Quantitative Metabolic Profiling of Second and Third Trimester Human Amniotic Fluid via High-Resolution MR Spectroscopy: Analysis for Biomarkers of Fetal Maturation

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Introduction

High-resolution MR spectroscopy of human amniotic fluid shows promise in the evaluation of fetal lung maturity, gestational diabetes, drug toxicity, birth defects, and normal fetal growth and development (1,2). Prior studies have identified numerous MR detectable metabolites in human amniotic fluid; though only a few of these have examined how metabolite concentrations change as a function of gestational age (3). Correlation of metabolite concentrations with gestational age provides normative data for future studies and information about normal developmental patterns. In the present study, ^{1}H high-resolution magic angle spinning (HR-MAS) spectroscopy was used to measure and compare the concentrations of 21 metabolites in second versus third trimester human amniotic fluid samples in a search for markers of lung and renal maturity.

Methods

Fifty amniotic fluid samples were obtained at amniocenteses for clinical evaluation of fetal lung maturity or karyotyping (mean: 27 weeks gestation, range: 16-39 weeks). ^{1}H HR-MAS spectroscopy was performed at 11.7 T (500 MHz for ^{1}H), 1°C, and a 2250 Hz spin rate using a Varian INOVA spectrometer equipped with a 4 mm gHX nanoprobe. 3.0 µl of D_2O containing 0.75% TSP was pipetted into a 35 µl zirconium rotor and weighed, after which the amniotic fluid was added to the rotor and weighed (mean: 22.53 mg, range: 19.01-26.15 mg). Quantitative 1D spectra were acquired with a 90° pulse, 4s presaturation, 2s acquisition (TR = 6s), 256 transients, 40,000 points, 20,000 Hz spectral width, time = 26 min. The Electronic REference To access In Vivo Concentrations (ERETIC) method was used for quantitation (4). A database containing quantitative spectra of 21 known metabolites was created. Metabolite concentrations were then determined using these reference spectra and an automated spectral fitting software algorithm (QUEST) (5). Concentrations were compared between 2nd (15-20 weeks gestation, N=20) and 3rd (34-39 weeks gestation, N=21) trimester samples using the Wilcoxon test, samples between 21 and 33 weeks gestation (N=9) were not grouped. A stepwise multiple linear regression model (step-wise forward procedure) was applied to all 50 samples.

Results

Figure 1 shows representative ^{1}H HR-MAS spectra of A) 2nd (16 weeks 6 days gestation) and B) 3rd trimester (36 weeks) amniotic fluid. Both spectra are scaled relative to lactate. The region between 2.95 and 3.35 ppm is magnified to illustrate the significant decrease in glucose concentrations between 2nd and 3rd trimester samples and the concurrent increase in betaine and creatinine. As shown in Table 1, the concentrations of 15 metabolites were significantly different ($p < 0.05$, Wilcoxon test) between 2nd and 3rd trimester samples. Table 2 displays results from a stepwise multiple linear regression model, which assessed the joint capability of five selected metabolites (alanine, betaine, choline, creatinine and glucose) to predict gestational age. These metabolites were selected *a priori* as representative compounds that may be related to fetal lung and renal maturity. Terms were retained or dropped at a significance level of 0.01. This model yielded a significant additive contribution from 3 of the 5 metabolites. When applied sequentially, the combination of alanine, creatinine and glucose create a linear prediction model that fits the data with an R-square value of 0.90. When this model fitting procedure was repeated for all 21 metabolites, these same three compounds were part of the final model (with a minor added contribution from 3-Hydroxybutyrate).

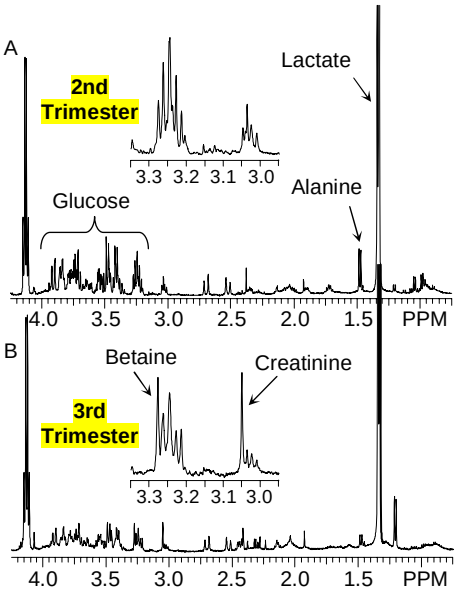

Figure 1: ^{1}H HR-MAS spectra of A) 2nd and B) 3rd trimester amniotic fluid samples.

Table 1: Concentrations of significantly different metabolites in 2nd versus 3rd trimester amniotic fluid samples.

	2nd Tri (mmolal)		3rd Tri (mmolal)		P
Metabolite	**mean**	**SD**	**mean**	**SD**	**(Wilcoxon)**
Alanine	0.881	0.217	0.236	0.129	<0.002
Betaine	0.00860	0.00200	0.0133	0.0060	0.011
Citrate	0.775	0.156	0.406	0.129	<0.002
Creatine	0.166	0.081	0.101	0.055	0.008
Creatinine	0.0123	0.0061	0.252	0.011	<0.001
GPC	0.0370	0.0150	0.0181	0.0161	<0.001
Glutamine	0.198	0.092	0.308	0.145	0.006
Glutamate	1.12	0.26	0.342	0.324	<0.001
Glucose	6.36	1.20	2.35	1.32	<0.001
Lactate	20.0	5.3	15.5	3.8	0.006
Leucine	0.0784	0.0316	0.0251	0.0259	<0.001
Lysine	0.740	0.163	0.291	0.222	<0.001
Pyruvate	0.0664	0.0092	0.0232	0.0216	<0.001
Succinate	0.0532	0.0646	0.0858	0.0726	0.006
Valine	0.829	0.305	0.304	0.179	<0.002

Table 2: Results of stepwise linear regression

Variable	Rsquare	Cp	ProbF
Alanine	0.77	54.18	<0.0001
Creatinine	0.83	29.79	0.0002
Glucose	0.90	5.84	<0.0001

Discussion and Conclusions

Metabolite profiles for 2nd and 3rd trimester amniotic fluid samples show clear differences with gestational age and are consistent with previous studies (3). Without adjusting significance thresholds to account for multiple comparisons, 15 of 21 metabolite concentrations differed significantly between 2nd and 3rd trimester groups ($p < 0.05$). Furthermore, when a Bonferroni correction was applied (i.e. a significance threshold of $p < 0.05/21 = 0.00238$), 10 metabolites (alanine, citrate, creatinine, glutamate, glucose, GPC, leucine, lysine, pyruvate and valine) were still significantly different. There was clear statistical evidence that decreasing glucose and amino acid concentrations were correlated with advancing gestational age, as well as concurrent increases in creatinine and betaine. This data provides excellent metabonomic evidence of pulmonary and renal maturation. Stepwise linear regression models suggest that gestational age can be accurately predicted using combinations of alanine, glucose and creatinine concentrations. These results provide key normative data for determining differences between normal and diseased states during fetal development, suggest future metabolic markers for assessing fetal maturation, and may provide the foundation for the development of in vivo MR spectroscopy as a future noninvasive test for evaluating fetal development.

References

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