Relationship of Octanoylcarnitine Concentrations to Age at Sampling in Unaffected Newborns Screened for Medium-Chain Acyl-CoA Dehydrogenase Deficiency

Javaria M. Khalid,1 Juliet Oerton,1 Guy Besley,2 Neil Dalton,4 Melanie Downing,4 Anne Green,5 Mick Henderson,6 Steve Krywawych,7 Veronica Wiley,8 Bridget Wilcken,8 and Carol Dezateux,1* on behalf of the UK Collaborative Study of Newborn Screening for MCADD

BACKGROUND: Although octanoylcarnitine (C8) concentrations measured from newborn screening dried blood spots are used to identify those at high risk of medium-chain acyl-CoA dehydrogenase deficiency (MCADD), age-related reference values are currently not available for unaffected newborn populations. Because age at sampling may vary within and between screening programs, variations in C8 concentrations by age may affect screening program performance. We determined whether C8 concentrations vary by age at sampling, sex, birth weight, or gestational age in unaffected newborns.

METHODS: We analyzed C8 concentrations from 227 098 unaffected newborns, including 179 729 from 6 English laboratories participating in a multicenter study and 47 369 from the single laboratory serving the New South Wales (NSW) Newborn Screening Program in Australia. In England, the majority of samples were collected at age 5–8 days and analyzed undervatized by use of tandem mass spectrometry (MS/MS); in NSW, samples were obtained at a median age of 3 days and analyzed derivatized by MS/MS. Information on infants’ sex, birth weight, gestation, hospitalization, and transfusion status was recorded at time of sampling.

RESULTS: C8 concentrations did not vary significantly by age at sampling, sex, birth weight, or gestational age and remained relatively constant during the first 2 weeks of life in unaffected babies being screened for MCADD.

CONCLUSIONS: Newborn MCADD screening programs using this biomarker for screening samples collected after the first day and during the first 14 days of life do not need to adjust cutoff values to account for postnatal age, prematurity, or size at birth. © 2010 American Association for Clinical Chemistry

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)9 leads to the accumulation of octanoylcarnitine (C8) in the blood and can be diagnosed by analysis of the concentration of acylcarnitines by use of tandem mass spectrometry (MS/MS) (1). The development of electrospray ionization MS/MS allows those at risk of MCADD to be identified by screening presymptomatic newborn infants using the same blood spot collected for analysis of phenylketonuria. The aim of newborn screening for MCADD is to prevent death and, in the longer term, neurological disability through early diagnosis and management.

Currently, many countries have newborn screening programs that use MS/MS for MCADD, including the Netherlands, Germany, Denmark, the US, and Australia; the programs vary by age at which blood samples are collected. Although C8 concentrations obtained from dried blood spots are widely used for the detection of MCADD, little attention has been paid to possible age-related variations in reference values in unaffected newborn populations. Because the age at testing may vary within and between screening programs, variations in C8 concentrations with age have a potential impact on screening program performance.

Our goals were to ascertain whether the concentration of C8 is affected by age at sampling, as has been suggested (2, 3), whether this change occurs in unaffected newborns as well as in those with MCADD (2),

1 MRC Centre of Epidemiology for Child Health, Institute of Child Health, University College London, London, UK; 2 Royal Manchester Children’s Hospital, Manchester, UK; 3 Guy’s Hospital, London, UK; 4 Sheffield Children’s Hospital, Sheffield, UK; 5 Birmingham Children’s Hospital, Birmingham, UK; 6 St James’ University Hospital, Leeds, UK; 7 Great Ormond Street Hospital for Children, London, UK; 8 The Children’s Hospital at Westmead, Sydney, Australia.

© Address correspondence to this author at: MRC Centre of Epidemiology for Child Health, Institute of Child Health, University College London, 30 Guilford St., London, WC1N 1EH UK. Fax +44-20-7905-2381; e-mail c.dezateux@ich.ucl.ac.uk.

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9 Nonstandard abbreviations: MCADD, medium-chain acyl-CoA dehydrogenase deficiency; C8, octanoylcarnitine; MS/MS, tandem mass spectrometry; NSW, New South Wales; MRM, multiple reaction mode; QA, quality assurance; VLBW, very low birth weight; IQR, interquartile range.
and whether thresholds need to take into account age at sampling, sex, or prematurity. Although potentially important for the optimal investigation and management of MCADD, age-related reference values of C8 are currently not available.

Materials and Methods

To determine C8 concentrations in the unaffected newborn population, we used data from the UK Collaborative Study of Newborn Screening for MCADD collected between November 2004 and April 2005 (inclusive) to examine the results of all infants who screened negative for MCADD. We also obtained data collected over the same time period in the Australian New South Wales (NSW) Newborn Screening Program, where screening is carried out at an earlier age than in the UK. We evaluated the variation in C8 concentrations measured by MS/MS with age at sampling, sex, birth weight, and gestational age.

Six centers participating in the UK Collaborative Study of MCADD10 screened approximately half the births in the UK (approximately 350 000 births per year). These centers were Guy’s Hospital, London; Great Ormond Street Hospital for Children, London; Royal Manchester Children’s Hospital, Manchester; Sheffield Children’s Hospital, Sheffield; Birmingham Children’s Hospital, Birmingham; and St James’ University Hospital, Leeds.

Heel-prick blood samples were collected onto Whatman or Schleicher and Schuell 903 (ID Biological Systems) bloodspot filter paper, with the majority collected between days 5 and 8 after birth (with day of birth counted as day 0) and analyzed underivatized by MS/MS with MRM acquisitions for quantification of octanoylcarnitine. All laboratories participated in an external quality assurance (QA) scheme. Screened values ≥0.40 μmol/L were repeated in duplicate using the same blood spot. A presumptively positive screening test for MCADD was defined by mean triplicate C8 values ≥0.50 μmol/L. All children with a presumptive positive test for MCADD were referred for diagnostic follow-up and treatment to a local specialist center.

During the data-collection period, the single laboratory serving the NSW Newborn Screening Program screened approximately 95 000 births per year, with a median age at sampling of 3 days; >99% of newborns were sampled before day 6 (4). Screening samples were collected on to Whatman BFC 180 bloodspot filter paper (Whatman Asia Pacific). Those with an initial C8 value ≥0.8 μmol/L were reassayed (same spot) and considered presumptively positive if the mean of these 2 assays was ≥1.0 μmol/L (5). Samples collected before 48 h of life were analyzed but deemed inappropriate for reporting, and repeat samples were collected from these babies. MS/MS with MRM acquisitions was used to quantify C8, and samples were, at that time, derivatized using butanolic HCl, a method adapted from Millington et al. (6).

In all centers, the midwife or health professional collecting the sample was asked to record infant birth weight, duration of gestation, and if the child was in a hospital or required a transfusion. In NSW, mothers were usually still in hospital on the day the sample was collected (at 48–72 h, day 2–3), whereas in the UK, mothers were usually discharged home by day 5–8, where a midwife would take the sample.

Information on C8 concentrations and age at sampling were provided by all screening centers and on birth weight, gestational age, hospital, and transfusion status by all but 1 English center. We excluded infants sampled before 4 days of age (n = 122) in the English dataset. In addition, those who exceeded the UK cutoff of C8 ≥0.5 μmol/L (n = 61 in England; n = 20 in NSW) as well as those born with a birth weight <0.5 kg (n = 4 in England; n = 5 in NSW) were excluded from both datasets. This gave a combined total of 227 098 samples (n = 179 729 and 47 369 from the English centers and NSW, respectively). For the purposes of this study, prematurity was defined as ≤37 completed weeks’ gestation, and very low birth weight (VLBW) as ≤1.5 kg.

We analyzed data with summary statistics using Stata/SE version 9.2 (Stata Corp.). We used Wilcoxon rank sum test with continuity correction to calculate 95% CIs for difference of medians using R version 2.4.1 (R Corp.).

Results

SCREENING CENTERS IN ENGLAND

During the data collection period, 92.9% [interquartile range (IQR) 90.5%–94.6%] of samples were collected.
by 8 days of age across all 6 screening centers. The median age at sampling ranged from 5 to 7 days, and median C8 concentrations, from 0.04 to 0.06 µmol/L between the centers.

Median C8 concentrations and their distributions did not vary by age at sampling by screening centre (Fig. 1). We therefore combined data from these 6 screening centers in all subsequent analyses. The interquartile values of the combined C8 data for newborns in England ranged from 0.04 to 0.07.

Overall, 51.1% of newborns tested were male, 1.2% VLBW, and 7.9% premature. At time of sampling, 7.9% were recorded as being in the hospital and 0.3% as having received a blood transfusion. The median C8 concentration was 0.06 (IQR 0.04–0.07) µmol/L in boys compared with 0.05 (IQR 0.04–0.07) µmol/L in girls. Similarly, median C8 concentrations were 0.07 and 0.05 µmol/L in those with VLBW or normal birth weight, respectively, and 0.06 and 0.05 µmol/L in those born prematurely or at full term, respectively. Median C8 concentrations based on samples from infants of known gestational age were not clinically different from those based on samples from infants with missing gestational age (data not shown).

ENGLISH AND NSW SCREENING CENTERS COMPARED

In NSW, median age at sampling ranged from 2 to 3 days, and median C8 concentrations, from 0.06 to 0.11 µmol/L (Table 1); 51.6% of newborns tested were male (1.2% missing), 1.0% VLBW, and 6.7% premature. At time of sampling, 92.1% were recorded as being in the hospital and 0.1% as having received a blood transfusion. The median C8 concentration was 0.09 (IQR 0.07–0.12) µmol/L in boys compared with 0.08 (0.06–0.11) µmol/L in girls. Similarly, median C8 concentrations were 0.09 and 0.08 µmol/L in those with VLBW or normal birth weight, respectively, and 0.08 and 0.09 µmol/L in those born prematurely or full term, respectively.

Comparing data from the 2 countries, the birth weight and gestation of the screened newborns were similar (Table 1). The IQR of age at sampling was 2–3 days in NSW compared with 5–7 days in England. Overall, the median concentration of C8 was only
slightly lower at 0.05 (IQR 0.04–0.07) μmol/L in England compared with 0.09 (0.06–0.11) μmol/L in NSW (95% CI of the difference of medians −0.04, 0.04 μmol/L, SE 1.92 × 10⁻⁷) (Table 1).

We were able to compare median C8 concentrations on day 4, when sufficient data were available from both countries for a combined total of 20,628 blood-spot samples (Table 2). At 4 days of age, in England, 4.0% of children were reported to be in the hospital compared with 88.6% of children screened on day 4 in NSW, reflecting country differences in postnatal care. A similar percentage of children received blood transfusions, were premature, or had VLBW [0.04% (3.8% missing) in England and 0.07% (0% missing) in NSW; 5.5% in England and 7.3% in NSW; 0.39% in England and 0.9% in NSW, respectively]. The minor differences in C8 concentration medians were not clinically important. These values did not vary with day of sampling between England and NSW and were relatively constant over the first 2 weeks of life (Fig. 2).

**Discussion**

This is the first multicenter cross-country study examining C8 concentrations in unaffected infants undergoing newborn screening for MCADD. Using large representative datasets from newborn screening programs in England and NSW, we demonstrated that C8 concentration does not vary by age at sampling within the first 2 weeks of life or by sex, birth weight, or length of gestation. Furthermore, clinically important differences in C8 concentrations were not observed between the 2 screening programs where the protocols for screening varied by derivatization technique.

Strengths of our study include the large number of prospectively collected study samples on which C8 concentrations and related demographic variables are based. Additionally, we were able to describe data from a large number of premature infants, a subgroup that is important to consider in newborn screening programs (7). Furthermore, we obtained comparable data from newborns in 6 English screening centers and 1 Australian center, all with a high level of completeness of coverage for the regions and time period of interest (8). This high level of coverage can be explained in part by these screening programs being free of charge and universal in both countries (8, 9), in contrast to programs offered in other developed countries, where access to health care may depend on parental ability to pay (10).

To our knowledge, there has only been 1 prospective study, by Chace et al. (1), examining C8 concentrations analyzed by a consistent method in unaffected newborns after 72 h of age; however, this was conducted in a small sample (n = 133). In these newborns, C8 concentrations were reported to be very low, with the median value being below detection limits. More recently, Rhead (11) tabulated means/medians of C8 concentrations in unaffected newborns taken from

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| a Data are median (IQR).  
b 20.0% missing birth weight.  
c 39.0% missing gestational age.  
d 0.3% missing birth weight.  
e 3.0% missing gestational age. |

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published reports of eight screening programs: these ranged from 0.05 to 0.30 µmol/L. However, some of the programs included had a sampling age of <72 h and are not directly comparable to the samples used in our study. Additionally, these studies do not allow conclusions to be drawn regarding variation in values by day of sampling.

Previous studies have reported that acylcarnitine profiles in premature and VLBW infants differ from profiles of babies born at term or of normal birth weight (7, 12). Were this the case, it might pose a problem in interpreting results from screening programs (4, 13). We have demonstrated, however, that, at the population level, C8 concentrations do not vary by prematurity or birth weight over the first 2 weeks of life. Similarly, no differences were found between sexes.

Several countries, including the US, collect newborn bloodspot screening samples between days 1 and 9, and in some places as early as 16 h after birth (14–16). These screening programs use different thresholds to define a positive screening result for MCADD owing to differences in screening criteria, health technologies, and case definitions (17). Such differences in thresholds may limit direct comparison of screening program performance between studies. In our study, we used data obtained using a common MS/MS protocol across the 6 English screening centers, all of which participated in an external QA scheme for C8. We combined these values with data analyzed in NSW by a protocol that differed by the day of sampling and derivatization method and found no clinically important differences in C8 concentrations. It is possible that the minor differences observed in median C8 values in England compared with NSW may be explained by the derivatization of the NSW samples. Derks et al. (3) have also reported the mean C8 concentration of underivatized blood samples to be slightly but not significantly lower than derivatized samples. In both the study by Derks and our study, however, the differences observed in C8 concentrations are negligible and not clinically significant for a newborn screening program. Consequently, we conclude that there is scope for making comparisons between screening programs that differ by age of sampling and derivatization method, as C8 appears to be a stable biomarker for MCADD.

We were unable to report data on C8 concentrations in the first 24 h of life or information on whether infants being tested were sick, both of which may have

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**Fig. 2. C8 concentrations in newborns without MCADD.**

Samples from NSW taken at age 1–9 days, English samples at age 3–14 days. Data exclude presumptive positives for MCADD (C8 values ≥0.5 µmol/L). For illustrative purposes, random noise (jitter) was applied to data points.
an impact on C8 concentrations. In addition, information on ethnicity was not available at the time in the datasets used in our study.

Our study is based on cross-sectional data from an unaffected population. However, there have been previous studies examining age variation in C8 concentrations among children with confirmed MCADD (1, 2, 4, 7), demonstrating that infants tested at <10 weeks of age have significantly higher (P < 0.05) C8 concentrations than those tested at later ages (2) and that a 3-fold decline of C8 concentrations is seen over the first weeks of life (11). Although C8 concentrations in infants with MCADD are reported to vary by gestational age (7), we did not observe such a fall in our study. Currently, the cutoff used to identify infants in whom MCADD is suspected varies considerably between international screening programs (including those reported from the US, Canada, Germany, Portugal, Italy, Switzerland, and Austria) and ranges from 0.2 to 1.0 μmol/L. These values are approximately 4–11 standard deviations above C8 concentrations seen in unaffected children (11). Additionally, results from a screening program in the Netherlands and from a retrospective study of dried blood spots in the UK described a mean value of 6.01 μmol/L (3) and 3.04 μmol/L (2), respectively, in affected children with MCADD. Because the C8 concentrations in the pathologic state of MCADD (C8 ≥0.5 μmol/L) are markedly higher than those observed in an unaffected population in the UK MCADD screening program, the small differences in C8 concentrations seen in our study are not clinically important.

We were unable to examine C8 concentrations according to heterozygosity for the common mutation c.985A>G, which has been reported to be associated with increased C8 concentrations in the first weeks of life (7, 12, 15, 18). We recently reported the heterozygote frequency of c.985A>G to be 1/65 in the UK newborn population (19). From this, we estimate that in this study we would expect 2722 c.985A>G heterozygotes among the 179,916 unaffected newborns screened in England over the study period (comprising 179,729 unaffected and 187 previously excluded infants). In contrast, only 7 infants were identified as heterozygous for c.985A>G among those who tested presumptively positive during the UK MCADD screening study in the same period. Therefore, we conclude that the population of newborns heterozygous for the c.985A>G mutation is not at a high risk of being detected in the UK screening program, where a threshold of C8 ≥0.5 μmol/L is used to define a presumptively positive screening result for MCADD.

Currently, there are no reports based on large-scale studies to determine the effect of mode of feeding on C8 concentrations: the laboratories were unable to provide us with data on feeding method. Approximately 70% of mothers initiate breastfeeding in England, but this declines rapidly in the first week of life (20). We were therefore unable to address the suggestion that C8 concentrations are increased in poorly breastfeeding newborns heterozygous for the c.985A>G mutation.

In summary, we have shown that C8 concentrations do not vary according to age at sampling, sex, birth weight, or gestational age, and that they remain relatively constant during the first 2 weeks of life in unaffected babies. These findings provide useful evidence for MCADD screening programs using C8 as a biomarker in samples obtained from newborns between the second and fourteenth days of life.

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References

2. Clayton PT, Doig M, Ghafari S, Meane C, Taylor...


