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Age-Related Decreases in Lymphocyte Protein Kinase C Activity and Translocation Are Reduced by Aerobic Fitness

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This study investigated the effects of advancing age and long-term aerobic fitness on lymphocyte protein kinase C (PKC) activity and translocation. Lymphocytes were obtained from young (20–36 years old) and older (61–78 years old) healthy men who were either aerobically conditioned or deconditioned. Both baseline PKC activity and the response of this enzyme to the direct PKC stimulating agent, phorbol 12-myristate, 13-acetate (PMA) or to the mitogen, phytohaemagglutinin (PHA), were measured in partially purified extracts of cytosolic and membranous fractions of lymphocytes. Basal PKC activity, PMA-induced redistribution of PKC, and PHA-induced enhancement of PKC activity were reduced among older subjects in both lymphocyte cytosolic and membranous fractions. However, the magnitudes of these reductions were smaller among the older subjects who were aerobically fit. Lymphocyte PKC activity and translocation may be biological markers of aging, and the maintenance of aerobic fitness into later life may serve to slow the rate at which activation of this enzyme declines during senescence.

PROTEIN kinase C (PKC) is a serine/threonine protein-phosphorylating enzyme. It is widely distributed throughout the brain and in peripheral tissues and is involved in a broad spectrum of cellular functions (1). Lipid second messengers, produced in response to hormones, neurotransmitters, or growth factors, regulate the activity of this enzyme. An important outcome of cellular stimulation induced by these substances is the translocation of PKC from cytosol to membrane (2–6). PKC plays an important role in regulating the release of neurotransmitters, such as acetylcholine (7), norepinephrine (7), glutamate (8), and serotonin (3), as well as in processes like synaptic potentiation that model various aspects of learning and memory (9).

Both PKC activity and its translocation are reported to change with advancing age in animal studies (3,5,10–11). Consistent with these observations, we have observed an age-dependent decrease in PKC activity and its translocation in postmortem human brains (12). Furthermore, we have found significant reductions in platelet PKC activity associated with both membranous and cytosolic cellular fractions among elderly men, as well as in the redistribution of platelet PKC activity that is elicited by stimulation of cell surface receptors (13). We also observed, however, that these age-related reductions were mitigated in older men who had sustained moderately high levels of aerobic fitness as they aged (13). Thus, PKC activity and its translocation may be biological markers of aging that are sensitive to interventions, such as physical exercise, that may serve to slow the aging process.

Age-related declines in lymphocyte-mediated immune function, defined as the proliferative response to mitogenic stimulation, have also been observed (14). This proliferation may involve the activation of PKC (15). In this article, we describe an extension of our work to include the effects of aging and aerobic fitness on PKC activity and its translocation in the immune system. To do so, we tested the effects of two known mitogenic agents, phorbol 12-myristate, 13-acetate (PMA) and phytohaemagglutinin (PHA), on lymphocytes obtained from young and older sedentary and aerobically fit males. PMA stimulates PKC activity directly, inducing a translocation of PKC from cytosol to membrane, thus redistributing intracellular PKC activity. The precise mechanism by which PHA elicits its mitogenic action is unknown.

Materials and Methods

Subjects

Fifty-eight men, ranging in age from 20 to 36 and 61 to 78 years, participated in this study. With the exception of one young nonexerciser and one older exerciser, this was the same sample we evaluated in our previous study of blood platelets (13). Subjects were identified on the basis of self-report as sedentary nonexercisers or as regular aerobic exercisers. For inclusion in the sample, young and older aerobic exercisers had to have trained systematically for a minimum of 2 or 10 years, respectively. These minimums were set to insure that subjects were currently training regularly and had done so for a reasonable period of time. The minimum of 10 years for the older subjects was selected to increase the likelihood that these subjects would have sustained exercise for a long enough time to have derived physiologic benefit. In practice, the participants in this study, both young and older, had been exercising regularly

for large portions of their lives: young and older subjects reported exercising regularly for an average of 8.5 years (range, 3–18 years) and 16.8 years (range, 10–40 years), respectively. Four groups of subjects were thus formed: (i) nine young nonexercisers, ranging in age from 23 to 34 years; (ii) 18 older nonexercisers, ranging in age from 62 to 78 years; (iii) 12 young exercisers, ranging in age from 20 to 36 years; and (iv) 19 older exercisers, ranging in age from 61 to 77 years.

Subjects were screened for medical exclusionary criteria in a two-step process. A candidate first responded to a newspaper advertisement or public announcement with a telephone call during which a comprehensive medical history was taken. Individuals taking any medication that acts on the central nervous system (CNS), reporting any current or past medical disorder that could affect the CNS, having histories of drug or alcohol abuse, or receiving nonsteroidal antiinflammatory drugs that could interact with lymphocytes, were eliminated in this phase of the screening process. Those candidates not excluded on the basis of these criteria completed a medical examination that included a history, physical, blood work, vision and hearing tests, and an exercise stress test. The stress test was performed using a stationary ergometer. It was stopped when the subjects achieved 75% of predicted maximum heart rate. This yielded an estimate of maximal oxygen consumption ($\dot{V}O_2max$) that provided an objective index of aerobic fitness to differentiate exercisers from nonexercisers. Candidates who passed the medical examination participated in the study. All participants were free of clinical evidence of cardiovascular or neurological illnesses or infectious diseases that could alter lymphocyte functioning.

PKC-Mediated Protein Phosphorylation and Enzyme Redistribution in Lymphocytes

Venous blood was collected into ethylenediaminetetraacetic acid (EDTA)-containing syringes (Luer-Monovette, Sarstedt, Inc, Newton, NC). Lymphocytes were isolated using Sigma Histopaque-1077, according to the manufacturer's instructions. The isolated lymphocytes were washed in 10 ml of phosphate-buffered saline (PBS) prior to PKC activity measurements, and then were incubated at 37°C in a total of 0.5 ml for 10 minutes in 0.32 M sucrose buffered with 5 mM N-2-hydroxyethylipiperazine-N′-2 ethanesulforic acid (pH 7.5), with 80 nM PMA, or with 40 mg/ml PHA. The concentrations of these agents were selected to approximate their respective EC_{50}s for PKC activity and mitogenic action. Reaction was stopped by the addition of 0.5 mM ethyleneglycol-bis (β-aminoethyl)-N,N,N′,N′-tetraacetic acid (EGTA)-containing buffer and cooling on ice. Fresh lymphocytes were harvested and homogenized in 1 ml, 0.32 M sucrose in 20 mM Tris-HCl (pH 7.5), with 2 mM EDTA, 0.5 mM EGTA, 50 mg/ml leupeptin, 0.2 mM phenylmethylsulfonyl fluoride, and 0.1% 2-mercaptoethanol and sonicated (Micro Cell Disrupter, Kontes, Vineland, NJ) prior to centrifugation at 25,000 × *g* for 15 minutes. The supernatant yielded after centrifugation is considered the cytosolic fraction. The pellet was resuspended in 200 μl of buffer containing 1% Nonidet P-40 and solubilized on ice for 1 hour. The sample was diluted to 1 ml and centrifuged at 25,000 × *g* for 15 minutes. The supernatant contains extracted enzymes from the membrane. Both cytosolic and membrane extracts were applied to 1 ml of diethylaminoethyl cellulose (DE52, Whatman, Rockland, MA) equilibrated in buffer, and the columns were washed, eluted with buffer containing 0.1 M NaCl, and used immediately for assessing PKC activity.

The standard assay mixture (250 μl) containing 24 mM Tris-HCl (pH 7.5), 20 mM NaCl, 0.1 mM EGTA, 0.4 mM EDTA, 0.03% 2-mercaptoethanol, 60 μg/ml leupeptin, 0.04 mM phenylmethylsulfonyl fluoride, 0.25 mg/ml histone type III-s (Sigma, St. Louis, MO), 1.2 mM $CaCl_2$, 20 μg/ml phosphatidyl-l-serine (PS), 80 nM PMA, 1 mM $MgAc_2$ and 0.03 mM [^{32}P]adenosine triphosphate (ATP) (DuPont, Boston, MA; 500,000 cpm) was preincubated at 30°C for 5 minutes, and the reaction was initiated by the addition of 50 μl of eluted protein. After 1 minute, the reaction was terminated by transferring 50 μl onto a 1 × 2 cm phosphocellulose (P81, Whatman, Rockland, MA) strip that was subsequently immersed in 75 mM phosphoric acid (10 ml per strip). The strips were washed 3 times (2 min per wash) in fresh phosphoric acid and air dried. They were then placed in scintillation fluid and radioactivity was determined by liquid scintillation spectrometry (LKB 1214 Rackbeta, Stockholm, Sweden). PKC activity was defined as the phosphorylation that occurred in the presence of phosphatidyl-l-serine (PS), and PMA and was expressed as pmol ^{32}Pi incorporated per unit protein of column eluate. Negligible phosphorylation was measured when the column eluates were assayed in the presence and absence of PS and PMA without added substrate. Protein was determined by the method of Lowry and colleagues. (16).

Statistical Analyses

To maximize the quality of our data and thus the validity of our analysis and interpretation, careful consideration was given to model assumptions (and the consequences of violation of assumptions). Where violations were observed, steps were taken to correct the data (or adjust the analysis). For example, violations of normality assumptions were corrected by data transformations. A general factorial analysis of variance (ANOVA) (17), weighted for between-group differences in sample size, was completed to determine the influence of the between-group factors for age and exercise groups on the dependent measures: age (in years), $\dot{V}O_2max$ (ml/min/kg), and cytosolic and membrane PKC activities in lymphocytes. Significant main effects ($p < .05$) were followed by post hoc multiple comparisons between means of a given factor. For these post hoc mean comparisons, Tukey's honestly significant difference procedure (18) provided the appropriate balance between statistical power and overall control of type I errors. Because this is an unbalanced design (number of cases in all the cells are not equal) with no empty cells, we used a specific approach to calculating sums of squares (type III). This method calculates the sum of squares of a specific effect in the design as the sum of squares adjusted for any other effects that do not contain it and orthogonal to any effects (if any) that do contain it. Results of the Tukey test are only given when they added information not provided by the tests of simple effects. The degrees of freedom for all of the analyses were 1 and 54.

Given this constancy in degrees of freedom across analyses, when a statistical result is shown, it will include only the F ratio and the exact p value (i.e., $p = .4358$). In cases where the p value is smaller than .0001, we will designate it as $p < .0001$.

Results

Age and $\dot{V}O_2$max

Analyses on age and $\dot{V}O_2$max were done to accomplish two ends. We wanted to determine (i) if subjects within each exercise group were matched on age and (ii) if subjects who identified themselves as exercisers had higher levels of measured aerobic fitness than subjects who described themselves as sedentary. Table 1 contains the ages and levels of $\dot{V}O_2$max for each of the four age-by-exercise groups. The percentage of exercisers was slightly greater among the young subjects (12 of 21) than among the older subjects (19 of 37). The possible confounding introduced by this small imbalance was avoided by the use of type III sums of squares in all two-way ANOVAs. Exercisers and nonexercisers (Exercisers, 52.9 ± 19.8, vs Nonexercisers, 57.0 ± 20.2; Exercise Group, $F = 0.62$, $p = .4358$) differed in $\dot{V}O_2$max within each age group. As expected, $\dot{V}O_2$max was significantly higher in exercisers than in nonexercisers (44.1 ± 10.0 vs 27.0 ± 7.5; Exercise Group, $F = 53.36$, $p < .0001$), with the difference being larger among young versus older subjects (significant Age Group × Exercise Group interaction, $F = 5.87$, $p = .019$; simple effects: young, $F = 64.86$, $p < .0001$; older, $F = 85.00$, $p < .0001$).

Effects of Age and Exercise on Basal Lymphocyte PKC Activity

A varied pattern of sensitivity to age and aerobic fitness was evident for basal lymphocyte PKC activity. Age-sensitive responses to aerobic fitness were apparent in both cytosolic and membrane-associated enzyme activities. Activity levels in both cell fractions were significantly higher in young than in older subjects (Age Group, [*cyt*] $F = 100.71$, $p < .0001$; [*memb*] $F = 62.96$, $p < .0001$) and in exercisers than in nonexercisers (Exercise Group, [*cyt*] $F = 5.23$, $p = .0269$; [*memb*] $F = 10.96$, $p = .0027$). However, as depicted in Figure 1, differences associated with aerobic fitness in the activity levels of both measures varied among the young and the older subjects (significant Age Group × Exercise Group interaction, [*cyt*] $F = 30.52$, $p < .0001$; [*memb*] $F = 27.07$, $p < .0001$). Tests of the simple effects revealed that young nonexercisers had higher cytosolic, but not membrane-associated, enzyme activity levels than young exercisers ([*cyt*] $F = 8.52$, $p = .0088$; [*memb*] $F = 3.18$, $p = .0904$); however, older exercisers had higher levels of activ-

Table 1. Mean Age and $\dot{V}O_2$max, Age × Exercise Groups

Group	*n*	Age (years)	$\dot{V}O_2$max
Young nonexercisers	9	29.0 ± 3.8	34.4 ± 7.3
		(23–34)	(25.1–43.7)
Old nonexercisers	18	70.0 ± 4.4	23.3 ± 4.3
		(62–78)	(19.4–32.8)
Young exercisers	12	29.6 ± 4.8	55.4 ± 4.4
		(20–36)	(48.2–61.9)
Old exercisers	19	67.7 ± 4.4	37.2 ± 4.9
		(61–77)	(27.2–45.8)

Notes: n = number of subjects; values not in parentheses are the means ± standard deviation for each group; values in parentheses on the line below the means are the range for the group.

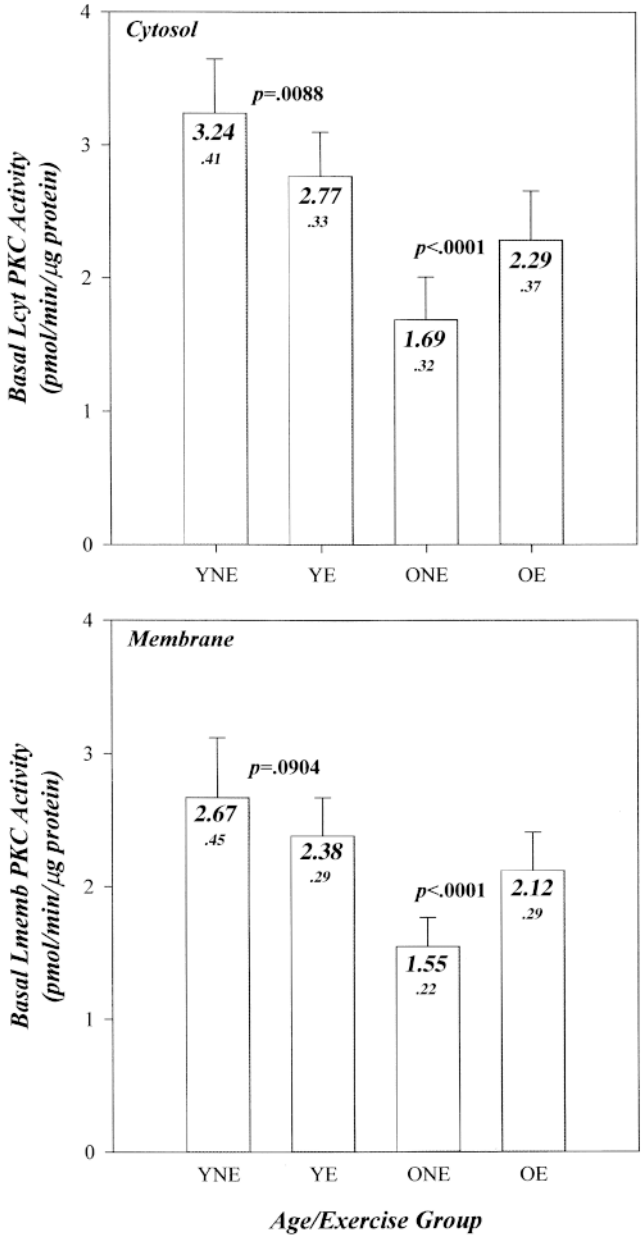

Figure 1. Combined effects of age and aerobic fitness on protein kinase C (PKC) activity in lymphocyte cytosol and membrane fractions. Lymphocytes from healthy young (Y) and older (O) nonexercisers (NE) and exercisers (E) were collected and sonicated, and cytosolic and membrane-associated PKC activities were determined. In this and all subsequent figures, error bars are shown to indicate the standard deviation, the absolute value for each measure for each group is given in the bar accompanied by the standard deviation, and the absolute p values are shown for the tests of the simple effects for the exerciser vs nonexerciser comparison within an age group. The p values for the interactions were <.0001 for both cytosol and membrane.

ity on both measures than older nonexercisers ([*cyt*] $F = 27.90$, $p < .0001$; [*memb*] $F = 45.91$, $p < .0001$).

Effects of Age and Exercise on PKC Translocation Induced by PMA

As noted previously, activation of PKC by the phorbol ester, PMA, produces a redistribution of PKC activity from cytosol to membrane. To evaluate the influences of age and aerobic fitness on this redistribution, we stimulated PKC with 80 nM of PMA. Although distribution of PKC in cytosolic and membranous fractions under basal conditions was not altered by age or aerobic fitness (Age Group, $F = 0.61$, $p = .4219$; Exercise Group, $F = 0.57$, $p = .4314$), both factors were associated with variations in the redistribution of PKC following stimulation by PMA. These variations were revealed in changes induced by PMA on the ratio of membrane-to-cytosolic PKC activity, an index that reflects enzyme translocation. Young subjects had higher ratios than older subjects (Age Group, $F = 56.89$, $p < .0001$), and exercisers had higher ratios than nonexercisers (Exercise Group, $F = 12.31$, $p = .001$). Although the direction of the difference between the two age groups did not vary with aerobic fitness, the Age Group × Exercise Group interaction was suggestive ($F = 2.33$, $p = .1331$). There is no extant literature, to our knowledge, that provides an a priori basis to support any prediction regarding differences in the magnitude of the combined influence of age and aerobic fitness on PMA-induced changes in PKC activity. Inspection of Figure 2 suggests, however, that differences in the magnitude of the difference in PKC activity between exercisers and nonexercisers may be larger among older than young subjects. This impression was confirmed in tests of the simple effects. They showed that the ratios did not differ significantly among young subjects ($F = 0.56$, $p = .4648$), whereas among older subjects, the ratio was significantly higher among the aerobically fit ($F = 17.45$, $p = .0002$).

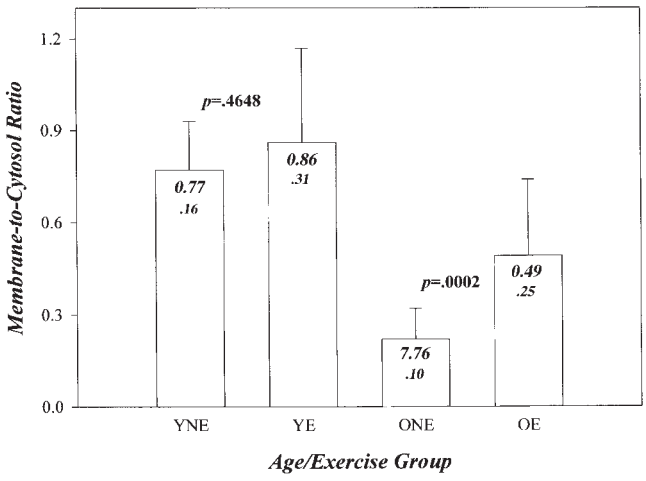

Figure 2. Combined effects of age and aerobic fitness on protein kinase C (PKC) translocation induced by PMA in lymphocytes. Lymphocytes from healthy young (Y) and older (O) nonexercisers (NE) and exercisers (E) were incubated for 10 min with 80 nM PMA, collected and sonicated. Cytosolic and membrane-associated PKC activities were then determined. The *p* value for the interaction was .1331.

Table 2. PMA-Induced PKC Activation Ratios of Membrane-to-Cytosol Activity, Age × Exercise Groups

	n	Young	*n*	Old
Nonexercisers				
PMA	9	0.77 ± 0.16 (0.61–0.98)	18	0.22 ± 0.10 (0.07–0.43)
Exercisers				
PMA	12	0.86 ± 0.31 (0.43–1.44)	19	0.49 ± 0.25 (0.17–1.09)

Notes: *n* = number of subjects; values not in parentheses are the mean cytosol-to-membrane ratios for each group ± standard deviation; values in parentheses on the line below the means are the ranges for the group. PMA = phorbol 12-myristate, 13-acetate; PKC = protein kinase C.

Thus, PMA-induced differences in the translocation of PKC between exercisers and nonexercisers were evident only among older subjects. This set of ratios is shown in Table 2.

Effects of Age and Aerobic Fitness on Cytosolic and Membranous PKC Activity Induced by PHA

The mitogen, PHA, increased lymphocyte PKC activity. We evaluated the influence of age and aerobic fitness on this enhancement by measuring the percent change in PKC activity induced by PHA in the four groups. These data are summarized in Table 3 and illustrated graphically in Figure 3. PHA induced a larger change in PKC activity among young than older subjects in both cytosol and membrane fractions (Age Group: [*cyt*] $F = 45.52$, $p < .0001$; [*memb*] $F = 26.07$, $p < .0001$). Likewise, it enhanced PKC activity to a greater extent among exercisers than nonexercisers in both cytosol and membrane (Exercise Group: [*cyt*] $F = 7.74$, $p = .007$; [*memb*] $F = 9.51$, $p = .003$). The influence of age on PHA-induced PKC activity in cytosol varied, however, with aerobic fitness (significant Age Group × Exercise Group interaction, $F = 5.53$, $p = .0224$; see Figure 3, top panel). Tests of the simple effects showed that aerobic fitness did not influence the degree of PKC activation induced in cytosol by PHA among young subjects ($F = 0.07$, $p = .7939$), but that this activation was reduced among older nonexercisers compared with exercisers ($F = 11.34$, $p = .0019$).

In contrast to the influence of aerobic fitness on age-related changes in PHA-induced PKC activation in cytosol, aerobic fitness did not appear to alter this activation differentially between the two age groups (Age Group × Exercise Group, $F = 2.70$, $p = .106$). Again, however, as was the case for PMA-induced changes in PKC activity, although there is no a priori basis on which to predict a specific age-by-exercise relationship, the interaction was suggestive and visual inspection of Figure 3 (bottom panel) reveals the possibility of age-related differences. Thus, we completed tests of the simple effects. They revealed that PHA-induced activation of PKC in membrane was comparable among young nonexercisers and exercisers ($F = 0.36$, $p = .5531$), but that it was reduced in older nonexercisers compared with exercisers ($F = 10.55$, $p = .0026$). Thus, differences between exercisers and nonexercisers in PHA-induced activation of PKC in both cytosol and membrane were evident only among older subjects. Here it is of interest to note that young nonexercisers and older exercisers did not differ in the

Table 3. Percent Change in PHA-Induced Enhancement of PKC Activity, Age × Exercise Groups

	n	Young	*n*	Old
Nonexercisers				
PMAcyt	9	16.01 ± 1.99 (13.10–18.80)	18	4.11 ± 4.57 (0.00–14.50)
PHAmemb	9	22.20 ± 5.42 (16.80–34.40)	18	7.76 ± 6.25 (0.00–25.30)
Exercisers				
PMAcyt	12	15.56 ± 4.80 (10.20–26.80)	19	9.67 ± 5.42 (−2.80–20.50)
PHAmemb	12	24.30 ± 7.73 (14.50–44.30)	19	16.25 ± 9.95 (−10.80–28.50)

Notes: *n* = number of subjects; values not in parentheses are the mean PHAcyt(osol) or PHAmemb(rane) for each group ± standard deviation; values in parentheses on the line below the means are the ranges for the group. PHA = phytohemagglutin; PKC = protein kinase C.

PHA-induced activation of PKC in membrane ($F = 2.81$, $p = .1056$).

Discussion

We found that age induces declines among men in both lymphocyte PKC activity and the activation of this enzyme in response to cellular stimuli, but that the magnitude of these declines is reduced by aerobic fitness. The levels of aerobic fitness characteristic of the young and older exercisers in this sample place them among the well conditioned for their respective age groups, whereas the levels of aerobic fitness characteristic of the young and older nonexercisers in this sample place them in the average range for their respective age groups (see references in [19]). Hence the exercisers and nonexercisers in this sample represent individuals with very different levels of aerobic conditioning. It is of interest to note that the age-dependent changes we found in PKC activity (both cytosolic and membrane-associated) and in the ability of the enzyme to translocate in response to stimuli, parallel changes previously reported in animal studies (3,5,10–11). Thus, although maintenance of aerobic fitness among older men may not preserve lymphocyte PKC activity and enzyme redistribution at levels comparable with those of the young, it may serve to slow the rate at which enzyme activation declines with advancing age. This observation accords well with a variety of other reports that have demonstrated that older aerobically fit subjects tend to fall between the young and the older aerobically deconditioned on many measures that have been evaluated (19–23). We observed an unexpected difference between young exercisers and nonexercisers on basal PKC activity in cytosol, however; namely, young nonexercisers had higher levels than young exercisers (whereas older exercisers had higher levels than older nonexercisers). Given the overall pattern of results in this and in our previous study of blood platelets (13), we expected no differences in baseline activity between the two young groups. This variation may reflect an important physiological difference between the two groups or it may simply reflect a statistical artifact emanating from the relatively small sample sizes of the two young groups.

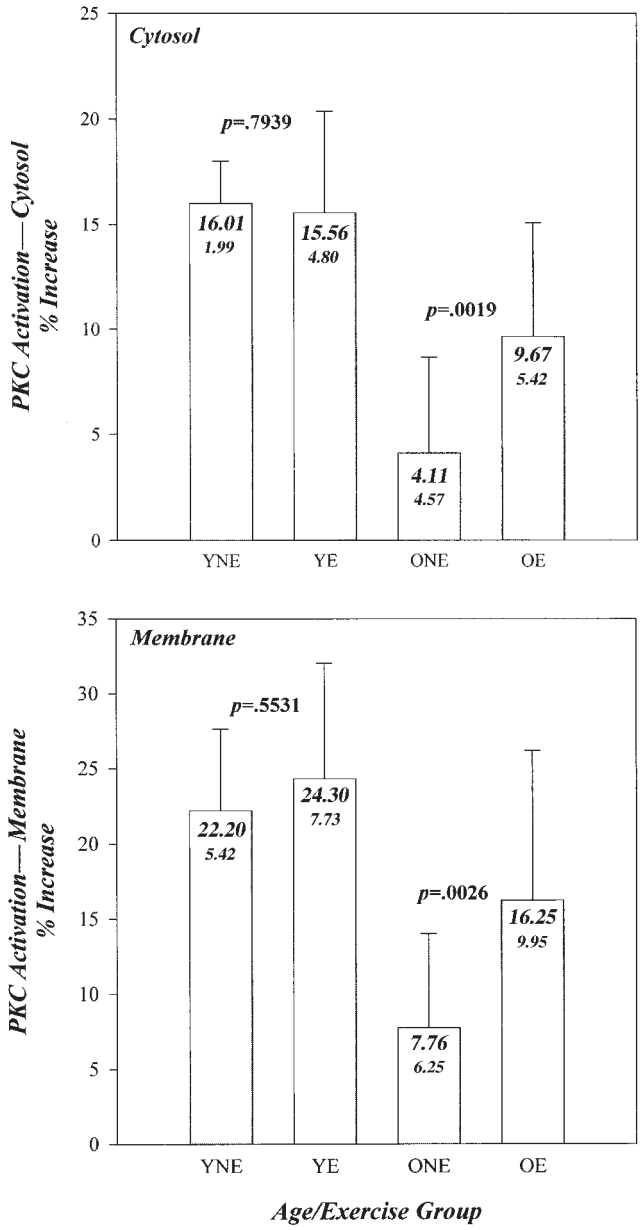

Figure 3. Combined effects of age and aerobic fitness on protein kinase C (PKC) activation induced by PHA in lymphocytes. Lymphocytes from healthy young (Y) and older (O) nonexercisers (NE) and exercisers (E) were incubated for 10 min with 40 mg/ml PHA, collected and sonicated. Cytosolic and membrane-associated PKC activities were then determined. The *p* values for the interactions for cytosol and membrane were .0224 and .1060, respectively.

We have no explanation for this difference, but are inclined to accept the latter explanation.

The activation of PKC seen in association with its translocation to membrane in response to cellular stimuli is dependent on membrane constituents, such as phospholipids and phospholipase C, and on the availability of Ca^{2+}. Thus, alteration in the metabolism of membrane phospholipids, changes in Ca^{2+} homeostasis, and changes in membrane fluidity during senescence may have a significant impact on the activation of PKC in lymphocytes (24–27). Alterna-

tively, posttranslational modification of the enzyme, such as phosphorylation or changes in the relative expression of PKC isozymes in peripheral blood T and B lymphocytes (28), may be responsible for the age-associated changes in the activation and translocation of PKC. In addition, recent data also indicate that age-dependent changes in the receptors for activated C kinase, or RACKs, may be responsible for some portion of the age-related decrease in PKC activation (29). It is possible that the differential activation of PKC isozymes in lymphocytes may be associated with age-related changes in immunological responses (14,30–32). This possibility is supported by data that show that an increase in αPKC level is coupled with a decrease in the proliferative response of splenic T cells (33).

Although the precise mechanism for age-related changes in lymphocyte PKC activation is still obscure, the data presented here and those in the previous reports on neuronal tissues reveal striking similarities in the effects of age (3,5). This suggests further that age-dependent change in PKC and its activation may result from alterations at a particular site or sites common to these cells. Reduced production of inositol-1,4,5-trisphosphate, a product of phosphoinositide turnover and Ca^{2+}-dependent PKC activation, has been found to be associated with an age-related decline in T cell function (32). Diminished diacylglycerol level, another product of phosphoinositide metabolism, may result in a decrease in PKC activity and translocation, as has been previously suggested in other cell types (11). More importantly, our data indicate that the age-related reduction in PKC activity and its intracellular redistribution in response to stimulation can be reduced in magnitude by aerobic fitness. Although the precise mechanisms by which aerobic fitness modifies physiological functions are not currently known, the present results, together with previous observations in other systems (35–38), may reflect a contribution aerobic fitness makes to maintaining functions that would otherwise decline at a faster rate with advancing age. The effects of aerobic exercise in reducing the age-dependent decrease in PKC—both its activity and translocation—may be related to age-associated alterations in membrane fluidity or phospholipid content and/or in Ca^{2+} homeostasis that are known to be affected by exercise (39–40).

Thus, the data we have presented suggest once again that sustained aerobic fitness may have beneficial effects on changes in basic biological processes that are induced by advancing age. We should underscore, however, that our sample was restricted to men. Hence, the differences we have reported here and in our earlier study (13) may not be generalizable to women. In addition, because our comparisons were only of young and older adults, we cannot draw any inferences about the timing of changes in PKC activity over the lifespan, about critical periods, if any, when these changes may express themselves, or about when in the lifespan the influence of aerobic fitness on PKC activity may be optimal. For example, because the older exercisers in our study had typically exercised most of their adult lives, we cannot conclude that initiation of aerobic activities later in life will produce changes in PKC activity like those we have found. We are left, then, with a variety of interesting possibilities to explore.

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