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AGE AND SEX SPECIFIC REFERENCE SERUM SELENIUM LEVELS ESTIMATED FOR THE ITALIAN POPULATION

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Summary. - *The association between the concentration of selenium in serum and the risk of degenerative processes of the cardiovascular apparatus or of neoplastic disease remains still uncertain. An inaccurate selection of the study populations, and above all the lack of age, sex and area of residence specific reference values could have contributed to create confusion on the biological relevance of selenium in human diseases. In our present work the serum selenium levels for the Italian population have been studied, adopting standardized methods. The study population (4201 adult subjects and 1217 children) was derived from samples of populations previously enrolled in epidemiological preventive programs. The mean observed values for the various adult populations examined varied between 87 and 93 µg/l and resulted approximately 5 µg/l higher than the mean observed values in ten European countries. The mean observed values for the paediatric population (< 15 years of age) were slightly lower (78-83 µg/l). A decreasing trend of the values with age, above 60 years, expecially in males, has been observed. No significant difference has been observed for sex and geographic area of residence. A preliminary study of the variations of the serum selenium levels during certain diseases has shown a sharp reduction in children with phenylketonuria and undergoing dietary restrictions, in subject with active systemic* Lupus erythematosus, *and in certain neoplasias.*

Riassunto (Valori di riferimento per il selenio sierico nella popolazione italiana in rapporto al sesso e all'età). - *L'associazione tra concentrazione sierica di selenio e rischio di insorgenza di processi degenerativi a carico dell'apparato cardiovascolare o di malattia neoplastica, rimane tuttora incerta. Una non accurata selezione della popolazione studiata e soprattutto la mancanza di valori di riferimento in rapporto ad età, sesso e area geografica di appartenenza potrebbero, a nostro avviso, aver contribuito a creare confusione sull'importanza biologica del selenio nell'ambito della patologia umana. Nel presente lavoro sono stati studiati i livelli di selenio sierico per la popolazione italiana, adottando metodologie standardizzate. I soggetti studiati (4201 adulti e 1217 bambini) provenivano da campioni di popolazioni originariamente arruolate per l'attuazione di programmi epidemiologici di prevenzione. I valori medi osservati per le varie popolazioni di adulti esaminate variavano da 87 a 93 µg/l e risultavano di circa 5 µg/l più alti della media dei valori osservati in dieci paesi europei. I valori medi osservati per la popolazione infantile (< 15 anni) erano leggermente più bassi (da 78 a 83 µg/l). E' stata osservata una tendenza alla diminuzione dei valori con l'età, oltre i 60 anni, soprattutto nei maschi. Nessuna differenza significativa è stata osservata in relazione al sesso e all'area geografica di provenienza. Uno studio preliminare delle variazioni dei livelli di selenio sierico in condizioni di malattia ha messo in evidenza una loro netta riduzione nei bambini fenilchetonurici sottoposti a dieta, nei soggetti affetti da* Lupus eritematoso *sistemico in fase attiva di malattia e in alcuni casi di neoplasie.*

Introduction

Selenium is a biologically essential micronutrient and its relevance has already been known for several decades. Schwarz and Foltz [1] demonstrated in 1957 that hepatocyte necrosis induced in rats by a vitamin E deficient diet is prevented by small doses of Se. In the following years an impressive number of studies appeared, aiming at demonstrating the essential dietary properties of Se and its beneficial effect on certain animal diseases. In 1973, Se has been identified in the catalytic sites of the glutathione peroxidase, an enzyme involved in the removal of organic peroxides, and therefore relevant in the protection of biologic membranes from oxidative damages [2]. During the past fifteen years, great efforts have been made to elucidate the nutritional relevance of Se and its role in human pathogenesis.

The quantity of Se in food directly depends from the amount of Se in soil: therefore, a high geographical variability both in dietary intake and in blood levels of Se is present, even if such variability is much less pronounced of what it would be expected, given the Se content in soil, because of the exchange of enormous amounts of foodstuffs from countries in different geographical areas. Many

doubts still persist about the daily dietary requirements of Se, even if in 1980 the Food and Nutrition Board of the National Research Council (USA) has proposed as safe and adeguate a daily intake varying from 10 to 200 μg according to the age.

The great number of diseases observed in animals kept with a Se deficient diet has given way to many hypotheses on the possible role of Se in different human pathological conditions.

So far, the only human disease that is certainly associated with Se is a myocardiopathy known as Keshan's syndrome, that is observed in children and in pregnant women living in certain areas of China, and that is promptly cured by administration of sodium selenite [3, 4]. Other cases of myocardiopathy associated with low blood Se levels have been reported in patients undergoing total parenteral nutrition [5, 6]. However, it has been reported that heart involvements do not necessarily occur, so that a necessary presence of concurrent causal factors can be supposed in the pathogenesis of the myocardiopathy. In the last years, following epidemiologic observations and *in vitro* experiments, many researches have been made on the possible role of Se as an anticancer agent [7, 8].

The possible relationship between Se and cancer was suggested for the first time by Shamberger *et al.* [9], who found an inverse correlation between blood Se levels and cancer mortality in certain areas of the USA. Even if low blood Se levels have been observed in cancer patients, Robinson *et al.* [10] believe that such a phenomenon is a result rather than a cause of the neoplastic process.

Finally, Salonen *et al.* [11] pointed out a possible relationship between low blood Se levels and an increased incidence of arteriosclerotic cardiovascular diseases. The reduced activity of the glutathione peroxidase in the platelets of patients with acute myocardial infarction and the high Se content in the platelets suggested to Kasperek *et al.* that a reduced Se dietary intake could increase the risk of thrombosis: this hypothesis was further supported by an observation of reduced prostacyclin synthesis in the aorta of rats treated with a Se deficient diet [12].

Anyway, despite the great amount of studies, the biological relevance of Se in human pathogenesis has not yet been sufficiently defined. Among many reasons, we point out an inaccurate patient selection and above all the lack of age, sex, and area of residence specific reference values.

In the past, very relevant analytical problems have impaired the determination of Se in biological fluids. Only the most recent technology in the field of atomic absorption spectrometry with graphite furnace has made it possible for various laboratories to correctly determine Se in blood. Since we have set up in our laboratory a method for the determination of Se in serum, we have been involved in trying to produce reference values, in healthy subjects, that could be of use in researches on the biological role of the element in the different human physiopathological conditions.

We also considered it interesting to start a study on the variation of serum Se levels in pathological conditions: we will report here also the preliminary results of this study.

Methods

In clinical chemistry, a reference value is defined as a series of measurements of a particular biochemical parameter, obtained on a sample group of individuals, intended to give meaning to a value subsequently observed in an individual similar to those in the examined group, taking into account the biological variability of the parameter of interest, in healthy or ill condition. Reference values have meaning only if the methods used for their production are adequately chosen and described. Parameters that need to be defined include: the selection criteria for the reference population; the stratification criteria; the procedures of sample collection; the analytical method; the statistical method for the estimate of the reference limits. Withhin this framework it is advisable to follow the specific "Recommendations" produced by the "Scientific Committees" of the International Federation of Clinical Chemistry [13-16] and of the French Society of Clinical Biology [17-24].

The serum Se reference values, in healthy condition, presented here, have been produced through the *a posteriori* (or retrospective) selection of individuals from sample populations originally enrolled in epidemiological programs for the study and the prevention of cardiovascular disease and cholelithiasis, with the exclusion of subjects affected by systemic diseases or under drug treatment.

Sample collection operations have been performed under standard conditions: avoidance of food, alcoholic beverages, coffee, tobacco, during the 12 hours before sample collection; venipuncture, with haemostatic loop, in the fold of the elbow; use of disposable syringes; serum separation from the sample immediately after collection, using a standard procedure; serum storage at + 4 °C for a maximum of 8 days; serum storage at - 20 °C or - 80 °C if longer periods of storage were needed.

In order to reduce as much as possible the analytical error, Se determination has been performed by using a method in atomic absorption spectrophotometry with graphite furnace that has been expressly studied and characterized as "known accuracy method" [25]. The analytical variability of the method has been constantly checked by the adoption of appropriate internal quality controls and by preparing control charts with measurements carried out on control samples with certified title (SRM 909, NBS) (Fig. 1).

Where necessary and feasible, we have proceeded to the separation of the reference values in subclasses (by area, age and sex).

The distributions (to point out deviations from the gaussian) have been examined: in certain cases statistical tests have been performed.

The interval that includes the central fraction of the 95th percentile of the distribution of the reference values has been conventionally adopted as reference interval. The fractiles 0.025-0.975 are the reference limits.

In the estimate of the reference interval by a non parametric method, the lower reference limit will be the value corresponding to the rank number of the 0.025

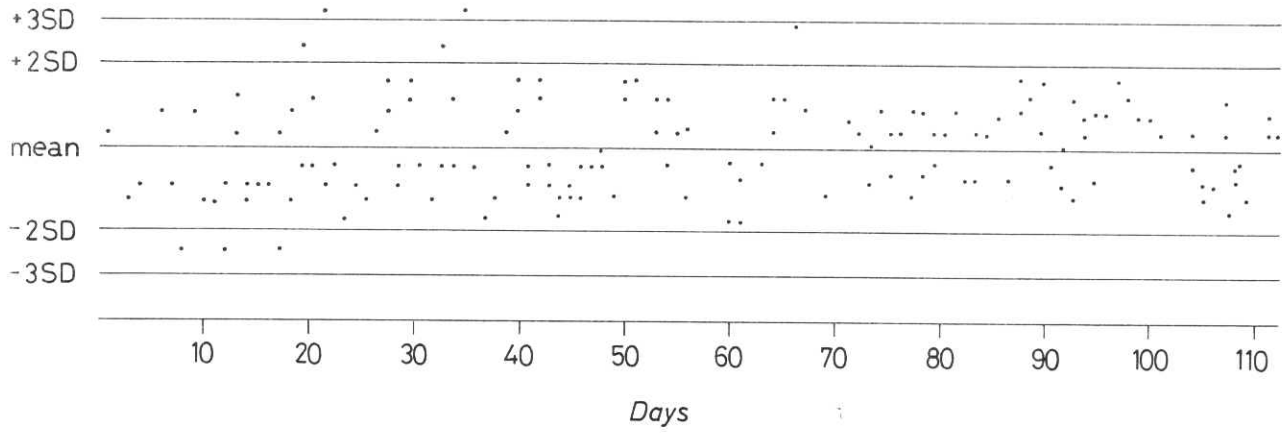

Fig. 1. - Control chart prepared for the internal quality control of serum selenium levels determination. Human serum SRM 909 of the National Institute of Standards and Technology was used. Analysis of this material yielded the value of 106 (SD 2.4) µg/l (expected value 106 µg/l).

fractile; the upper limit will be the value corresponding to the 0.975 fractile.

In the estimate by a parametric method, the reference limits are given by: $x \pm 1.96$ SD (x = arithmetic mean of the reference values; SD = standard deviation).

Study population

Sezze group. - 1804 subjects, males and females, age between 20 and more than 69 years, enrolled in the Sezze municipality (province of Latina), for the Di.S.Co. Project (Sezze district, communitary control of the degenerative diseases).

Priverno group. - 1709 subjects, males and females, age between 20 and more than 69 years, enrolled in the Priverno municipality (province of Latina), for the Di.S.Co. Project.

Lenola group. - 243 subjects, males and females, age between 20 and 69 years, enrolled in the Lenola municipality (province of Latina) for the MoniCA Project (monitoring of the cardiovascular diseases).

Tivoli group. - 274 subjects, males and females, age between 30 and 69 years, random subsample of about 2000 individuals enrolled in a project for the study of the prevalence of the cholelithiasis (MICOL project, Italian cholelithiasis multicentre study).

Crevalcore group and Montegiorgio group. - 80 and 91 subjects respectively, age between 65 and 84 years, males, random subsamples of the population in that age group (approximately 900 subjects) from the municipalities of Crevalcore (province of Bologna) and of Montegiorgio (province of Ascoli Piceno): they are the survivors of the population enrolled in 1960 (1712 subjects in total) for the "seven countries study", an epidemiological investigation on cardiovascular diseases.

Latina I group. - 187 children, males and females, age between 9 and 13 years, random subsample of a group of children (848 subjects) from the elementary and junior high schools of Latina, enrolled for the "health project" designed to study the epidemiology and the prevention of arteriosclerosis during childhood.

Latina II group. - It includes all the 848 children described above: the children were examined when their age was between 11 and 15 years.

Naples group. - 182 subjects, males and females, age between 6 and 11 years, random subsample of a group of children (approximately 500 subjects) enrolled in the city of Naples for a study on the epidemiology of hepatitis B.

Subjects affected by several diseases

Group of 23 children affected by phenylketonuria, undergoing a phenylalanine restricted diet, of age between 6 months and 8 years.

Group of 17 subjects affected by clinical coronarosclerosis with X-ray evidence.

Group of 18 subjects affected by seropositive rheumatoid arthritis.

Group of 91 subjects affected by systemic *Lupus erythematosus* (SLE), 10 of these were in serious condition.

Group of 47 subjects affected by different neoplasms (cancer of the stomach, prostate, kidney, colon, lung, breast, rectum, bladder); these patients were in fairly good general condition.

Results

Reference values in good health condition

The distributions of the reference values of the serum Se levels, means and standard deviations, medians, minimum and maximum values, observed in the different population groups examined, are shown in Figs 2-9 and in Table 1.

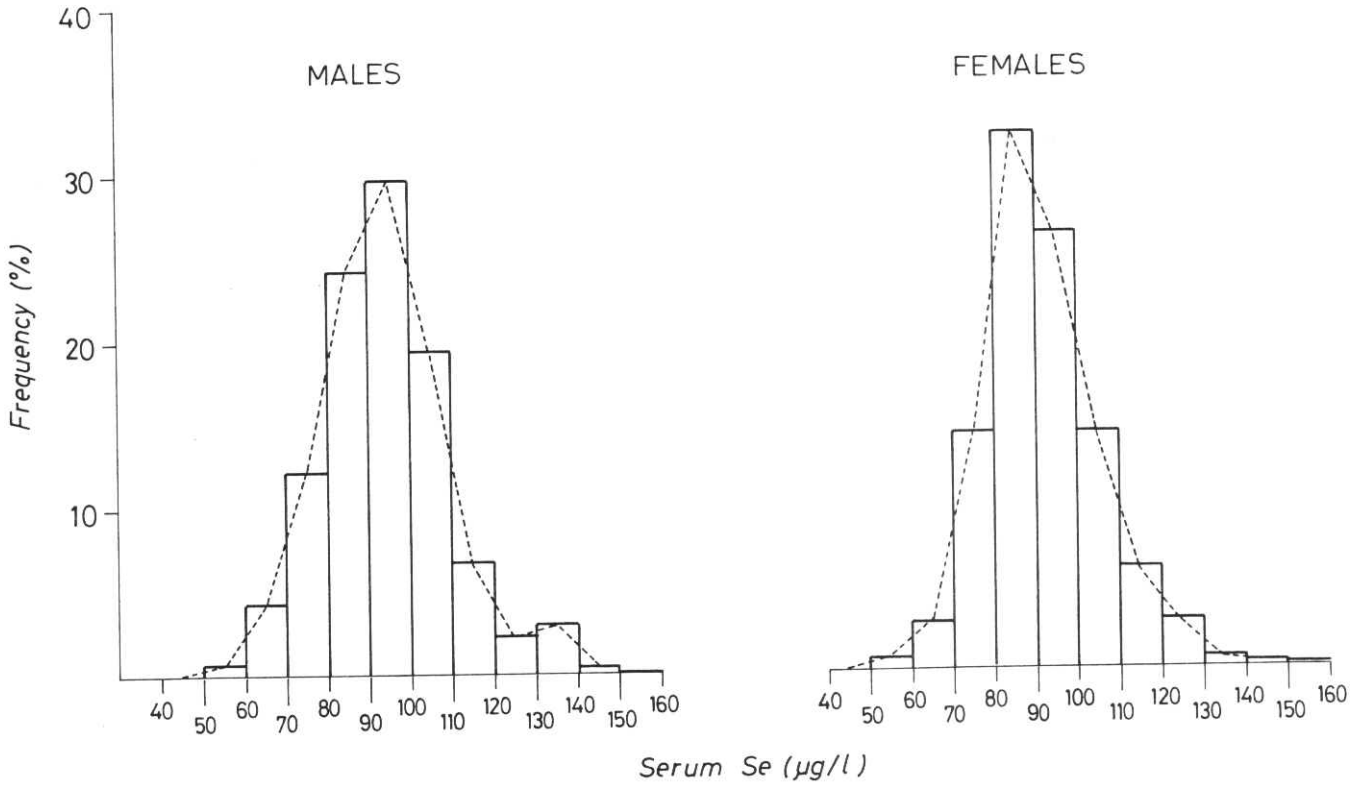

Fig. 2. - Serum Se values distribution observed in the Sezze group (adults).

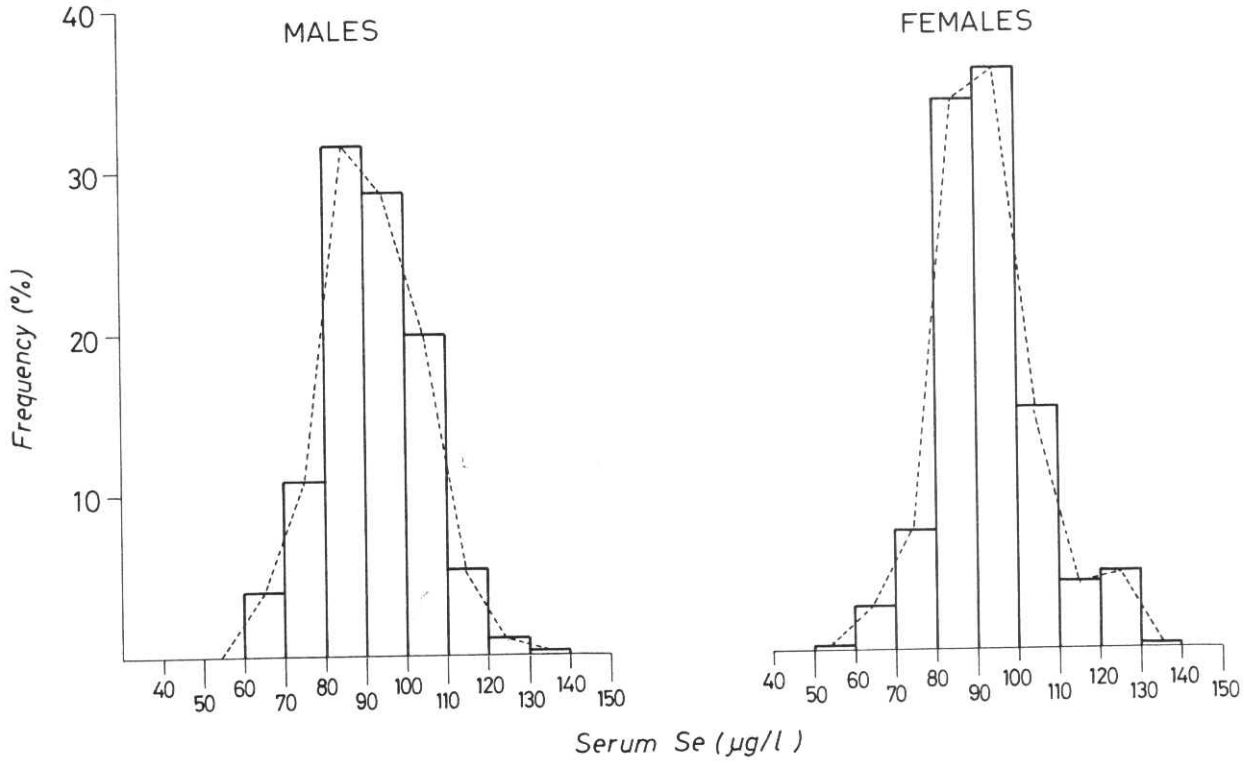

Fig. 3. - Serum Se values distribution observed in the Priverno group (adults).

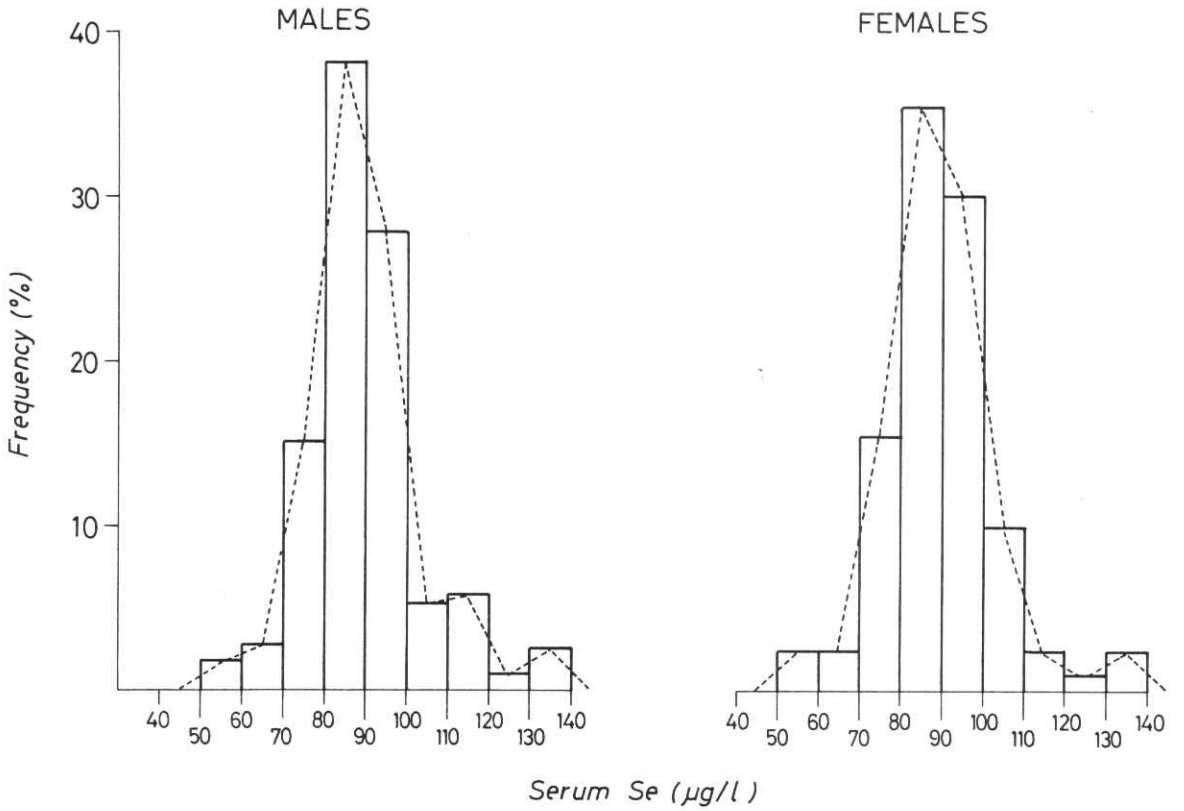

Fig. 4. - Serum Se values distribution observed in the Lenola group (adults).

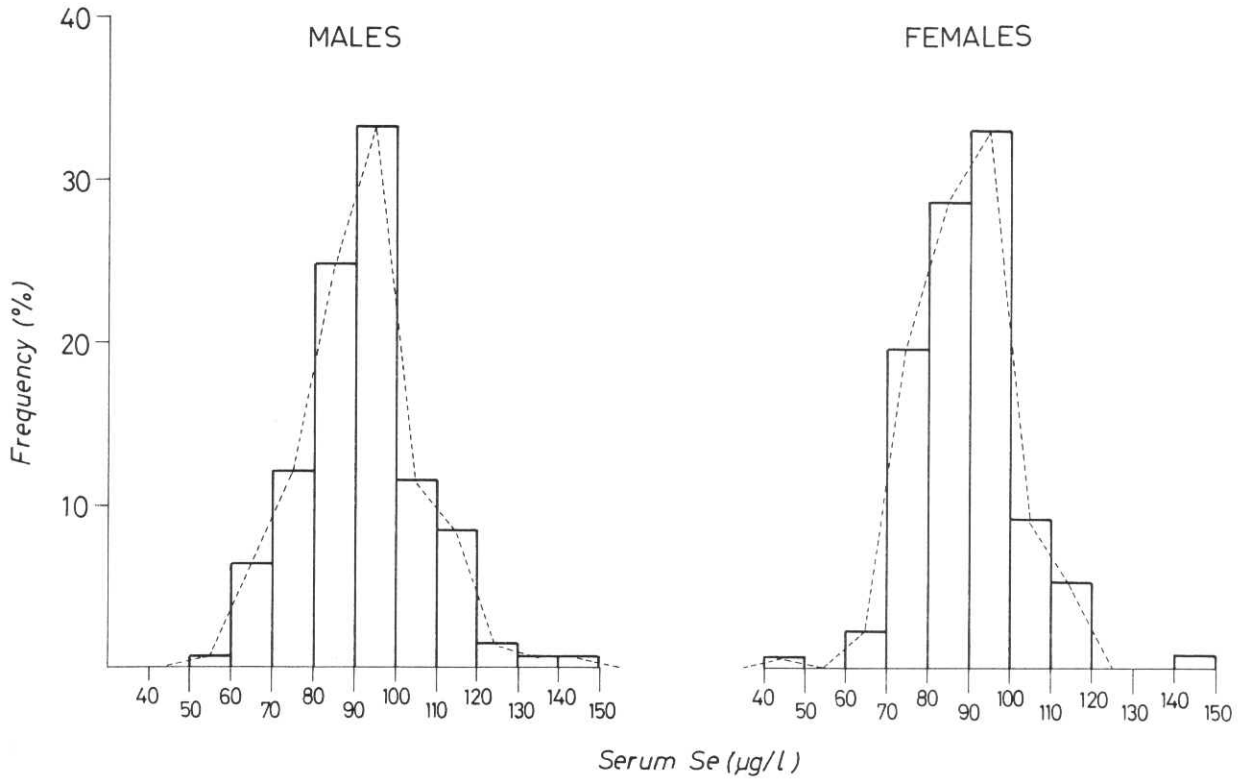

Fig. 5. - Serum Se values distribution observed in the Tivoli group (adults).

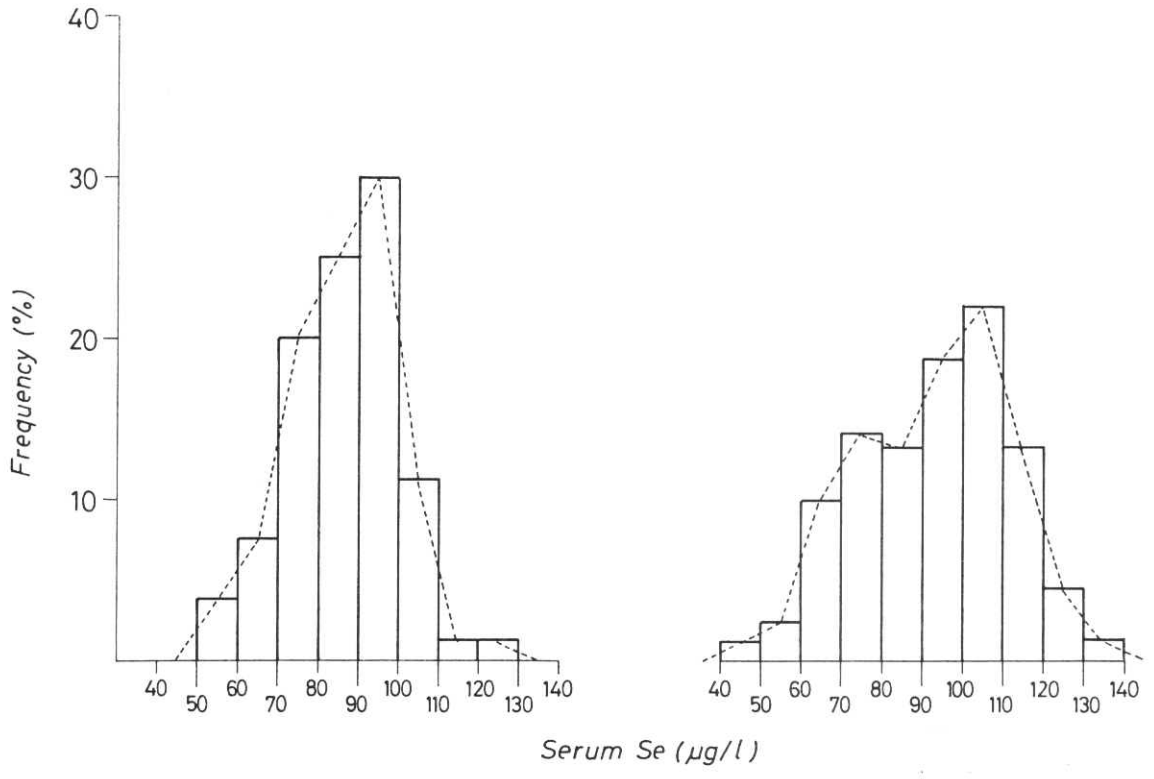

Fig. 6. - Serum Se values distribution observed in the Crevalcore group, left, and the Montegiorgio group, right (adult males, aged subjects).

Table 1. - *Serum Se levels (µg/l) and male-female difference observed in the different population groups examined*

Group		n	Mean	SD	Male-female difference	Median	min.	max.
Sezze	M	813	92.7	14.5	t = 2.15 (p < 0.032)	93	54	154
adults	F	991	91.3	14.1		90	49	150
Priverno	M	753	91.4	12.6	n.s.	91	56	130
adults	F	956	90.8	11.2		91	49	138
Lenola	M	119	88.5	13.8	n.s.	88	55	138
adults	F	124	88.9	13.6		88.5	55	138
Tivoli	M	141	91.3	14.4	n.s.	91.4	57.5	149
adults	F	133	89.1	13.2		89.5	49	141
Crevalcore	M	80	86.9	13.1		88.2	50.7	120
elderly								
Montegiorgio	M	91	92.9	18.7		96.9	45.8	130
elderly								
Latina I	M	97	81.9	9.9	t = 2.83 (p < 0.005)	81.2	52.5	104
children	F	90	77.9	9.3		78.1	59.7	104
Latina II	M	442	82.6	10.2	n.s.	81.5	48	120
children	F	406	82.7	11.2		82	48	126
Naples	M + F	182	82.1	12.5		81	55	133
children								

n.s. = not significant

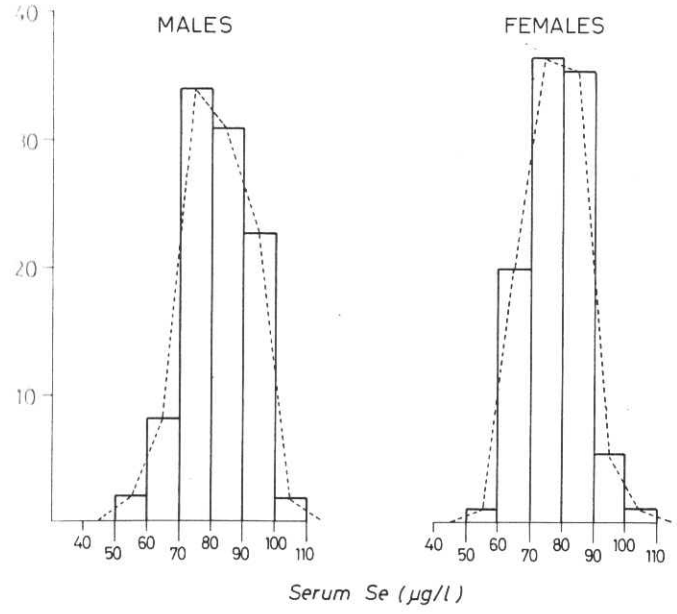

Fig. 7. - Serum Se values distribution observed in the Latina I group (children 9-13 years old).

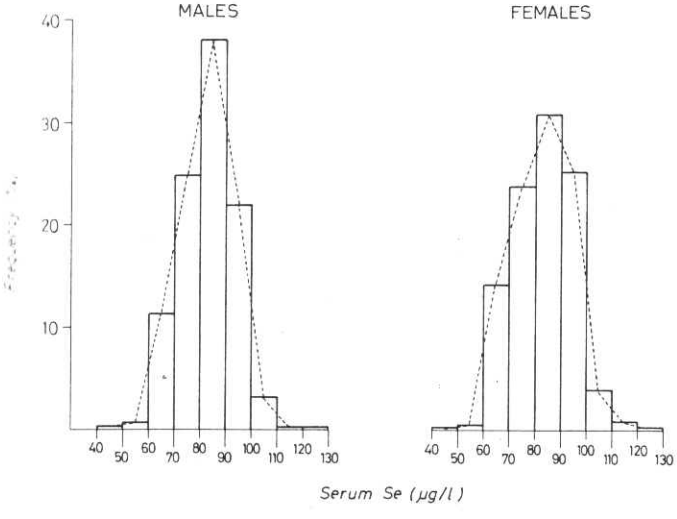

Fig. 8. - Serum Se values distribution observed in the Latina II group (children 11-15 years old).

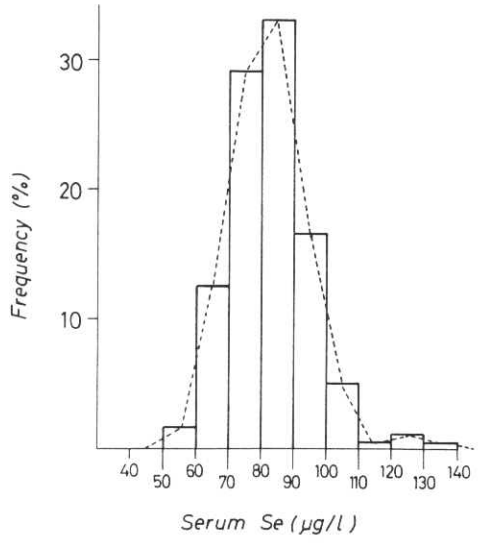

Fig. 9. - Serum Se values distribution observed in the Naples group (children, males and females, 6-11 years old).

The data presented in Table 1 do not show relevant differences of the mean values of serum Se levels in males and females. These differences (slightly lower values in females) are statistically significant in the Sezze group ($p = 0.032$) and in the Latina I group ($p = 0.017$).

Also the intervals of the serum Se levels in males and females, in all the examined groups, are very similar.

Instead, in children the mean levels are considerably lower than in adults.

Also the mean level observed in a group of aged subjects (Crevalcore group) is slightly reduced if compared to that of middle-aged adults. In the other group of aged subjects (Montegiorgio group) the mean value of the serum Se levels does not differ from the mean values of the middle-aged adults.

No considerable differences can be found between the mean values of the four adult groups - Sezze, Priverno, Tivoli, and Lenola - even if the mean values of this last group are 2-3 units lower than those of the other groups.

Also in the three groups of children the mean values do not seem to be different.

The reference intervals (Table 2), computed for the four adult groups and separately for males and females, are all, with good approximation, comparable.

The reference intervals computed for the three children groups show an upper reference limit that is lower than that of the adults, while the reference intervals for the aged subjects show a lower reference limit below that of the adults.

Tables 3 and 4 show the reference values, stratified by age group and sex, concerning the Sezze and Priverno groups, the only groups with a numerosity that allows such an analysis of the data. It is clear, for the males of both groups, a trend towards lower reference values when age increases above 60-65 years. In the Priverno group (Table 4) the serum Se values in subjects of age > 60 years are significantly lower than those of the subjects with age < 60 years ($t = 2.08$; $p = 0.038$). In the Sezze group (Table 3), only the values for the age group > 69 years are statistically different from the global values of the subjects with age ≤ 64 years ($t = 3.28$; $p = 0.0013$).

The statistical analysis of the distributions observed in the Sezze, Priverno, and Lenola groups (Figs 2-4) show significant differences from the normal distribution both with the t test on the Kurtosi and skewness, and with the Kolmogorov-Smirnov test. However, it has to be noted that such difference is not particularly relevant, since the distributions are substantially symmetrical and there are no systematic deviations (i.e., a tendency towards a log-normal distribution). Such lack of relevance is confirmed, for all the distributions, by the excellent agreement of the mean and median values (Table 1) and by the good agreement between the values of the reference limits worked out with the non-parametric and parametric methods, the latter ones being computed from the original distribution (Table 2).

Table 2. - *Reference intervals of serum Se levels (μg/l) computed with the non-parametric and parametric methods*

Group		Non-parametric method (2.5 - 97.5 percentiles)	Parametric method (mean ± 1.96 SD)
Sezze	M	64 - 122	63.7 - 121.8
adults	F	67 - 121	69.1 - 119.4
Priverno	M	66 - 116	66.2 - 116.7
adults	F	69 - 114	68.4 - 113.2
Lenola	M	66 - 127	61 - 116.1
adults	F	66 - 127	61.6 - 116.1
Tivoli	M	64 - 120.6	62.4 - 120.2
adults	F	66.7 - 119	63.1 - 115.9
Crevalcore	M	58 - 110	60.8 - 113.1
elderly			
Montegiorgio	M	56 - 121.6	55.5 - 130.3
elderly			
Latina I	M	64 - 99	61.4 - 101
children	F	62.5 - 97.8	59.3 - 96.5
Latina II	M	63 - 105	62.2 - 102.9
children	F	60 - 105	60.3 - 105
Naples	M + F	62 - 106	57 - 107
children			

Table 3. - *Age and sex specific serum Se levels (μg/l) observed in the Sezze population*

Age group (yrs)	Males					Females				
	n	Mean	SD	min.	max.	n	Mean	SD	min.	max.
20-24	39	92.0	10.50	73	112	48	87.4	10.75	70	121
25-29	59	94.4	11.90	72	121	78	90.8	10.68	55	116
30-34	63	98.3	18.40	75	154	78	91.9	11.44	66	129
35-39	81	96.4	11.39	67	127	90	89.9	14.23	64	148
40-44	79	95.3	11.76	64	127	92	91.8	13.97	54	150
45-49	81	93.5	14.69	60	137	122	90.9	12.45	49	137
50-54	73	94.8	14.45	67	143	111	92.9	12.94	60	129
55-59	89	92.7	14.23	60	133	125	93.2	13.99	58	130
60-64	120	90.0	13.75	54	121	129	89.2	14.68	53	133
65-69	92	87.1	15.47	55	148	82	93.3	15.99	56	149
> 69	32	80.7	16.09	54	126	31	87.6	13.41	66	131

n = number of subjects examined

Table 4. - *Age and sex specific serum Se levels (μg/l) observed in the Priverno population*

Age group (yrs)	Males					Females				
	n	Mean	SD	min.	max.	n	Mean	SD	min.	max.
20-24	30	94.5	9.91	64	110	46	90.4	9.65	69	115
25-29	54	96.6	11.22	76	121	37	90.6	10.40	72	117
30-34	61	94.3	10.57	72	115	67	91.2	8.83	69	112
35-39	59	94.8	9.20	79	118	77	91.0	11.41	63	121
40-44	56	91.6	11.26	65	118	84	90.1	10.45	72	117
45-49	83	94.3	9.84	77	119	109	92.0	10.94	66	138
50-54	88	92.2	14.28	59	128	104	92.0	11.29	66	127
55-59	99	90.8	12.99	62	130	128	92.5	12.11	49	126
60-64	111	87.1	12.70	61	125	162	89.6	11.58	60	122
65-69	89	86.5	10.93	63	123	106	88.9	11.86	62	122
> 69	21	84.0	14.41	56	113	34	89.2	11.90	59	118

n = number of subjects examined

Serum Se levels in several diseases

Tables 5 and 6, and the diagrams in Figs 10-14 show, in an absolutely preliminary way, the values and the distributions of the serum Se levels determined in groups of subjects affected by several different diseases.

As it is shown in Table 5 and in the diagram of Fig. 10, a dramatic reduction of the Se levels has been observed in children of 6-12 months of age with phenylketonuria; a less drastic reduction has also been observed in children of > 1 year of age affected by the same disorder. The very low serum levels of this micronutrient observed in these two groups are due to their specific diet that demonstrates to be inadequately supplemented with Se, while the difference between the two groups has to be ascribed to the different dietetic restrictions.

The serum Se levels are also sharply reduced in the subjects affected by SLE in an active phase of disease. For these subjects an improvement of the clinical condition is accompanied by a return to normal values of the serum Se levels (Table 6).

Table 5. - *Serum Se levels (μg/l) observed in groups of subjects affected by different diseases*

Disease	n	Mean	SD	Median	min.	max.
Phenilketonuria						
6 - 12 months	11	28.5	9.3	32	10	40
1 - 8 years	11	61.6	16.8	63	40	93
Coronarosclerosis	17	84.7	10.5	84.5	60	105
Rheumatoid arthritis	18	85.1	13.3	87.3	60	105
Cancer	43	83.2	18.3	82	40	115
SLE (all the cases)	91	88.1	15.8	87	40	135
SLE (patients in serious conditions)	10	67.9	2.7	69.5	66	76

n = number of patients examined

Table 6. - *Serum Se levels (μg/l) in ten SLE patients before and after 12 weeks of steroid therapy*

Patients	Before therapy	After therapy
1	68	118
2	68	107
3	70	101 *(a)*
4	70	99 *(a)*
5	70	98 *(a)*
6	69	92
7	68	90
8	72	89
9	66	86
10	76	82 *(a)*
Mean	67.9	96.7
SD	2.7	10.7

(a) patients with inactive disease

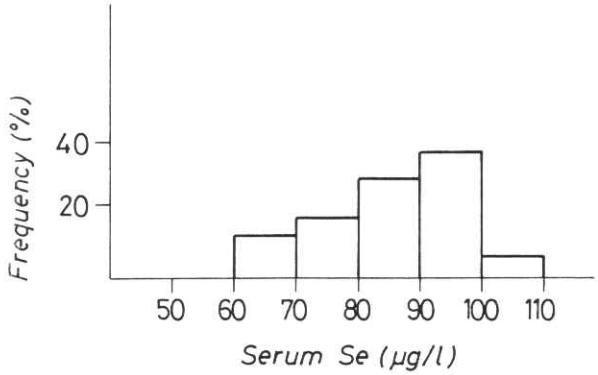

Fig. 10. - Serum Se values distribution observed in the group of children with phenylketonuria.

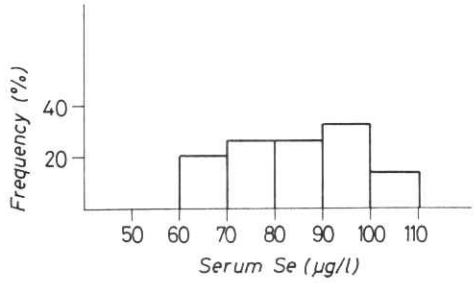

Fig. 11. - Serum Se values distribution observed in the group of subjects with coronaropathy.

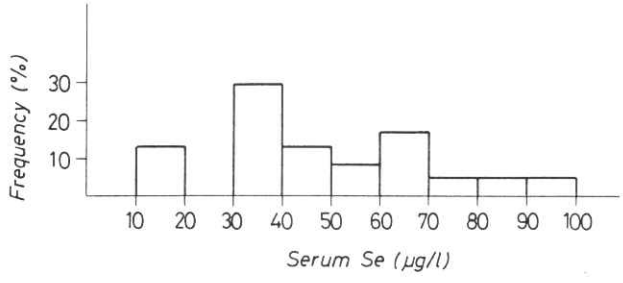

Fig. 12. - Serum Se values distribution observed in the group of subjects with rheumatoid arthritis.

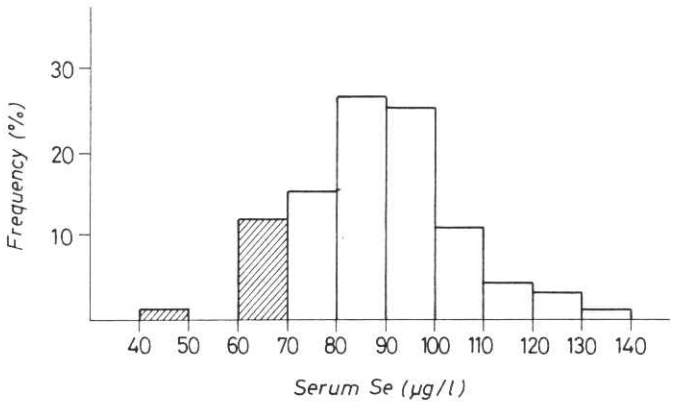

Fig. 13. - Serum Se values distribution observed in the group of subjects affected by systemic *Lupus erythematosus*. The shaded diagram describes subjects with a more serious clinical history.

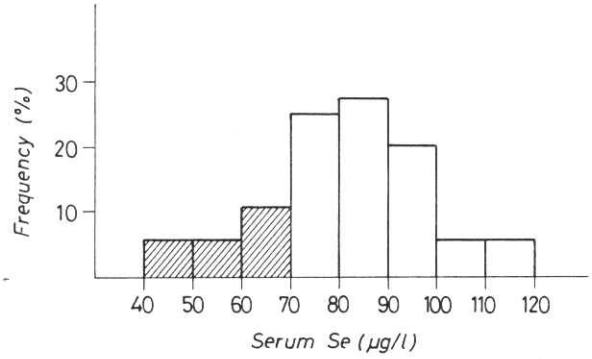

Fig. 14. - Serum Se values distribution observed in the group of subjects with cancer. The shaded diagram describes active cancers and/or liver metastases.

Only limited reductions of the Se levels are observed in subjects affected by rheumatoid arthritis or by coronarosclerosis.

Cancer patients, as a whole, show a limited reduction of the Se levels. However it has been noted, even from the scanty data collected so far, that subjects with colon, stomach, pancreas, ovary, and lung cancer often show much more marked reductions, while no significant reduction has been observed in patients with breast cancer.

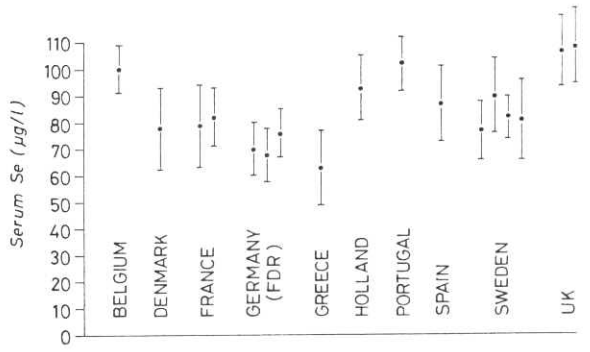

Fig. 15. - Serum Se values distribution observed in population groups in different European areas (modified from Thorling and Overvad [27]).

Conclusions

The results we have obtained allow us to draw the following conclusions concerning the serum Se levels in Italy:

1) the average levels of Serum Se in the Italian population seem to be between the low levels reported for New Zealand and Finland and the high levels observed in the United States of America and in Venezuela [26];

2) the average levels of the adult Italian population are approximately 5 units higher than the average of the values observed in 10 European countries (Fig. 15) by Thorling and Overvad [27] in groups of adult subjects of 20-60 years of age;

3) variations of serum Se levels in the Italian population, deriving from different geographical areas of residence, do not seem to occur, even if the number of different examined areas is limited;

4) there are no differences between serum Se levels in males and females;

5) limited reductions of the serum Se levels can occur in subjects above 65 years of age;

6) the serum Se levels of children (< 15 years of age) are approximately 15 units lower than those observed in the adults.

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