

Effect of age on human neutrophil function

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Abstract: Neutrophil phagocytosis, reactive oxygen intermediate production (intra- and extracellular), neutrophil bactericidal activity, and chemotaxis/chemokinesis were assessed in three age groups: 21–36, 38–56, and 62–83 years. A significant age-dependent reduction in the number of phagocytized *Escherichia coli* per neutrophil (measured by acridine orange staining) and *Staphylococcus aureus* phagocytosis (measured by flow cytometry) was seen ($r = 0.669$ and $r = 0.684$, $P < 0.001$ for both). These findings correlated with an age-dependent increase in intracellular calcium concentrations in resting neutrophils ($r = 0.698$, $P < 0.001$) and a reduced hexose uptake ($r = 0.591$, $P < 0.01$). In addition, a significant reduction in the intracellular reactive oxygen production was seen after stimulation with *S. aureus* ($P < 0.001$) with increasing age. In contrast, no differences between the groups in reactive oxygen production was seen after stimulation with *E. coli*. The neutrophil bactericidal activity was impaired with increasing age ($64 \pm 4\%$ of the phagocytized bacteria were killed in group 1; 66 ± 2 in group 2, and 59 ± 6 in group 3; $P < 0.01$). In addition, a trend toward a reduced neutrophil chemotaxis was seen with increasing age ($P = 0.022$). The findings suggest that increased intracellular calcium concentrations in resting neutrophils and/or a reduced hexose uptake result in reduced phagocytic ability and decreased bactericidal activity of neutrophils in the elderly. *J. Leukoc. Biol.* 67: 40–45; 2000.

Key Words: phagocytosis · intracellular calcium · hexose uptake · reactive oxygen production

INTRODUCTION

Elderly subjects suffer higher rates of morbidity and mortality from infectious diseases [1]. Because neutrophils are the leukocytes that respond most rapidly to invasion by pathogens, an age-related decline in neutrophil function may be partially responsible for this increased susceptibility to infections. Although the elderly possess a normal number of neutrophils, a decrease of their function has been demonstrated. Using a variety of stimuli, age-related decreases have been demonstrated in superoxide production, phagocytic ability [2–4], cytotoxicity, and enzyme release [5–10]. In addition, reduced neutrophil adhesion and age-dependent reductions of Fc gamma

receptor-mediated neutrophil effector function have been described [11–15]. The cytosolic concentration of free calcium ($[Ca^{2+}]_i$) plays an important role in the control of many neutrophil functions. A wave of elevated cytosolic calcium usually spreads through human neutrophils during phagocytosis [16], mediating the phagosome-lysosome fusion [17] by control of the actin network [18]. In addition, increases in paraphagosomal $[Ca^{2+}]_i$ were closely related to neutrophil superoxide production [19, 20]. A diminished ability of calcium mobilization in stimulated neutrophils has been linked to reduced chemotaxis, superoxide anion production, and lytic enzyme activity [21]. In addition to calcium concentrations the carbohydrate metabolism of neutrophils was shown to affect their function [22, 23].

In this study we analyzed the effect of age on cytosolic free calcium concentration and hexose transport in relation to neutrophil phagocytosis, intra-, and extracellular reactive oxygen production, neutrophil bactericidal ability, and chemokinesis/chemotaxis.

METHODS

Subjects

Thirty-four healthy subjects from three age groups were studied for percent phagocytosis, number of *Escherichia coli* per neutrophil, bactericidal ability, extracellular reactive oxygen production, hexose and calcium metabolism, and chemotaxis/chemokinesis: group 1 (21–36 years; mean \pm SD 27 ± 5 ; $n = 11$, 5 female, 6 male); group 2 (38–56 years; mean \pm SD 45 ± 7 ; 6 female, 6 male; $n = 12$); and group 3 (62–83 years; mean \pm SD 71 ± 7 ; 5 female, 6 male; $n = 11$).

In addition, 47 healthy subjects [group 1 (22–36 years; mean \pm SD 27 ± 4 ; $n = 12$, 6 female, 6 male); group 2 (38–57 years; mean \pm SD 46 ± 6 ; 8 female, 8 male; $n = 16$); and group 3 (64–92 years; mean \pm SD 74 ± 6 ; 9 female, 10 male; $n = 19$)] had phagocytosis and intracellular reactive oxygen production assessed by flow cytometry after stimulus with *E. coli* or *Staphylococcus aureus*. These two pathogens were chosen as stimuli to elucidate potential differences between the neutrophil response to gram-negative and gram-positive bacteria. All subjects were free of diseases affecting the immune system, cancer, or infections and were taking no antiinflammatory drugs, corticosteroids, or calcium antagonists, and were selected according to the SENIEUR protocol [24].

Neutrophil isolation

Neutrophils were isolated from venous blood of healthy subjects according to Nauseef et al. [25] and Metcalf et al. [26]. Ten milliliters of Ficoll-Paque

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(Pharmacia LKB Biotechnology, Uppsala, Sweden) were overlaid by 10 mL of whole blood anticoagulated with 10 µL of heparin sodium (Liquemin Roche iv., Hoffmann-La Roche, Basel, Switzerland). After approximately 45 min, most of the erythrocytes sedimented into the Ficoll layer. Granulocytes, monocytes, lymphocytes, and thrombocytes in the plasma supernatant were separated on a Percoll (Pharmacia) density gradient. After isolation of the neutrophil band, the cells were washed twice with the buffer used in the *in vitro* test and counted under the microscope. The viability of the neutrophil population obtained by this procedure was greater than 95%, as determined by the trypan blue (Sigma, St. Louis, MO) exclusion test.

Phagocytosis and intracellular killing (microscopy assay)

Phagocytosis and intracellular killing of opsonized *E. coli* were performed as described by Moiola [27]. The *E. coli* strain ATCC 25922 was grown overnight in 3 mL of Columbia Broth (Life Technologies, Paisley, UK). After harvesting and washing the cells with Hanks' buffer (with Ca/Mg), 10^8 *E. coli*/mL were opsonized by incubation with 1/10 volume of autologous serum for 30 min at 37°C. Four hundred microliters containing 2×10^6 neutrophils were incubated with 2×10^7 *E. coli* for 30 min at 37°C. Thereafter, 1 mL of ice-cold Hanks' buffer was added, centrifuged for 7 min at 160 *g*, and the supernatant removed. The pellet was stained by addition of 200 µL acridine orange (1.44 mg/100 mL Hanks' buffer) for 1 min. After addition of 1 mL ice-cold Hanks' buffer, 500 µL of this suspension was centrifuged (cytocentrifuge, 5 min, 10,000 rpm) and the supernatant was removed. Phagocytosis and intracellular killing were evaluated under the fluorescence microscope: the DNA of living *E. coli* cells react with acridine orange to fluoresce green, whereas killed cells appear orange. All tests were performed in duplicate. Data are expressed as percentage of bacteria phagocytized and killed by neutrophils.

Oxidative metabolism (cytochrome *c* reductase)

Reactive oxygen intermediate (ROI) production by neutrophils was determined by measuring superoxide dismutase-inhibitable reduction of cytochrome *c* according to Nauseef et al. [25]. Neutrophils were resuspended in Hanks' buffer at a concentration of 3×10^6 cells/mL. One 250-µL cell suspension per tube was preincubated for 5 min at 37°C. Thereafter 10 µL of buffer or phorbol myristate in a final concentration of 1.3×10^{-8} M was added at 37°C for 15 min. Fifty microliters of cytochrome *c* (80 µmol) and 150 µL of Hanks' buffer were added and further incubated for 30 min at 37°C in a shaking water bath. The tubes were centrifuged at 300 *g* for 15 min at 4°C. The supernatant was decanted and read at 550 nm. Data are expressed as nmol/L O_2^- produced by the 2×10^5 cells. The calculation was made using the molar extinction coefficient of 29.9×10^3 mol/L.

Intracellular Ca^{2+} concentrations and hexose transport

The basal levels of intracellular $[Ca^{2+}]_i$ in neutrophils were measured with Fura-2/AM, using a Perkin-Elmer fluorometer, model LS 5B (Perkin-Elmer, Norwalk, CT). The details of this method have been reported by Alexiewicz et al. [28]. The dissociation constant for Ca^{2+} -Fura-2 was assumed to be 225 mM, and the calculation of $[Ca^{2+}]_i$ was made using the Grykiewicz equation [29]. The hexose transport was determined before and after stimulation of the neutrophils as described [22]. In addition, the percentage increase was calculated. All tests were performed in duplicate. Data are expressed as nanomoles per liter.

Chemotaxis and chemokinesis assay

Neutrophils were resuspended in Hanks' buffer at a concentration of 1×10^6 cells/10 µL. *N*-formyl-methionyl-leucyl-phenylalanine (fMLP; Sigma) was used as a chemoattractant. The assay was performed using the under-agarose method [29]. The agarose plates were incubated for 100 min at 37°C. Then the cells were fixed with methanol and paraformaldehyde and stained with Giemsa. The distance the cells migrated under the agarose was measured under the microscope. Chemotaxis, chemokinesis, and effective chemotaxis (chemotaxis-chemokinesis) were assessed.

Phagocytosis and oxidative metabolism (FACS-analysis)

Phagocytosis and ROI production by neutrophils was determined by flow cytometry according to Wenisch et al. [30]. Briefly, heat-killed *Staphylococcus aureus* ATCC 25923 or *E. coli* ATCC 25922 (10^8 /mL) were labeled with fluorescein isothiocyanate (FITC) as previously described [30]. DHR 123 was purchased from Molecular Probes Inc. (Eugene, OR) and dissolved in *N,N*-dimethyl formamide (Sigma Chemicals, Munich, Germany) at a concentration of 3 µg/mL. Phagocytic capacity was assessed by adding 10 µL of pre-cooled FITC-labeled *S. aureus* or *E. coli* to 100 µL of heparinized whole blood incubated for 10 min at 37°C. Thereafter the samples were washed twice in phosphate-buffered saline (PBS, pH 7.4). Finally, 2 mL FACS-lysing solution (Becton Dickinson) were added. After 20 min the samples were washed again and resuspended in 100 µL of PBS containing propidium iodide (PI) at a concentration of 50 µg/mL for DNA staining and kept on ice until analysis. For analysis of ROI production blood samples were stimulated with 25 µL of *E. coli* or *S. aureus* (10^8 /mL; ATCC 25922 or ATCC 25923, respectively, not labeled) at 37°C. After 10 min 25 µL of the DHR solution was added. After another 10 min at 37°C 2 mL of FACS-lysing solution was added and incubated for 20 min at room temperature. Thereafter the samples were washed with PBS and resuspended with 100 µL of PBS containing PI at a final concentration of 50 µg/mL for DNA staining. The cells were analyzed on a standard FACScan flow cytometer (Becton Dickinson). For each measurement, 10,000 events were collected. To exclude cell debris and non-phagocytized bacteria, a live gate was set on PI-stained leukocytes during acquisition in FL2. For analysis of the ROI production, the shift to the right in FL1 (green) was determined. The amount of cleaved substrate was estimated by the mean fluorescence using the statistical option of the FACScan software. Similarly, the amount of phagocytized bacteria was assessed by a shift in mean fluorescence to the right (FL1). The mean fluorescence of both assays was compared with unstimulated controls. Daily alignment and calibration of the instrument was done using fluorescence beads (Calibrite, Becton Dickinson). The beads were put into the same histogram channel every day.

Statistical analysis

Differences between groups were calculated using one-way analysis of variance (ANOVA). Pearson's correlation was used for correlations. All the analyses were two-sided and differences with a *P* value less than 0.01 were considered significant.

RESULTS

Neutrophil phagocytosis and bactericidal function

The effect of age on neutrophil phagocytosis and bactericidal ability (fluorescence microscopy assay and flow cytometry assay) is depicted in **Table 1**.

In the fluorescence microscopy assay ($n = 34$), the percent of phagocytizing cells and the number of *E. coli* per neutrophil were significantly reduced ($P < 0.001$ and $P = 0.002$, respectively) with increasing age. This reduction correlated with the age of the participants ($r = 0.669$ and 0.762 , respectively, **Fig. 1**, $P < 0.01$ for both).

In addition, an age-dependent reduction in neutrophil bactericidal ability was seen ($n = 34$, $P = 0.006$, Table 1).

By flow cytometry ($n = 47$), a significant reduction was seen in the phagocytosis of *S. aureus* ($P < 0.001$, **Table 2**) as well as a trend following stimulation with *E. coli* ($P = 0.068$, Table 2). In addition, the reduction in neutrophil phagocytosis of *S. aureus* correlated with the age of the participants ($r = 0.684$, **Fig. 2**, $P < 0.01$).

TABLE 1. Age and Indices of Neutrophil Function

Age group	Phagocytosis (percent phagocytizing cells)	Phagocytosis (<i>E. coli</i> per neutrophil)	Bactericidal activity (percent killed bacteria)	Burst (basal, nmol/L)	Burst (stimulated, nmol/L)	Burst (percent increase)
1 (21–36, <i>n</i> = 11)	91 ± 2.5	7.1 ± 1.7	64 ± 4	0.37 ± 0.2	14.9 ± 0.8	97 ± 1
2 (38–56, <i>n</i> = 12)	89 ± 2.6	6.4 ± 0.9	66 ± 2	0.34 ± 0.2	15.6 ± 1.5	97 ± 1.6
3 (62–83, <i>n</i> = 11)	74 ± 1.2	4.9 ± 1.1	59 ± 6	0.51 ± 0.2	15.0 ± 0.9	96 ± 1.3
<i>P</i> (ANOVA)	<0.001	0.002	0.006	0.131	0.392	0.134

Neutrophil function indices are: neutrophil phagocytosis of *E. coli* and bactericidal activity (percent killed *E. coli*) analyzed by fluorescence microscopy (mean ± SD); and extracellular respiratory burst (cytochrome *c* reduction assay, mean ± SD).

Reactive oxygen production

A significant reduction in neutrophil intracellular respiratory burst was seen after stimulation with *S. aureus* with increasing age (flow cytometry, *n* = 47, *P* < 0.001, Table 2). In contrast, no differences between the age groups were observed in the extracellular (basal, stimulated, and percentage increase, cytochrome reduction assay, *n* = 34, Table 1) or intracellular reactive oxygen intermediate production assessed by flow cytometry after stimulation with *E. coli* (*n* = 47, Table 2). In addition, no relation between extracellular reactive oxygen production and neutrophil bactericidal ability was seen (*P* > 0.05 for all comparisons).

Intracellular calcium concentrations and hexose transport

The results of intracellular calcium concentrations and hexose transport in the three age groups are shown in Table 3. A significant age-dependent increase in intracellular calcium concentrations was seen in resting neutrophils (*n* = 34, *P* < 0.001 by ANOVA, Table 3; and *r* = 0.698, by Pearson correlation, Fig. 3, *P* < 0.01). After stimulation with fMLP, the influx of intracellular calcium (percentage increase) was reduced by increasing age (*P* = 0.003) and a trend toward a diminished intracellular calcium concentration after stimulation was seen with increasing age (*n* = 34, *P* = 0.057 by ANOVA, Table 3). Basal intracellular calcium concentrations were inversely related to the number of phagocytized *E. coli* per neutrophil (*r* = 0.557, *n* = 34, *P* < 0.01 by Pearson's

correlation) and percent phagocytosis (*r* = 0.622, *n* = 34, *P* < 0.01 by Pearson's correlation).

Basal, stimulated (fMLP), and percentage increase of intracellular hexose was reduced in an age-dependent manner (*n* = 34, *P* < 0.001, *P* < 0.001, and *P* = 0.006, respectively, by ANOVA, Table 3). In addition, the basal and stimulated hexose uptake correlated with age (*r* = 0.503 and 0.592 by Pearson's correlation, respectively, *P* < 0.01 for both). In addition, significant correlations between basal and stimulated hexose transport and percent phagocytosis and number of phagocytized *E. coli* was observed (*n* = 34, *P* < 0.01 for all comparisons, respectively). In addition, the trend to a relation between the hexose transport and neutrophil bactericidal ability (*r* = 0.469, *P* = 0.054) was seen.

Chemokinesis/chemotaxis

The results of the chemokinesis/chemotaxis assays are shown in Table 4. No differences between the age groups were seen in neutrophil chemokinesis. However, a trend toward a reduction in neutrophil chemotaxis and effective chemotaxis was seen with increasing age (*n* = 34, *P* = 0.022 and *P* = 0.023, respectively, by ANOVA, Table 4).

DISCUSSION

Age-related decreases in cellular function have been attributed to reductions in transmembrane signaling efficiency in a variety of cell types, including lymphocytes [31–35], parotid cells [36–38], neurons [39], and pituitary cells [40]. Previously, in neutrophils from aged individuals, reduced chemotactic peptide-induced transmembrane signaling was shown to lead to a reduction in chemotaxis [41], superoxide anion production [42], and lytic enzyme activity [42]. These results have been

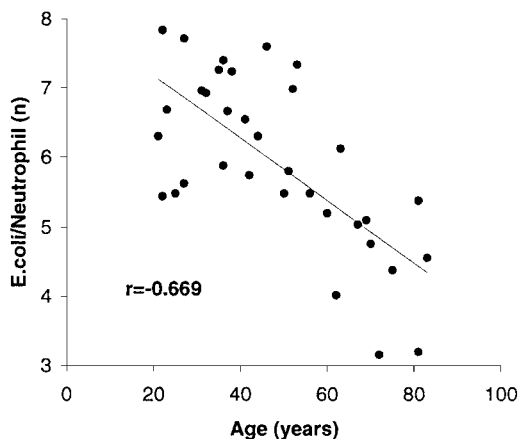


Fig. 1. The correlation between age and number of phagocytized *E. coli* per neutrophil analyzed by fluorescence microscopy (*P* < 0.01, Pearson's correlation) is shown.

TABLE 2. Age and Indices of Neutrophil Function

FACS age group	Phagocytosis		Respiratory burst	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
1 (22–36, <i>n</i> = 12)	3071 ± 613	240 ± 63	12.2 ± 1.3	43.4 ± 17.8
2 (38–57, <i>n</i> = 16)	2341 ± 705	226 ± 84	14.3 ± 1.2	45.9 ± 13.1
3 (64–92, <i>n</i> = 19)	1845 ± 421	177 ± 84	11.9 ± 0.7	48.9 ± 14.8
<i>P</i> (ANOVA)	<0.001	0.068	<0.001	0.652

Indices of neutrophil function are: phagocytosis of *S. aureus* and *E. coli* and intracellular reactive oxygen production after stimulation with *S. aureus* or *E. coli* analyzed by flow cytometry (the mean fluorescence channel ± SD are shown).

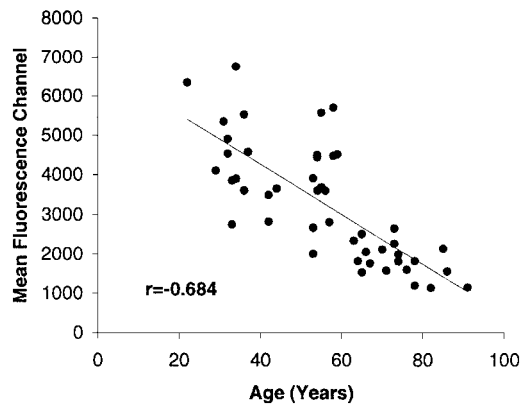


Fig. 2. The correlation between age and neutrophil phagocytosis of FITC-labeled *S. aureus* analyzed by flow cytometry ($P < 0.01$, Pearson's correlation) is shown.

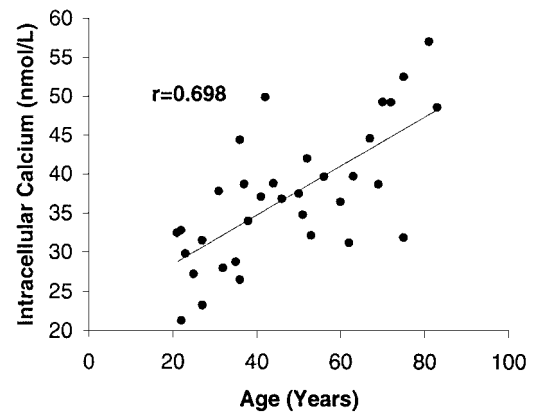


Fig. 3. The correlation between age and an increase of intracellular calcium levels in resting neutrophils ($P < 0.01$, Pearson's correlation) is shown.

linked to a diminished ability of calcium mobilization in fMLP-stimulated neutrophils from aged persons [21].

We found an age-dependent decrease in neutrophil phagocytic ability, which was related to both increased basal levels of intracellular calcium and an age-dependent reduced hexose transport. In addition, we found a trend toward reduced chemotaxis with increasing age, which corresponds to previous findings [41]. A significant reduction in the intracellular reactive oxygen production was seen after stimulation with *S. aureus*. In contrast, we did not see adverse effects of aging on the intra- or extracellular production of reactive oxygen species after stimulation with *E. coli*.

The latter corresponds to the findings of Lipschitz et al. [43], Rossi et al. [44], and Peveri et al. [45], who demonstrated that aging had no effect on the reduction of the second messenger phosphatic acid, which has been shown to serve as the major activator of the NADPH oxidase. However, after stimulation by fMLP, neutrophils from elderly individuals generated significantly less diacylglycerol and inositol triphosphate than neutrophils from young donors [43]. The missing age-related decrement in superoxide generation could thus be related to the compensation by adequate amounts of phosphatic acid production, which is not significantly compromised with age [43]. The difference in the response to the different organisms (*E. coli* and *S. aureus*) with respect to reactive oxygen production might relate to differences in bacterial cell wall components and their interaction with neutrophil surface receptors. In contrast to *S. aureus*, the internalization of *E. coli* by a CD14-dependent, calcium-independent mechanism [46], which is not affected by aging, could be an explanation for the age-independent reactive oxygen production in neutrophils after phagocytosis of *E. coli*.

In addition, other studies suggest that the response of the aged host to gram-negative bacteria might be increased and associated with a poor outcome. This could be related to the priming effect of bacterial lipopolysaccharide involving heterogeneity in calcium-mediated signal transduction [47].

A negative effect of elevated intracellular calcium on neutrophil phagocytic ability has previously been demonstrated in patients with diabetes mellitus and/or patients receiving long-term hemodialysis [48, 49]. Herein, neutrophils evince a smaller increase in calcium levels after cellular stimulation [48]. In our study, the individuals of all age groups had no evidence for renal impairment and/or diabetes mellitus. However, the effect of increased basal concentrations of intracellular calcium on neutrophil phagocytosis in the elderly corresponds to the findings in patients with diabetes and/or hemodialysis. From a clinical point of view, it could be speculated that the reduced *S. aureus*-stimulated intracellular reactive oxygen production in patients with elevated intracellular calcium concentrations leads to the particular susceptibility to infections with *S. aureus* in these patients.

How might increased intracellular calcium levels in neutrophils impair their function? Normally, neutrophil activation is dependent on a rapid, phospholipase C-mediated increase in intracellular calcium levels from less than 50 nmol/L to micromolar concentrations [50]. Thus an intriguing hypothesis is that increased calcium levels in resting neutrophils is also associated with a lesser increase of intracellular calcium levels after cellular stimulation. In addition, it has been shown that fMLP-triggered calcium extrusion is decreased with aging [51].

Indeed, in another study nifedipine partially corrected intracellular calcium levels in neutrophils from patients with

TABLE 3. Age and Indices of Neutrophil Function

Age group	Calcium (basal, nmol/L)	Calcium (stimulated, nmol/L)	Calcium (percent basal/stimulated)	Hexose (basal, nmol/L)	Hexose (stimulated, nmol/L)	Hexose (percent basal/stimulated)
1.: 21–36 a, n = 11	29 ± 4.4	452 ± 156	93 ± 2.0	12,038 ± 3104	35,260 ± 9796	65 ± 7
2.: 38–56 a, n = 12	39 ± 4.8	522 ± 111	92 ± 2.5	14,539 ± 3857	31,468 ± 8510	52 ± 11
3.: 62–83 a, n = 11	44 ± 8.6	425 ± 60	89 ± 2.6	6917 ± 1355	20,363 ± 3299	65 ± 7
p-value (ANOVA)	<0.001	0.057	0.003	<0.001	<0.001	0.006

Indices of neutrophil function are: intracellular calcium and hexose transport (mean ± SD).

TABLE 4. Age and Indices of Neutrophil Function

Age group	Chemokinesis	Chemotaxis	Effective chemotaxis
1 (21–36, <i>n</i> = 11)	0.64 ± 0.2	5.06 ± 0.76	4.42 ± 0.65
2 (38–56, <i>n</i> = 12)	0.49 ± 0.1	4.39 ± 0.65	3.9 ± 0.62
3 (62–83, <i>n</i> = 11)	0.56 ± 0.14	4.08 ± 0.76	3.52 ± 0.74
<i>P</i> (ANOVA)	0.087	0.022	0.023

Indices of neutrophil function are: chemokinesis, chemotaxis, and effective chemokinesis (μm , mean \pm SD).

and without diabetes who were receiving hemodialysis and substantially improved the cellular phagocytic ability [52]. However, because increased calcium levels in patients who receive hemodialysis are largely attributable to abnormalities in parathyroid hormone metabolism, extrapolation of these findings to neutrophils from elderly patients may not be appropriate. Furthermore, caution is in order because verapamil not only inhibits neutrophil calcium influx but also inhibits activation of neutrophil oxidative metabolism [53].

In addition to altered intracellular calcium levels, decreased hexose transport has been seen with aging in both resting and stimulated neutrophils in this study. Again, a decreased hexose uptake has been described in patients receiving long-term hemodialysis [22]. In this study [22], the elimination of dialyzable factors improved, but did not restore, neutrophil hexose uptake. Because neutrophil phagocytosis is an energy-dependent process, a decreased hexose uptake in the elderly could in part explain the reduced phagocytic ability. This hypothesis is supported by the demonstration of a reduced activity of the hexose monophosphate shunt pathway in patients with end-stage renal disease leading to decreased neutrophil phagocytosis [54].

In conclusion, we found an age-related decreased hexose uptake and intracellular calcium levels in resting neutrophils, which were related to an impairment of neutrophil phagocytosis and bactericidal ability. These findings were similar to previous findings in patients with diabetes mellitus and/or patients receiving hemodialysis [48, 54]. In addition, a diminished *S. aureus*-stimulated intracellular reactive oxygen was found that could explain the particular susceptibility to this pathogen in these patients. It may be tantalizing to speculate that pharmacological modulation of intracellular calcium levels and/or hexose uptake may improve neutrophil function and reduce the risk for infection in elderly patients.

REFERENCES

- National Center for Health Statistics (U.S.) (1990) *Vital Statistics of the University States*, Washington, DC: US Government Printing Office, 81–93.
- Bruce, I. N., McNally, J. A., Rea, I. M., Bell, A. L. (1977) Age-related changes in non-receptor dependent generation of reactive species from phagocytes of healthy adults. *Mech. Ageing Dev.* 94, 135–144.
- Nagel, J. E., Pyle, R. S., Chrest, F. J., Adler, W. H. (1982) Oxidative metabolism and bactericidal capacity of polymorphonuclear leukocytes from normal young and aged adults. *J. Gerontol.* 37, 29–34.
- Mege, J. L., Capo, C., Michel, B., Gastaut, J. L., Bongrand, P. (1988) Phagocytic cell function in aged subjects. *Neurobiol. Aging* 9, 217–220.
- Suzuki, K., Swenson, C., Sasagawa, S., Sakatani, T., Watanabe, M., Kobayashi, M., Fujikura, T. (1983) Age-related decline in lysosomal enzyme release from polymorphonuclear leukocytes after *N*-formyl-

- methionyl-leucyl-phenylalanine stimulation. *Exp. Hematol.* 11, 1005–1013.
- Antonaci, S., Jirillo, E., Ventura, M. T., Garofalo, A. R., Bonomo, L. (1984) Non-specific immunity in aging: deficiency of monocyte and polymorphonuclear cell-mediated functions. *Mech. Ageing Dev.* 24, 367–375.
- Fulop, T., Jr., Komaromi, I., Foris, G., Worum, I., Leovey, A. (1986) Age-dependent variations of intralysosomal release from human PMN leukocytes under various stimuli. *Immunobiol.* 171, 302–310.
- Winocour, P. H., Lenton, J., Puxty, J. A., Anderson, D. C. (1988) Leukocyte microbicidal activity assessed by chemiluminescence in elderly non-insulin dependent diabetes mellitus. *Diabetes Res.* 9, 73–75.
- Emanuelli, G., Lanzio, M., Anfossi, T., Romano, S., Anfossi, G., Calcamuggi, G. (1986) Influence of age on polymorphonuclear leukocytes in vitro: phagocytic activity in healthy human subjects. *Gerontol.* 32, 308–316.
- Nagel, J. E., Han, K., Coon, P. J., Adler, W. H., Bender, B. S. (1986) Age differences in phagocytosis by polymorphonuclear leukocytes measured by flow cytometry. *J. Leukoc. Biol.* 39, 399–407.
- Fulop, T., Jr., Foris, G., Worum, I., Leovey, A. (1985) Age-dependent alterations of Fc gamma receptor-mediated effector functions on human polymorphonuclear leukocytes. *Clin. Exp. Immunol.* 61, 425–432.
- Fulop, T., Jr., Foris, G., Worum, I., Paragh, G., Leovey, A. (1985) Age related variations of some polymorphonuclear leukocyte functions. *Mech. Ageing Dev.* 29, 1–8.
- Fulop, T., Foris, G., Worum, I., Leovey, A. (1985) Age-dependent changes of the Fc gamma-receptor-mediated functions of human monocytes. *Int. Arch. Allergy Appl. Immunol.* 74, 76–79.
- Tortorella, C., Ottolenghi, A., Pugliese, P., Jirillo, E., Antonaci, S. (1993) Relationship between respiratory burst and adhesiveness capacity in elderly polymorphonuclear cells. *Mech. Ageing Dev.* 69, 53–63.
- Perskin, M. H., Cronstein, B. N. (1992) Age-related changes in neutrophil structure and function. *Mech. Ageing Dev.* 64, 303–313.
- Schwab, J. C., Leong, D. A., Mandell, G. L. (1992) A wave of elevated intracellular free calcium spreads through human neutrophils during phagocytosis of zymosan. *J. Leukoc. Biol.* 51, 437–443.
- Jaconi, M. E., Lew, D. P., Carpentier, J. L., Magnusson, K. E., Sjogren, M., Stendahl, O. (1990) Cytosolic free calcium elevation mediates the phagosome-lysosome fusion during phagocytosis in human neutrophils. *J. Cell Biol.* 110, 1555–1564.
- Bengtsson, T., Jaconi, M. E., Gustafson, M., Magnusson, K. E., Theler, J. M., Lew, D. P., Stendahl, O. (1993) Actin dynamics in human neutrophils during adhesion and phagocytosis is controlled by changes in intracellular free calcium. *Eur. J. Cell Biol.* 62, 49–58.
- Murata, T., Sullivan, J. A., Sawyer, D. M., Mandell, G. L. (1987) Influence of type and opsonization of ingested particle on intracellular free calcium distribution and superoxide production by human neutrophils. *Infect. Immun.* 55, 1784–1791.
- Thiel, M., Bardenheuer, H. (1992) Regulation of oxygen radical production of human polymorphonuclear leukocytes by adenosine: the role of calcium. *Pfluegers Arch.* 420, 522–528.
- Lipschitz, D. A., Udupa, K. B., Boxer, L. A. (1988) The role of calcium in the age-related decline of neutrophil function. *Blood* 71, 659.
- Haag-Weber, M., Hable, M., Fiedler, G., Blum, I., Schollmeyer, P., Kreusser, W., Horl, W. H. (1991) Alterations of polymorphonuclear leukocytes glycogen metabolism and glucose uptake in dialysis patients. *Am. J. Kidney Dis.* 17, 562–568.
- Haag-Weber, M., Schollmeyer, P., Horl, W. H. (1991) Neutrophil carbohydrate metabolism in patients with essential hypertension and uremia. *Adv. Exp. Med. Biol.* 297, 151–160.
- Lighthart, G. J., Corberand, J. X., Fournier, C., Galanaud, P., Hijmans, B., Kennes, H. K. (1988) Müller-SENIEUR protocol. *Mech. Ageing Dev.* 28, 47–55.
- Nauseef, W. M., Metcalf, J. A., Root, P. K. (1983) Role of myeloperoxidase in the respiratory burst of human neutrophils. *Blood* 61, 483–492.
- Metcalf, J. A., Gallin, J. I., Nauseef, W. M., Root, R. K. (1986) *Laboratory Manual of Neutrophil Function* New York: Raven Press, 2–5.
- Moiola, F. (1992) Phagocytosis, F-actin polymerization and cell volume of bovine neonatal neutrophils. A comparative study with adult cattle. University of Bern, dissertation.
- Alexiewicz, J. M., Smogorzewski, M., Fadda, G. Z., Massry, S. G. (1991) Impaired phagocytosis in patients. Studies on mechanisms. *Am. J. Nephrol.* 11, 102–111.
- Grykiewicz, G., Poenic, M., Tsien, R. Y. (1985) A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260, 3440–3450.
- Wenisch, C., Graninger, W. (1995) Are soluble factors relevant for polymorphonuclear leukocyte dysregulation in septicemia? *Clin. Diagnost. Labor Immunol.* 211, 241–245.

31. Proust, J. J., Filbrun, C. R., Harrison, S. A., Buchholz, M. A., Nordin, A. A. (1987) Age-related defect in signal transduction during lectin activation of murine T lymphocytes. *J. Immunol.* 139, 1472–1475.
32. Kennes, B., Hubert, C., Brohee, D., Neve, P. (1981) Early biochemical events associated with lymphocyte activation in ageing I. Evidence that Ca²⁺-dependent processes induced by PHA are impaired. *Immunol.* 42, 119–121.
33. Miller, R. A., Jacobson, B., Weil, G., Simons, E. R. (1987) Diminished calcium influx in lectin-stimulated T cells from old mice. *J. Cell Physiol.* 132, 337–341.
34. Murasko, D. M., Weiner, P., Kaye, D. (1987) Decline in mitogen induced proliferation of lymphocytes with increasing age. *Clin. Exp. Immunol.* 70, 440–445.
35. Segal, J. (1986) Studies on the age-related decline in the response of lymphoid cells to mitogens: Measurements of concanavalin A binding and stimulation of calcium and sugar uptakes in thymocytes from rats of varying ages. *Mech. Ageing Dev.* 33, 295–298.
36. Ishikawa, Y., Gee, M. V., Ambudkar, I. S., Bodner, L., Baum, B. J., Roth, G. S. (1988) Age-related impairment in rat-parotid cell, α -adrenergic action at the level of inositol trisphosphate responsiveness. *Biochim. Biophys. Acta* 968, 203–208.
37. Ito, H., Baum, B. J., Uchida, T., Hoopes, M. T., Bodner, L., Roth, G. S. (1982) Modulation of rat parotid α -adrenergic responsiveness at a step subsequent to receptor activation. *J. Biol. Chem.* 246, 9532–9537.
38. Gee, M. V., Ishikawa, Y. I., Baum, B. J., Roth, G. S. (1986) Impaired adrenergic stimulation of rat parotid cell glucose oxidation during aging: The role of calcium. *J. Gerontol.* 41, 331–336.
39. Roth, G. S., Hess, G. D. (1982) Changes in the mechanisms of hormone and neurotransmitter action during aging: Current status of the role of receptor and post-receptor alterations. A review. *Mech. Ageing Dev.* 20, 175–179.
40. Chuknyiska, R. S., Blackman, M. R., Roth, G. S. (1987) Ionophore A23187 partially reverses LH secretory defect of pituitary cells from old rats. *Am. J. Physiol.* 253, 233–239.
41. Niwa, Y., Kasama, T., Miachi, Y., Kanoh, T. (1989) Neutrophil chemotaxis, phagocytosis, and parameters of reactive oxygen species in human aging: Cross-sectional and longitudinal studies. *Life Sci.* 44, 1655–1661.
42. Suzuki, K., Swenson, C., Sasagawa, S., Sakatani, T., Watanabe, M., Kobayashi, M., Fujikura, T. (1983) Age-related decline in lysosomal enzyme release from polymorphonuclear leukocytes after *N*-formyl-methionyl-leucyl-phenylalanine stimulation. *Exp. Hematol.* 11, 1005–1009.
43. Lipschitz, D. A., Udupa, K. B., Indelicato, S. R., Das, M. (1991) Effect of age on second messenger generation in neutrophils. *Blood* 78, 1347–1354.
44. Rossi, F., Grzeskowiak, M., Della Bianca, V., Calzetti, F., Gandini, G. (1990) Phosphatidic acid and not diacylglycerol generated by phospholipase D is functionally linked to the activation of the NADPH oxidase by fMLP in human neutrophils. *Biochem. Biophys. Res. Commun.* 168, 320–327.
45. Peveri, P., Curnette, J. T. (1990) Phosphatidic acid may be a physiologic activator of human neutrophil NAPH oxidase. *Blood* 76, 190–196.
46. Grunwald, U., Fan, X., Jack, R. S., Workalemahu, G., Kallies, A., Stelter, F., Schutt, C. (1996) Monocytes can phagocytose gram-negative bacteria by a CD-14-dependent mechanism. *J. Immunol.* 157, 4119–4125.
47. Yee, J., Christou, N. V. (1993) Neutrophil priming by lipopolysaccharide involves heterogeneity in calcium-mediated signal transduction. *J. Immunol.* 150, 1988–1997.
48. Alexiewicz, J. M., Smogorzewski, M., Fadda, G. Z., Massry, S. G. (1991) Impaired phagocytosis in dialysis patients: studies on mechanisms. *Am. J. Nephrol.* 11, 102–111.
49. Alexiewicz, J. M., Kumar, D., Smogorzewski, M., Klin, M., Massry, S. G. (1995) Polymorphonuclear leucocytes in non-insulin-dependent diabetes mellitus: abnormalities in metabolism and function. *Ann. Intern. Med.* 123, 919–924.
50. Densen, P., Clark, R. A., Nauseef, W. M. (1995) Granulocytic phagocytes. In *Principles and Practices of Infectious Diseases, 3rd ed.* (G. L. Mandell, J. E. Bennett, R. Dolin, eds.) New York: Churchill Livingstone, 78–101.
51. Fulop, T., Jr., Hauck, M., Worum, I., Foris, G., Leovey, A. (1987) Alterations of the fMLP-induced Ca²⁺ efflux from human monocytes with aging. *Immunol. Lett.* 14, 283–286.
52. Weiss, J., Kao, I., Victor, M., Eslbach, P. (1985) Oxygen-independent intracellular and oxygen-dependent extracellular killing of *Escherichia coli* S15 by human polymorphonuclear leukocytes. *J. Clin. Invest.* 76, 206–212.
53. The Diabetes and Complication Trial Research Group (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications of insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 329, 977–986.
54. Vanholder, R., De Smet, R., Jacobs, V., Van Landschoot, N., Waterloos, M. A., Vogeleere, P., Ringoir, S. (1994) Uraemic toxic retention solutes depress polymorphonuclear response to phagocytosis. *Nephrol. Dial. Transplant.* 9, 1271–1278.