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Natural Killer Activity and Thyroid Hormone Levels in Young and Elderly Persons

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Key Words
Aging, human · Natural killer activity · Thyroid hormones

Abstract
Background: On the basis that (1) multiple interactions exist between the hormonal and immune systems, and (2) aging is accompanied by changes in thyroid hormone metabolism and responsiveness, we postulate that thyroid hormones may be involved in the observed decrease in natural killer (NK) activity in a population of apparently healthy elderly subjects. The purpose of the study is to compare NK cytotoxic activity and serum concentrations of TSH and thyroid hormones in healthy old and young people, and to assess in vitro the effects of triiodothyronine (T_3) on NK activity. ***Materials and Methods:*** Sixteen of the 47 healthy old people (mean age 64 ± 5.2) were classified as optimally healthy, and the remainder as 'almost healthy' (according to the criteria of the Senieur protocol) [Ligthart et al., Mech Ageing Dev 1984;28:47–55]; the mean age of the healthy young people was 23.3 ± 2.3 years. NK cytotoxic activity of peripheral blood mononuclear cells was assessed using ^{51}Cr release from K562 target cells. The cutoff level for defining low and high NK responses was set at a value of 20%. Serum concentrations of TSH, total thyroxine (T_4) and total triiodothyronine (T_3) were measured by radioimmunoassay. ***Results:*** NK activity in the 'optimally healthy' elderly was high (mean 41 ± 12%, SE), whereas 'almost healthy' subjects showed low NK activity (mean 6 ± 5%). Serum T_4 and TSH levels, but not T_3 concentrations were similar in both the young and old. We observed a significant correlation ($r = 0.53$, $n = 21$, $p < 0.05$) between the serum total T_3 level and the NK activity in the elderly individuals. Under in vitro conditions exogenous T_3 significantly increased NK activity in the elderly subjects who had serum T_3 values at the lower end of the reference range. However, no effect of T_3 on NK activity was observed in peripheral blood mononuclear cells obtained from either old or young individuals who had serum T_3 levels at the midpoint of the range. ***Conclusion:*** Decreased serum concentrations of total T_3 may contribute to low NK activity in the 'almost healthy' subgroup of the elderly.

The immunological status of the elderly is characterized by the impairment of cell-mediated immunity that is reflected in decreased proliferative responses to T cell [1] and B cell [1, 2] mitogens. However, natural killer (NK) activity, an important component of antiviral and antitumor immunity, has been found to be unchanged [3], increased [4] or dependent on the health status [5–7] during physiological aging. In an attempt to resolve these dis-

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crepancies, by implementing a detailed protocol to assess the health status of elderly subjects [Senieur protocol (SP); 8] we were able to show that the NK activity of healthy old female subjects was similar to that of younger people [5], and that elderly high NK responders were characterized by an optimal health status and a strong proliferative response to mitogens [9]. Nevertheless, we found that even in the population of apparently healthy old people (who did not, however, fulfil *all* SP criteria), NK activity was lower than that in young people [7]. These observations suggested that additional factors, and not only old age per se, could be responsible for the age-related preponderance of low NK activity in the healthy elderly. We decided therefore to look at the indices of thyroid hormone homeostasis, as it is known that multiple interactions exist between the immune system and the hypothalamus-pituitary-thyroid axis [10], and because aging is accompanied by alterations in thyroid hormone metabolism and responsiveness [11]. Disturbances of thyroid hormone homeostasis also take place more often in the elderly than in young or middle-aged people [12]. Age-related changes in serum thyroid hormone levels in rats, similar to those found during human aging [12], have been observed by us [13] and other authors [11]. Although there is indirect evidence that triiodothyronine (T_3) or thyroxine (T_4) treatment may influence NK activity in protein starvation [14], or in aging mice [15], there are no data relating serum concentrations of thyroid hormones of the NK activity in human aging. Therefore, we compared serum levels of thyrotropin (TSH), total T_4, and total T_3 in healthy young and old people who differed in their NK activity.

Although there is indirect evidence that T_3 or T_4 treatment may influence NK activity in protein starvation [14], or in aging mice [15], there are no data relating serum concentrations of thyroid hormones to the NK activity in human aging. Therefore, we compared serum levels of TSH, total T_4, and total T_3 in healthy young and old people who differed in their NK activity.

Methods

Subjects

Forty-seven elderly volunteers (17 males and 30 females, mean age 64 ± 5.2) were recruited from the Geriatric Outpatients Department in Gdansk and 20 young individuals (10 males and 10 females, mean age 23 ± 3.2) from the Gdansk student population. All volunteers gave informed consent to the study. In the preceding 3 years, the older volunteers had undergone a full medical examination every 12 months by the physicians from the Geriatric Outpatients Department. The participants of the young group had also undergone a detailed annual medical (clinical, hematological, biochemical) examination. The laboratory tests were carried out at the Department of Clinical Biochemistry of the Medical University of Gdansk.

All the elderly volunteers were recruited to the study according to the SP criteria. They were then divided into two groups: (1) optimally healthy subjects (designated 'completely healthy') who fulfilled all the SP criteria (n = 15; 9 females and 6 males, mean age 62 ± 6.5 and 65 ± 9.1, respectively) and (2) those not fulfilling all the SP criteria (designated 'almost healthy': n = 32; 19 females and 13 males, mean age 64.7 ± 3.1 and 62 ± 5.6, respectively). Subjects in both elderly groups led active and independent lives in their own homes. Participants of both groups were well nourished but not overweight. They regarded themselves as healthy and visited the Outpatients Department only occasionally for checkups. Their hematological and biochemical parameters were in the normal range for the elderly [16]. Some of these data are shown in table 1. There were no differences between the older individuals and the young group in the values of red and white cells and platelet counts, hemoglobin concentration, fasting blood glucose, plasma electrolytes and serum creatinine (data not shown). There were no statistically significant differences between the completely healthy and the almost healthy elderly in regard to the values of triglycerides, cholesterol (total, HDL, LDL fractions), fasting blood glucose, plasma electrolytes and serum creatinine (data not shown). Both groups of elderly subjects had a similar body mass index (24.1 ± 2.10 and 23.07 ± 2.28 kg/m^2 for completely healthy and almost healthy, respectively). None of the participants of the study had ever been hospitalized, or suffered from chronic inflammatory or autoimmune diseases, or from cancer. Ten (4 female) members of the almost healthy group had a sporadically elevated blood pressure (systolic in the range 160–180 mm Hg, diastolic 90–100 mm Hg) that required no drug therapy. Twenty-one representatives of the almost healthy group had degenerative changes of the skeleton without any serious pain and limitation of movement. None of the subjects smoked cigarettes or received drugs known to affect the immune system or metabolism of thyroid hormones. At the time of the study they had no symptoms of any disease and were not receiving any medication.

The young participants led very active lives and were in perfect physical condition. They had never been hospitalized, did not suffer from chronic inflammatory or autoimmune disease, or cancer. They very rarely suffered from acute infectious diseases and, at the time of the study, had no symptoms of any disease and were not on any drug therapy.

Sera

Sera were frozen and kept at –80 °C until used. In 21 elderly subjects blood was collected on the same day both for hormone level measurements and for the determination of NK activity. In the remainder of the elderly, both sampling procedures took place within 7 days.

Isolation of Peripheral Blood Mononuclear Cells

Human peripheral blood mononuclear cells (PBMC) were obtained by centrifugation of heparinized blood samples on a Lymphoprep gradient (Nyegaard, Oslo, Norway). After three washings with phosphate-buffered saline the cells were suspended in culture medium RPMI 1640 (Life Sciences, Warsaw, Poland) supplemented with 5% fetal calf serum (FCS; Serva, Warsaw, Poland).

Table 1. Some biochemical parameters, thyroid hormone and TSH concentrations in the blood serum of young and elderly patients with low and high NK activity

Age/NK activity	Young control		Elderly	
	low NK	high NK	almost healthy, low NK	completely healthy, high NK
Age, years	22 ± 3.1 (10)	24 ± 4.2 (10)	64.1 ± 4.2 (32)	63.2 ± 6.5 (15)
Total T_3, nmol/l	1.83 ± 0.27 (10)	1.93 ± 0.17 (9)	1.19 ± 0.14 (32)*,+	1.83 ± 0.09 (15)
Total T_4, nmol/l	98 ± 3.9 (10)	91 ± 2.2 (9)	100.1 ± 2.4 (32)	102.9 ± 5.6 (15)
TSH, mU/l	1.75 ± 0.33 (10)	1.90 ± 0.36 (9)	1.41 ± 0.24 (32)	1.83 ± 0.33 (15)
Triglycerides, mg/dl	89 ± 9.1 (10)	91 ± 7.9 (10)	155 ± 17.2 (32)+	139.7 ± 14.7 (13)+
Cholesterol, mg/dl	161.0 ± 10.2 (10)	164.0 ± 8.1 (10)	231.0 ± 12.1 (32)+	216.1 ± 14.3 (15)+
Total protein, g/l	72.10 ± 7.1 (10)	73.4 ± 5.9 (10)	64.6 ± 8.3 (32)	67.0 ± 10.5 (15)
Albumin, g/l	42.1 ± 3.9 (10)	43.4 ± 3.3 (10)	38.0 ± 3.2 (32)*,+	41.5 ± 2.1 (15)
Albumin, %	58.4 ± 0.6 (10)	59.5 ± 0.4 (10)	58.8 ± 0.7 (32)	61.9 ± 1.3 (15)

Data represent means ± standard error (SE), number is given in parentheses. * $p < 0.05$ vs. elderly group with high NK activity (unpaired t test); + $p < 0.05$ vs. young people with low or high NK activity (unpaired t test).

Assessment of NK Cytotoxic Activity

NK cytotoxic activity was measured by a standard (^{51}Cr) chromium release assay as described previously [7]. K562 (human erythroleukemic cell line NK-sensitive) cells were used as targets. PBMC were used as effector cells. K562 cells were cultured in RPMI 1640 medium containing 5% FCS, 100 μg/ml streptomycin, 100 U/ml penicillin, 2 m*M* *L*-glutamine (Life Sciences, Warsaw, Poland) and 100 m*M* pyruvate (Sigma, Poznań, Poland). PBMC were suspended in culture medium containing 90% RPMI 1640 and 10% FCS at a concentration of 1×10^6/ml. Effector PBMC and target K562 cells were mixed in a ratio of 25:1 (2×10^5 cells/100 μl), centrifuged and incubated for 4 h in a humidified atmosphere containing 5% CO_2 at 37 °C. The percentage of ^{51}Cr release (% of cytotoxicity) was calculated according to the formula:

$$\% \text{ cytotoxicity} = 100\% \times \frac{\text{experimental } ^{51}\text{Cr release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}}$$

Definition of 'Low' and 'High' NK Activity

NK activity in our study was arbitrarily designated as high or low on the basis of our long-term experience measuring NK activity in more than 600 elderly people [5–7, 9]. The cutoff level for defining low and high responses was set at the value of 20%.

Modulation of NK Cytotoxic Activity by T_3

The stock solution of T_3 was prepared by dissolving 1 mg of crystalline 3,3′,5-triiodo-*L*-thyronine (free acid, Sigma) in 1 ml of 0.01 *M* NaOH. Serial dilutions of T_3 were made from the frozen stock solution with serum-free RPMI and added to the cytotoxicity assay at a final concentration of 10^{-6}, 10^{-7} or 10^{-8} *M* for 48 h.

Determination of Total T_3, Total T_4 and TSH Serum Levels

Total T_3 total T_4 and TSH serum concentrations were measured by the use of specific radioimmunoassays (Farmos Diagnostica, Turku, Finland; normal ranges: 1.0–3.0 mmol/l, 50–150 nmol/l and 0.3–5.0 mU/l, respectively).

Data Analysis

The significance of differences between groups was evaluated by the means of the unpaired and paired t test, and regression analysis using the SigmaPlot program (Jandel Scientific, Erkrath, Germany). Differences with $p < 0.05$ were considered significant.

Results

Level of NK Cytotoxic Activity in the Elderly Differing in Health Status

We found (fig. 1) that all the elderly subjects who had low NK activity (n = 32) belonged to the subgroup of almost healthy people. In contrast, all the elderly subjects demonstrating high NK activity (n = 15) belonged to the completely healthy group. The young people with low (n = 10) and high (n = 10) NK activity were completely healthy. There were no sex-related differences in either age group.

Biochemical and Hormonal Characteristics of the Young and Elderly Differing in NK Activity

We next divided both age groups into two subgroups – those with low and high NK potential – and analyzed the data for each subgroup separately. Table 1 shows that in the group of young people half of the individuals had low NK activity whereas in the elderly group there were twice as many with low NK activity. Serum cholesterol levels were significantly higher in the elderly regardless of the level of the NK activity in relation to both subgroups of young people. The total protein serum concentration was

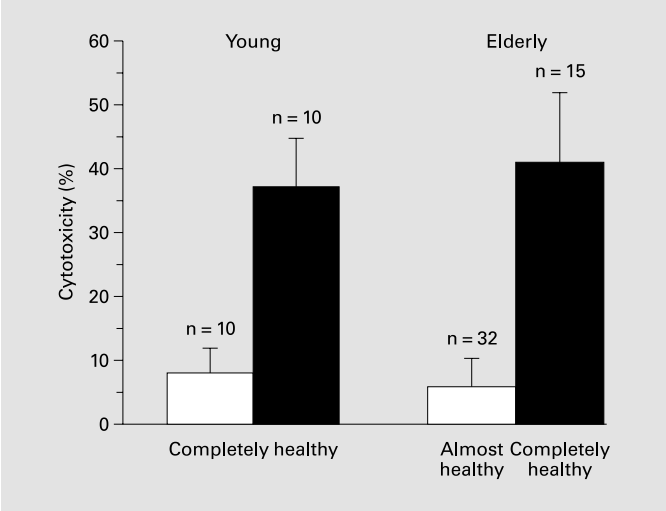

Fig. 1. NK activity of the young and elderly differing in health status. The values are expressed as percent of cytotoxicity; they represent arithmetic means ± SE (standard error). □ = Low NK activity; ■ = high NK activity.

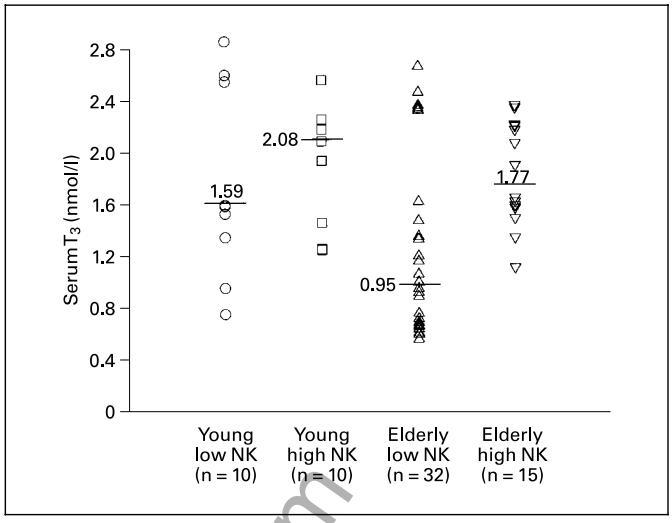

Fig. 2. Individual values of total T_3 serum concentration in young and elderly subjects differing in NK activity. Figures above short horizontal lines denote median values of serum total T_3 concentration.

similar in both age groups independent of NK activity. However, the serum albumin concentration was significantly lower in the old people with low NK activity than in other groups. The serum total thyroxin and TSH concentrations were similar in both the young and old. In contrast, serum total T_3 concentration in the elderly individuals characterized by low NK activity was significantly lower (at the lower limit of the range) than in the old people with high NK activity, or in the young people who had serum total T_3 level in the middle of the range. The results did not differ significantly between females and males in any of the groups (data not shown).

T_3 Serum Concentrations in Young and Elderly Subjects Differing in NK Activity

Figure 2 shows individual T_3 serum concentrations in young and elderly low and high NK responders. Almost all the low NK responders in the elderly subgroup had the lowest serum total T_3 levels of all the groups investigated.

Relationship between T_3 Serum Concentration and NK Activity in the Elderly

The results obtained thus far prompted us to investigate the relationship between the T_3 serum level and the NK activity in the elderly. A statistically significant correlation between T_3 serum concentration and NK activity was observed in the elderly group (fig. 3) – no such correlation was observed in the young group (data not shown).

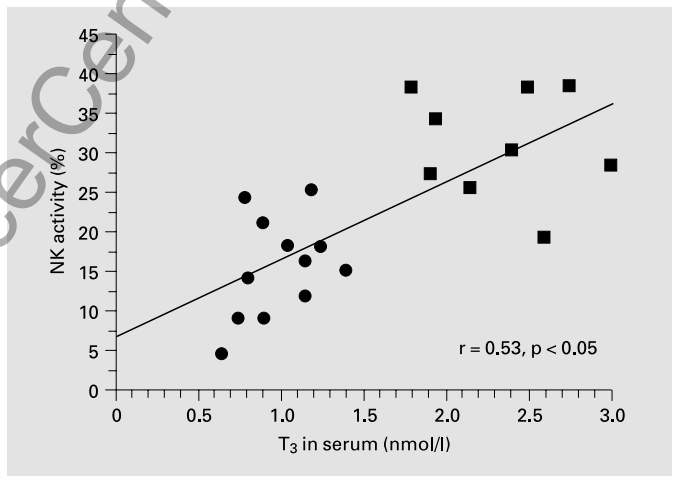

Fig. 3. Correlation between serum T_3 concentration and NK activity in the elderly. ● = Elderly with low NK activity; ■ = elderly with high NK activity. Only the data obtained from blood samples taken on the same day for the measurements of NK and hormone concentrations were used for the analysis (n = 21).

In vitro Effects of T_3 on the NK Activity in Subjects with Different Serum Total T_3 Levels

To investigate whether the observed correlation between the T_3 level and NK activity was due to the direct effect of T_3 on NK activity, we decided to perform an in vitro experiment. PBMC were isolated from young and

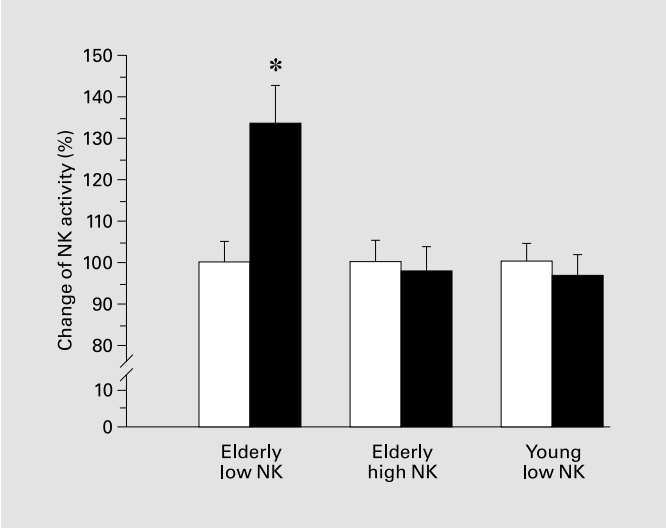

Fig. 4. In vitro effect of exogenous T_3 on the NK activity of PBMC of subjects with different levels of NK cytotoxic activity. PBMC were incubated for 48 h with T_3 (10^{-7} *M*) and the NK activity was measured thereafter as described in Methods. □ = NK activity of the control cells, not incubated with T_3 expressed as 100%; ■ = NK activity of cells incubated with T_3 (isolated from the same subjects). Bars show arithmetic means ± SE (n = 4 for each group). * $p < 0.05$ vs. control PBMC (paired t test).

elderly individuals and T_3 (10^{-6}, 10^{-7} or 10^{-8} *M*) or 0.9% NaCl (control) was added at the beginning of the 48-hour culture of PBMC. Thereafter the cytotoxicity assay was performed in the usual manner. Figure 4 shows that a significant increase in NK activity was observed in PBMC isolated from patients belonging to the old low NK responders, who had serum T_3 values at the lower limit of the range (mean serum level 1.22 ± 0.31 nmol/l, n = 4) when 10^{-8} or 10^{-7} *M* exogenous T_3 was used (increased by 28 ± 7.2 and 33 ± 8.2%, respectively, as compared to control cells; the effect of T_3 at 10^{-6} *M* was not significant, increased by 8 ± 8.6%). Moreover, the cytotoxic activity of PBMC from the old high NK responders (serum T_3 concentration 1.83 ± 0.44 nmol/l, n = 4) was unaffected by T_3 at all concentrations used; low and high NK responders in the young group were similarly unaffected (serum T_3 concentrations 1.85 ± 0.22 and 1.91 ± 0.15 nmol/l, respectively, n = 4) (fig. 4). It is worth noting that culturing of PBMC for 48 h without exogenous T_3 did not alter the level of NK activity in all the groups examined in relation to the initial values characteristic of freshly isolated cells.

Discussion

Since the hormonal status may significantly influence functions of the immune system, we decided to compare serum levels of total T_3, total T_4 and TSH in a carefully selected population of healthy young and old people who differed in the levels of NK activity. We found that old people with low NK activity had significantly lower serum T_3 concentrations than age-matched individuals with high NK, or young subjects with low or high NK activity. The correlation observed in the elderly between serum T_3 levels and NK activity may suggest the existence of a causal relationship. Indeed, our in vitro experiments showed that exogenous T_3 increased NK activity in PBMC of old subjects who had a relatively low concentration of serum total T_3 but did not effect NK activity of PBMC isolated from old or young individuals who had higher serum levels of total T_3. The mechanisms by which low NK activity of the elderly may be linked to a relatively low total T_3 serum concentration are at present unknown. Lymphocytes possess thyroid hormone binding sites in the cytoplasm and in the nucleus [17], and some metabolic processes such as an increase in cytoplasmic calcium [18] were shown to be stimulated in vitro by T_4 or T_3. It has been demonstrated that thyroid hormones are able to influence selected immune responses such as cell-mediated immunity, differentiation of B lymphocytes and the activity of NK cells [for review, see 19]. In vivo T_4 administration to old mice (with total T_4 serum levels 50% lower than in young animals) caused a significant, 2.2-fold increase in endogenous NK activity, which approached the values observed in young animals, while in young mice T_4 did not modify NK activity [15]. Other authors have found that supplementing a protein-deficient diet with T_3 increased the NK activity of splenic mononuclear cells in two strains of mice [14]. It remains to be elucidated how T_3 increases, under in vitro conditions, the NK activity of elderly individuals with borderline serum T_3 levels. However, the lack of its stimulatory effect on the NK activity in subjects with serum T_3 levels in the midrange (and high NK activity) suggests that the maintenance of a basal serum T_3 level is necessary for high NK activity in the elderly. Some immunoregulatory factors, e.g. cytokines, may significantly contribute to this phenomenon. We have recently observed that low NK cytotoxic activity in the elderly was associated with a lack of full health and overactivity of tumor necrosis factor α (TNFα) but not interleukin-6 (IL-6) [7, 20]. Interestingly, it has been shown that decreased serum T_3 levels correlate with increased plasma concentration of TNFα [21] and

IL-2 [22] in old patients with nonthyroid illness. These data indicate that the immunomodulatory activity of some cytokines has to be considered in determining the relationship between thyroid hormones and NK activity in the course of normal aging.

The lower total T_3 serum levels in a low NK subgroup of the elderly could not be a manifestation of the so-called 'low T_3 syndrome' because our investigations were performed on healthy elderly subjects, in whom no symptoms of extrathyroid illness were present. Although in the general population the range of total T_3 serum values is relatively wide (1.2–3.0 nmol/l), more than half of the investigated old people had serum total T_3 values at the lower limit of the range. Interestingly, those subjects belonged to the group of almost healthy elderly who did not fulfil all the criteria of the SP. We could not definitively point to the cause of this phenomenon as the serum levels of total T_3 or T_4 reflect a balance of many different phenomena such as hormone production rate in the thyroid gland, peripheral T_4 to T_3 conversion and binding of thyroid hormones by serum proteins. Similar levels of serum total T_4 in both groups of NK responders point rather to the last-mentioned processes. Although decreased T_4 to T_3 conversions in peripheral tissues has been shown in old rats [11], no direct experimental data are available in relation to human aging. However, the observed decreased serum albumin concentration in the elderly group of low NK responders might explain, at least partially, lower serum total T_3 levels because up to 20% of the total T_3 is bound to albumin [23]. A highly significant correlation between serum albumin levels and total (or free) T_3 concentration in a group of elderly subjects with minor disease has been found [24]. Although the health status of all participants of our study had been carefully evaluated to exclude sick persons, the existence of some undetected pathophysiological processes, reflected in a slightly but significantly lower plasma level of albumin, cannot be ruled out. It has been found in a large-scale study that serum albumin concentration decreases with increasing age [25]. The reason(s) for this tendency is not clear, but this phenomenon has been linked to the increasing permeability of the filtration membrane in the aging kidney [26]. The potential effects of undernutrition which lead to declined serum levels of albumin could be excluded as both completely healthy and almost healthy subjects showed a similar body mass index.

In summary, we found a correlation between serum total T_3 concentration and the level of NK activity in the elderly persons. The increase by exogenous T_3 of the NK activity in the elderly people with borderline low serum total T_3 levels suggests that T_3 may constitute one of the endogenous modulators of NK activity in this subgroup of the elderly. Moreover, this subgroup of the elderly should be carefully monitored as their suboptimal health status may deteriorate in the future.

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