

**Table 1. UFC in 50 apparently healthy adult volunteers (25 men and 25 women).**

	Cortisol, $\mu\text{g}/\text{day}$		Cortisol/Creatinine ratio, $\mu\text{g}/\text{g creatinine}$	
	Male	Female	Male	Female
Mean	30.9	22.6	14.9	15.5
Median	22.3	20.2	11.8	15.9
SD	20.8	14.3	8.2	5.3
95th percentile	60.7	43.6	27.8	24.2
5th percentile	9.4	9.3	6.7	7.8
Range	5.7–90.0	8.5–77.2	3.3–51.9	6.7–50.2

nisolone produced a weak signal at a slightly different retention time. At the evaluated concentration of 5000  $\mu\text{g}/\text{L}$ , prednisolone produced a signal equivalent to 20  $\mu\text{g}/\text{L}$  cortisol, representing a 0.4% interference. Fenofibrate generated parent ions at  $m/z$  361 and 363 in an abundance ratio of 3:1, a distinctive pattern related to the presence of chlorine in the chemical composition. In MS/MS mode, fenofibrate produced a  $m/z$  363 $\rightarrow$ 121 transition that interfered with the quantitative transition of cortisol, but it did not produce the  $m/z$  363 $\rightarrow$ 97 transition. Thus, switching to the secondary transition for quantification eliminated interference from this drug. In addition, the elution time of the drug was  $\sim$ 30 s longer than that of cortisol.

Taylor et al. (1) noted isotopic exchange between the deuterated IS and hydrogen-containing vapors in an APCI ion source. Unlike the observations of Taylor et al., we have not observed isotopic exchange with our APCI interface. The reason for this difference is unclear, but it may be related to ion source conditions. The good agreement obtained between our method and the comparison LC-MS/MS method suggests that isotopic exchange did not affect the proposed method.

Apparently healthy adult volunteers (25 males and 25 females; age range, 19–53 years) collected 24-h urine samples without preservative. The volunteers were asked to keep samples under refrigeration during the collection. Statistical results for this study are presented in Table 1.

To evaluate agreement between the established reference interval with UFC values in the population, we

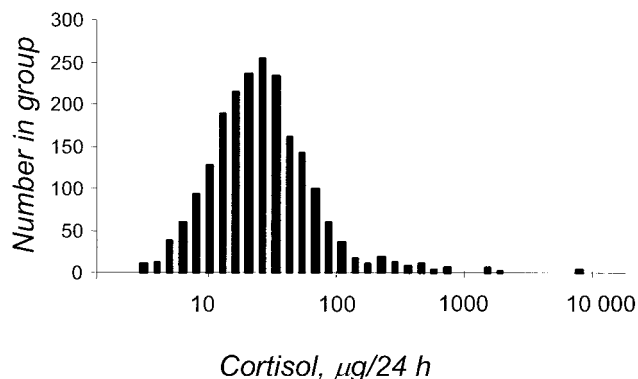


Fig. 1. Cortisol distribution in patient samples (n = 2089).

evaluated results for 2089 random 24-h urine specimens analyzed with the proposed method (Fig. 1). The mean (SD) value for log-transformed UFC excretion in 24 h was 1.26 (0.24) log  $\mu\text{g}/24$  h (minimum and maximum, 0.3 and 3.84 log  $\mu\text{g}/24$  h, respectively), and skewness and kurtosis for the distribution were 1.51 and 5.57, respectively.

The stability of cortisol in urine was evaluated in the presence of acetic (15 mmol/L), boric (15 mmol/L), and hydrochloric (30 mmol/L) acid. Two samples without added acid were stored and analyzed under the same conditions as the samples stored with the acids. Samples were stored at room temperature, 4 °C, and  $-20$  °C and analyzed every 4–7 days during 1 month of storage. Cortisol concentrations in samples stored with the acids were higher by  $\sim$ 30% than in samples stored without acid, possibly as a result of partial hydrolysis of glucate and glucuronide conjugates.

In conclusion, the rapid LC-MS/MS method for UFC analysis appears to be free from interference and agrees closely with a HPLC-MS/MS method that uses sample extraction. The method has been demonstrated reliable in a high-volume clinical laboratory environment.

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**Pediatric Concentrations of S100B Protein in Blood: Age- and Sex-related Changes**, Diego Gazzolo,<sup>1</sup> Fabrizio Michetti,<sup>2\*</sup> Matteo Bruschetti,<sup>1</sup> Nora Marchese,<sup>3</sup> Mario Lituania,<sup>1</sup> Salvatore Mangraviti,<sup>3</sup> Enrico Pedrazzi,<sup>4</sup> and Pierluigi Bruschetti,<sup>1</sup> (<sup>1</sup> Department of Pediatrics, <sup>3</sup> Clinical Chemistry Laboratory, Giannina Gaslini Children's University Hospital, I-16147 Genoa, Italy; <sup>2</sup> Institute of Anatomy and Cell Biology, Catholic University, I-00168 Rome, Italy; <sup>4</sup> Clinical Chemistry Laboratory, Acqui Terme Hospital, I-15011 Acqui Terme, Italy; \* address correspondence to this author at: Institute of Anatomy and Cell Biology, Catholic University, Largo Francesco Vito, 1, I-00168 Rome, Italy; fax 39-06-30154813, e-mail fabrizio.michetti@rm.unicatt.it)

The term S100 refers to members of a multigenic family of calcium-modulated proteins, mostly of low molecular mass ( $\sim$ 10 000 Da), first identified as a protein fraction detectable in the brain and called S100 because of its solubility in a solution of 100 g/L ammonium sulfate (1). The protein seems to be most abundant in glial cells,

although its presence in neuronal subpopulations has also been reported (2, 3).

The biological role of this protein within the cell populations that contain it has not been completely elucidated. The possibility of an extracellular biological role for S100B, which, secreted by astrocytes as a cytokine, may have a neurotrophic effect during both development and nerve regeneration at physiologic (nmol/L) concentrations, appears particularly interesting (4–7). Recent studies conducted in perinatal medicine that showed a correlation between S100B protein measured in several biological fluids (i.e., amniotic fluid, cord blood, and urine) and gestational age (8–10) appear consistent with a neurotrophic role for the protein.

The present study offers a reference curve for S100B protein in peripheral blood from the postnatal period to 15 years of age in healthy pediatric patients.

Between April 1997 and July 2000, we routinely collected blood samples for S100B measurement from healthy children admitted to our Institute for routine day-hospital investigations. All of the children were delivered at term without perinatal complications, and their clinical history, from birth to the time of blood sampling, was negative for neurologic abnormalities and comorbidities. We recruited a total of 1004 healthy children (males,  $n = 482$ ; females,  $n = 522$ ) whose ages ranged from 1 month to 15 years of age (mean, 8 years).

On admission to the study, all of the patients were checked against routine clinical and laboratory indices, and height growth velocity was assessed according to the normograms for our population (11).

Exclusion criteria included multiple pregnancies; intrauterine growth retardation; gestational hypertension, diabetes, or infections; fetal malformations; chromosomal abnormalities; perinatal asphyxia or dystocia; obesity; pediatric infections or neuromuscular diseases; endocrine diseases; and congenital heart disease.

The Ethics Committee of the Giannina Gaslini Children's Hospital, Genoa University, approved the study protocol, and the parents of the children who were examined gave informed consent.

For sampling, blood (1.5 mL) was drawn from the antecubital vein. Heparin-treated blood samples were immediately centrifuged at 900g for 10 min, and the supernatants were stored at  $-70^{\circ}\text{C}$  until measurement. The S100B protein concentration was measured in all samples by a commercially available immunoluminometric assay (Lia-mat Sangtec 100; AB Sangtec Medical). This assay is specific for the  $\beta$ -subunit of the protein, which is known to be predominant (80–96%) in the human brain (12, 13). Each measurement was performed in duplicate according to the manufacturer's recommendations, and the averages were reported. The lower limit of detection of the assay was  $0.02\ \mu\text{g/L}$ . The intraassay imprecision (CV) was  $<5\%$ , and the interassay CV was  $<10\%$ .

S100B concentrations are expressed as the median and interquartile ranges. Statistical analysis was performed by comparing the groups by use of the Kruskal–Wallis one-way ANOVA and Mann–Whitney  $U$ -test when the

data did not follow a gaussian distribution. The correlation between the blood concentrations of S100B and age at sampling was assessed by linear regression analysis. Reference curves for S100B values and age at sampling for all of the cases admitted to the study were assessed by use of a polynomial regression analysis. A  $P$  value  $<0.05$  was considered significant.

All of the patients admitted to the study were in healthy clinical condition, and no overt neurologic injury was observed on discharge from the hospital. Routine clinical analytes recorded at sampling were within the appropriate reference intervals.

S100B protein concentrations in blood were measurable in all of the cases examined. We found that the concentrations of the protein in children 0–15 years of age presented a pattern consisting of a decrease from 0 to 7 years followed by an increase from 7 to 13 years and, finally, by a second decrease in S100B from 14 to 15 years of age. The median and interquartile range for S100B at 0–1 year of age (median,  $0.95\ \mu\text{g/L}$ ; 25th–75th percentiles,  $0.44$ – $2.55\ \mu\text{g/L}$ ) were substantially higher than those from 2 to 7 years of age (median,  $0.73\ \mu\text{g/L}$ ; 25th–75th percentiles,  $0.44$ – $1.06\ \mu\text{g/L}$ ;  $P < 0.01$  for all). The largest difference was recorded at 4 years (median,  $0.63\ \mu\text{g/L}$ ; 25th–75th percentiles,  $0.36$ – $1.06\ \mu\text{g/L}$ , respectively). S100B concentrations at 0–1 year were also higher than those found at 11–12 years of age (median,  $0.45\ \mu\text{g/L}$ ; 25th–75th percentiles,  $0.39$ – $0.45\ \mu\text{g/L}$ , respectively;  $P < 0.01$ ) and significantly lower than those measured at 9–11 years (median,  $1.65\ \mu\text{g/L}$ ; 25th–75th percentiles,  $0.91$ – $1.74\ \mu\text{g/L}$ ) and at 13–14 years of age (median,  $1.23\ \mu\text{g/L}$ ; 25th–75th percentiles,  $1.12$ – $2.01\ \mu\text{g/L}$ ;  $P < 0.01$ ). Moreover, blood S100B concentrations at 9–11 and 13–14 years were significantly higher than those measured at 2–7 and 11–12 years ( $P < 0.01$  for all).

We found no significant differences in S100B concentrations between 0 and 1 year, and between 14 and 15 years of age (median,  $0.78\ \mu\text{g/L}$ ; 25th–75th percentiles,  $0.5$ – $0.87\ \mu\text{g/L}$  respectively;  $P > 0.05$ ; Fig. 1). The highest individual concentrations were observed in the first year of life and between and 9 and 10 years of age.

The S100B blood concentrations at different ages after correction for sex are shown in Table 1. Blood S100B concentrations in pediatric patients, monitored from birth to 15 years of age, were significantly higher in females ( $P < 0.05$ ). Median concentrations of the protein in females were higher in the first 3 years of life and statistically significant only at 2–3 years of age ( $P < 0.05$ ). These differences were also found at 6–7 years and from 13 to 15 years of age ( $P < 0.05$  for all).

We found a negative correlation between blood S100B protein concentrations and gestational age both for all the cases admitted to the study ( $r = -0.29$ ;  $P < 0.001$ ) and when the data were corrected for sex (male,  $r = -0.21$ ; female,  $r = -0.32$ ;  $P < 0.001$  for both). We also found significant correlations between blood S100B concentrations and height growth velocity values, both in all of the cases monitored ( $r = 0.33$ ) and when the data were

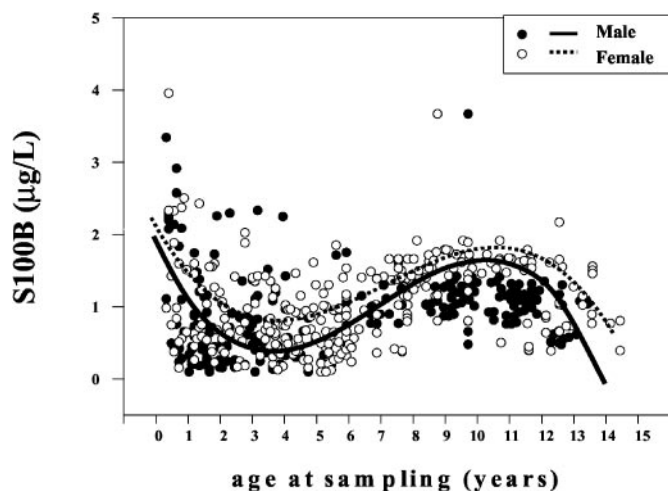


Fig. 1. Correlation between individual S100B protein concentrations in peripheral blood ( $\mu\text{g/L}$ ) and age at sampling (months) in male (●) and female (○) pediatric patients.

The filtered curve was obtained by use of a third-degree polynomial regression analysis. There was a significant negative correlation between age at sampling and blood S100B concentrations in both males ( $r = -0.51$ ;  $P < 0.001$ ; solid line) and females ( $r = -0.59$ ;  $P < 0.001$ ; dashed line).

corrected for sex (male,  $r = 0.30$ ; female,  $r = 0.34$ ;  $P < 0.001$  for all).

The reference curve was finally assessed by use of a polynomial regression analysis and showed a significant correlation between S100B and age at sampling both for all the cases studied ( $r = -0.49$ ) and when the data were

corrected for sex (male,  $r = -0.51$ ; female,  $r = -0.57$ , respectively;  $P < 0.001$  for all; Fig. 1).

The present data provide a reference curve indicating S100B concentrations in peripheral blood at different ages in the two genders. All of the patients showed measurable S100B concentrations in peripheral blood, in agreement with recent observations (14–16).

It is interesting that blood S100B concentrations were correlated with age at sampling and showed significant gender differences in pediatric patients. The presence of S100B in pediatric patients, albeit in a smaller number of cases (85 vs 1004 in our study), was also reported by Portela et al. (16). Their data are essentially consistent with the present findings, although there are significant differences in the median concentrations of the protein. These discrepancies could be largely a result of the limited number of cases studied ( $n = 44$  vs  $n = 1004$ ), as well as the reduced frequency of monitoring time points. These limitations may also be responsible for the lack of gender differences (16). The correlation between blood S100B concentrations and gestational age at sampling is in agreement with previous studies at prenatal, perinatal, and adult ages (7–10, 16, 17).

Our observation of higher S100B concentrations in children during the first year of age and in adolescence than at other ages is intriguing. One explanation for this phenomenon during the first year of age could involve a possible difference in the permeability of the blood–brain barrier and cerebral circulation, although this issue is still a matter of debate. Notwithstanding this, some involvement of the functional role of the protein should also be considered. Among the numerous functions attributed to this protein, all of which are still a matter of debate, the possibility that S100B acts as a cytokine with neurotrophic effects at physiologic concentrations appears to be interesting and relevant to the present study. Seen in this light, the progressive decrease in blood S100B concentrations after 2 years of age could reflect a reduced release of the trophic factor at a more advanced stage of brain maturation. The progressive increase in concentrations of the protein in peripheral blood from 7 years onward, with a peak at 12–13 years, is in agreement with that shown by earlier anthropometric studies (11). The correlation between height growth velocity values, an index suggestive of nerve elongation, and blood protein patterns at different monitoring ages is consistent with the possibility of a neurotrophic role of S100B in the pediatric period, as previously demonstrated in the perinatal period. In this respect, it has been extensively reported that the two periods under consideration (i.e., the first year of life and adolescence) are those in which height growth velocity is at its peak, with a standard index of growth of  $\sim 15$  and 9 cm per year, respectively (11). Additional support for this hypothesis is offered by the presence of S100B in peripheral nerves and by its stimulating action on neurite outgrowth (5, 6, 18, 19).

The present findings, pointing to a role of S100B as a trophic cytokine, suggest the possibility of reciprocal modulation of the protein and growth hormones: future

**Table 1. S100B blood concentrations ( $\mu\text{g/L}$ ) in healthy male and female pediatric patients at different ages of sampling expressed in years<sup>a</sup>**

Age at sampling, years (M/F)	Males (n = 482)			Females (n = 522)			<i>P</i> <sup>b</sup>
	Median	25th	75th	Median	25th	75th	
0–1 (43/42)	0.81	0.44	1.93	0.95	0.45	2.24	0.27
1–2 (46/40)	0.72	0.32	1.33	0.77	0.49	1.37	0.35
2–3 (52/48)	0.62	0.39	0.97	0.76	0.59	1.61	<b>&lt;0.05</b>
3–4 (52/47)	0.70	0.32	1.27	0.66	0.37	0.89	0.70
4–5 (41/53)	0.61	0.31	1.00	0.74	0.44	1.10	0.29
5–6 (34/48)	0.68	0.32	1.24	0.56	0.36	1.10	0.59
6–7 (37/49)	0.60	0.43	0.96	0.86	0.65	1.17	<b>&lt;0.05</b>
7–8 (28/39)	0.90	0.65	0.96	0.90	0.39	0.96	0.77
8–9 (35/52)	1.37	1.34	4.56	1.41	1.03	4.60	0.95
9–10 (41/32)	1.44	0.91	1.75	1.67	1.44	2.03	0.45
10–12 (24/18)	1.45	0.81	2.63	1.74	0.97	2.10	0.71
11–12 (16/16)	0.42	0.39	0.45	0.45	0.41	0.48	0.38
12–13 (10/8)	1.23	1.2	2.00	1.25	0.96	2.21	0.66
13–14 (17/18)	1.13	0.99	1.89	1.35	1.16	2.23	<b>&lt;0.05</b>
14–15 (6/12)	0.66	0.45	0.72	0.91	0.52	0.97	<b>&lt;0.05</b>
Total 1–15 (482/522)	0.80	0.44	1.49	0.95	0.58	1.62	<b>&lt;0.05</b>

<sup>a</sup> Data are shown as median and interquartile ranges.

<sup>b</sup> Bold font indicates statistical significance.



studies using clinical and/or experimental approaches will address this hypothesis, regarding which little information is currently available. Similarly, we have no useful information at present regarding possible interactions between S100B and sex hormones, which could also be hypothesized on the basis of the gender differences found in S100B concentrations. More generally, the growing body of evidence that indicates a biological role of S100B as a cytokine points to the usefulness of future studies on possible interactions between this protein and individual hormonal patterns.

The most probable origin of S100B in peripheral blood, as previously reported in several studies, is nervous tissue, although we cannot exclude the possibility that it may also be released from other sites of concentration, such as adipose tissue (20). However, data on the presence of the protein in adipose tissue at the ages studied here are not conclusive.

Finally, the different peaks of protein concentration in the two sexes could, in common with other clinical and anthropometric studies (i.e., height/weight growth reference curves), suggest the possibility that brain maturation in the pediatric period differs in males and females, as it does in the intrauterine and adult periods (9, 21). In this respect it could be relevant that genetically modulated overexpression of S100B has been reported to affect the behavior of female mice without causing any appreciable effects in males in experimental models (22).

In conclusion, the reference curve for S100B protein in peripheral blood in healthy pediatric patients constitutes a useful tool to evaluate pathologic alterations of the protein during this period and also suggests an approach for future investigations into the suggested neurotrophic role of the protein, which could potentially be related to the process of maturation, including hormone concentrations.

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**Quantitative Spectrophotometric Microplate Assay for Angiotensin-converting Enzyme in Cerebrospinal Fluid,** J. Alan Erickson,<sup>1\*</sup> Roxann Cousin,<sup>2</sup> James T. Wu,<sup>1,3</sup> and Edward R. Ashwood<sup>1,3</sup> (<sup>1</sup> ARUP Institute for Clinical and Experimental Pathology, LLC, Salt Lake City, UT 84108; <sup>2</sup> ARUP Laboratories, Special Chemistry Section, Salt Lake City, UT 84108; <sup>3</sup> Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT 84132; \* address correspondence to this author at: ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108; fax 801-584-5109, e-mail ericksja@aruplab.com)

Angiotensin-converting enzyme (ACE; EC 3.4.15.1) catalyzes the formation of angiotensin II by cleaving the C-terminal histidylleucine dipeptide from angiotensin I (1). Indications are that ACE is affiliated with an autonomous renin-angiotensin system of the brain that participates in physiologic processes inside the brain (2, 3). In addition, studies suggest that changes in ACE concentrations in brain tissue, caused by various neurologic disorders, are reflected by alterations in ACE activity in cerebrospinal fluid (CSF) (4). For example, increased ACE concentrations in CSF are associated with neurosarcoidosis (4–7), with affected patients generally having activities approximately twofold or more higher than those of healthy individuals (4, 6, 7). Increased CSF ACE has also been implicated in neurologic diseases, such as bacterial and viral meningitis and Behcet disease (4–7). Decreased concentrations have been reported in patients with Alzheimer disease, Parkinson disease, and progressive supranuclear palsy (8, 9).