SERUM TESTOSTERONE AND ESTRADIOL IN SUDDEN INFANT DEATH

MICHAEL J. EMERY, PHD, HENRY F. KROUS, MD, JULIE M. NADEAU-MANNING, MSW, BRETT T. MARCK, BA, AND ALVIN M. MATSUMOTO, MD

Objective To test the hypothesis that among infants who die unexpectedly, testosterone and/or estradiol levels are elevated in those diagnosed with SIDS versus those with known causes of death (controls).

Study design Postmortem blood was collected and coded from infant autopsies, and serum was prepared and frozen until assayed for total testosterone and estradiol by fluoroimmunoassay. Subject information was then collected from the medical examiner's report.

Results Testosterone, but not estradiol, was significantly higher in 127 SIDS cases versus 42 controls for both males (4.8 ± 0.4 vs 2.2 ± 0.4 nmol, respectively; $P < .005$) and females (2.4 ± 0.2 vs 1.6 ± 0.2 nmol, respectively; $P < 0.03$).

Conclusions Higher testosterone levels in infant victims of unexpected, unexplained death may indicate a role for testosterone or related steroids in SIDS. Further research is needed to understand the potential utility of testosterone as an indicator of SIDS risk. *(J Pediatr 2005;147:586-91)*

Physiological and biochemical studies of cases of sudden infant death syndrome (SIDS) suggest that affected infants experience ventilatory and/or cardiac arrest.[1-4] Potential mechanisms leading to such deaths include abnormal autonomic control during sleep,[5] perhaps due to abnormal development of the brainstem, including neurons involved in chemosensitivity and cardiorespiratory control.[6-9] However, limited evidence exists for specific mechanisms that cause SIDS, and known risk factors (eg, age, ethnicity, prematurity at birth, face-down placement in the crib) do not identify specific individuals destined for SIDS. Several compelling lines of evidence suggest potential links between the gonadal/adrenal-derived steroid hormones, testosterone and estradiol, and SIDS. There is a striking coincidence between the postnatal rise in gonadal steroid levels (testosterone and estradiol in males and estradiol in females) and the age range of risk for SIDS, with both events reaching a peak between about 1 and 5 months.[10-13] Preterm infants are at increased risk for SIDS[13,14] and have substantially elevated gonadal- and adrenal-derived androgen levels in their first year of life compared with term infants.[11,12]

Testosterone administration decreases ventilation and ventilatory drive during sleep in adult humans and infant primates,[15-17] and increased androgen levels are associated with sleep-disordered breathing in human adult males and females.[15,16,18] Gonadal steroid production is increased at night in primate infants,[19] and sensitivity of respiratory control to testosterone administration is found to be greater in weanling female rats than in adults.[20] Moreover, testosterone and estradiol alter the development and regulation of serotonergic and muscarinic (central and peripheral) neurons,[21-25] both of which are associated with neuromodulatory systems that regulate chemosensitivity in the medulla and may be abnormal in some SIDS cases.[6-8]

We tested the hypothesis that SIDS infants have elevated testosterone and estradiol levels compared with infants with a known cause of death (controls). Studies of living infants have found that testosterone levels are very low in females, but estradiol levels are similar in both sexes.[10-12] We further analyzed the results to assess potential influences of several uncontrolled variables that may affect levels of these hormones, including age at death,[10-12] postmortem interval (due to time-dependent sample degradation), time of

GA	Gestational age	SIDS	Sudden infant death syndrome

From the Departments of Physiology & Biophysics, Medicine (Pulmonary and Critical Care Medicine), and Pediatrics (Neonatology), University of Miami School of Medicine, Miami, Florida; Departments of Pathology and Pediatrics, Children's Hospital of San Diego and University of California San Diego School of Medicine, San Diego, California; Department of Medicine (Gerontology and Geriatric Medicine), University of Washington School of Medicine and GRECC, Veteran's Affairs Puget Sound Health Care System, Seattle, Washington.

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Reprint requests: Michael J. Emery, PhD, Department of Neurology, Box 356465, University of Washington School of Medicine, Seattle, WA 98195. E-mail: mjemery@u.washington.edu.

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death (due to possible diurnal variations in hormone levels and SIDS incidence),[13,19] and preterm birth.[11,12]

METHODS

Serum Sample Collection

Serum was prepared from whole blood collected at sequential autopsies of nearly all infants accessioned by the medical examiner offices serving San Diego and Seattle over a 3-year period, and Los Angeles over a 1-year period. The sites of blood sampling (most were central venous or arterial blood) were at the discretion of the examiner, with a goal of obtaining samples from every case. Each sample was assigned a study number, and serum was separated from other blood components by suction pipette after centrifugation (10 to 20 min, 2000 *g*, room temperature). Each specimen was placed in a sealed polypropylene tube labeled with the study number and stored frozen (<−10°C) until submitted (without further identification) for analysis of total testosterone (ether-extracted) and estradiol by highly specific time-resolved fluoroimmunoassay (Delfia; Wallac Oy, Turku, Finland).[26] The lower limits of detection for testosterone and estradiol were 0.5 nmol and 50 pmol, respectively. Mean intraassay and interassay coefficients of variation were 3.1% and 4.5% for testosterone and 2.4% and 3.8% for estradiol. No attempt was made to select samples for quality (eg, hemolysis) or other criteria, and the testosterone assay was given priority when sample quantity was a limiting factor.

Collection of Subject Information

Subject information collected from investigative and autopsy reports included age, sex, preterm birth (<37 weeks gestational age [GA]), time of death (estimated midway between the last time confirmed alive and when death was pronounced), postmortem interval (time of death to initiation of autopsy), and assigned cause of death. Infants outside of the SIDS age range (ie, those age <1 week and >1 year) were excluded from further analysis. Times were estimated to the nearest hour.

A diagnosis of SIDS was made according to the National Institute of Child Health and Human Development definition,[1] whereas control diagnoses were based on positive findings from medical history, scene investigation, and postmortem examination. Cases lacking a known cause of death after autopsy but not meeting the SIDS definition for various reasons were labeled as "undetermined" or "unknown" in the final medical examiner reports. Information concerning subjects' parents or other family members was outside of the study guidelines and thus was not assessed. All procedures were reviewed and approved by the Seattle Children's Hospital and Regional Medical Center Human Subjects Committee.

Statistical Analysis

Statistical comparisons of testosterone and estradiol levels were made by unpaired 2-tailed *t*-tests between same-sex SIDS and control groups. Results from preterm infants were removed for another unpaired comparison of SIDS versus control group hormone levels, to assess their influence on overall group results. The significance of changes in serum hormone levels as a function of infant age was first assessed by analysis of variance for male and female SIDS and control subgroups versus the null hypothesis of no effect. Unpaired comparisons of SIDS versus control hormone levels were also made for subgroups defined by deaths occurring during peak SIDS incidence (age 1 week to 5 months) and during the period of lower risk (age >5 months to 1 year), samples taken after relatively short and long postmortem intervals (same sex; "short" ≤ median postmortem interval < "long"), and deaths occurring during the night (18:01 to 06:00) and day (06:01 to 18:00). Unpaired comparisons were also made to assess the significance of changes in hormone levels within the SIDS and control groups due to age and time of death.

RESULTS

Samples from 221 of the original 277 cases contained sufficient serum for analysis. Excluded cases included 15 infants found to be out of the study age range, 19 who were resuscitated but later died after varying time and treatment courses in the hospital, and 18 with an unknown cause of death. Thus 169 cases were used in this study, including 4 (3 male, 1 female) that yielded sufficient sample volume to measure only testosterone. Specific cause of death, sex, age, and serum testosterone levels for the 42 control cases are given in Table I (also available online at www.jpeds.com). Unless otherwise stated, all results herein are expressed as mean ± standard error under the mean.

Serum Testosterone and Estradiol Levels

Testosterone levels were significantly higher in both male SIDS (4.8 ± 0.4 nmol) versus controls (2.2 ± 0.4 nmol; $P < .005$) and female SIDS (2.4 ± 0.2 nmol) versus controls (1.6 ± 0.2 nmol; $P < 0.03$) (Figure 1A). Estradiol levels (Figure 1B) did not differ between SIDS and control males (0.12 ± 0.02 nmol) and females (0.14 ± 0.02 nmol). Variance in testosterone levels was substantially greater for SIDS males (13.4 nmol) and females (2.4 nmol) than for control males (3.1 nmol) and females (0.7 nmol).

Age and Postmortem Interval

Male controls (age 155 ± 25 days; n = 18) were significantly older than SIDS males (97 ± 7 days; n = 71; $P < .01$), and control females (134 ± 14 days; n = 24) were significantly older than SIDS females (86 ± 7 days; n = 56; $P < .01$). Postmortem interval was significantly longer for the male control group (52 ± 10 hours) than for the male SIDS group (30 ± 3 hours; $P < .01$), but did not differ significantly between the female control (47 ± 7 hours) and female SIDS (39 ± 4 hours) groups.

Effect of Age

Testosterone decreased significantly with infant age for both the male and female SIDS subgroups but not for controls