Density of Lumbar Cerebrospinal Fluid in Pregnant and Nonpregnant Humans

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Background: Dextrose-free local anesthetics and opioids, alone and in combinations, are being used increasingly to provide subarachnoid anesthesia and analgesia. These dextrose-free drugs have been described as hypobaric by some and isobaric by others. To accurately classify anesthetics with regard to baricity, the density of cerebrospinal fluid (CSF) must be known. The authors sought to determine the exact density of human CSF, and determine whether CSF density is altered by pregnancy.

Methods: Density measurements accurate to 0.00001 g/ml were made at 37.00°C, using a mechanical oscillation resonance frequency density meter. Cerebrospinal fluid samples were obtained from 44 patients during spinal anesthesia. Five groups were studied: men, and premenopausal, postmenopausal, term pregnant, and postpartum women.

Results: Mean CSF densities in men (1.00064 ± 0.00012 g/ml), postmenopausal women (1.00070 ± 0.00018 g/ml), and nonpregnant premenopausal women (1.00049 ± 0.00004 g/ml) were significantly greater than in term pregnant (1.00030 ± 0.00004 g/ml) and postpartum (1.00034 ± 0.00005 g/ml) women. Cerebrospinal fluid density did not correlate with age.

Conclusions: Mean CSF density varies in different patient subpopulations. Pregnancy and the immediate postpartum period are associated with the lowest CSF densities. In addition, the cutoff values defining hypobaricity (mean CSF density minus three standard deviations) are greater than previously reported. Accurate CSF density values should be used when considering baricity as a mechanism for clinical observations of dextrose-free intrathecal local anesthetics and opioids. Gestational status also should be considered. (Key words: Anesthesia: spinal. Cerebrospinal fluid: density; Pregnancy: human.)

THE density difference between an intrathecal anesthetic solution and cerebrospinal fluid (CSF) is a major determinant of extent of spinal block.[1] Densities of nondextrose-containing intrathecal local anesthetics and opioids have been determined recently and are thought to be very close in value to the density of human CSF.[2,3] The values for the density of human CSF derive from two earlier reports.[4,5] These values have been useful for comparison with dextrose-containing intrathecal solutions that are clearly hyperbaric. However, accuracies of mass, volume, and temperature measurements were limited by the techniques used in both studies. The studies also reported different means, each with large standard deviations (1.00100 ± 0.00030[4] and 1.00030 ± 0.00030[5] g/ml). The techniques used in earlier CSF density studies limit comparisons with current spinal anesthetic solution densities. As a result, dextrose-free solutions continue to be described as hypobaric by some and isobaric by others.[6,7] In addition, both previous reports restricted study subjects to particular population subsets, neither including pregnant women. Although CSF composition has been compared in pregnant and nonpregnant women,[8,9] CSF density in pregnancy is unknown. We obtained CSF from individuals in several patient subpopulations and measured density with greater accuracy than was reported previously (±0.00001 g/ml). We then compared densities among the subgroups, which included women during and immediately after pregnancy.

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Materials and Methods

After approval from the human subjects review board and informed consent, 44 healthy ASA physical status 1 and 2 patients undergoing spinal anesthesia for various procedures were studied. Specific groups studied were men (n = 10), postmenopausal women (n = 8), nonpregnant premenopausal women (n = 6), women undergoing postpartum tubal ligation (n = 10), and parturients at term gestation undergoing elective Ce-

sarean section (n = 10). Parturients with preeclampsia were excluded. Patient age was noted, as were gestational age and time since delivery, when applicable. Lumbar CSF was collected *via* a 25-G Whitacre needle at the time of induction of spinal anesthesia. After confirming free flow of clear CSF and before intrathecal injection of spinal anesthetic, 2 ml clear CSF was collected for immediate analysis.

The density of CSF at 37°C was determined using a density meter (DA-310, Mettler-Toledo, Hightstown, NJ) that uses the mechanical oscillation resonance frequency technique. Samples are injected into a glass measuring cell. The cell is stimulated to determine the oscillation period generated at its natural oscillation frequency. The oscillation period (T) depends on the density, volume, and temperature of the sample. A cell factor (F) is calculated from the oscillation periods of two different standard substances (desiccated air and distilled water) at a specific temperature (37.00 ± 0.01°C). The density (d) of an unknown sample is calculated when its oscillation period (T) is determined:

$$d = d_A - F(T_A^2 - T^2)$$

where d_A is the density of desiccated air and T_A is the oscillation period of desiccated air. Calibration was verified before and after each CSF density measurement, using desiccated air and distilled deionized water (d_A = 0.99114 g/ml and d_w = 0.99333 g/ml at 37°C, respectively). After the calibration procedure recommended by the manufacturer (Mettler-Toledo), density measurements are accurate to ±0.00001 g/ml in the range of 0–3 g/ml. An electronically controlled thermistor maintained temperature of the sample and the measuring cell at 37.00°C, with a temperature control accuracy of ±0.01°C.

Cerebrospinal fluid densities are expressed as mean (g/ml) ± SD. The upper limit of hypobaricity was calculated for each group as defined previously (mean CSF density minus three standard deviations of the mean).[1,4] Comparisons between groups were made using one-way analysis of variance. Pair-wise comparisons were then made, using Tukey's *post hoc* test method of multiple comparisons, with a 5% overall error rate. The subgroups of women selected in the design of this study were likely to have significantly different mean ages (*e.g.*, pregnant women are more likely to be much younger than postmenopausal women), making age a potential confounding variable. Therefore, we performed analysis of covariance controlling for group, then for age, to determine whether density differences are explained by age. Linear regression was performed to analyze the relation between density and time between delivery and CSF sampling. For all determinations, $P < 0.05$ determined significance.

Results

Individual CSF density measurements are shown in figure 1. Mean CSF densities, standard deviations of measurements, and upper limits of hypobaricity for each group are presented in table 1. Analysis of variance revealed significant differences in CSF density between groups ($P < 0.0001$). Pair-wise comparisons revealed that CSF density during the peripartum period (term pregnant and postpartum groups) is significantly less than in men and nonpregnant pre- and postmenopausal women (table 1). Means, standard deviations, and ranges of ages in each group are also shown in table 1. Density differences are not explained by age, because analysis of covariance controlling for group revealed no effect of age on density. Controlling for age, analysis of covariance showed the groups to differ still ($P = 0.02$). There was no correlation between density and time from delivery to CSF sampling in postpartum women, which ranged from 9 to 70.5 h (mean 28.1 ± 17.5 h).

Discussion

The density of a spinal anesthetic solution relative to human CSF (*i.e.*, baricity) is an important determinant of extent of subarachnoid block.[1,10,11] Stienstra and colleagues showed that small differences in density (0.00060 g/ml) influence intrathecal distribution of local anesthetics both clinically[12] and in an *in vitro* model.[13] Several authors recently proposed that small density differences between CSF and dextrose-free intrathecal agents could explain the rapid, high sensory block observed after administration of local anesthetics and/or lipid-soluble opioids for labor analgesia.[10,11,14–16] Baricity also was proposed as an explanation for postural differences in extent of block produced by dextrose-free intrathecal anesthetics.[6,17] Hypobaric intrathecal solutions have been defined as those with densities less than three standard deviations below mean human CSF density.[1,4] Previous CSF density studies suggest that this upper limit of hypobaricity is 0.99980 g/ml[4] or 0.99940 g/ml[5] (fig. 2). Most authors quote the latter.[2,3] Our data suggest that actual values are greater (1.00016–1.00037 g/ml), and vary for dif-
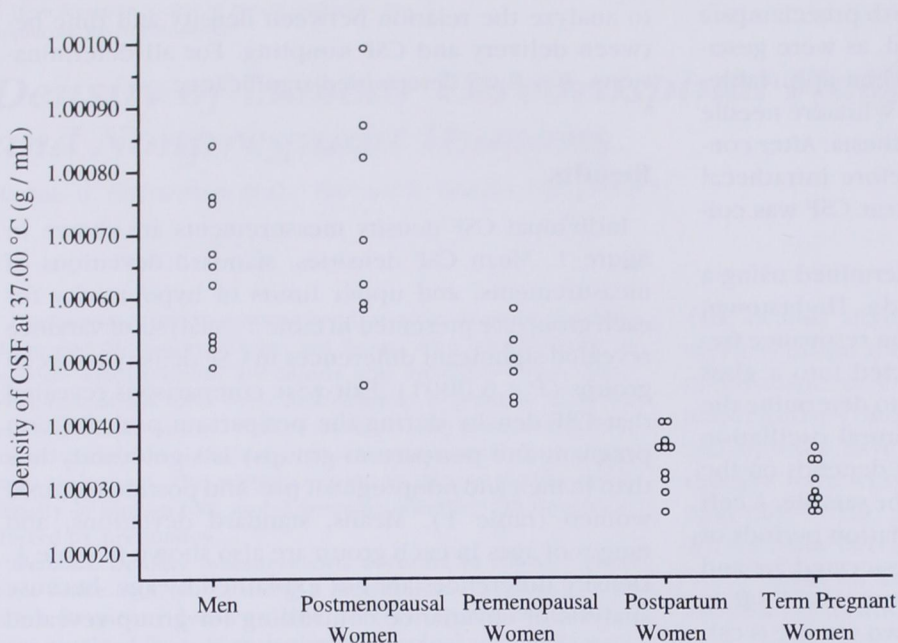
M. G. RICHARDSON AND R. N. WISSLER

Fig. 1. Density measurements of cerebrospinal fluid from 44 patients.

ferent subgroups of patients (table 1, fig. 2). Therefore, density differences between dextrose-free intrathecal drugs and CSF are greater than previously reported.[2,3] These findings are consistent with hypobaricity as a mechanism to explain postural effects on extent of sensory block and side effects produced by dextrose-free intrathecal anesthetics.[10,11,14-16]

Two groups of investigators previously measured the density of human CSF at 37°C,[4,5] each reporting different means (fig. 2). Davis and King[4] studied 9 patients (7 males, 2 females) undergoing pneumoencephalography for central neurologic disorders, and Levin *et al.* studied 9 healthy male volunteers.[5] Densities were calculated from mass and volume measured at 37°C. Both studies reported 1.7- to 7.5-fold larger standard deviations than we observed (table 1, fig. 2). Reduced ac-

curacy and unspecified precision of mass, volume, and temperature measurements associated with their techniques limit the findings of both. In addition, restriction of determinations to specific subpopulations (individuals with neurologic disorders[4] and male volunteers[5]) limits application of their results to all patients. These differences in technique and patient selection likely account for the differences between our results and those of the previous reports.

Our data indicate that CSF density is significantly lower in the peripartum period. The chemical basis of this finding remains to be elucidated. Two previous studies failed to demonstrate a significant difference in CSF total protein content between pregnant and nonpregnant patients,[8,9] although a wide range of values was reported in both groups. Progesterone may be a physiologic mediator of

Table 1. Cerebrospinal Fluid (CSF) Densities in Five Groups of Patients

Group	No. of Patients	Age [mean ± SD (range) (yr)]	CSF Density [mean ± SD (g/ml)]	Upper Limit of Hypobaricity* (g/ml)
Men	10	43 ± 13 (28–65)	1.00064 ± 0.00012	1.00028
Postmenopausal	8	67 ± 11 (53–84)	1.00070 ± 0.00018	1.00016
Premenopausal (nonpregnant)	6	36 ± 6.9 (27–43)	1.00049 ± 0.00004	1.00037
Postpartum	10	28 ± 6.0 (21–37)	1.00034 ± 0.00005†	1.00019
Term pregnant	10	29 ± 4.5 (23–40)	1.00030 ± 0.00004†	1.00018

* Mean density minus three standard deviations.[1,4]

† Different from mean densities in men, postmenopausal, and premenopausal (nonpregnant) groups ($P < 0.05$).

Fig. 2. Comparison of human cerebrospinal fluid density measurements reported by Davis and King[4] and Levin *et al.*,[5] and those measured in this study. Densities are presented as mean ± three standard deviations (g/ml). Mean density minus three standard deviations has been defined as the upper limit of hypobaricity,[1,4] previously taken to be 0.99940 g/ml,[3,5] and determined by us to range from 1.00016 to 1.00037 g/ml.
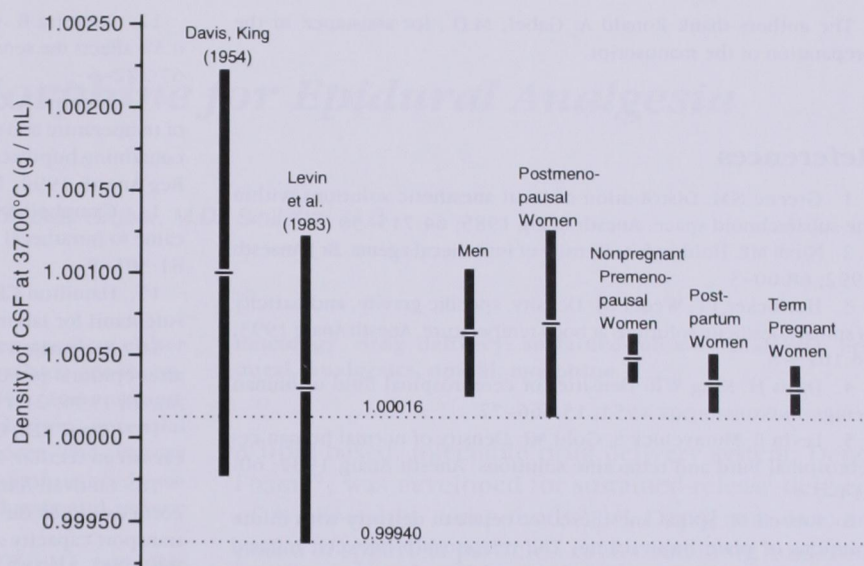
altered CSF densities during human pregnancy, because progesterone treatment of estrogen-primed nonpregnant rabbits significantly alters Na^+, K^+-ATPase activity in isolated choroid plexi.[18] This enzymatic activity is the primary driving force for CSF production.[19]

We chose the mechanical oscillation technique to measure density because of its well-established accuracy (±0.00001 g/ml) and high level of precision.[20–23]‡ Pycnometry predated the mechanical oscillation technique for density determination, and despite its limitations, had been the method of choice for density determination.[22] However, soon after its introduction in 1966, the mechanical oscillation technique supplanted pycnometry as the preferred method for density determination for fluids (including gases, aqueous and nonaqueous liquids, organic and inorganic solutions, and colloidal suspensions) in a variety of industries, for both research and quality applications.[20]‡ This method has been compared to pycnometry, and is simpler, requires less time and smaller samples, and gives more reproducible results.‡

Although all samples were inspected visually for gross blood contamination, we did not perform microscopic examination of the CSF samples. Although nonvisible amounts of blood may impact on density measurements, this source of error would be expected

to confound results of previous studies as well. Such contamination also would be expected to influence density measurements in each of the five groups evaluated in this study, which does not appear to be the case, given the intergroup differences in both density means and standard deviations of the means. Because the density of human blood is significantly greater than that of CSF,[20,23] blood contamination of CSF samples would be expected to result in significantly greater density values. However, mean CSF density values of all five groups were found to be intermediate between the two previously reported values. The most striking finding is the exceedingly high reproducibility in each of the five groups studied.

In summary, we determined values for the density of human CSF. The ranges of values measured are much narrower than previously reported, likely because of the greater accuracy and precision of the measurement technique used. The values defining hypobaricity of intrathecal solutions are greater than previously proposed. In addition, we observed significant differences in mean CSF density among subgroups studied, with density significantly lower at term gestation and immediately after pregnancy as compared with men and nonpregnant women. Consideration of baricity as a mechanism for observed differences in extent of block using different dextrose-free intrathecal solutions should take into account differences in CSF densities between patient subgroups.

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M. G. RICHARDSON AND R. N. WISSLER

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