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ORIGINAL ARTICLE

Chronobiologic study of the GH-IGF1 axis and the ageing immune system

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Summary
One of the many systems that weakens as we age is our immune system and there is a reduction in the GH-IGF1 axis activity with increasing age. In this study we evaluated the immune system and the GH-IGF1 axis function in healthy ageing. CD3, CD4, CD20, CD25, HLA-DR and GH showed acrophase during the night, whereas CD8, CD16 and TCRγδ expressing cells showed acrophase during the day. MESOR of CD3 was higher in the old aged subjects, MESOR of CD20 and CD20 values at 14:00h and at 02:00h were higher in the young middle aged subjects, MESOR of CD25 and CD25 values at 10:00 were higher in the elderly subjects, MESOR of HLA-DR was higher in the young middle aged subjects, whereas MESOR of DR+T cells and HLA-DR at 02:00h were higher in the elderly subjects, MESOR of TCRγδ bearing cells was higher in the elderly subjects, GH value at 18:00h was also higher in the elderly subjects, and MESOR of IGF1 was higher in the young middle aged subjects. There was a statistically significant difference for the acrophases of CD25, HLA-DR and IGF1. There were different and opposing correlations among lymphocyte subpopulations and GH-IGF1 axis hormones in young and middle aged subjects in comparison with old aged subjects. Linear regression evidenced a statistically significant positive trend between age and the 24h mean of CD3 and CD25 and a statistically significant negative trend between age and the 24h mean of CD20 and GH. In conclusion, ageing is associated with an altered GH and IGF1 secretion, with decreased peripheral B cell compartment, increased peripheral T cell compartment and alterations of circadian rhythmicity.

Key words: GH; IGF1; ageing; neuro-endocrine-immune system; circadian; lymphocyte; somatopause; immunomodulation

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INTRODUCTION

The age-associated decrease in growth hormone (GH) secretion and insulin-like growth factor (IGF) 1 production by the liver and other tissues in response to GH has been termed the somatopause or hyposomatotropism of ageing (Gusenoff et al. 2001, Rehman et al. 2001, Russel-Aulet et al. 2001). IGF1 is one of the most important growth factors for normal cell proliferation: it acts as an endocrine

hormone via the blood and as a paracrine and autocrine growth factor locally. An autocrine or paracrine GH-IGF1 system has been found in lymphoid tissues, capable of influencing lymphopoiesis and the immune function and in particular, IGF1 assists the maturation of lymphocytes in bone marrow and their function in the periphery (Auernhammer et al. 1995).

A characteristic phenomenon of ageing is the involution or disappearance of the thymus gland, that is the director and activator of the immune system. It secretes hormones such as thymosin and thymopietin, which regulate the immune system. GH is able to reverse the thymic atrophy of old rats so that their thymus glands became as large and robust as the thymus glands of healthy young rats (Morrhaye et al. 2009). It has been demonstrated that GH and IGF1 promote hematopoiesis, particularly the megakaryocyte and erythroid lineages, both *in vitro* and *in vivo* and promote early B cell and natural killer (NK) cell development, which occurs in the bone marrow, induce B cell proliferation and immunoglobulin (Ig) production and promote the survival of T cell progenitors and T cell development in the thymus. GH and IGF1 have been found to promote T cell chemotaxis and therefore may play a role in normal lymphocyte circulation to the lymphnodes and spleen (Clark 1997). The rate of GH secretion from the anterior pituitary is highest around puberty, and declines progressively thereafter. The cause of the normal age-related decrease in GH secretion is not well understood. Evidence suggests the existence of a relationship between declining GH and IGF1 levels and age-related changes in body composition and physical function. Associated with these physiological changes is a clinical picture often referred to as the somatopause: frailty, muscle atrophy, relative obesity, increased frequency of fractures, disordered sleep, decreased immune function (Toogood 2003). Immunological response decreases in most elderly people and immunosenescence is a process that affects all cell compartments of the immune system. Ageing associated changes have been demonstrated not only in T lymphocytes but also in different aspects of the innate immunity including natural killer (NK) cells (Born et al. 1995, Ginaldi et al. 2000).

The aim of our study was to evaluate differences among healthy young-middle aged subjects and old aged subjects in the GH-IGF1 axis and lymphocyte subpopulations.

MATERIALS AND METHODS

Subjects gave written informed consent and the study was approved by the local Scientific and Ethical Committee. Peripheral blood samples were collected at intervals of four hours for twenty four hours from fifteen healthy young-middle aged male subjects (YMA subjs, age range 36–55 years, mean age±SE 44.1±1.8) and fifteen healthy old aged male subjects (OA subjs, age range 67–79 years, mean age±SE 68.5±1.3). Inclusion criteria were age (<65 years for YMA subjs, ≥65 and <80 years for OA subjs), BMI (>25 and <30), non smoker, normal physical activity level, no psychiatric disorder, no alcohol intake, no chronic conditions, and normal blood pressure level) In all subjects healthy status was assessed by medical history and physical examination, basal screening blood and urine tests, ECG, chest X-ray, and upper and lower abdominal ultrasound scan. All subjects were studied in our Department and were submitted to the same social routine (light/dark cycle and mealtimes). Sleep was allowed between 23:00h (lights off) and 07:00h (lights on). During daytime (between 07:15h and 20:15h), subjects stayed in the Department and standardized meals were provided at appropriate times for breakfast (07:30h), lunch (12:30h), and dinner (18:30h).

In each blood sample we measured GH and total IGF1 on serum and analyzed lymphocyte subpopulations: CD3 (total T cells), CD4 (T helper/inducer), CD8 (T cytotoxic/suppressor), CD16 (natural killer), CD20 (total B cells), CD25 (activated T cells with the expression of the alpha chain of the IL2 receptor), HLA-DR (B cells and activated T cells), TcRδ1 (TCRγδ expressing cells) on peripheral blood anticoagulated with sodium ethylenediamine tetraacetic acid (EDTA). To measure hormone serum concentrations blood samples were centrifuged immediately after collection and frozen at –20 °C for later determination. All samples were analyzed in duplicate in a single assay; the intrassay and interassay coefficients of variation were below 5% and 3% for GH, and 3% and 8% for IGF1. We measured GH by immunoenzymometric assay (AIA-PACK HGH, Tosoh, Japan), IGF1 by radioisotopic assay (IGF I 100T Kit, Nichols Institute Diagnostics, San Clemente, USA). Analyses of lymphocyte subpopulations were performed on unfixed cell preparations with a multicolor fluorescence activated cell sorter (FACScan, Becton-Dickinson FACS Systems, Sunnyvale, USA) and a panel of monoclonal antibodies (mAbs) to lymphocyte surface antigens (Ortho Diagnostic Systems, Inc., Raritan, USA: OKT3, OKT4, OKT8, OK-NK, OKB20, OKT26a, OK-DR; Medical

Systems, Thermo Fisher Scientific Inc. Rockford, USA: TcRδ1). Briefly, mAbs were directly conjugated with phycoerythrin (PE) and to fluorescein isothiocyanate (FITS) and 10 μl mAbs were added to 100 ml EDTA blood in Trucount tubes (BD Biosciences, San José, USA). After a 15-min incubation at room temperature the erythrocytes were disintegrated and after centrifugation the supernatants were washed with PBS. Non-lymphocytic cells contaminating the preparations were excluded from analysis using scatter gates set on the 90° light scatter profile. At least 10,000 cells were acquired on the FACScan. Absolute counts of T cell subsets were calculated based on the proportion of the respective T cell subpopulation and on absolute counts obtained by the procedure. The number of fluorescent cells was expressed as a percentage of the total lymphocytes.

Statistical analysis

The statistical evaluation of hormone serum levels and lymphocyte subpopulation values was performed by non-inferential descriptive biometric analyses, including one-way ANOVA performed between the timepoints for each variable and each group on original data and on data transformed as a percentage of their individual 24h mean to look for a time-effect, Pearson's product moment correlation coefficients calculated for hormone serum levels at each sampling time to assess temporal relationships between their variations, linear regression between age and the 24h mean of each variable, Student's *t* test and Mann-Whitney rank sum test, where appropriate, on MESOR, amplitude and acrophase values. The data were also analyzed by an inferential temporal descriptive biometric analysis using the methods named Single Cosinor and Population Mean Cosinor, based on a least-squares fit of a cosine curve to individual and grouped time series data, testing the occurrence of a 24h rhythm and quantifying the parameters MESOR, amplitude and acrophase of the rhythm. MESOR is the acronym for Midline Estimating Statistic of Rhythm and defines the rhythm-determined average. Amplitude is the measure of one half the extent of rhythmic change in a cycle estimated by the function used to approximate the rhythm. Acrophase, measure of timing, is the phase angle of the crest time in the function appropriately approximating a rhythm, in relation to the specified reference timepoint (Nelson et al. 1979). Chronobiologic analysis was performed and chronobiologic graphs were created with Cosinor 2.2. ANOVA, Pearson's product moment correlation, linear regression, Student's *t* test and Mann-Whitney rank sum test were performed with SigmaPlot11.0. We used the significance level 2α=0.05.

RESULTS

In YMA subjects a clear circadian rhythm was validated for the time-qualified changes of all the factors studied, with the exception of IGF1, with Cosinor analysis, and a time effect for all the factors studied was evidenced with ANOVA performed on data transformed as a percentage of their individual 24h mean. CD3, CD4, CD20, CD25, HLA-DR and GH showed acrophase during the night, whereas CD8, CD16 and TCRγδ expressing cells showed acrophase during the day. In OA subjs a clear circadian rhythm was validated with Cosinor analysis for the nyctohemeral changes of CD3, CD8, CD16, CD20 and GH and a time effect was evidenced with ANOVA performed on data transformed as a percentage of their individual 24h mean for CD4, CD8, CD16, CD20, CD25, DR+T cells and GH.

Our data evidenced that MESOR of CD3 was higher in OA subjects (statistically significant), MESOR of CD20 and CD20 values at 14:00h and at 02:00h were higher in YMA subjects (statistically significant), MESOR of CD25 and CD25 values at 10:00 were higher in elderly subjects (statistically significant), MESOR of HLA-DR was higher in YMA subjectss (statistically significant) whereas MESOR of DR+ T cells and HLA-DR at 02:00h were higher in elderly subjects (statistically significant), MESOR of TCRγδ bearing cells was higher in elderly subjects (statistically significant), GH value at 18:00h was higher in elderly subjects (statistically significant), MESOR of IGF1 was higher in YMA subjects (statistically significant). There was a statistically significant difference for the acrophases of CD25 (statistically significant), HLA-DR (statistically significant) and IGF1 (statistically significant). Pearson's product moment correlations showed that in YMA subjects at 06:00h HLA-DR correlated positively with GH (r=0.882, statistically significant); at 22:00h CD8 correlated positively with IGF1 (r=0.972, statistically significant); at 02:00h CD8 correlated positively with IGF1 (r=0.992, statistically significant). In OA subjects at 06:00h HLA-DR correlated negatively with GH (r=–0.914, statistically significant), TcRδ1 correlated negatively with GH (r=–0.875, p=0.05); at 22:00h CD8 correlated negatively with IGF1 (r=–0.938, statistically significant); at 02:00h CD20 correlated negatively with GH (r=–0.941, statistically significant), CD25 correlated positively with IGF1 (r=0.913, statistically significant), TcRδ1 correlated negatively with GH (r=–0.876, statistically significant). Linear regression evidenced a statistically significant trend between age and the 24h mean of CD3, CD20, CD25 and GH.

Table 1. **Chronobiological summary** of data derived from best fitting (fitted period: 24 hours=360°) and *F* and *p* value from ANOVA performed between the time points for each variable.

	Young middle aged subjects								Old aged subjects							
	p #	MESOR[a]	Amplitude	Acrophase	ANOVA				*p* #	MESOR[a]	Amplitude	Acrophase	ANOVA			
					Original units		% of mean						Original units		% of mean	
					F	*p*	*F*	*p*					*F*	*p*	*F*	*p*
CD3	0.002	78.0±0.1[a]	1.1±0.2	01:40±0:50	0.08	0.99	2.3	0.015	0.002	84.9±0.2*	1.0±0.0	04:45±0:12	0.71	0.61	2.1	0.090
CD4	0.001	45.2±0.8	3.1±1.1	00:13±1:38	0.42	0.82	5.6	0.001	0.145	45.1±0.8	3.1±1.2	02:13±1:28	0.89	0.50	2.6	0.047
CD8	0.003	29.5±0.2	1.9±0.2	12:05±0:10	0.13	0.98	4.2	0.001	0.005	29.2±0.2	3.2±0.5	12:36±0:49	1.50	0.22	4.3	0.001
CD16	0.030	6.2±0.4	0.8±0.2	14:08±1:25	0.32	0.89	2.7	0.030	0.001	8.0±0.3	2.4±0.4	12:45±0:30	0.68	0.69	3.1	0.020
CD20	0.002	13.2±0.2*	1.5±0.1	22:27±0:49	0.25	0.93	1.0	0.005	0.001	8.3±0.1	1.1±0.1	19:09±1:26	0.79	0.56	3.0	0.029
CD25	0.002	3.8±0.0	0.6±0.2	00:37±0:28	0.29	0.91	2.9	0.033	0.060	7.1±0.1*	1.0±0.2	16:45±0:49*	0.17	0.96	1.7	0.045
DR+T cells	0.005	3.2±0.3	0.8±0.2	00:49±3:25	0.24	0.34	1.1	0.020	0.057	5.1±0.3*	1.7±0.2	9:24±0:45*	0.34	0.42	2.7	0.040
HLA-DR	0.010	16.2±0.2*	1.3±0.3	22:10±0:45	0.74	0.60	2.9	0.050	0.297	13.2±0.1	1.2±0.9	12:20±2:13*	0.73	0.60	1.8	0.150
TcRδ1	0.002	2.1±0.0	0.6±0.1	10:38±1:57	0.55	0.73	1.0	0.020	0.210	4.2±0.1*	0.3±0.1	12:48±2:02	0.39	0.54	0.9	0.176
GH	0.001	0.3±0.1	0.3±0.1	01:01±0:54	2.41	0.06	2.2	0.018	0.015	0.3±0.0	0.3±0.0	23:57±0:13	1.34	0.28	4.2	<0.01
IGF1	0.086	229.4±1.3*	17.2±1.2	08:28±0:25	1.39	0.26	3.6	0.013	0.267	212.3±3.1	2.5±4.7	12:04±8:01*	0.06	0.99	1.1	0.388

[a] mean±SE

Units: % for lymphocyte subpopulations, ng/ml for GH, ng/ml for IGF1, hours:minutes for acrophase; all parameters analyzed in all the subjects. *p* # value from an *F*-test of the null amplitude rejection hypothesis (for a rhythm with a chosen period τ); * statistically significant.

Table 1 shows chronobiological data derived from best fitting cosine curves, *F* and *p* value from ANOVA performed between the timepoints for each variable and *p* values from *t*-test and Mann-Whitney rank sum test, as indicated performed between MESOR, amplitude and acrophase values. Figs 1 and 2 show the fitting cosine curves of rhythm of lymphocyte subpopulations, GH and IGF1 in YMA and OA subjects, Figs 3 and 4 show the 24-hour profiles of lymphocyte subpopulations, GH and IGF1 in YMA subjects and OA subjects, Fig. 5 shows the regression lines between age and the 24h mean of CD3, CD20, CD25 and GH.

DISCUSSION

Biological rhythms in different frequency ranges characterize the mammalian body and this phenomenon is particularly evident when we consider the neuro-endocrine and immune system functions, characterized by a multifrequency time structure (Besedovski and del Rey 1996, Haus 1996, Haus and Smolensky 1999). In the healthy organism, rhythms of the same frequency may have the same phase or different phases and usually show a well defined time-relation to each other. The loss of the array of rhythms or a change of their functional interactions may alter the organism's time structure leading to chronodisruption and internal desynchronization. The alteration of the organism's time structure may lead to functional disturbances and to alteration of the anatomic integrity (Haus et al. 1983).

Circadian rhythmicity of variation characterizes innate and adaptive immunity as well as their humoral factors and cellular effectors (Abo and Kumagai 1978, Abo et al. 1981, Canon et al. 1985, Born et al. 1997, Arjona et al. 2004). The nyctohemeral variations of physiological phenomena are controlled by a complex system comprising of a master circadian clock in the suprachiasmatic nuclei (SCN), which are entrained by environmental timing cues (light-darkness and/or the activity-rest pattern), extra-SCN cerebral clocks and peripheral oscillators. At a molecular level circadian rhythms are regulated by transcriptional and post-translational feedback loops generated by a set of interplaying clock proteins and as many peripheral tissues and cells, leukocytes also rhythmically express clock genes (Teboul et al. 2005, Fukuya et al. 2007, Berger 2008). Lymphocyte subpopulations present circadian variation of some of their subsets and this variation may influence magnitude and expression of the immune responses (Lévi et al. 1985, Mazzoccoli et al. 1997). The circadian variation of lymphocyte subsets has been related to circadian changes in cell production, release and destruction and to cortisol and epinephrine influence on cell redistribution to the bone marrow, mobilization and migration to lymphoid and non lymphoid organs and peripheral tissues (Dimitrov et al. 2009). The phenomenon of lymphocyte subpopulation redistribution may be more complex and may involve other hormones, monoamines and cyto/chemochines. The contribution of the immune system to healthy ageing and longevity is still an open question and immunosenescence is a process that affects all cell compartments of the immune system. Aging of the immune system function may be related to alteration of circadian rhythmicity with loss of interaction among key lymphocyte subsets, immunomodulating hormones and cytokines/ chemokines as well.

The results obtained in our study show interesting differences between the studied groups in hematic levels and temporal organization of some investigated factors. Young and middle aged subjects have higher levels of total B cells and show a clear circadian rhythm and a customary temporal architecture of many studied factors. As evidenced in our study, peripheral blood lymphocytes show circadian variations of specific subpopulations and the T helper/inducer and the T suppressor/cytotoxic subsets change with circadian rhythmicity but in an opposite phase, showing a temporal organization of lymphocyte functions. The variations of total T cells, T helper/inducer subset, DR+ B cells and activated T cells, total B cells and activated T cells with expression of the alpha chain of IL-2 receptor show circadian rhythmicity with acrophase at night, synchronized with those of GH, in antiphase with the rhythm of T suppressor/cytotoxic lymphocytes, natural killer cells and γδTCR expressing cells.

There is a general agreement in the international literature about the presence of circadian rhythmicity of variation of the total number of lymphocytes, with the zenith during the night in antiphase with the rhythm of cortisol secretion, whereas the reports concerning the single lymphocyte subsets are conflicting. Some papers describe a circadian rhythm only for CD4 with acrophase during the night (Kawate et al. 1981, Ritchie et al. 1983, Kronfol et al. 1997, Suzuki et al. 1997), whereas other reports describe an ultradian rhythm of CD8 (Lévi et al. 1988a) and a clear circadian rhythm of CD4/CD8 ratio (Lévi et al. 1983). This discrepancy may be related to the large interindividual and seasonal variability of lymphocyte rhythmicity that may hamper the statistical interpretation of the data from different studies and from different groups of subjects (Canon et al. 1986, Lévi et al. 1988b).

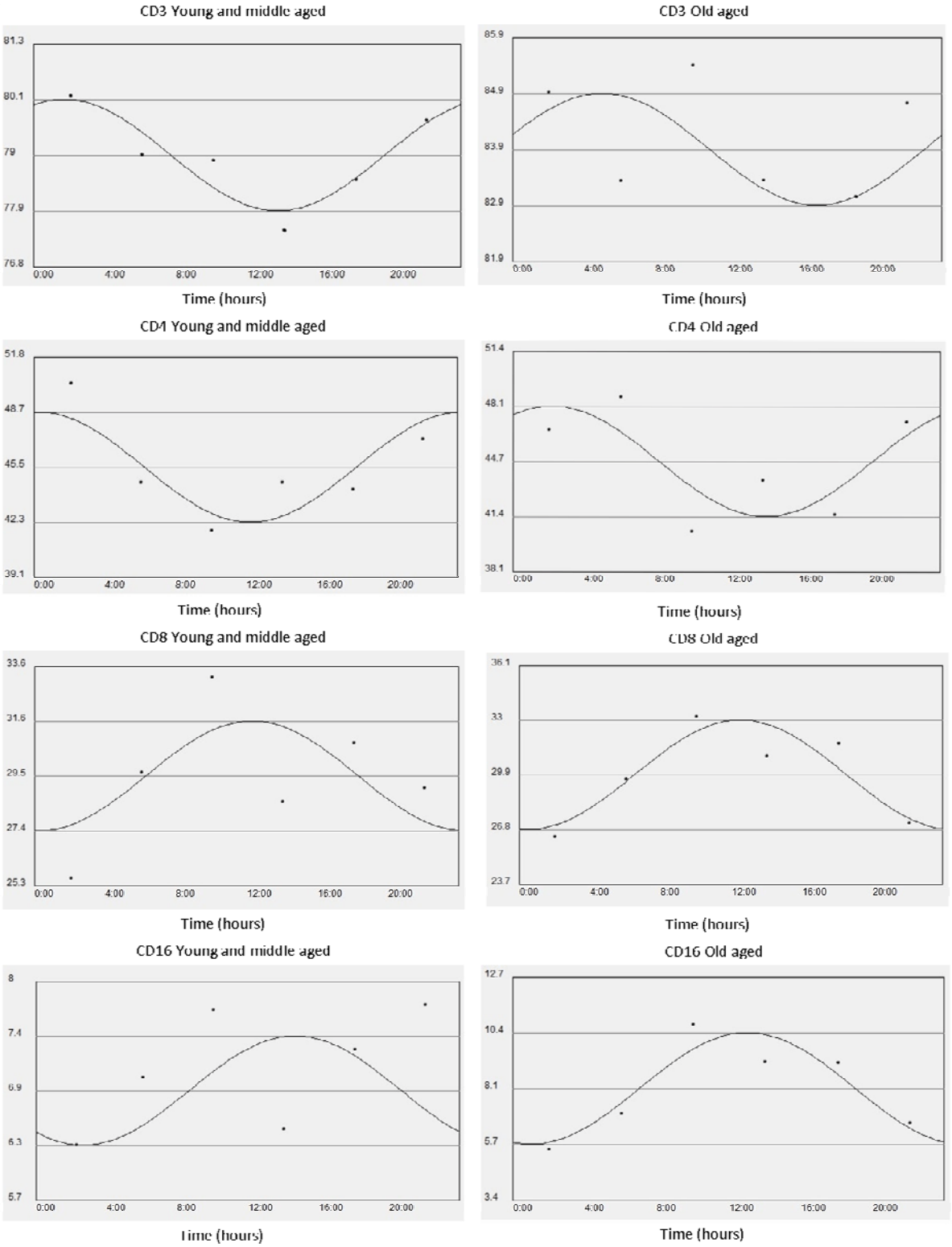

Fig. 1a. **x-y plots showing from top to bottom the fitted cosine curve of rhythm** (continous line) **superimposed on raw data** (dots), the MESOR and 95% confidence limits of CD3, CD4, CD8, CD16 expressing cells in Young middle aged subjects (left) and Old aged subjects (right). Units: % for lymphocyte subpopulation on y axis.

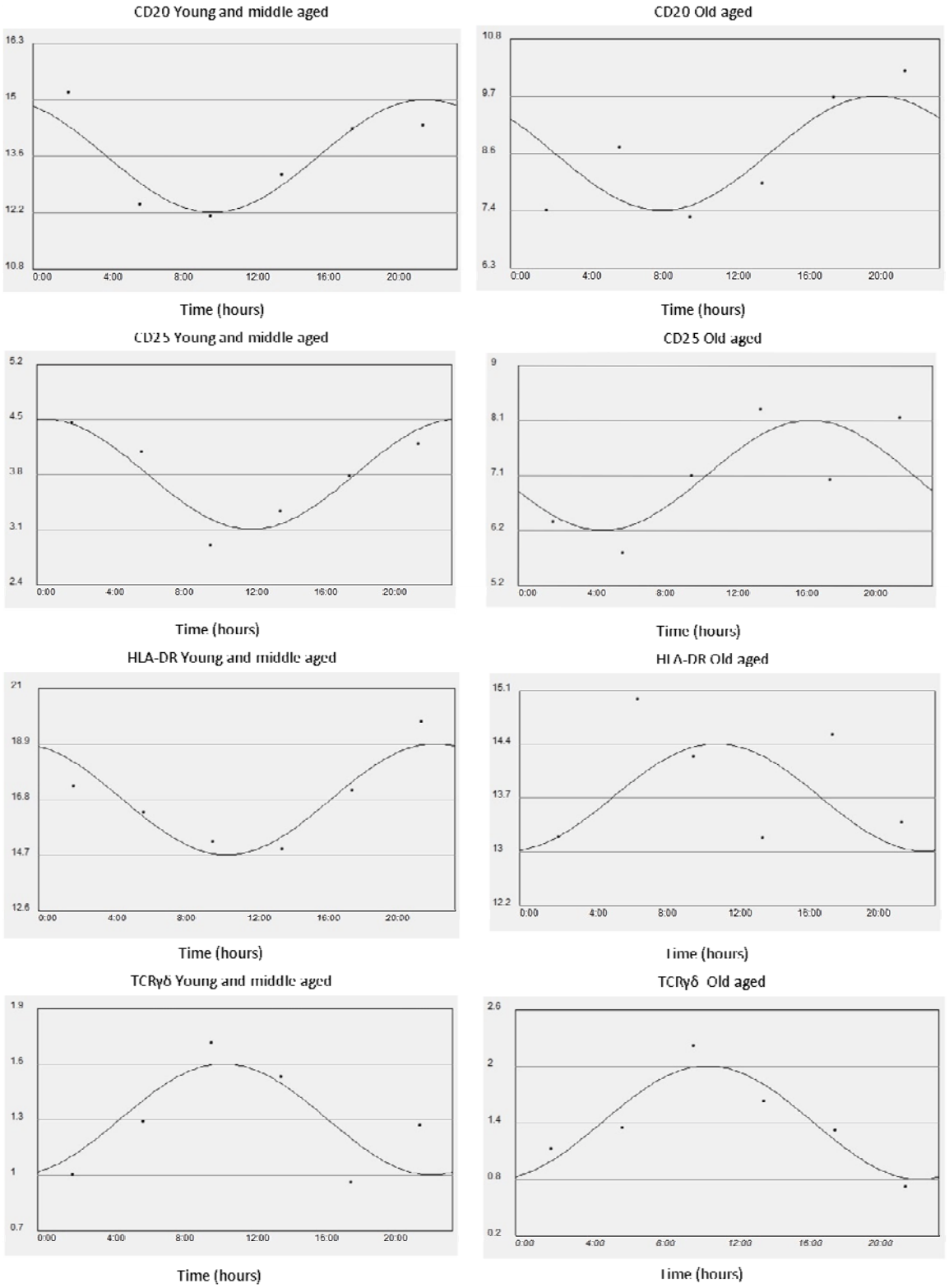

Fig. 1b. **x-y plots showing from top to bottom the fitted cosine curve of rhythm** (continous line) **superimposed on raw data** (dots), the MESOR and 95% confidence limits of CD20, CD25, HLA-DR, γδTCR expressing cells in Young middle aged subjects (left) and Old aged subjects (right). Units: % for lymphocyte subpopulation on y axis.

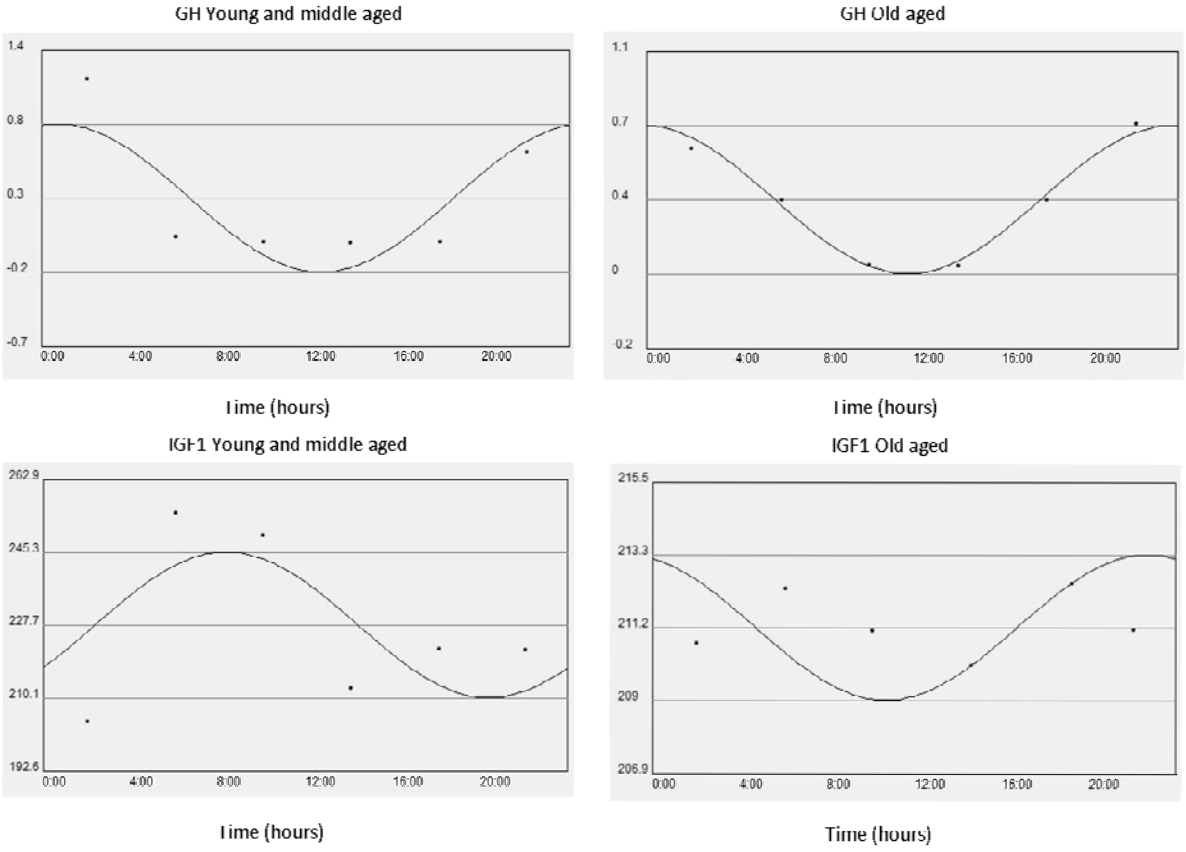

Fig. 2. **x-y plots showing from top to bottom the fitted cosine curve of rhythm** (continous line) **superimposed on raw data** (dots), the MESOR and 95% confidence limits of GH and IGF1in Young middle aged subjects (left) and Old aged subjects (right). Units: ng/ml for GH and IGF1 on y axis.

Our study found significant differences in the pattern of GH and IGF1secretion between the studied groups with old aged subjects presenting higher GH levels in the evening, but decreased mean levels and different acrophase of IGF1 in comparison with younger subjects. This phenomenon may be caused by an alteration in the GH-IGF1 axis regulation. Elderly subjects in our study had higher levels of CD3+ lymphocytes, DR+ T cells, activated T cells with expression of the alpha chain of IL-2 receptor and γδTCR expressing cells and we have found that in these subjects the circadian rhythm of CD25 subset is phase advanced, the circadian rhythm of total T cells is phase delayed and the nyctohemeral variations of T helper/inducer subset, DR+ B cells and activated T cells, total B cells, TCRγδ expressing cells do not show circadian periodicity. The MESOR of TCRγδ bearing cells is increased in our elderly subjects and this might be an important finding, because TCRγδ complex is mainly expressed at the cell surface of cellular elements temporally and maybe functionally related to cytotoxic T lymphocytes. Previous studies have shown that this complex is involved in T cell activation and that activated γδ expressing cells frequently exhibit cytotoxic activity against multiple target cell lines including neoplastic cells, thereby playing a key role in immunosurveillance (Bensussan et al. 1989, Macintyre and Sigaux 1989, Scott et al. 1990, Sleasman et al. 1990).

The alteration of circadian rhythmicity found in our old aged subjects may be responsible for altered correlations among the lymphocyte subpopulation and hormone time-related variations, such as are found for the altered correlation of CD8+ lymphocytes with IGF1 and HLA-DR+ cells with GH, and maybe the expression of the loss of physiological timed windows of interaction with occurrence of new anomalous interactions. Nocturnal GH levels were lower in our old aged subjects, but this result does not reach statistical significance ($p=0.06$), maybe because in humans GH secretory dynamics decline from high values in young adults to be virtually absent after the age of 50–60 years (Corpas et al. 1993, Touitou and Haus 2000) and we preferred to include in the study

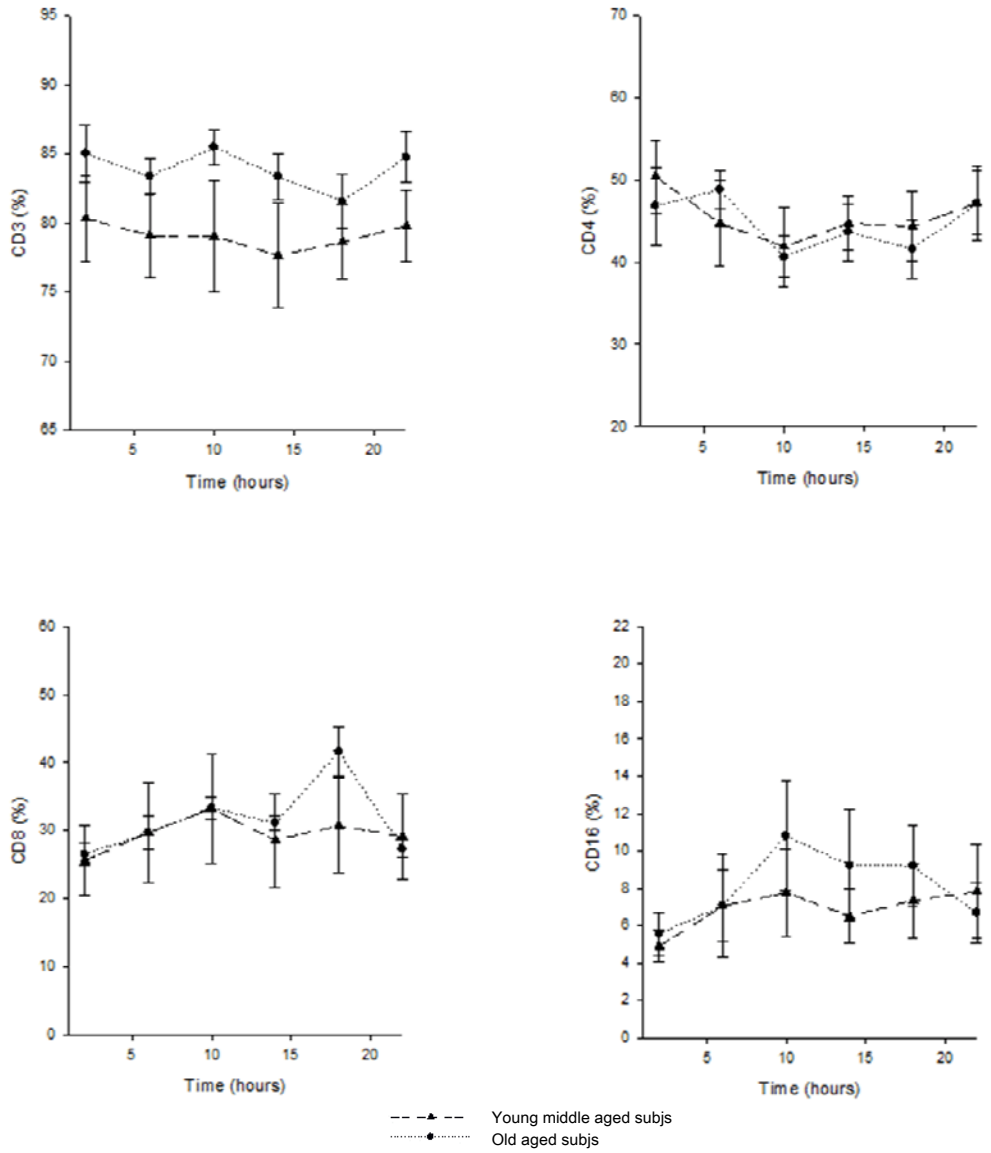

Fig. 3a. **x-y plots showing 24-hour profiles of CD3, CD4, CD8 and CD16 expressing cells** in fifteen Young middle aged and fifteen Old aged subjects; * statistically significant.

middle aged (45–65 years) and to leave out more than 80 years aged volunteers to evaluate age ranged subjects with closer physiological characteristics. We enrolled able-bodied and generally healthy old subjects and their slightly lower GH serum levels confirm the need to distinguish GH deficient from non GH deficient old individuals. Studies conducted on GH-deficient patients have demostrated that different time treatment schedules of GH administration have different effects on IGF1 serum levels and the closest similarity to normal hormone and metabolite patterns and relationships is reached by GH injection in the evening (Copeland et al. 1980, Jorgensen et al. 1988, 1990, Laursen et al. 1995, Oscarsson et al. 1997). In the young, GH is secreted in a pulsatile fashion mainly during the first hours of sleep (stages 3 and 4), whereas ageing is associated with a severe decrease in both frequency and amplitude of the pulses, leading to a decline of plasma GH levels, in part as a consequence of decreased responsiveness of the pituitary to GH-releasing factor and leading to reduced peripheral IGF1 production. In our elderly subjects IGF1 levels are significantly decreased and the GH secretion seems to be higher in the late afternoon than during the night (phase advanced), maybe in relationship to

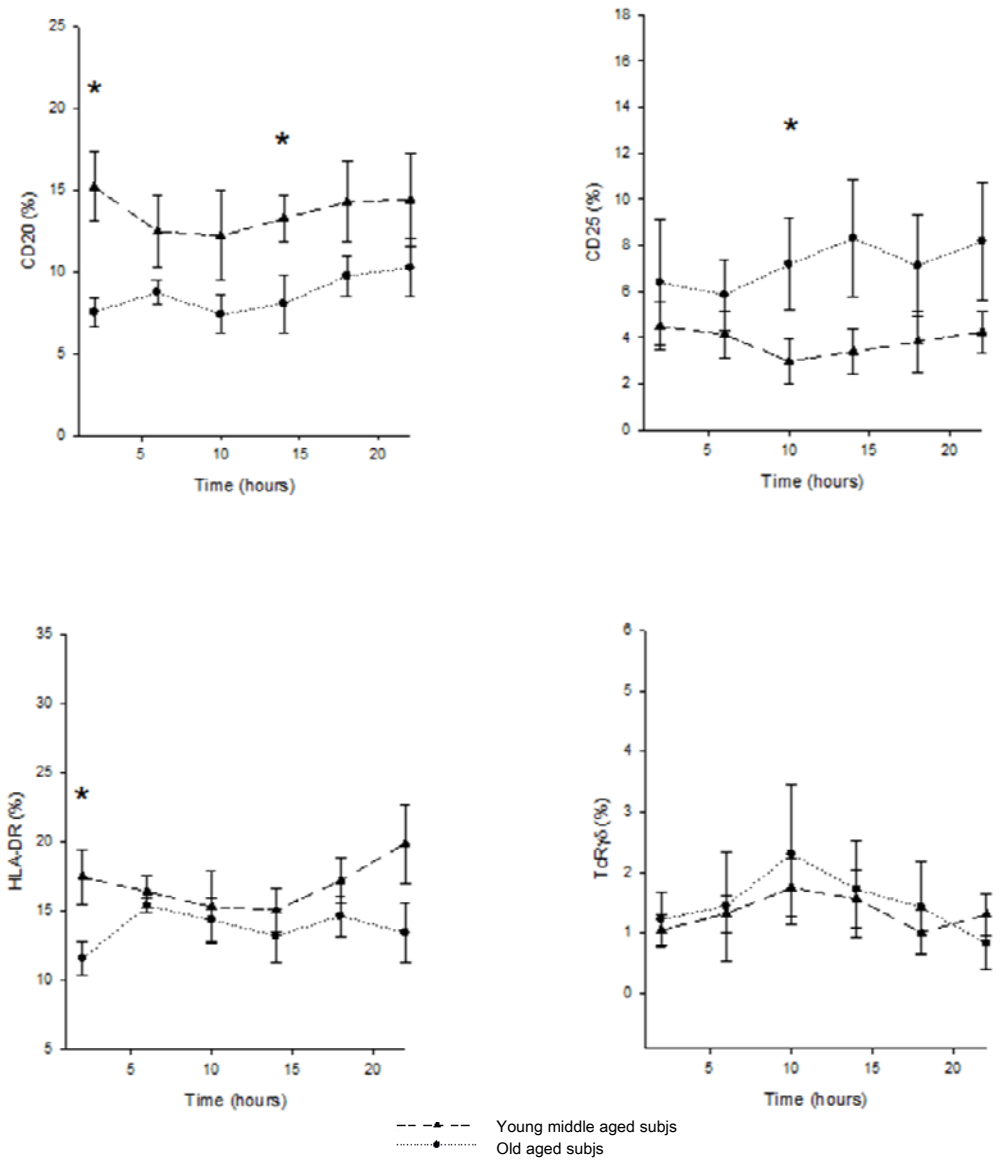

Fig. 3b. **x-y plots showing 24-hour profiles of CD20, CD25, HLA-DR, TcR expressing cells** in fifteen Young middle aged and fifteen Old aged subjects; * statistically significant.

an advance in the phase of the sleep-wakefulness cycle, the most common change of sleep pattern with age (Duffy and Czeisler 2002). This finding is in agreement with the alteration of GH secretion in the elderly reported in precedent studies (Touitou and Haus 1994, Touitou et al. 1997) and confirms the importance of normal circadian rhythmicity of GH secretion for the preservation of hormone action.

Increased classical signs of T cell activity are the level of soluble interleukin 2 receptor in serum and up-regulation of HLA-DR and interleukin 2 receptor on circulating T lymphocytes. IL-2 plays a pivotal role in regulating the adaptive immune system by controlling the survival and proliferation of regulatory T cells, which are required for the maintenance of immune tolerance. Of crucial importance for the delivery of IL-2 signals to regulatory T cells is the expression of CD25, which confers high affinity binding to IL-2 (Schwartz 2003, Letourneau et al. 2009). Our data show that important alterations of the immune system function occur during ageing. The decrease of B cells (CD20 and HLA-DR+ B cells), lymphocytes that play a key role in the humoral immune response, may be responsible for a decreased response to exogenous antigens, included vaccines and adjuvants (Saurvein-Teissla et al. 1998, de Bruijn

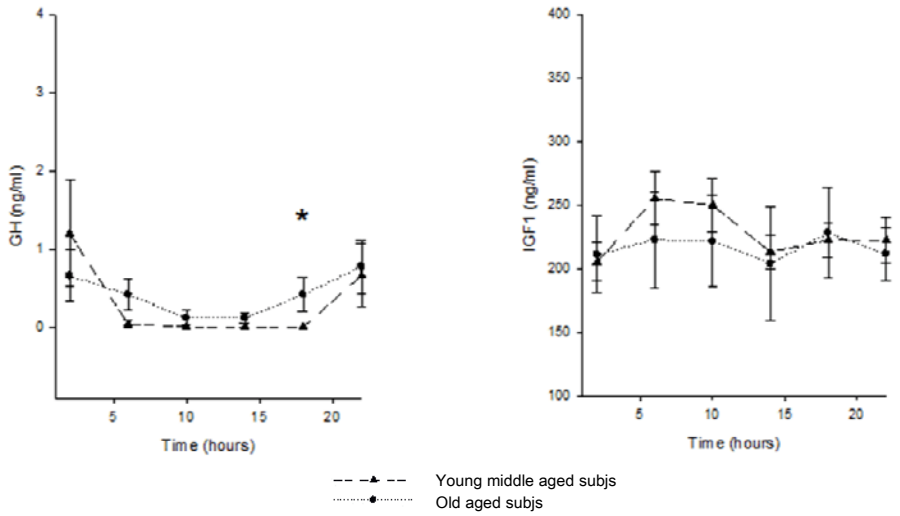

Fig. 4. **x-y plots showing 24-hour profiles of GH and IGF1 serum levels** in fifteen Young middle aged and fifteen Old aged subjects; * statistically significant.

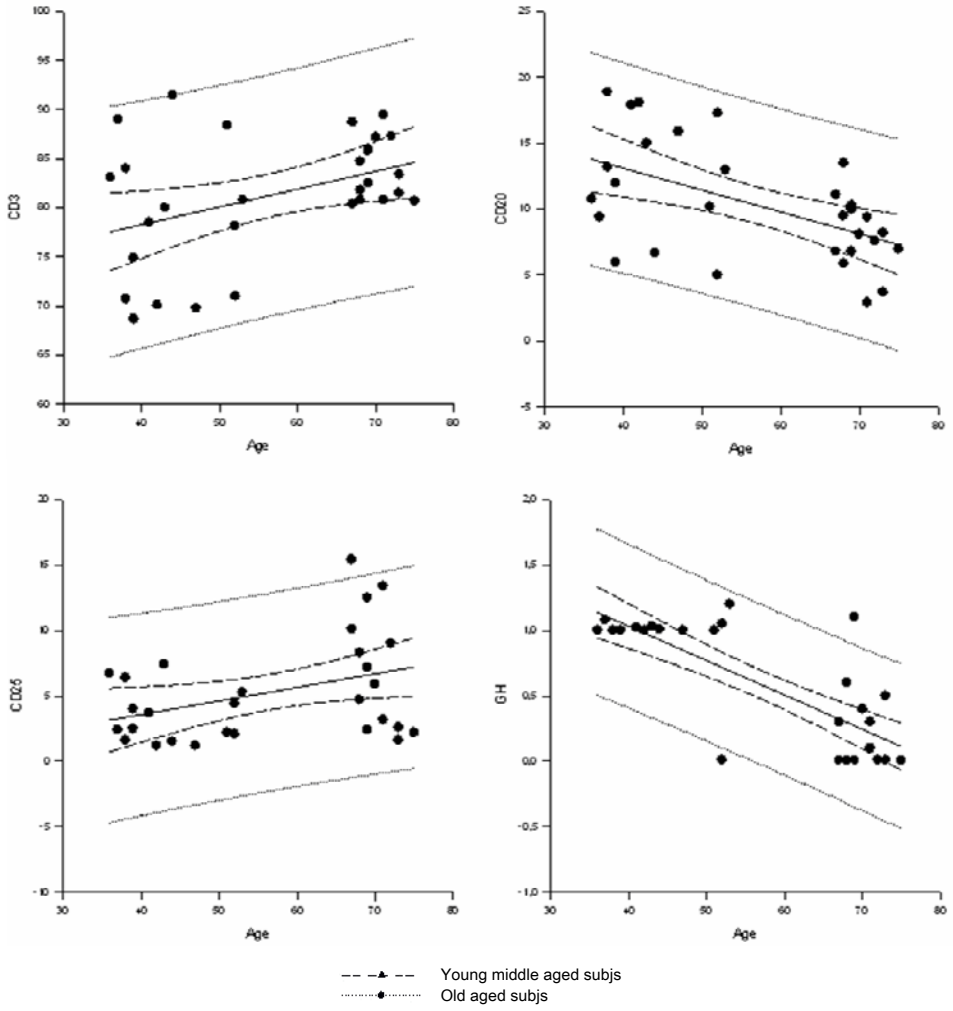

Fig. 5. **x-y plots showing regression lines with 95% confidence limits between age and the 24h mean of CD3, CD20**, **CD25**, **GH** (all statistically significant).

et al. 2004). In addition, the increase of activated T cells (CD25 and DR+ T cells) may be associated with an increased frequency of autoimmune phenomena and to an altered regulation of immune function and in our study we have documented that the circadian rhythmicity of these subsets is severely altered in elderly subjects.

In conclusion, elderly people present a decrease of peripheral B cell compartment, an increase of the peripheral T cell compartment, an alteration of circadian rhythmicity and an alteration of GH-IGF1 axis function, that may be responsible for altered integration between the neuro-endocrine and immune system.

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