

Supplementary Materials



Figure S1. Map of the areas selected for the recruitment: SIN Brescia-Caffaro area (northern Italy, blue circle), SIN Sacco River Valley (central Italy, green circle) and the northern area of city of Naples, “Land of Fires” (southern Italy, orange circle).

Table S1. General characteristics of the study population reported for each area as mean value \pm standard deviation.

| | BSC | LF | SRV | q value ¹ |
|------------------------------|-----------------|-----------------|-----------------|----------------------|
| N | 144 | 137 | 51 | - |
| Age (year) | 20.0 \pm 1.2 | 18.8 \pm 1.3 | 18.2 \pm 0.5 | 0.0001 |
| Weight (Kg) | 70.6 \pm 9.0 | 71.6 \pm 9.7 | 73.4 \pm 8.7 | n.s. |
| Height (cm) | 177.3 \pm 5.9 | 175.6 \pm 6.5 | 177.3 \pm 6.8 | n.s. |
| BMI (Kg m ⁻²) | 22.4 \pm 2.3 | 23.2 \pm 2.7 | 23.4 \pm 3.0 | 0.0318 |
| Abdominal circumference (cm) | 83.5 \pm 5.8 | 88.2 \pm 7.2 | 83.0 \pm 7.7 | 0.0001 |

¹p-values were calculated with the Kruskal-Wallis Test; n.s.: not significant p value (>0.05)

Table S2. Lifestyle factors (PREDIMED and IPAQ scores) and semen quality parameters of subjects in each area reported as mean value \pm standard deviation

| | BSC | LF | SRV | q value ¹ |
|---|-----------------|-----------------|-----------------|----------------------|
| PREDIMED score | 6.9 \pm 2.1 | 7.4 \pm 2.3 | 7.2 \pm 2.5 | n.s. |
| IPAQ score (Met) | 2380.4 \pm | 2858.9 \pm | 3414.6 \pm | n.s. |
| | 2105.7 | 3219.5 | 2729.0 | |
| Volume (mL) | 2.9 \pm 1.4 | 2.5 \pm 1.1 | 2.9 \pm 1.6 | n.s. |
| pH | 8.1 \pm 0.4 | 7.9 \pm 0.1 | 7.8 \pm 0.2 | 0.0001 |
| Spermatic concentration (10⁶ ml⁻¹) | 67.3 \pm 45.9 | 47.6 \pm 32.7 | 52.3 \pm 37.9 | 0.001 |
| Total Motility (%) | 39.8 \pm 20.0 | 42.7 \pm 26.2 | 26.7 \pm 21.6 | 0.0002 |
| Progressive motility (%) | 27.1 \pm 18.1 | 28.9 \pm 20.5 | 16.8 \pm 15.6 | 0.0008 |
| Cells with normal morphology (%) | 6.4 \pm 4.4 | 7.1 \pm 7.3 | 5.0 \pm 3.2 | n.s. |

¹p-values were calculated with the Kruskal-Wallis Test; n.s.: not significant p value (>0.05)

Table S3. Limit of detection (LOD) and limit of quantification (LOQ) in the final sample expressed in $\mu\text{g/L}$.

| | Unit | Ca | Mg | Na | K | Cu | Fe | Mn | Ni | Se | Zn | Al | As | Ba |
|------------|-----------------|-----|-----|-----|-----|------|-----|-----|------|-----|-----|-----|-----|------|
| LOD | $\mu\text{g/L}$ | 200 | 200 | 200 | 200 | 4.4 | 3.4 | 0.4 | 4.2 | 1.1 | 1.8 | 1.2 | 0.2 | 6.6 |
| LOQ | $\mu\text{g/L}$ | 500 | 500 | 500 | 500 | 11.0 | 8.5 | 1.0 | 10.5 | 2.8 | 4.5 | 3.0 | 0.5 | 16.5 |
| | Unit | Be | Cd | Co | Cr | Hg | Li | Pb | Rb | Sb | Sn | Sr | U | V |
| LOD | $\mu\text{g/L}$ | 2.6 | 0.2 | 0.4 | 0.8 | 0.2 | 0.4 | 0.1 | 3.0 | 0.2 | 0.2 | 0.2 | 0.2 | 1.8 |
| LOQ | $\mu\text{g/L}$ | 6.5 | 0.5 | 1.0 | 2.0 | 0.5 | 1.0 | 0.2 | 7.5 | 0.5 | 0.5 | 0.5 | 0.5 | 4.5 |

Table S4. Blood serum sample below limit of detection (LOD) expressed in %.

| | Whole cohort | | BSC | | LF | | SRV | |
|-----------|--------------|------|-----|--------|-----|--------|-----|--------|
| | n | % | n | median | n | median | n | median |
| Ca | 332 | 0 | 144 | 0 | 137 | 0 | 51 | 0 |
| Mg | 332 | 0 | 144 | 0 | 137 | 0 | 51 | 0 |
| Na | 332 | 0 | 144 | 0 | 137 | 0 | 51 | 0 |
| K | 332 | 0 | 144 | 0 | 137 | 0 | 51 | 0 |
| Cu | 332 | 0 | 144 | 0 | 137 | 0 | 51 | 0 |
| Mn | 332 | 13,9 | 144 | 13,9 | 137 | 0 | 51 | 51,0 |
| Ni | 332 | 100 | 144 | 100 | 137 | 100 | 51 | 100 |
| Se | 332 | 0 | 144 | 0 | 137 | 0 | 51 | 0 |
| Zn | 332 | 0 | 144 | 0 | 137 | 0 | 51 | 0 |
| Al | 332 | 100 | 144 | 100 | 137 | 100 | 51 | 100 |
| As | 332 | 0 | 144 | 0 | 137 | 0 | 51 | 0 |
| Ba | 332 | 21,4 | 144 | 10,4 | 137 | 24,8 | 51 | 43,1 |
| Be | 332 | 78,0 | 144 | 76,4 | 137 | 72,3 | 51 | 98,0 |
| Cd | 332 | 56,6 | 144 | 72,9 | 137 | 37,2 | 51 | 62,8 |
| Co | 332 | 100 | 144 | 100 | 137 | 100 | 51 | 100 |
| Cr | 332 | 100 | 144 | 100 | 137 | 100 | 51 | 100 |
| Hg | 332 | 43,4 | 144 | 34,0 | 137 | 35,8 | 51 | 90,2 |
| Li | 332 | 2,5 | 144 | 5,6 | 137 | 0 | 51 | 0 |
| Pb | 332 | 4,2 | 144 | 5,6 | 137 | 4,4 | 51 | 0 |
| Rb | 332 | 0 | 144 | 0 | 137 | 0 | 51 | 0 |
| Sb | 332 | 12,0 | 144 | 4,9 | 137 | 17,5 | 51 | 17,6 |
| Sn | 332 | 15,4 | 144 | 33,3 | 137 | 0,73 | 51 | 3,9 |
| Sr | 332 | 0 | 144 | 0 | 137 | 0 | 51 | 0 |
| U | 332 | 41,9 | 144 | 50,0 | 137 | 29,9 | 51 | 51,0 |
| V | 332 | 100 | 144 | 100 | 137 | 100 | 51 | 100 |

Table S5. Semen sample below limit of detection (LOD) expressed in %.

| | Whole cohort | | BSC | | LF | | SRV | |
|-----------|--------------|------|-----|--------|-----|--------|-----|--------|
| | n | % | n | median | n | median | n | median |
| Ca | 268 | 0 | 113 | 0 | 100 | 0 | 55 | 0 |
| Mg | 268 | 0 | 113 | 0 | 100 | 0 | 55 | 0 |
| Na | 268 | 0 | 113 | 0 | 100 | 0 | 55 | 0 |
| K | 268 | 0 | 113 | 0 | 100 | 0 | 55 | 0 |
| Cu | 268 | 0 | 113 | 0 | 100 | 0 | 55 | 0 |
| Mn | 268 | 0 | 113 | 0 | 100 | 0 | 55 | 0 |
| Fe | 268 | 0 | 113 | 0 | 100 | 0 | 55 | 0 |
| Ni | 268 | 40,7 | 113 | 3,5 | 100 | 93,0 | 55 | 21,8 |
| Se | 268 | 0 | 113 | 0 | 100 | 0 | 55 | 0 |
| Zn | 268 | 0 | 113 | 0 | 100 | 0 | 55 | 0 |
| Al | 268 | 100 | 113 | 100 | 100 | 100 | 55 | 100 |
| As | 268 | 0,37 | 113 | 0,88 | 100 | 0 | 55 | 0 |
| Ba | 268 | 23,9 | 113 | 56,6 | 100 | 0 | 55 | 0 |
| Be | 268 | 72,0 | 113 | 65,5 | 100 | 67,0 | 55 | 94,6 |
| Cd | 268 | 94,8 | 113 | 87,6 | 100 | 100 | 55 | 100 |
| Co | 268 | 100 | 113 | 100 | 100 | 100 | 55 | 100 |
| Cr | 268 | 100 | 113 | 100 | 100 | 100 | 55 | 100 |
| Hg | 268 | 33,3 | 113 | 20,4 | 100 | 19,0 | 55 | 70,9 |
| Li | 268 | 0,37 | 113 | 0,88 | 100 | 0 | 55 | 0 |
| Pb | 268 | 4,1 | 113 | 9,7 | 100 | 0 | 55 | 0 |
| Rb | 268 | 0 | 113 | 0 | 100 | 0 | 55 | 0 |
| Sb | 268 | 60,4 | 113 | 41,6 | 100 | 66,0 | 55 | 89,1 |
| Sn | 268 | 3,0 | 113 | 0,88 | 100 | 6,0 | 55 | 1,8 |
| Sr | 268 | 0 | 113 | 0 | 100 | 0 | 55 | 0 |
| U | 268 | 97,0 | 113 | 96,5 | 100 | 96,0 | 55 | 100 |
| V | 268 | 100 | 113 | 100 | 100 | 100 | 55 | 100 |

Table S6. Precision, accuracy and recovery data evaluated on blood serum samples.

| | Unit | C_{obs} | C_{spike} | CV% | Rec % |
|-----------|-------------|------------------------|--------------------------|------------|--------------|
| Ca | mg/L | 187 | - | 5.6 | - |
| Mg | mg/L | 35.2 | - | 9.0 | - |
| Na | mg/L | 227 | - | 2.8 | - |
| K | mg/L | 4392 | - | 4.8 | - |
| Cu | µg/L | 60.4 | 100 | 20 | 81 |
| Mn | µg/L | - | 100 | 28 | 92 |
| Ni | µg/L | 8.8 | 100 | 22 | 112 |
| Se | µg/L | 3.6 | 100 | 14 | 80 |
| Zn | µg/L | 37.4 | 100 | 7.0 | 96 |
| Al | µg/L | - | 100 | 11 | 104 |
| As | µg/L | - | 100 | 25 | 85 |
| Ba | µg/L | - | 100 | 13 | 116 |
| Be | µg/L | - | 100 | 19 | 98 |
| Cd | µg/L | - | 100 | 21 | 93 |
| Co | µg/L | - | 100 | 11 | 82 |
| Cr | µg/L | - | 100 | 12 | 107 |
| Hg | µg/L | 0.8 | 100 | 27 | 95 |
| Li | µg/L | 12.2 | 100 | 16 | 116 |
| Pb | µg/L | - | 100 | 17 | 93 |
| Rb | µg/L | 11.1 | 100 | 23 | 116 |
| Sr | µg/L | 1.3 | 100 | 25 | 118 |
| V | µg/L | - | 100 | 14 | 119 |

¹ C_{obs}, observed concentration in unfortified blood serum sample² C_{spike}, known added spiked concentration³ CV%, mean value of coefficient of variation⁴ Rec%, mean recovery based on spiked concentration

Table S7. Precision, accuracy and recovery data evaluated on semen samples.

| | Unit | C _{obs} | C _{spike} | CV% | Rec % |
|-----------|------|------------------|--------------------|-----|-------|
| Cu | µg/L | 8.0 | 100 | 24 | 90 |
| Mn | µg/L | 0.9 | 100 | 25 | 86 |
| Ni | µg/L | - | 100 | 2 | 105 |
| Se | µg/L | 3.7 | 100 | 10 | 88 |
| Zn | mg/L | 4.6 | - | 5 | - |
| Al | µg/L | - | 100 | 8 | 115 |
| As | µg/L | 3.2 | 100 | 15 | 85 |
| Ba | µg/L | - | 100 | 5 | 118 |
| Be | µg/L | - | 100 | 5 | 94 |
| Cd | µg/L | - | 100 | 4 | 82 |
| Co | µg/L | - | 100 | 3 | 114 |
| Cr | µg/L | - | 100 | 10 | 108 |
| Fe | µg/L | 165 | 100 | 6 | 120 |
| Hg | µg/L | 0.5 | - | 22 | - |
| Li | µg/L | 1.4 | 100 | 24 | 118 |
| Pb | µg/L | - | 100 | 4 | 85 |
| Rb | µg/L | 75 | 100 | 5 | 116 |
| Sr | µg/L | 3.5 | 100 | 9 | 114 |
| V | µg/L | - | 100 | 9 | 114 |

¹ C_{obs}, observed concentration in unfortified blood serum sample² C_{spike}, known added spiked concentration³ CV%, mean value of coefficient of variation⁴ Rec%, mean recovery based on spiked concentration**Table S8.** Recovery data evaluated on CRMs.

| | | | | This work | |
|------------------|-----------|------------------------------|---------------|-------------------------|-------------------------|
| | Unit | Certified value ¹ | | Mean value ² | Recovery % ³ |
| BCR - 304 | Ca | mmol/L | 2.201 ± 0.019 | 2.496 ± 0.255 | 113 |
| BCR - 304 | Mg | mmol/L | 1.85 ± 0.03 | 1.67 ± 0.15 | 90 |
| BCR - 304 | Li | mmol/L | 0.985 ± 0.029 | 1.126 ± 0.105 | 114 |
| BCR - 638 | Se | µg/L | 104 ± 7 | 111 ± 18 | 107 |
| BCR - 638 | Zn | µg/L | 1430 ± 210 | 1602 ± 273 | 112 |

¹ unweighed mean value and expanded uncertainty; ² unweighed mean value and standard deviation of 5 replicates; ³ unweighed mean value of 5 replicates

Table S9. Reference Values and biomonitoring data expressed as µg/L for macro and essential trace elements in blood serum.

| | WHO [1,2] | | ISS 10/22 [3] | | ISS 11/9 [4] | | SIVR [5] | |
|-----------|-------------------------|------|---------------|------|----------------------|------|-------------------------|------|
| | range | year | range | year | range ^{a,b} | year | range ^b | year |
| Ca | - | - | 52577 - 70632 | 2005 | - | - | - | - |
| Mg | - | - | 14643 - 20255 | 2005 | - | - | - | - |
| Cu | 800 – 1200 ^c | 1996 | 601 - 1373 | 1990 | - | - | 500 - 1250 | 2005 |
| | - | - | 648 - 1301 | 2005 | - | - | 600 – 1600 ^d | 2011 |
| Mn | 0.5 - 1 | 1996 | 0.3 - 0.9 | 1990 | 0.47 - 1.38 | - | 0.1 - 1.1 | 2005 |
| | - | - | 0.31 - 1.02 | 2005 | - | - | - | - |
| Ni | < 1 - 2 | 1996 | 0.10 - 1.25 | 2005 | 0.09 - 0.95 | - | 0.1 - 1 | 2005 |
| | - | - | 0.26 - 0.75 | 2006 | - | - | - | - |
| Se | 75 - 120 | 1996 | 56 - 105 | 1990 | - | - | 50 - 130 | 2005 |
| | 39 - 197 | 2005 | - | - | - | - | - | - |
| Zn | 800 - 1100 | 1996 | 587 - 1215 | 1990 | - | - | 600 - 1080 | 2005 |
| | - | - | 597 - 1028 | 2005 | - | - | 800 - 1600 | 2011 |

^a data referred to adults aged between 18 and 35; ^b data expressed as 5° - 95° percentiles; ^c referred to males; ^d referred to adults

Table S10. Reference Values and biomonitoring data expressed as µg/L for additional and not essential trace elements in blood serum.

| | WHO [1,2] | | ISS 10/22 [3] | | ISS 11/9 [4] | | SIVR [5] | |
|-----------|-------------|------|---------------|------|----------------------|------|--------------------------|------|
| | range | year | range | year | range ^{a,b} | year | range ^b | year |
| Al | - | - | 0.03 – 7.5 | 1990 | - | - | 1.0 – 6.0 | 2005 |
| | - | - | 0.43 – 5.29 | 2005 | - | - | - | - |
| As | < 1 - 5 | 1996 | - | - | < 0.15 - 2.80 | - | - | - |
| Ba | - | - | 0.32 - 1.37 | 2005 | - | - | 0.2 - 1.2 | 2005 |
| Be | - | - | 0.03 - 0.27 | 1990 | < 0.022 - 0.086 | - | 0.06 - 0.25 | 2005 |
| | - | - | 0.06 - 0.43 | 2005 | - | - | - | - |
| Cd | 0.1 - 0.2 | 1996 | 0.04 - 0.36 | 1990 | 0.032 - 0.272 | - | 0.1 - 0.15 | 2005 |
| | - | - | 0.03 - 0.20 | 1990 | - | - | - | - |
| Co | - | - | 0.08 – 0.4 | 1990 | 0.074 – 0.485 | - | 0.05 – 0.30 | 2005 |
| | - | - | 0.06 – 0.42 | 2005 | - | - | - | - |
| Cr | 0.14 – 0.15 | 1996 | 0.04 – 0.41 | 1990 | 0.054 – 0.292 | - | 0.1 – 0.20 | 2005 |
| | - | - | 0.07 – 0.28 | 2005 | - | - | - | - |
| Hg | < 1 | 1996 | 0.6 - 3.8 | 1990 | 0.14 - 1.88 | - | 0.2 - 1.5 | 2005 |
| Li | 0.2 - 0.8 | 1996 | 0.36 - 2.20 | 2005 | - | - | - | - |
| Pb | < 1 | 1996 | 0.1 - 0.5 | 1990 | 0.05 - 0.68 | - | 0.05 - 0.35 | 2005 |
| | - | - | 0.20 - 0.98 | 2005 | - | - | 0.01 - 0.25 ^c | 2011 |
| Rb | 0.1 - 0.2 | 1996 | 78 - 317 | 1990 | - | - | - | - |
| Sb | - | - | 0.02 - 0.22 | 2005 | 0.028 - 0.340 | - | - | - |
| Sr | - | - | 23.0 - 61.5 | 2005 | - | - | - | - |
| V | 0.1 - 1.0 | 1996 | 0.07 - 1.1 | 1990 | 0.020 - 0.115 | - | 0.03 - 0.1 | - |
| | - | - | 0.03 - 0.11 | 2005 | - | - | - | - |

^a data referred to adults aged between 18 and 35; ^b data expressed as 5° - 95° percentiles; ^c referred to plasma

Table S11. Schematic representation of trace elements found in human semen and their functions.

| Elements | Role / Findings | Ref |
|----------|---|----------|
| Ca | Steroidogenesis; Acrosome reaction; Hyperactivation; Sperm quality; Chemotaxis | [6] |
| Cu | Sperm quality | [6–9] |
| Fe | Co-factor of Catalase | [7,9,10] |
| K | Sperm quality; Sperm capacitation | [6] |
| Mg | Spermatogenesis; Sperm quality | [6,9] |
| Mn | Sperm quality | [6] |
| Na | Spermatogenesis; Acrosome reaction; Sperm quality; Sperm capacitation | [6] |
| Se | Spermatogenesis; Sperm quality | [6,7] |
| Zn | Steroidogenesis; Spermatogenesis; Testicular development; Sperm quality; Sperm capacitation; Chemotaxis | [6–9,11] |
| As | Spermatogenesis ↓ ; Sperm maturation ↓ ; Sperm motility ↓ | [12] |
| Ba | Sperm motility ↓ | [13] |
| Be | Chromosomal aberrations ↑ ; Sperm abnormalities ↑ | [14] |
| Cd | Testicular morphology ↓ ; Spermatogenesis ↓ ; Sperm Quality ↓ ; Sperm Morphology ↓ | [8] |
| Li | Sperm motility ↓ | [15] |
| Hg | Testicular morphology ↓ ; Spermatogenesis ↓ ; Sperm Quality ↓ ; Sperm motility ↓ ; Sperm Morphology ↓ | [16,17] |
| Ni | Sperm motility ↑ | [18] |
| Pb | Testicular morphology ↓ ; Spermatogenesis ↓ ; Sperm Quality ↓ ; Sperm Morphology ↓ | [8,19] |
| Sr | Sperm motility ↑ ; Sperm capacitation ↑ ; Acrosome reaction ↑ | [20] |
| V | Sperm motility ↓ ; Sperm capacitation ↓ | [19] |

An up arrow (↑) represents an increment of that function, while a down arrow (↓) represents a decrease of that function.

Table S12. Ratio between Zn and Cu in blood serum.

| | WRC | BSC | LF | SRV |
|--------------------|------|------|------|------|
| Zn/Cu Serum | 1,43 | 1,64 | 1,32 | 1,15 |

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