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Change in Arginine Vasopressin Concentrations with Age

To the Editor:

There have been conflicting reports on changes in the concentrations of plasma arginine vasopressin (AVP) with age (1, 2). Difficulties become apparent when interpreting results in older subjects, who are rarely used to determine the reference range because of their medical complications and unavailability to the laboratory.

Plasma AVP was measured for 2 years in 68 healthy volunteers (ages 53-87 years) and compared with our reference range for 45 subjects (ages 21-51 years), which had been established 2 years previously as part of the weekly diagnostic service. To ensure continuity of the reference range, we routinely included in each assay three plasma quality-control samples. The study was approved by the hospital ethical committee. The elderly subjects (older than 75 years) were classified as healthy on the basis of the absence of significant abnormalities as shown by clinical assessment, a full blood count, serum electrolytes, renal and liver function tests, a chest x-ray, an electrocardiograph, and two-dimensional transesophageal echocardiography. No subject was prescribed medication known to influence the concentration of plasma AVP.

Plasma was applied to Sep-Pak C₁₈ columns (Millipore Waters, Milford, MA), and eluted AVP was measured by radioimmunoassay as described previously (3). The sensitivity (detection limit) of the method, measured as the minimum amount of AVP that could be statistically distinguished from zero at 95% confidence, was 0.1 pg/tube (0.45 ng/L, or 0.42 pmol/L). The mean within-run CV was 11.1% for three plasmas with AVP at 3, 5, and 10 ng/L examined 10 times in one day; the mean between-run CV was 11.7% for three plasmas with AVP at 3, 4, and 6 ng/L assayed over 12 days.

By unpaired t-test, the mean (±SE) AVP concentration in the plasma of the 68 older subjects was significantly higher (4.7 ± 0.6 ng/L) than that found in the younger group (2.1 ± 0.2 ng/L, n = 46; t = -3.54, P < 0.001). In all subjects studied, we observed a significant correlation between plasma AVP and age (r = 0.29, P = 0.002) (Figure 1) and between the plasma AVP to osmolality ratio and age (r = 0.29, P = 0.002). However, because serum osmolality did not change significantly with age, this means that, in healthy subjects, aging is accompanied by an increase in AVP synthesis

Fig. 1. Correlation between plasma AVP concentrations and age in 113 healthy adults


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Utility of Unconjugated Estriol in Screening for Down Syndrome is Not Proven

To the Editor:

In an editorial (1) to our paper on unconjugated estriol (ue₃) assays in Down syndrome screening (2), Cuckle asserts that ue₃ should continue to be used in Down syndrome screening because ue₃ is important because of fetoprotein (AFP) may be increased in cases of trisomy 18 with neural tube defects (NTD) or ventral wall defects, and that "modified parameters" have been reported that significantly improve detection when ue₃ is used (3).
He states that in a statistically modeled population, based on using the new parameters, addition of $u_E^a$ to the AFP + human chorionic gonadotropin (HCG) screen adds as much as 10% to the detection rate of Down syndrome for a fixed false-positive rate of 5%.

We believe that $u_E^a$ has not been proven to be of value in Down syndrome screening (4–7) and note that in the second trimester, AFP or ultrasound is extremely effective in detecting cases of anencephaly, NTD, or ventral wall defect and that these conditions in themselves indicate further investigations. We have already described effects of the use of modified kits on the distribution of results (2) and wish to emphasize that underestimation of standard deviations and correlations with other analytes will produce important effects on statistical modeling of screening.

To evaluate the efficacy of $u_E^a$ in second-trimester Down syndrome screening, we examined the serum results for 506 randomly selected unaffected pregnancies and 52 Down syndrome-affected pregnancies. The unaffected results and 27 of the Down syndrome results came from the University Hospital of Wales (UHW); the remaining 25 Down syndrome results came from Old Church Hospital. The Down syndrome samples were all collected prospectively and have not been previously reported. In-house assays of AFP and total HCG were used at the UHW, and in-house AFP and Kodak Diagnostics (Rochester, NY) 2nd-Trimester HCG kits were used at Old Church Hospital. All $u_E^a$ assays were performed with the Kodak Diagnostics 2nd-Trimester $u_E^a$ kit. The median multiples of the median (MoMs) for unselected pregnancies were AFP, 0.94; HCG, 0.85; $u_E^a$, 1.05; for affected pregnancies they were AFP, 0.7; HCG, 2.07; $u_E^a$, 0.74.

Likelihood ratios were calculated by using the original population parameters published in 1988 (8) and, because all of the pregnancies were ultrasound-dated, by using the "new" population parameters appropriate for ultrasound-dated pregnancies (3). To determine composite detection and false-positive rates, we calculated the distribution of risks in Down syndrome and unaffected pregnancies by numerically integrating the distribution of age and the bivariate or trivariate gaussian density functions over a grid of values of age and the corresponding variables as described by Wald et al. (8). The distribution of ages in Down syndrome and unaffected pregnancies was derived by applying the age-specific risk for a Down syndrome term pregnancy (9) to the number of pregnant women of different ages in England and Wales in 1986–88. Likelihood ratios for combinations of AFP + HCG and AFP + HCG + $u_E^a$ with use of old and new parameters were entered into the model to derive false-positive and detection rates for cutoff risks of 1:100–1:300. Our usual false-positive rate for a 1:300 cutoff when using the old parameters and AFP + HCG to derive risks is 4.7%. This approach estimates standardized detection rates and false-positive rates that are not influenced by the maternal age distribution of the samples. If the detection and false-positive rates were determined directly from the data and the age distribution reflected predominantly older women, the detection rate would appear greater than if the sample contained predominantly younger women.

The receiver-operating characteristic (ROC) plot of the data (Figure 1) shows that using the "old parameters" gives detection and false-positive rates for two- and three-analyte combinations that are roughly identical, indicating that $u_E^a$ adds nothing. When the "new parameters" are used, there is no significant change for the AFP + HCG combination but the line for the triple combination is shifted to the right, indicating a significant decrease in the detection rate for a constant false-positive rate. Adding extra analytes to the risk calculation algorithm results in an increase in the imprecision of the resulting risk with a consequent broadening of the confidence limits of the result (5). This serves to reduce the effectiveness of the triple combination as compared with a double test and can be expected to decrease the triple test detection rate in a real population in comparison with a modeled population.

Our study and the studies of Spencer et al. (7) and Crossley et al. (5) comprise 191 cases of Down syndrome in total. Although our data are at variance with a prospective study that included 35 Down syndrome cases and showed a loss of detection when $u_E^a$ was removed (10), we must remember that all current studies of Down syndrome screening are small and therefore of low statistical power. We must accordingly take care to ensure that excess weight is not placed on small numbers of cases.

Furthermore, our data (2), by inference, cast doubt on the use of $u_E^a$ in the first trimester. Currently no assay kits available can reliably measure $u_E^a$ at the concentrations found in the first trimester; therefore any reports of its utility as a marker in the first trimester based on the use of modified second-trimester assays must be viewed with considerable skepticism. We conclude that further research is necessary before $u_E^a$ can be considered an essential component of any Down syndrome screening program.

References
5. Crossley J, Aitken D, Connor J. Second
trimester unconjugated oestriol levels in maternal serum from chromosomally abnormal pregnancies using an optimized assay. Prenatal Diagn (in press).

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The author of the Editorial responds:

To the Editor:

In maternal serum screening for Down syndrome at 15–20 weeks of gestation, the marker of first choice is human chorionic gonadotropin (hCG). The discriminatory powers of α-fetoprotein (AFP) and unconjugated oestriol (uE₃) are similar to each other, although both are much less than hCG. Because of its use in screening for neural tube defects, AFP rather than uE₃ is chosen as the second marker in most centers. The data presented by Reynolds et al. (1) and in the recent publication of Spencer et al. (2) bring into focus the controversy regarding the measurement of uE₃ as a possible third marker. In the two se-

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* Based on the multivariate gaussian model method described in reference 4 but applied to the maternal age distribution of England and Wales in 1989–90 (10).

Table 1. Estimated Increase in Down Syndrome Detection Rate for a 5% False-Positive Rate When uE₃ is Considered in Addition to hCG and AFP According to Different Sets of Parameters

world” data. There is a general tendency for this to happen with statistical models, and as a consequence they overestimate the detection rate. This has been examined for Down syndrome screening and the likely deviation from fit was thought to be small (11, 12). However, the expected increase in detection with the addition of uE₃ is relatively small and it is possible that a poor fit may negate it. Detailed examination of data from series showing discrepant results may pinpoint the deviation and indeed suggest a better model.

The principal reason for measuring uE₃ in second-trimester maternal serum is the detection of Down syndrome. It is also of benefit in the detection of Edward syndrome (13) and triploidy (14), but that is only a marginal consideration. A more important issue is the choice of markers for screening earlier than 15 weeks’ gestation. Most centers test at 15–20 weeks gestation, when the AFP measurement can also be used to screen for neural tube defects. However, there are obvious advantages to the patient in obtaining an earlier diagnosis and many centers are now confident enough in routine ultrasound screening for anomalies at 18–20 weeks’ gestation to rely on it rather than AFP for neural tube defect screening. With the establishment of free β-hCG (15) and pregnancy-associated placental protein A (16–19) as very discriminatory early pregnancy markers, such centers can now consider Down syndrome screening before 15 weeks of gestation. Provided that sensitive-