesterol

Introduction

Hypercholesterolemia decreases the bioactivity and synthesis of endothelium-derived nitric oxide (EDNO) and impairs endothelial vasodilator function (1–5). However, notwithstanding the long-term consequences on the development of atherosclerosis, few immediate functional consequences of hypercholesterolemia have been described. We have reported that EDNO contributes to exercise hyperemia and is a determinant of aerobic exercise capacity in mice (6). In that study, the nitric oxide synthase (NOS) inhibitor, L-nitroarginine, attenuated post-exercise urinary nitrate (NO_x) excretion (a measure of EDNO production during exercise), hindlimb exercise hyperemia and aerobic exercise capacity. In hypercholesterolemic Apo E deficient mice, we observed similar reductions in post-exercise urinary NO_x, hindlimb blood flow and aerobic exercise capacity. The data suggest that conditions of reduced EDNO synthesis or activity cause inadequate exercise hyperemia that is rate limiting to oxygen transport and exercise capacity. This hypothesis is further supported by our observation that supplementation of E^- mice with the EDNO precursor, L-arginine, restores aerobic capacity (7). The effect of L-arginine is associated with increased post-exercise urinary NO_x, reflecting enhanced NO synthesis. These findings are consistent with the finding that Apo E deficient mice exhibit increased levels of the circulating NOS inhibitor ADMA (8). The present study was designed to determine the extent to which aerobic and anaerobic components of exercise capacity are affected, to establish the temporal and quantitative relationship of hypercholesterolemia with exercise dysfunction, and to determine which of the 3 major components of aerobic capacity: functional capacity, transport capacity and oxidative capacity are most affected by hypercholesterolemia.

Materials and Methods

Animals

Eight week old female wild type (E^+, n = 123) and E^- (9) (E^-, n = 79) C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were entered into experimental protocols after a 1 week period of acclimation. All mice were inspected prior to the study by a veterinarian and monitored daily by technicians and investigators. Mice were housed 4 per cage, maintained on a 12 hour light/dark cycle, given unlimited access to food and water, handled daily and taught to run on a treadmill, but were otherwise confined to cages for the duration of the study. All experimental protocols were approved by the Administrative Panel on Laboratory Animal Care of Stanford University and conforms with *the Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and with the recommendations of the American Association for the Accreditation of Laboratory Animal Care.

Experimental protocol

In preliminary studies performed to determine serum cholesterol levels at various ages for the two strains while fed a chow diet, E^+ and E^- were sacrificed at 4, 6, 8, 10, 12 and 20 weeks for serum cholesterol measurements. From this data, 8, 12 and 20 weeks of age were selected to examine the indices and determinants of exercise capacity.

Eight-week-old E^+ mice and E^- mice that had been receiving a chow diet since weaning were randomized into 4 dietary groups. One group of E^+ mice (E^+, n = 49) was fed regular chow while a second group (E^+_{chol}, n = 22) received a high cholesterol diet (modified Thomas-Hartroft). One group of E^- mice (E^-, n = 24) received regular chow whereas the other E^- group (E^-_{chol}, n = 10) received a high fat diet (10).

At 8 weeks (before initiating dietary intervention), 12 weeks and 20 weeks of age, randomly selected mice from each group underwent treadmill-testing and urinary NO_x measurement. At 12 weeks of age, mice were randomly chosen from each group and sacrificed following treadmill testing by overdose of methoxyflurane (Pitman-Moore, Mundelein, IL) inhalation anesthesia. The aorta was harvested for studies of vascular reactivity and stimulated aortic NO_x production. Blood was collected from the right atrium for measurement of serum total and HDL cholesterol levels. The heart was removed by transecting the major vessels at the base. After fat was removed, the heart was blotted dry and weighed then snap frozen and stored at −80°C for histologic examination. Gastrocnemius and vastus medialis muscles were collected for measurements of arteriolar and capillary density and citrate synthase activity.

Indices of Exercise Capacity and Treadmill Testing

The indices used to determine aerobic exercise capacity were maximal oxygen uptake (V_{O2max}), anaerobic threshold (AT), and aerobic work capacity (AWC). The distance run to exhaustion ($DIST_e$) provides a good indicator of overall exercise capacity (aerobic as well as anaerobic). Respiratory quotient (RQ) was also determined to assess anaerobic work performance and to assess the form of substrate utilization. The definitions and methods of measurement of these indices, and methods of treadmill testing have been described previously (6). Briefly, mice were treadmill tested (Exer-4 Treadmill, Columbus Instruments, Columbus, OH) using shock-plate incentive at a constant 8° angle at an initial speed of 10 m/min which was incrementally increased 1 m/min every minute until the mouse reached exhaustion. Data on V_{O2}, V_{CO2}, RQ, and $DIST_e$ were collected and stored on hard disk (Oxymax software, Columbus Instruments).

Vascular Reactivity

A 7 mm segment (ring) of thoracic aorta was dissected free of connective tissue and immediately placed in cold physiologic saline solution (PSS). Aortic segments were quickly mounted on wire stirrups, hung from force transducers and submerged in oxygenated PSS at 37°C. Over the course of 60 minutes, the segments were progressively stretched to the optimum point of their length-tension relationship (determined previously to be 3 g). Subsequently, the concentration of norepinephrine inducing half-maximal response (EC_{50NE}) was determined by exposing the segments to increasing concentrations of norepinephrine (in half-log increments from 10^{-9} to 10^{-4} M). Once a maximal response was obtained, the segments were washed repeatedly with fresh PSS for 60 minutes until the tension returned to the previous baseline value. Responses to the vasodilating agents, nitroglycerine (NTG; in ½ log incremental doses from 1×10−10 to 5 × 10−5) and acetylcholine (ACh; in ½ log incremental doses from 1×10−10 to 1 × 10−5), were studied after precontracting the segments with EC_{50NE}. After a stable contraction was obtained, the segments were exposed to increasing doses of vasodilator.

Measurement of Aortic NO_x

A 7 mm segment from the abdominal aorta was removed and placed in ice-cold PSS. After the removal of connective tissue, the segment was bisected longitudinally and incubated in 300μl HBSS medium (Irvine Scientific, Santa Ana, CA) containing calcium ionophore A23187 which stimulates endothelial NO release (final concentration of 10^{-6} M) and L-

arginine (100μM/L) at 37°C. After 120 min, the medium was centrifuged at 15,000 rpm for 5 min and the supernatant was stored at −80°C. NO_x was measured with a commercially available chemiluminescence apparatus (model 2108, Dasibi Corp., Glendale, CA) as previously described (11). The samples were injected (50μl) into boiling acidic vanadium (III) chloride. Signals from the detector were analyzed by computerized integration of curve areas.

Measurement of Urinary NO_x

At 8 and 12 weeks of age, mice were placed in metabolic chambers for 2 hours for basal and post-exercise urinary NO_x collection. Previous work has demonstrated that urinary NO_x excretion correlates with cGMP excretion and is a reflection of microvascular EDNO production (11, 12). For the basal state, mice were confined to cages for greater than 24 hours and for the post-exercise state, mice were treadmill exercised over 22 minutes to a final treadmill speed of 32 m/min. Metabolic chambers were constructed from 250ml Plexiglas utility boxes. Each metabolic chamber drained into a test tube containing 100μl of isopropyl alcohol cooled by ice water for the duration of the 5-hour urine collection. Urine was centrifuged at 4,000rpm for 5 min and the supernatant was collected and stored at −80°C for measurement of NO_x and creatinine. Urine NO_x was measured in an identical fashion to that of aortic NO_x except that samples were diluted 1:9 in deionized, distilled water. Urine creatinine was measured using a kit from Sigma-Aldrich (12).

Hematology, Biochemistry and Histology

Blood samples were collected in serum separator tubes at the time of sacrifice and centrifuged at 3,000rpm for 15 minutes. The serum was separated and stored at −80°C until analysis. Total serum and HDL cholesterol were analyzed using the enzymatic method of Allain *et al* (13).

To measure muscle oxidative capacity, gastrocnemius and vastus medialis muscles were removed, frozen in liquid nitrogen and stored at −80°C until assayed. Maximal citrate synthase activity was assayed on muscle homogenates by the method of Srere (14). Values were expressed as an average of both muscles.

Hearts were fixed in 10% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Sections (16 per animal; n=6 in each group) were examined by microscopy for evidence of necrosis, fibrosis, contraction bands or other changes representing myocardial ischemia by an experienced cardiac pathologist blinded to the experimental groups.

To determine microvessel density, gastrocnemius muscles were fixed in 10% formalin. The specimens were then sectioned once transversely and the two pieces were embedded in paraffin such that each section would contain a transverse and longitudinal cut. For arteriolar density determination, sections (5μm) were stained by Avallone's modification of the Jones silver methenamine method for staining basement membranes. For capillary density, sections (2μm) were stained using toludine blue. Arteriolar and capillary density measurements were determined separately using stereologic analysis.

Diets & Drugs

Three diets were used in these experiments; the regular chow diet (0.022% cholesterol, 11% total fat by weight, Purina, Richmond, IN), the modified Thomas-Hartroft diet (1.3% cholesterol, 15% fat from cocoa butter, Dyets, Bethlehem, PA (10)), and the Western-type diet (0.15% cholesterol, 21% fat from butterfat, Dyets (15,16)).

Physiologic saline solution was composed of NaCl, 118(mM); KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2, $NaHCO_3$, 25; Na_2EDTA, 0.026; dextrose, 11.1; L-arginine, 0.1. All solutions were prepared in distilled water except for oxaloacetic acid and 5,5'-dithiobis-(2-nitrobenzoate) (DTNB) which were prepared in 0.1M and 1M Tris-HCl, respectively. Acetyl Coenzyme A, DTNB, oxaloacetic acid, norepinephrine bitartrate, ACh, calcium ionophore A23187, L-arginine were purchased from Sigma Chemical Co. (St. Louis, MO). NTG was obtained from DuPont Chemicals (Wilmington, DE).

Data Analysis

Data are expressed as mean ± standard error of the mean (SE). Comparisons of means from multiple populations were made by one and two factor analysis of variance (ANOVA) followed by Fisher's Protected Least Significant Difference. Multivariate regression analysis was used for simultaneous comparisons of relationships. Comparisons of multiple means from repeat-measures experiments were made by multivariate analysis of variance (MANOVA). Comparisons of multiple covariate-adjusted means were made by one and two factor analysis of covariance (ANCOVA). A p-value less than 0.05 was accepted as statistically significant.

Results

Aerobic Capacity in Relation to Onset of Hypercholesterolemia

At 8 weeks of age, all 5 measures of exercise capacity were similar (Table 1). However, the E^- mice demonstrated a decline in the 4 indices of exercise capacity that have aerobic capacity as component (V_{O2max}, AT, $DIST_e$ and AWC) as they aged from 8 to 12 and 20 weeks (Table 1; Figure 1). The decline in aerobic capacity was associated with an increase in cholesterol over time in the E− animals (Figure 2). By comparison, E^+ mice maintained their aerobic capacity with a slight downward trend in V_{O2max}, AT and AWC but an upward trend in $DIST_e$. By contrast, the RQ of all mice at all three ages were similar suggesting little impact on anaerobic capacity.

Aerobic Capacity in Relation to Degree and Origin of Hypercholesterolemia

The combinations of 2 diets and 2 genetic strains resulted in 4 groups with significantly different total serum cholesterol levels ($p < .0001$) that increased in the order of $E^+ < E^+_{chol} < E^- < E^-_{chol}$ and ranged from 153 ± 21 mg/dl to 2154 ± 223 mg/dl. The HDL cholesterol levels of each of the groups were not significantly different from each other.

At 12 weeks, the V_{O2max} of the 3 hypercholesterolemic groups was significantly less than that of E^+ ($p < .001$) and decreased across groups with increasing cholesterol levels (Figure 3). Similar inverse trends across groups were observed with AT and AWC with the values of the E^- groups being significantly lower than that of E^+ ($p < .005$ and $p < .01$ respectively). As the mice aged from 8 weeks to 12 weeks, the E^+ group improved in running distance ($\Delta DIST_e$) while the other three groups demonstrated a progressive decline in running distance with age.

Independence of Association of Hypercholesterolemia with Aerobic Capacity

We examined several known physical and biochemical determinants of aerobic capacity in comparison to total serum cholesterol of the mice at 12 weeks of age. These determinants included body weight, cardiac mass, and oxidative capacity (Table 2). We also examined the confounding variables of genetic strain and diet. Only the E^-_{chol} group demonstrated a significantly higher average body weight than the other groups at 12 weeks of age ($p < .001$). Similarly, only the E^- demonstrated significantly greater cardiac mass when indexed to body weight ($p < .005$). There were no differences between groups with respect to citrate

synthase activity. By multivariate analysis, both total serum cholesterol and body weight demonstrated inverse relationships with the indices of exercise capacity. Weak correlations were observed with strain, cardiac mass and diet. However, after adjusting for serum cholesterol level, differences in indices of exercise capacity between groups disappeared indicating that, of these variables, total serum cholesterol level was the strongest predictor of aerobic exercise capacity.

Mechanisms of Aerobic Exercise Impairment by Hypercholesterolemia

Effect on endothelial function—1) Vascular reactivity: The response to endothelium-independent vasorelaxation to NTG of aortic rings was similar in all groups at 12 weeks of age except for an attenuation in E^- at three intermediate doses of NTG ($p = 0.01$ by MANOVA, Figure 4). In contrast, endothelium-dependent vasodilation to ACh was significantly impaired in all three of the hypercholesterolemic groups compared with E^+ ($p < 0.0001$ by MANOVA). 2) EDNO activity: There was an inverse trend between aortic NO_x activity and cholesterol level ($r = -.32$, $p = .06$) as well as a direct correlation of aortic NO_x activity with measures of aerobic capacity; $\Delta DIST_e$ ($r = .55$, $p < .01$) and AT ($r = .43$, $p < .05$). Likewise, there was a trend toward a decline across all 4 groups in basal urinary NO_x excretion which became more pronounced in the post-exercise measures.

Effect on cardiac function, angiogenesis and oxidative capacity—To exclude myocardial injury as a mechanism for reduced aerobic capacity, myocardial sections were examined by light microscopy. No histologic evidence for myocardial ischemia, injury or necrosis was found in either the E^+ or E^- groups at 20 weeks of age. We also considered an effect of hypercholesterolemia on developmental angiogenesis and oxidative capacity of skeletal muscles. There were no differences in arteriolar or capillary density or in citrate synthase levels however.

Discussion

The salient findings of this study are;

1. Aerobic dysfunction is observed in murine models of genetic and diet induced hypercholesterolemia
2. The aerobic dysfunction is associated with a disturbance of endothelium-derived nitric oxide activity and vasodilatory function whereas no evidence of disturbances in other determinants of aerobic capacity was found.

This study confirms our previous observation that lipid-induced reduction in exercise capacity is a true aerobic dysfunction. Indeed, 4 indices of exercise capacity which have an aerobic component; oxygen uptake, anaerobic threshold, overall work performance and running distance, all declined in proportion to the degree of hypercholesterolemia. This occurred whether the elevation in serum cholesterol was genetically determined or diet-induced.

To examine if factors other than serum cholesterol might be contributing determinants of impaired aerobic capacity, we examined the contribution of several known physical and biochemical determinants of exercise capacity with our measures of aerobic capacity. Body weight, cardiac mass and heart volume have all been shown to correlate with aerobic capacity (17–19). In our model, only the E^-_{chol} group demonstrated a significantly higher average body weight and cardiac mass (when indexed to body weight) than the other groups. However, an increase in cardiac mass would be expected to augment cardiac performance and aerobic capacity rather than reduce it as occurred in the E^-_{chol} group. Oxidative enzyme activity in running muscle is also a known determinant of aerobic capacity. Oxidative

enzyme capacity is commonly measured by assessing mitochondrial citrate synthase activity. Mitochondrial citrate synthase is a highly inducible enzyme that is upregulated with other mitochondrial enzymes in trained skeletal muscle mitochondria (20). However, in our study there were no significant differences in running muscle citrate synthase activity amongst any of the groups. By multivariate analysis, effects of genetic strain, diet, body weight and cardiac mass were not significant determinants of aerobic capacity when total serum cholesterol was included in the analysis. Taken together, these data suggest that total serum cholesterol level is the strongest determinant of impaired aerobic capacity in our model. However, one limitation of this study is that two different diets are typically employed to induce hypercholesterolemia in wild-type and apoE deficient mice. Accordingly, it is possible that other components of the two diets may have contributed to the observed impairments in aerobic capacity and endothelial function in the hypercholesterolemic mice.

We investigated potential mechanisms by which hypercholesterolemia might adversely affect exercise capacity. Hypercholesterolemia can potentially affect any or all of the three major components of aerobic capacity (functional capacity, transport capacity and oxidative capacity (19)). One of these components, oxygen transport capacity, is perhaps most limited by vascular conduction and distribution of blood flow (21,22). Our finding of reduced EDNO activity as measured by aortic and urinary NO_x production, and attenuated endothelium-dependent vasodilation coupled with our previous findings of attenuated exercise hyperemia in hypercholesterolemic mice supports a disturbance in this component (7). One limitation of our approach was that aortic vascular reactivity and aortic NO release were measured in different segments of the aorta, the thoracic and abdominal aorta specifically, regions of the aorta that are known to have differences in vascular response.

Oxygen transport capacity can also be limited by blood vessel density. Vessel density might be altered in development by a depression in angiogenesis, which is modulated by EDNO activity (23). Indeed, E^- mice have been shown to have an age-dependent impairment in angiogenesis in response to surgically-induced ischemia (24). Indeed, we have shown that the impaired angiogenesis in response to hindlimb ischemia is a function of lipid-induced elevations in plasma levels of ADMA, the endogenous NOS inhibitor (8). Hypercholesterolemia could act by inhibiting angiogenesis in response to training. However, at a time when there was significant impairment of aerobic capacity (12 weeks), there was no significant reduction in microvascular density in the hypercholesterolemic mice. It is of course possible that such a deficiency develops at a later time course.

Hypercholesterolemia may also affect the functional capacity of the cardiovascular system by causing cardiac ischemia and dysfunction, particularly during intense exercise. However, other investigators have demonstrated that hemodynamically significant disease in the coronary arteries of E^- mice is not observed at this age (16). Indeed, we observed no histological evidence for ischemic insults to the myocardium.

Finally, hypercholesterolemia could alter oxidative capacity by effecting nitric oxide activity within the skeletal myocyte. Nitric oxide modulates mitochondrial respiration via the inhibition of cytochrome c oxidase, the terminal component, and rate-limiting step of the electron transport chain (25). However, a reduction in skeletal muscle nitric oxide activity would increase oxygen utilization as observed *ex vivo* (26) or down-regulate oxidative enzymes such as citrate synthase. Our data does not support a lipid-induced disinhibition of mitochondrial respiration as the hypercholesterolemic mice had reduced VO_{2max}.

Based on these findings as well as those from our previous report, we conclude that hypercholesterolemia adversely affects aerobic capacity by impairing EDNO synthesis

resulting in depressed vascular conductance and distribution (i.e. oxygen transport capacity). It is well documented that hypercholesterolemia impairs endothelial vasodilator function in the microvasculature as well as the conduit vessels (27–30). These alterations could disrupt the normal mechanisms that redistribute blood flow to exercising muscle (31). That depressed EDNO activity attenuates exercise-induced hyperemia is controversial. In two studies, administration of NOS inhibitors did not affect exercise-induced hyperemia in the human forearm, nor hyperemia in response to electrically stimulated contractions in dogs (32,33). However, others have reported a diminution of exercise hyperemia in the human forearm and in certain rat hindlimb muscles with infusion of NOS inhibitor (34,35). Still other studies suggest that EDNO is involved at low-intensity but not at high intensity exercise (36) or only partly responsible for exercise hyperemia (37).

An interdependence of physical performance, cholesterol fractions and coronary heart disease risk has been observed in multiple clinical studies. Notable is the direct relationship of $V_{O_{2max}}$ and exercise endurance with HDL cholesterol (38,39), ApoA-1 (40) and HDL/LDL ratio (41) and the inverse relationship with total cholesterol (38) and plasma triglyceride levels (42). In all of these studies, the investigators assumed that $V_{O_{2max}}$ or exercise endurance reflected the subjects' level of physical activity and conditioning and that serum cholesterol was a dependent variable in this relationship. They concluded that, as a consequence of greater energy expenditure, physically active individuals have lower cholesterol levels. Whereas this may be true in part, our studies suggest that the causality of the relationship may be ambiguous. Indeed, elevated levels of serum cholesterol appear to reduce $V_{O_{2max}}$. In this scenario, $V_{O_{2max}}$ is not only determined by physical activity habits, but also by serum cholesterol and its effects upon endothelial function.

Our observations take on greater clinical relevance with the recent finding that treatment with HMG coA-reductase inhibitors can improve treadmill exercise time in patients with peripheral arterial disease (43,44). In these trials, the improvement in treadmill exercise time was associated with a reduction in total and LDL-cholesterol. The beneficial effect of statins on walking distance in PAD is not likely due to a hemodynamic effect related to the regression of lesions. The effect of statins on conduit vessel luminal area is quite modest (45). Furthermore, the improvement in walking time occurred in the absence of any increase in ankle-brachial index in the treated patients (44). Accordingly, it seems plausible that the reduction of cholesterol in these studies, combined with lipid-independent effects of statins on the NO synthase pathway (46), may have improved vascular reactivity of the conduit, collateral and/or arteriolar vessels sufficiently to improve limb blood flow and oxygen transport capacity.

To conclude, hypercholesterolemia is associated with an aerobic dysfunction in mice as assessed by several indices of exercise performance. A lipid-induced defect in the NOS pathway may impair exercise capacity by reducing oxygen transport capacity. The impairment of exercise capacity is observed in diet-induced as well as genetically determined hypercholesterolemia. These studies may provide insight into recent clinical trials showing a benefit of statins on exercise capacity in patients with peripheral arterial disease.

Abbreviations

$\mathbf{E^+}$	wild type mice on a regular chow diet
$\mathbf{E^+_{chol}}$	wild type mice on a Thomas-Hartroft high cholesterol diet
$\mathbf{E^-}$	apoE deficient mice on a regular chow diet

$\mathbf{E^-_{chol}}$	apoE deficient mice on a high fat diet
$\mathbf{V_{O2max}}$	Maximal oxygen uptake
$\mathbf{V_{CO2}}$	Rate of carbon dioxide exhaled
AT	Anaerobic threshold
$\mathbf{DIST_e}$	Distance run to exhaustion
$\mathbf{\Delta DIST_e}$	Change in distance run to exhaustion from 8 weeks
AWC	Aerobic work capacity
RQ	Respiratory quotient
NTG	Nitroglycerin
PSS	Physiologic saline solution
$\mathbf{NO_x}$	Nitrogen oxide (NO_2 and NO_3)
EDNO	Endothelium-derived nitric oxide

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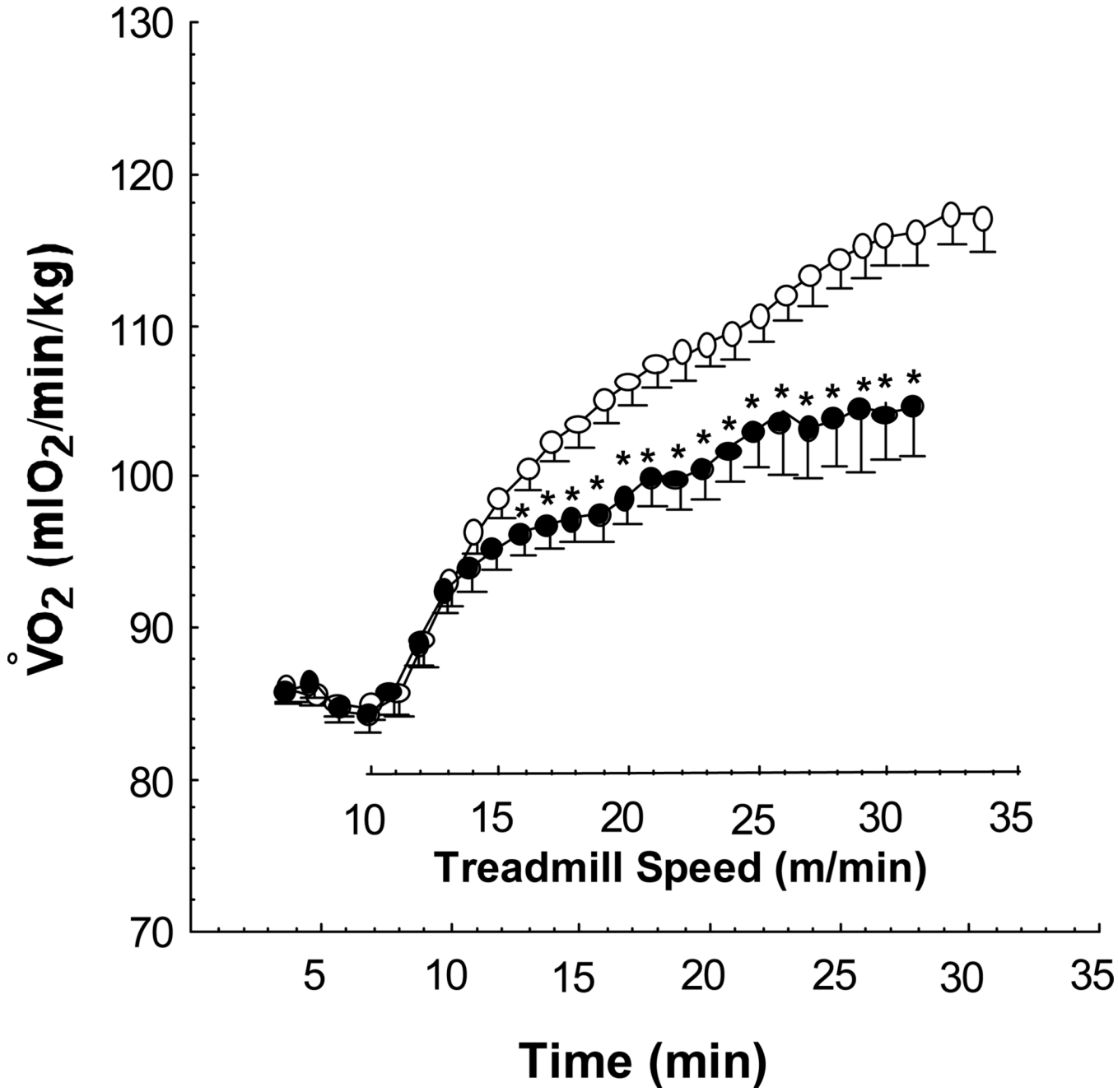

Figure 1.

Mean minute V_{O_2} of 12 week old E^+ (n = 32, open circles) and E^- (n = 22, solid circles) over the course of treadmill testing. After 7 minutes of basal measurements, the treadmill is started at 10 m/min and advanced 1 m/min every minute until exhaustion. ** $P < .05$ vs. E^+ by MANOVA.

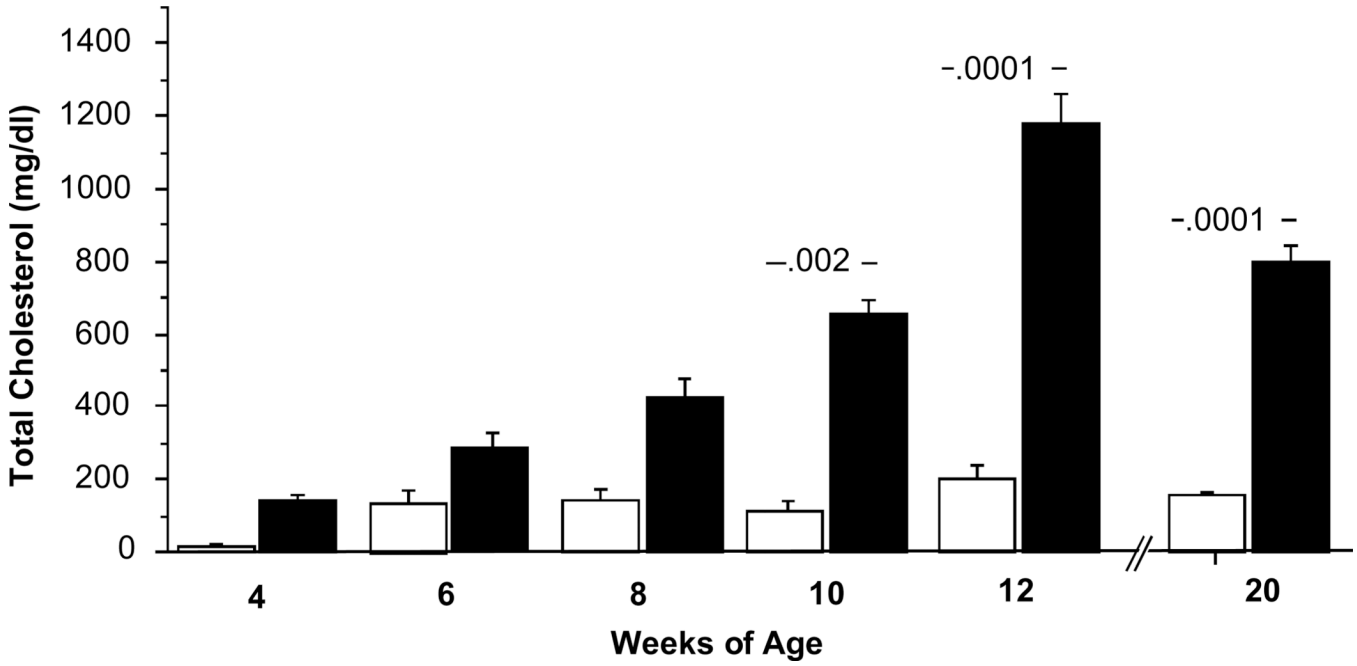

Figure 2.

Bar graph of total serum cholesterol over time. Cholesterol means ± SE of E^+ (n = 10, 5, 5, 5, 59 and 16 respectively, open bars) and E^- (n = 5, 4, 5, 4, 30 and 20, solid bars) at 4, 6, 8, 10, 12 and 20 weeks. Numbers over bars = p value vs. E^+ by ANOVA.

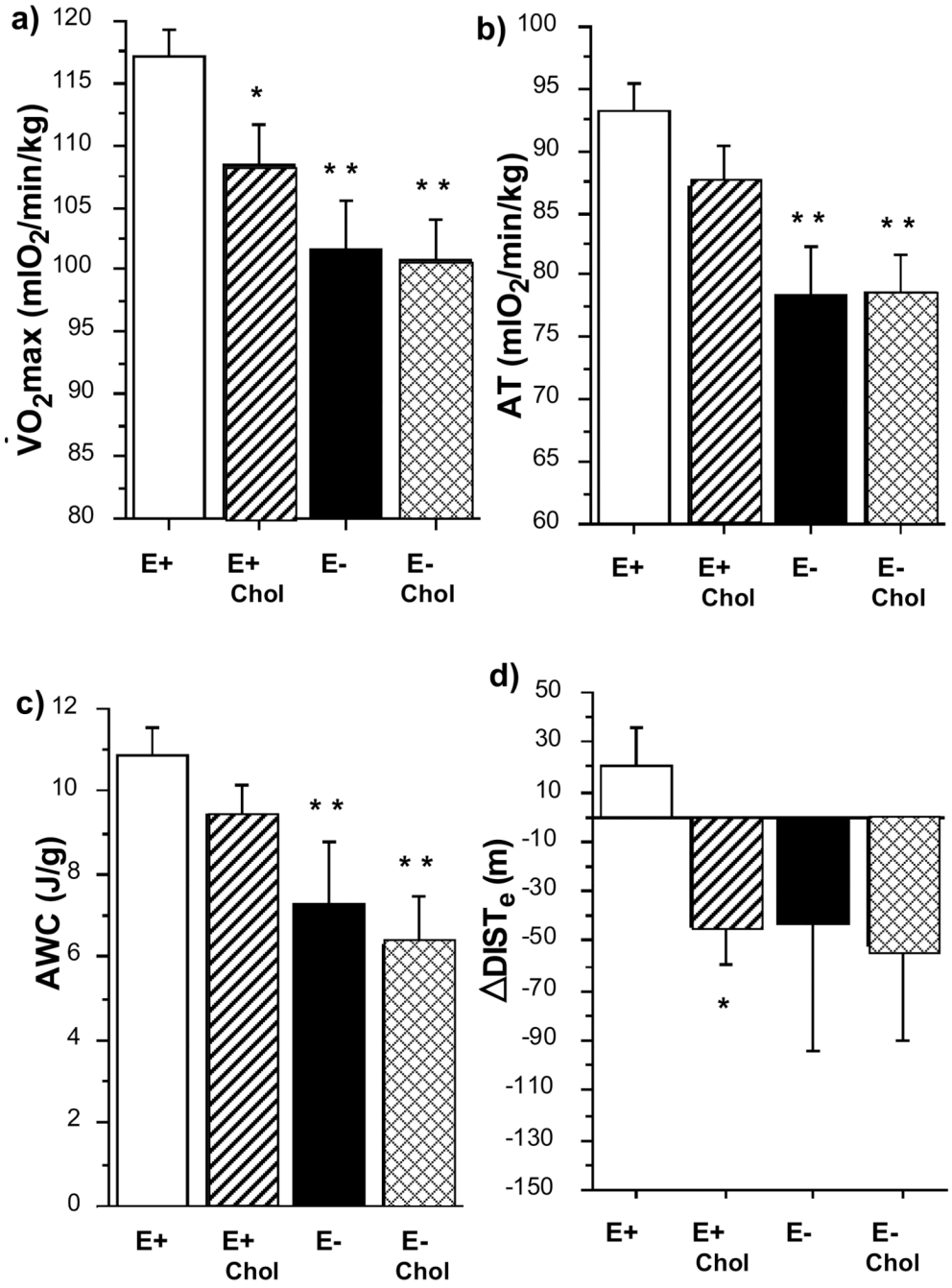

Figure 3.

Bar graphs of exercise indices of 4 cholesterol groups at 12 weeks. a) V_{o2max}, b) AT, c) $\Delta DIST_e$ and d) AWC of E^+ (n = 32, open bars), E^+_{chol} (n = 22, striped bars) and E^- (n = 14, solid bars) and E^-_{chol} (n = 9, cross-hatched bars). Expressed in mean ± SE. * $p < .05$ and ** $p < .01$ vs. E^+. Numbers over bars = p value vs. E^+ by ANOVA.

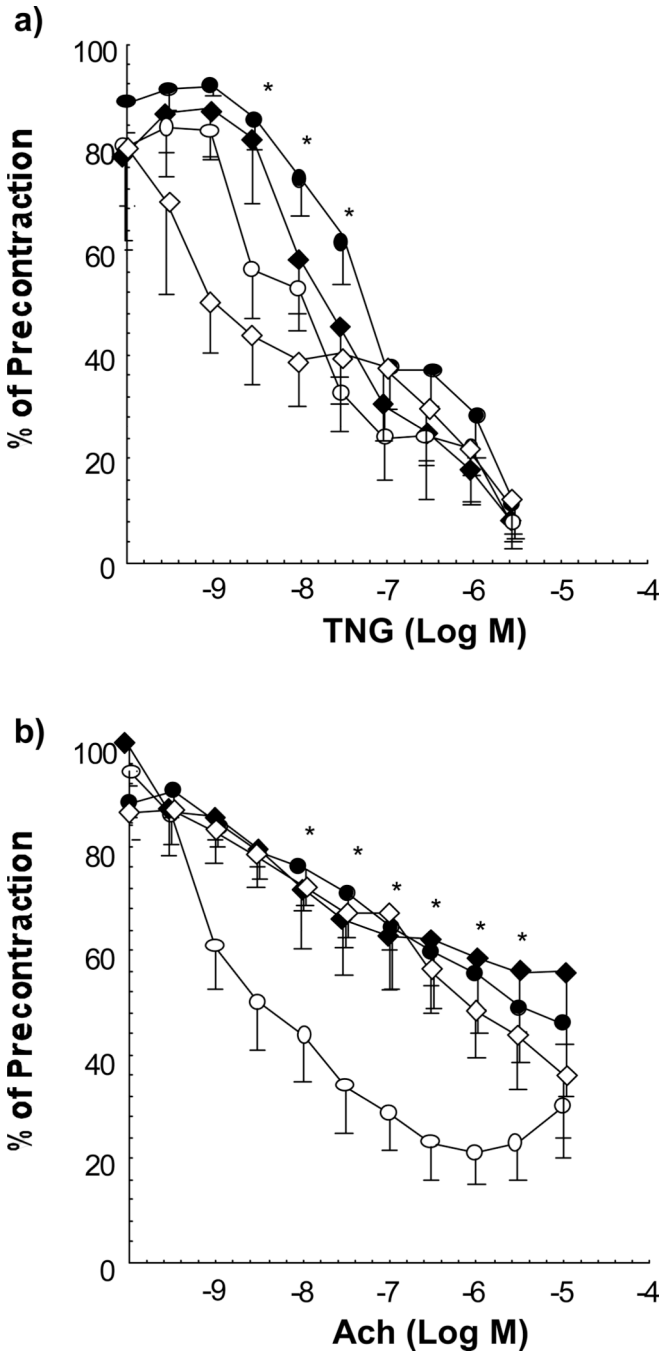

Figure 4.

Vascular reactivity studies represented by concentration-effect curves of rings of aortae from 12 week old mice. a) endothelium-independent vasorelaxation in response to NTG of segments of aorta from E^+ (n = 6, open circles), E^+_{chol} (n = 6, open diamonds), E^- (n = 6, filled circles) and E^-_{chol} (n = 9, solid diamonds). The E^- curve demonstrates significant attenuation of vasorelaxation at the middle three doses. This effect was not observed in the other two high cholesterol groups. b) Concentration-effect curves demonstrating endothelium-dependent vasorelaxation in response to ACh. The curves of the three high cholesterol groups demonstrate significant attenuation of relaxation by MANOVA. * $P < 0.05$ vs. E^+ at the same dose.

Table 1

Physical, Biochemical and Exercise Characteristics of E^+ and E^- Mice at 8 (n = 25 & 25), 12 (n = 32 & 15) and 20 (n= 17 & 9) Weeks of Age

Measurement	Age	E^+	E^-
Body Weight (g)	8 Week	20.8 ± 0.5	20.2 ± 0.5
	12 Week	22.4 ± 0.5	22.9 ± 0.8
	20 Week	23.1 ± 0.4	30 ± 1**
Total Cholesterol (mg/dl)	8 Week §	131 ± 19	422 ± 53
	12 Week	153 ± 21	1175 ± 107**
	20 Week	157 ± 2	807 ± 57**
Basal Urinary NO_x (PM NO/mg creatinine)	8 Week	181± 21	284 ± 43
	12 Week	279 ± 65	187 ± 26
Post-Exercise Urinary NO_x (PM NO/mg creatinine)	8 Week	202 ± 43	346 ± 52 **
	12 Week	429 ± 105	165 ± 20 **
$V_{O_{2max}}$ (mlO_2/min/kg)	8 Week	122 ± 3	123 ± 4
	12 Week	117 ± 2	102 ± 4**
	20 Week	110 ± 2	97 ± 2*
Anaerobic Threshold (mlO_2/min/kg)	8 Week	100 ± 4	96 ± 3
	12 Week	93 ± 2	78 ± 4 **
	20 Week	77 ± 2	71 ± 3
$DIST_e$ (m)	8 Week	456 ± 23	468 ± 23
	12 Week	476 ± 17	453 ± 42
	20 Week	524 ± 24	306 ± 32 **
AWC (J/g)	8 Week	12 ±1	13 ± 1
	12 Week	10.9 ± 0.6	7.0 ± 1**
	20 Week	8.0 ± 0.7	4.8 ± 0.7**
RQ_e	8 Week	1.07 ± .03	1.02 ± .01
	12 Week	1.00 ± .01	1.00 ± .02
	20 Week	1.03 ± .02	1.02 ± .01

Values are mean ± SE.

§based on smaller n

* $p < .05$,

** $p < .01$ vs. E^+

Table 2

Physical, & Biochemical Characteristics of 12 Week Old Mice

Measurement	**E^+ (n = 32)**	**E^+_{chol} (n = 22)**	**E^- (n = 15)**	**E^-_{chol} (n = 10)**
Body Weight (g)	22.4 ± 0.5	21.9 ± 0.5	22.9 ± 0.8	26.5 ± 0.6**
Total Cholesterol (mg/dl)	153 ± 21	306 ± 40	1175 ± 107**	2154 ± 223**
HDL Cholesterol §	60 ± 21	80 ± 19	17 ± 9	38 ± 17
Cardiac Mass Index (mg heart/g body)	5.3 ± .2	5.0 ±.2	6.3 ± .3 **	5.5 ± .1
Arteriolar density (#/mm2)	102 ± 3		99 ± 10	
Capillary density (#/mm2)	690 ± 114		661 ± 122	
Citrate Synthase (μg/min/g muscle)	34 ± 1	34 ± 1	32 ± 1	34 ± 2
Basal Urinary NO_x (pM/mg creatinine)	279± 65	289± 51	187 ± 26	200 ± 40
Post-Exercise Urinary NO_x (pM/mg creatinine)	429 ± 105	354 ± 40	165 ± 20 **	140 ± 40 **
Aortic NO_xActivity (pM/mm aorta)	1139 ± 253	937 ± 104	419 ± 106	532 ± 84
RQ_e	1.00 ± .01	0.99 ± .02	1.00 ± .02	0.99 ± .01

Values are mean ± SE.

* $p < .05$,

** $p < .01$ vs. E^+,

§ based on smaller n