**β-Adrenergic Receptors in Lymphocyte Subsets After Exercise**

**Alterations in Normal Individuals and Patients With Congestive Heart Failure**

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Dynamic exercise increases the number of β-adrenergic receptors in mixed lymphocytes by a mechanism that is incompletely understood. In a set of in vivo studies, we have investigated the effects of dynamic exercise on the subset distribution of circulating lymphocytes and on the number of β-adrenergic receptors in each of these subsets in two groups of patients. In healthy subjects, exercise increased plasma norepinephrine and epinephrine and caused lymphocytosis. Whereas the number of Thelper cells increased only modestly, the number of Tsuppressor/cytotoxic and natural killer cells more than tripled. The number of β-adrenergic receptors varied among subsets but was not significantly altered by dynamic exercise in any subset except natural killer cells (35% increase, \( p=0.0302 \)). In a group of patients with congestive heart failure, dynamic exercise increased plasma norepinephrine but did not alter plasma epinephrine and did not cause significant lymphocytosis. We did not detect any significant alterations of circulating leukocyte subsets or β-adrenergic receptors in any of these subsets after exercise. A combined analysis of healthy patients and heart failure patients revealed a significant correlation between increases in plasma epinephrine and increases in circulating lymphocytes. We conclude that the exercise-induced increase in β-adrenergic receptors of mixed lymphocytes is predominantly caused by a redistribution of circulating cell subsets that differ in their β-adrenergic receptor number. This appears to be mediated by epinephrine rather than norepinephrine. *(Circulation 1990;82:2003–2010)*

It is well documented that prolonged exposure to agonists decreases the number of β-adrenergic receptors in cultured cells and solid tissues of animals and humans in vitro and in vivo.\(^1\)–\(^3\) Such downregulation also occurs in human lymphocytes after prolonged treatment with β-adrenergic agonists.\(^4\)–\(^7\) Acute in vivo exposure to catecholamines, however, increases the number of β\(_2\)-adrenergic receptors in circulating lymphocytes. This increase can be elicited by exogenous catecholamines\(^8\)–\(^11\) or by acute elevation of the endogenous sympathetic activity by dynamic exercise\(^12\)–\(^20\) or mental arithmetic.\(^21\)

Despite numerous investigations, the mechanism of the increases in lymphocyte β\(_2\)-adrenergic receptors after acute agonist exposure is still incompletely understood. Earlier work has proposed that acute exposure to catecholamines might reveal cryptic β-adrenergic receptors by altering membrane fluidity\(^22\) or shutting β-adrenergic receptors from an intracellular pool to the plasma membrane.\(^9\) Both explanations are quite unlikely because the pool of intracellular β\(_2\)-adrenergic receptors does not appear to exceed 5% of the total lymphocyte β\(_2\)-adrenergic receptors\(^5\)–\(^6\) and the percentage of intracellular ("sequestered") lymphocyte β\(_2\)-adrenergic receptors is similar before and after exercise.\(^9\) Moreover, catecholamine infusions do not alter the number of β-adrenergic receptors in other tissues such as circulating monocytes and platelets.\(^10\) Therefore, more recent models that focused on the heterogeneity of circulating lymphocytes that are composed of several distinct subsets expressing different numbers of β-adrenergic receptors,\(^7\)\(^10\)\(^23\) These models were sup-
ported by the observation that sympathetic stimulation in vivo can alter the subset composition of circulating lymphocytes, possibly by selective release of distinct lymphocyte subsets from the spleen. van Tits et al. have recently demonstrated that infusion of exogenous isoproterenol does not only alter the subset distribution of circulating lymphocytes but also increases the number of \( \beta \)-adrenergic receptors in each subset.

The present study was designed to assess the role of the endogenous sympathoadrenal system in lymphocyte subset redistribution and increases in lymphocyte \( \beta \)-adrenergic receptors. For this purpose, we have used dynamic exercise that involves release of endogenous catecholamines and also of other neurotransmitters such as neuropeptide \( \Upsilon \). Additionally, we have studied the effect of dynamic exercise on circulating lymphocyte subsets and their \( \beta \)-adrenergic receptors in patients with congestive heart failure because previous work has indicated that exercise testing and assessment of the \( \beta \)-adrenergic receptor/adenylate system in lymphocytes might provide additional information regarding adrenergic responsiveness in these patients.

**Methods**

**Subjects**

Nine male healthy and drug-free subjects (age range, 23–44 years; mean±SEM age, 32.2±2.5 years) participated in our study after having given informed written consent. The study protocol had been approved by the Human Subjects Committee of the University of California San Diego.

Eleven male patients from the San Diego Veterans Administration Medical Center (age range, 42–76 years; mean±SEM age, 60.8±3.8 years) with chronic stable congestive heart failure (CHF) (New York Heart Association functional classes II and III) were studied. Each patient had a clinical history of CHF for at least 6 months. Five patients were known to have occlusive coronary artery disease by coronary angiography, or a previously well-documented myocardial infarction, and six were classified as idiopathic. The left ventricular ejection fraction, determined by equilibrium-gated radionuclide ventriculography, averaged 24.7±2.8%. No patients suffered from significant valvular dysfunction or was limited in exercise by angina pectoris. All patients were receiving digoxin and diuretics, and most were on long-term therapy with vasodilator drugs (nitrates, \( n = 4 \); angiotensin converting enzyme inhibitors, \( n = 5 \); flosequian, \( n = 4 \)). No patient had received adrenergic agonists or antagonists for at least 3 weeks. All medications were withdrawn for 12–24 hours before study.

**Protocol**

After having given informed consent, the control subjects reported in a fasting state to the exercise laboratory where an indwelling catheter was placed in a forearm vein. They lay supine in a quiet room for 20 minutes before a venous blood sample was drawn. Then both groups exercised on a treadmill until exhaustion. This was performed with increases of speed and slope of the treadmill every 3 minutes according to the Bruce (control subjects) or with constant speed and an increasing slope of the treadmill every 3 minutes according to the Balke-Ware protocol (CHF patients). On completion of the treadmill test, the subjects immediately reassumed the supine position and another venous sample was obtained. Twelve electrocardiographic leads were recorded during and for 6 minutes after the conclusion of the exercise test. A mercury sphygmomanometer was used at 3-minute intervals during and after exercise to measure arterial blood pressure. Indexes of myocardial oxygen consumption were the product of heart rate and systolic arterial pressure, the amount of metabolic equivalents of oxygen consumed during exercise (METS) obtained, derived from the calculated maximal oxygen consumption.

**Preparation of Lymphocyte Subsets**

Subsets of circulating lymphocytes (T
\(_{\text{helper}}\), T
\(_{\text{suppressor/cytotoxic}}\), and B lymphocytes—natural killer cells) and monocytes were prepared as recently described. Briefly, blood was anticoagulated with sodium citrate (0.38% final) and diluted with phosphate-buffered saline solution, and lymphocytes were separated by Ficoll gradient centrifugation. Lymphocyte subsets were identified by sequential positive selection by using the following specific monoclonal antibodies (Becton Dickinson, Mountain View, Calif.): T
\(_{\text{helper}}\) cells with anti-Leu-3a, T
\(_{\text{suppressor/cytotoxic}}\) cells with anti-Leu-2a, B cells with anti-Leu-12, natural killer cells with anti-Leu-7, anti-Leu-11, and anti-Leu-19, and monocytes with anti-Leu-M3. The sequential subset isolation was performed over 2 days, with selection of T
\(_{\text{helper}}\), T
\(_{\text{suppressor/cytotoxic}}\), and natural killer cells on day 1, and B cells and monocytes on day 2. To gain maximal purity and yield, slightly different isolation protocols were used for each subset as has been detailed. After removal of unconjugated antibody, dynal magnetic beads (4.5 \( \mu \)m diameter; coated with sheep anti-mouse immunoglobulin G [IgG], Robbins Scientific, Mountain View, Calif.) were added. After 1 hour, the incubation tube was placed in a magnetic particle concentrator for 90 seconds, during which all cells bound to beads were sedimented, and the supernatant was removed. A cocktail of three different antibodies was used to identify natural killer cells (anti-Leu-7, anti-Leu-11, and anti-Leu-19). The attachment of antibodies to natural killer cells yielded a very low recovery of conjugated cells. Thus, we conjugated the beads with the monoclonal antibodies before addition of cells. This protocol yields lymphocyte subsets of high purity (>95%) and with a good yield, and the preparation techniques do not affect the estimates of \( \beta \)-adrenergic receptor number or interfere with hormonal stimulation of cyclic AMP (cAMP) accumulation; the apparent \( K_d \) of the lymphocyte \( \beta \)-adrenergic receptor for the radioligand,
however, is slightly increased in the presence of the immunomagnetic beads.7

Flow-Cytometric Analysis of Lymphocytes

We assessed the number of cells in each subset by flow-cytometric analysis. For this purpose, monoclonal antibodies were directly conjugated with either fluorescein (FITC) or phycoerythrin (PE). We used a combination of anti–Leu-3a FITC and anti–Leu-2a PE or anti–Leu-7 FITC and anti–Leu-2a PE with two-color immunofluorescence, or anti–Leu-4 FITC or anti–Leu-11 FITC with single-color immunofluorescence. A negative control consisting of IgG, FITC and IgG, PE was included for each set of cells stained. Immunofluorescence was measured by using a FACScan instrument (Becton Dickinson) equipped with a 15-mW argon ion laser and interfaced with a Hewlett-Packard Model 310 computer. Data analysis was performed by using the CONSORT 30 DATA MANAGEMENT program supplied by the manufacturer. Five thousand cells were analyzed per sample. Electronic “gating” of the lymphocyte population was performed based on forward and sidescatter parameters. Percentage of positive fluorescence of the gated population was determined relative to the fluorescence of the negative control cells. This method yielded the relative proportion of each subset as a percentage of the total lymphocytes counted. The absolute number of cells in each subset was calculated by multiplying the fraction of cells in each subset times the absolute lymphocyte count derived from the white blood cell and differential count (S plus IV Coulter, Hialeah, Fla.).

Radioligand Binding

Conditions for radioligand binding have previously been described in detail.9 Briefly, intact lymphocytes were incubated with varying concentrations of \( ^{125}\text{I} \) (-)-iodopindolol (IPIN) (9–300 pM) at 4°C overnight. Nonspecific IPIN binding was determined by using 1 μM (-)-propranolol, and then subtracted from the total binding to yield specific IPIN binding. The affinity of IPIN for \( \beta \)-adrenergic receptors in lymphocyte subsets was typically between 20 and 40 pM, and did not differ between subsets, control subjects, and CHF patients, or between preexercise and postexercise determinations (data not shown).

Cyclic AMP Accumulation

cAMP accumulation was determined in T\(_{\text{helper}}\), T\(_{\text{suppressor/cytotoxic}}\) and natural killer cells; no AMP assays could be performed for B cells and monocytes because cells isolated on day 2 were unresponsive with regard to cAMP accumulation.7 The final reaction mixture contained Dulbecco’s modified Eagle’s medium supplemented with 20 mM HEPES, 100 μM isobutylmethylxanthine, and 100 μM Ro 20-1724 to inhibit cyclic nucleotide phosphodiesterase, 10 μg/ml each of superoxide dismutase and catalase to prevent oxidation of isoprotenerol,27 and in some tubes, 10 μM isoproterenol or 10 μM prostaglandin E\(_1\). Incubations were begun by adding 0.1 ml of cells (2–10\(\times\)10\(^2\), ice cold) to 0.9 ml of medium at 37°C. The reactions were terminated after 2 minutes by centrifuging at 10,000g, aspirating the supernatant, resuspending the pellet in 100 μM of 50 μM sodium acetate (pH 4.0) containing 0.2 mM isobutylmethylxanthine, and placing the tubes in a boiling water bath for 5 minutes. The tubes were then frozen and aliquots were later assayed for cAMP by using a commercially available radioimmunoassay (Amer sham). All assays were performed in quadruplicate.

Miscellaneous

Plasma catecholamines were measured radioenzymatically by the method of Durrett and Ziegler.28

Data Analysis

Density (Bmax) and affinity (Kd) of \( \beta \)-adrenergic receptors were calculated by fitting the experimental data to a rectangular hyperbolic function by using nonlinear regression analysis (GRAPHPAD, GRAPH-PAD Software, San Diego, Calif.). Values are given as mean±SEM of \( n \) experiments. The significance of differences between data obtained before and after exercise was assessed by two-tailed paired \( t \) tests.

Results

Studies on Healthy Subjects

Control subjects had a maximal exercise time of 12.3±6 minutes on the Bruce protocol, and for this exercising time, the calculated oxygen consumption was 14.6±0.5 METS. During exercise, plasma nor-epinephrine concentration increased fourfold from 304±37 to 1,505±316 pg/ml, and the epinephrine concentrations increased twofold from 43±8 to 132±38 pg/ml.

Under resting conditions, lymphocyte subsets differed with regard to the number of \( \beta \)-adrenergic receptors and cAMP formation in response to hormonal stimulation. Natural killer cells had the highest number of \( \beta \)-adrenergic receptors (1,934±122 sites/cell, \( n =9 \)); monocytes, B, and T\(_{\text{suppressor/cytotoxic}}\)

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\text{FIGURE 1. Bar graph showing } \beta \text{-adrenergic receptor density (sites per cell) in lymphocyte subsets before and after exercise. Values are given as mean±SEM of nine individuals. NK, natural killer cells.}
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cells had somewhat fewer β-adrenergic receptors, and \( \text{T}_{\text{helper}} \) cells had the fewest (449±76 sites/cell, \( n=9 \) [Figure 1]). Isoproterenol-stimulated cAMP accumulation was highest in \( \text{T}_{\text{suppressor/cytotoxic}} \) cells (15.4±3.4 pmol/10^6 cells), slightly lower in natural killer cells, and lowest in \( \text{T}_{\text{helper}} \) cells (1.8±0.4 pmol/10^6 cells [Figure 2, left panel]). Similarly, prostaglandin \( E_1 \)-stimulated cAMP accumulation was highest in \( \text{T}_{\text{suppressor/cytotoxic}} \) and natural killer cells and lowest in \( \text{T}_{\text{helper}} \) cells (Figure 2, right panel).

The previously reported increase in number and responsiveness of β-adrenergic receptors in mixed lymphocytes after exercise could be caused by alterations of the subset composition of circulating lymphocytes, by altered values in one or more subsets, or by both. Therefore, we determined the effect of exercise on subset distribution and on β-adrenergic receptors and hormone-stimulated cAMP accumulation in each subset. We did not detect any significant alteration of the number of β-adrenergic receptors in \( \text{T}_{\text{helper}} \), \( \text{T}_{\text{suppressor/cytotoxic}} \), or B cells but found a significant increase in β-adrenergic receptors in natural killer cells (1,934±122 before vs. 2,617±289 sites/cell after exercise, \( n=9; \ p=0.0302 \) [Figure 1]). Similarly, the isoproterenol-stimulated cAMP accumulation increased significantly only in natural killer cells (12.1±2.7 before vs. 34.1±7.5 pmol/10^6 cells after exercise, \( n=7; \ p=0.0218 \) [Figure 2, left panel]). The prostaglandin \( E_1 \)-stimulated cAMP accumulation was also elevated in natural killer cells after exercise, but this increase did not reach statistical significance levels (22.7±5.9 before vs. 41.6±11.6 pmol/10^6 cells after exercise, \( n=7; \ p=0.2053 \) [Figure 2, right panel]). Basal cAMP accumulation was not significantly altered by dynamic exercise (data not shown).

During exercise, the number of total white blood cells increased from 7,611±486 to 10,156±726 cells/µl and that of lymphocytes from 1,987±165 to 4,016±242 cells/µl. Among the lymphocytes, \( \text{T}_{\text{helper}} \) cells did not increase, B cells (+42%) and monocytes (+57%) increased modestly, and \( \text{T}_{\text{suppressor/cytotoxic}} \) (+159%) and natural killer cells (+272%) increased markedly (Figure 3). Accordingly, the \( \frac{\text{T}_{\text{helper}}}{\text{T}_{\text{suppressor/cytotoxic}}} \)-cell ratio decreased from 1.3±0.2 to 0.6±0.1 (data not shown). We have used the number of circulating cells and of β-adrenergic receptors in each subset to back-calcu-

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**Figure 2.** Bar graphs showing cyclic AMP (cAMP) accumulation (pmol/10^6 cells) in response to 10 µM isoproterenol (left panel) and 10 µM prostaglandin \( E_1 \) (right panel) in \( \text{T}_{\text{helper}} \), \( \text{T}_{\text{suppressor/cytotoxic}} \), and natural killer (NK) cells of healthy subjects before and after exercise. Data are mean±SEM of nine individuals.

**Figure 3.** Bar graph showing subsets of circulating lymphocytes (cell number/mm^3) in healthy subjects before and after exercise. The Leu-4 antibody identifies pan T cells, the Leu-3a antibody identifies the \( \text{T}_{\text{helper}} \) cells, the Leu-2a antibody identified the \( \text{T}_{\text{suppressor/cytotoxic}} \) cells, the Leu-9, Leu-7, and Leu-1 antibodies identify natural killer cells, the Leu-12 antibody identifies B cells, and the M3 antibody identifies monocytes. Values are given as mean±SEM of nine individuals.

**Figure 4.** Bar graph showing β-adrenergic receptor density (sites per cell) in lymphocyte subsets of patients with congestive heart failure before and after exercise. Values are given as mean±SEM of 11 individuals.
late the increase in β-adrenergic receptors in mixed lymphocytes. These computations show that the observed redistribution of circulating subsets alone can explain an apparent increase in β-adrenergic receptors of 19%, and if the elevated receptor number in natural killer cells is considered, the redistribution can explain an increase of 36% in mixed lymphocytes (data not shown). Such increases are well in the range previously observed in mixed lymphocytes by us and other investigators.9,12–20

Studies on Heart Failure Patients

As expected, CHF patients had a short maximal exercise time (7.8±1.2 minutes on the Balke-Ware protocol) and a small calculated oxygen consumption (4.8±0.9 METS). The resting plasma norepinephrine concentration doubled from 529±93 to 953±171 pg/ml during exercise; plasma epinephrine was not significantly elevated during exercise (32±8 before and 38±8 pg/ml after exercise).

The number of β-adrenergic receptors differed among lymphocyte subsets under resting conditions. Similar to the lymphocytes of healthy subjects, we found high numbers of β-adrenergic receptor in Tsuppressor/cytotoxic cells, B cells, natural killer cells, and monocytes, and low numbers of β-adrenergic receptor in Thelper cells (Figure 4). Isoproterenol-stimulated and prostaglandin E1-stimulated cAMP accumulation was higher in natural killer and Tsuppressor/cytotoxic cells than in Thelper cells (Figure 5). Maximal exercise did not significantly alter the number of β-adrenergic receptors or hormone-stimulated cAMP accumulation in any lymphocyte subset including natural killer cells (Figures 4 and 5).

During exercise, only small increases in the white blood cell count (7,566±357 before and 8,612±666 cells/µl after) and the number of circulating lymphocytes (1,671±220 before and 1,968±253 cell/µl after) were achieved. A subanalysis of the cell numbers in lymphocyte subsets did not reveal any significant alterations of Thelper, pan T, or B cells, and only small increases in Tsuppressor/cytotoxic and natural killer cells (Figure 6). Accordingly, the Thelper/Tsuppressor/cytotoxic-cell ratio decreased only slightly from 3.2±0.9 to 2.4±0.9 (data not shown).

Relation Between Plasma Catecholamines and Lymphocyte Subset Alterations

To analyze the relation between sympathetic activation and alterations of circulating lymphocyte subsets in more detail, we analyzed the combined data from the healthy subjects and the CHF patients (Figure 7). This analysis demonstrated that exercise-induced increases in circulating lymphocytes significantly correlated with elevations of plasma epinephrine (r=0.6247, n=16; p=0.0097) but not with those of plasma norepinephrine (r=0.4083, n=16; p=0.1164).

Discussion

Lymphocytes are widely used as a model system to study the regulation of β-adrenergic receptors in humans because they are an easily accessible tissue with a homogeneous population of β2-adrenergic receptors that couple to stimulation of adenylate cyclase.2,29,30 Recent studies, however, have revealed

![Figure 5](http://circ.ahajournals.org/)

**Figure 5.** Bar graph of cyclic AMP (cAMP) accumulation (pmol/10⁶ cells) in response to 10 µM isoproterenol (left panel) and 10 µM prostaglandin E₁ (right panel) in T<sub>helper</sub>, T<sub>suppressor/cytotoxic</sub>, and natural killer (NK) cells of patients with congestive heart failure before and after exercise. Values are given as mean ± SEM of 11 individuals.

![Figure 6](http://circ.ahajournals.org/)

**Figure 6.** Bar graph of subsets of circulating lymphocytes (cell number/mm<sup>3</sup>) in patients with congestive heart failure before and after exercise. The Leu-4 antibody identifies pan T cells, the Leu-3a antibody identifies the Thelper cells, the Leu-2a antibody identifies the T<sub>suppressor/cytotoxic</sub> cells, the Leu-9, Leu-7, and Leu-1 antibodies identify natural killer cells, the Leu-12 antibody identifies B cells, and the M3 antibody identifies monocytes. Values are given as mean ± SEM of 11 individuals.
the following three major limitations of this model system. First, alterations of lymphocyte $\beta_2$-adrenergic receptors only poorly reflect alterations of $\beta_1$-adrenergic receptors in other tissues. Second, lymphocytes are composed of distinct subsets of cells, and redistribution of these subsets might mimic or conceal ongoing receptor regulation. Third, exposure of $\beta$-adrenergic receptors to agonists in solid tissues is followed by desensitization and downregulation, but acute in vivo exposure of lymphocytes to catecholamines increases $\beta_2$-adrenergic receptor number and responsiveness; the mechanism of this phenomenon is still incompletely understood.

It is well documented that the number of lymphocyte $\beta_2$-adrenergic receptors increases after dynamic exercise by a $\beta_2$-adrenergic mechanism. The increase can be mimicked by acute administration of exogenous $\beta$-adrenergic agonists such as isoproterenol and epinephrine but not by the $\beta_2$-selective norepinephrine, and it can be blocked by $\beta$-adrenergic antagonists such as propranolol and the $\beta_2$-selective ICI 118,551 but not by the $\beta_2$-selective bisoprolol.

The previous data, however, do not explain why catecholamines can increase the number of $\beta$-adrenergic receptors in mixed lymphocytes but failed to do so in other tissues. The understanding of this phenomenon is complicated by the fact that different cell populations are sampled from peripheral blood before and after catecholamine exposure. Thus, dynamic exercise markedly increases the number of circulating lymphocytes (mostly due to $T_{\text{suppressor/cytotoxic}}$ and natural killer cell increases) but only marginally elevated the number of $T_{\text{helper}}$ cells. This differential regulation lowered the $T_{\text{helper}}/T_{\text{suppressor/cytotoxic}}$ cell ratio.

In patients with CHF, however, we did not observe any redistribution of circulating lymphocyte subsets after dynamic exercise until exhaustion. Two possibilities might explain this. First, the $\beta$-adrenergic receptor mediating the lymphocyte subset redistribution might be desensitized in CHF patients. We consider this to be unlikely because the redistribution of circulating lymphocyte subsets is mediated by $\beta_2$-adrenergic receptors, and $\beta_2$-adrenergic receptor desensitization (in contrast to that of $\beta_1$-adrenergic receptors) is not a general feature of CHF.

Second, the increase in lymphocyte $\beta$-adrenergic receptors is mediated by a $\beta_2$-adrenergic receptor, which is physiologically activated by epinephrine but not by norepinephrine. Exercise resulted in little or no increase in plasma epinephrine levels in CHF patients in both our present and previous studies. Moreover, we have now detected a significant correlation between increases in plasma epinephrine and increases in the number of circulating lymphocytes. Thus, not releasing the necessary stimulant, epinephrine, is the most likely reason for the failure of dynamic exercise to increase lymphocyte $\beta$-adrenergic receptors in patients with CHF.

The present study confirms previous observations that lymphocyte subsets differ in their number of $\beta$-adrenergic receptors. Specifically, $T_{\text{helper}}$ cells have only few $\beta$-adrenergic receptors, whereas $T_{\text{suppressor/cytotoxic}}$ and natural killer cells have many. The isoproterenol-stimulated cAMP generation differs among lymphocyte subsets in a similar way. Because dynamic exercise reverses the ratio of $T_{\text{helper}}/T_{\text{suppressor/cytotoxic}}$ and increases the percentage of natural killer cells, a redistribution of lymphocyte subsets can at least partly explain the observed increase in the $\beta$-adrenergic receptor number of mixed lymphocytes after exercise.

With the exception of natural killer cells, we did not detect significant changes in $\beta$-adrenergic receptor number in any lymphocyte subset after dynamic exercise. As natural killer cells are only a minor fraction of the total circulating lymphocytes and as the elevation of natural killer cell $\beta$-adrenergic receptors was not greater than 30%, these alterations cannot explain the doubling of total lymphocyte $\beta$-adrenergic receptors after dynamic exercise that we and others have observed previously by using this and similar exercise protocols. It is possible, however, that similar increases have also occurred in other lymphocyte subsets but have artifactually been reversed during the sequential purification process. We consider this possibility to be unlikely because we have previously demonstrated.
that our purification protocol does not alter the number of detectable \(\beta\)-adrenergic receptors.\(^7\)

In conclusion, our data suggest that the increased number of \(\beta\)-adrenergic receptors of mixed lymphocytes after dynamic exercise is predominantly caused by a redistribution of circulating lymphocyte subsets; increases in the number of \(\beta\)-adrenergic receptors in one or more lymphocyte subsets do not appear to play a major role in this phenomenon. This conclusion differs in part from previously reported data on the effects of infusions of exogenous catecholamines.\(^{10}\) Such infusions increased the number of lymphocyte \(\beta\)-adrenergic receptors to an extent not entirely explained by redistribution of lymphocyte subsets. Moreover, it has been shown directly that infusion of isoproterenol substantially increases the number of \(\beta\)-adrenergic receptors in various lymphocyte subsets.\(^{10}\) The following are two major differences that exist between our exercise protocol and the previously described isoproterenol infusions.\(^{10}\) First, endogenous and exogenous catecholamines might differ in their effects due to compartmentalization. Second, isoproterenol infusion is a mixed \(\beta_1/\beta_2\)-adrenergic stimulus, whereas exercise is predominantly associated with release of the \(\beta_1\)-selective norepinephrine and only a modest elevation of plasma epinephrine (present study and References 36 and 37), which acts on both \(\beta_1\) and \(\beta_2\)-adrenergic receptors.

Two further conclusions regarding differences between healthy subjects and patients with CHF can also be derived from the present data. First, under resting conditions and after acute activation of the sympathoadrenal system, the subset distribution of circulating lymphocytes differs between patients with CHF and control subjects. Although no attempt was made to age-match CHF patients and control subjects in this study, we have made similar observations in an age-matched comparison and also in healthy volunteers after a 7-day treatment with the \(\beta\)-adrenergic agonist terbutaline sulfate.\(^{24}\) The pathophysiological relevance of this immunological alteration in patients with CHF or other disease states, or both, of heightened sympathoadrenal activity, remains to be assessed.

Second, under resting conditions, we did not observe alterations in the number or responsiveness of \(\beta\)-adrenergic receptors in any lymphocyte subset in CHF patients compared with healthy subjects. This extends our previous observation that the number and responsiveness of \(\beta\)-adrenergic receptors is similar in mixed lymphocytes from age-matched CHF patients and controls.\(^{16}\) Similarly, Måki et al\(^{16}\) failed to detect significant differences in the number of \(\beta\)-adrenergic receptors of mixed lymphocytes between control subjects and patients with CHF. Two other groups, however, have reported desensitization of lymphocyte \(\beta\)-adrenergic receptors in patients with CHF.\(^{19,38}\) As treatment modalities, severities, and etiologies of CHF of these studies differ, it is difficult at present to assess the reason for these divergent results. Taken together, however, the data support the view, that whether \(\beta\)-adrenergic receptors desensitize under chronic CHF conditions depends specifically on the tissue studied and the form of CHF under investigation.\(^{39}\)

**Acknowledgments**

We thank S. Carter for his skillful technical assistance and Dr. M.G. Ziegler for determination of plasma catecholamines.

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**KEY WORDS** • nervous system, sympathetic • β-adrenergic receptors • lymphocytes • congestive heart failure
Beta-adrenergic receptors in lymphocyte subsets after exercise. Alterations in normal individuals and patients with congestive heart failure.
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Circulation. 1990;82:2003-2010
doi: 10.1161/01.CIR.82.6.2003

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