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Influence of maternal nutrition in pregnancy

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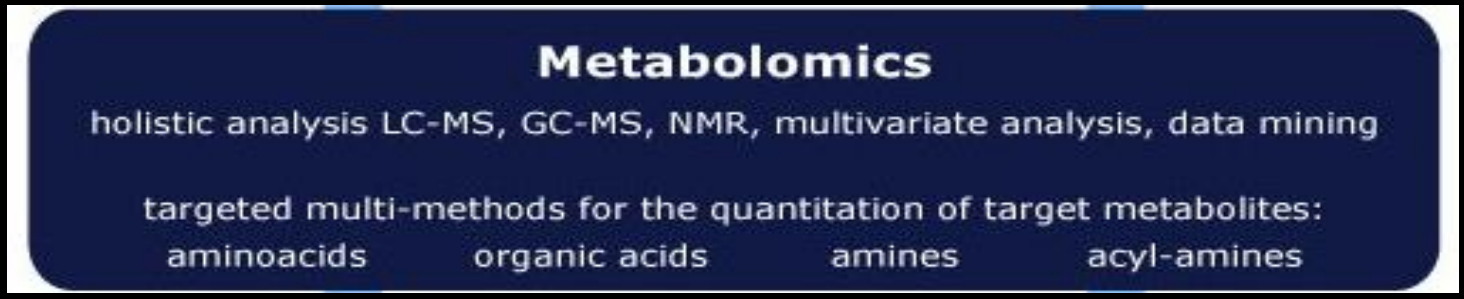
Metabolomics as a tool for the assessment of

A. the embryo growth and viability in *in vitro* fertilization and

B. The influence of maternal nutrition on

1. Fetal and neonatal growth

2. maternal blood and amniotic fluid composition



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OPERATIONAL PROGRAMME
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investing in knowledge society
MINISTRY OF EDUCATION, LIFELONG LEARNING AND RELIGIOUS AFFAIRS
MANAGING AUTHORITY
NSRF 2007-2013
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Department of Obstetrics & Gynecology, Aristotle University of Thessaloniki

Department of Food Science & Technology, School of Agriculture, Aristotle University of Thessaloniki

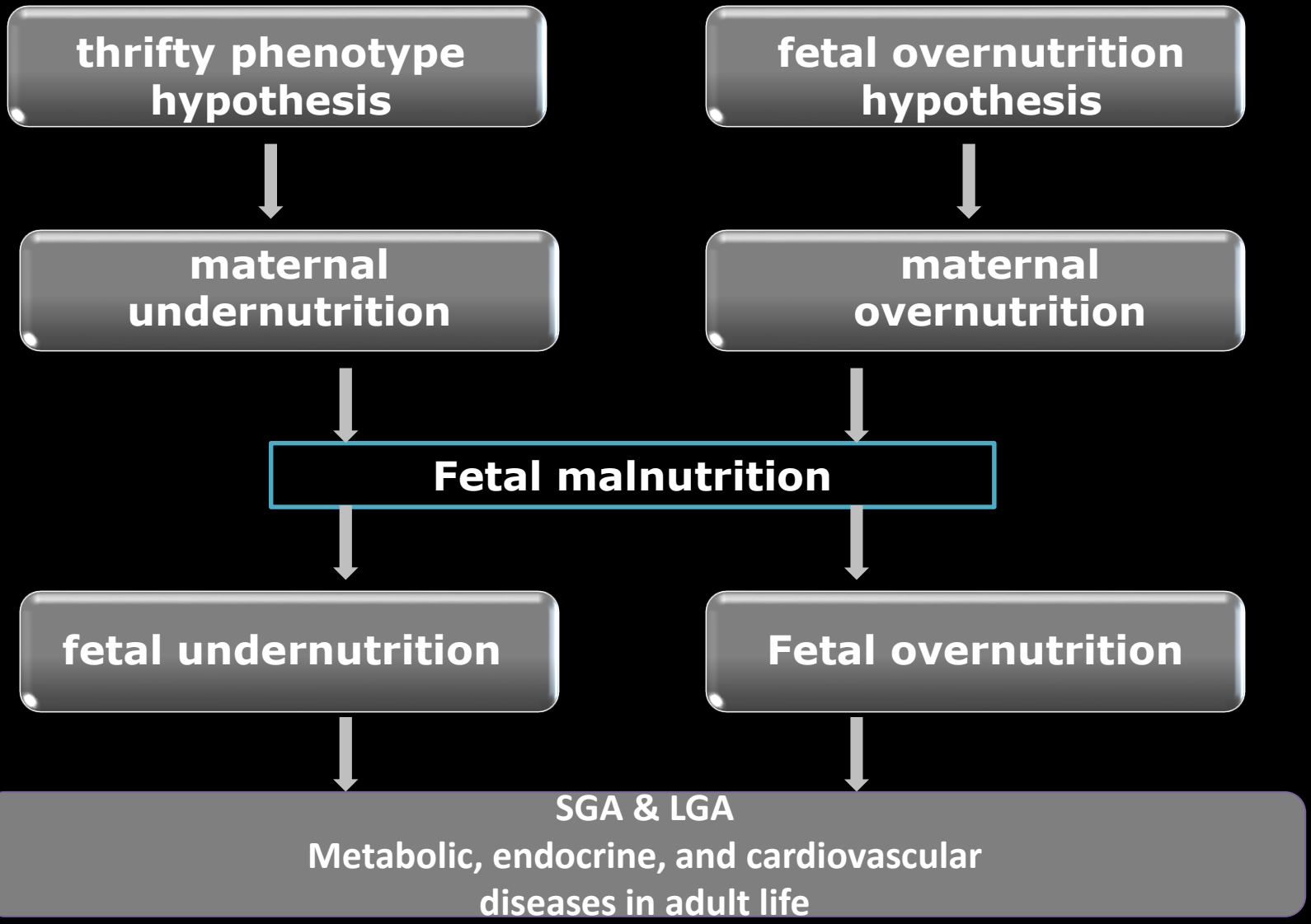
Department of Chemistry, Aristotle University of Thessaloniki

Obstetrics and Gynecology University Clinic, University of Ioannina

Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation

FETAL PROGRAMMING

alteration of gene expression during critical periods of development



Hales, C. N., & Barker, D. J.: *British medical bulletin*, 2001

Koepp UMS, et al: Acta Obstet Gynecol Scand, 2012

Influence of maternal nutrition in pregnancy

1. Relation of maternal pre-pregnancy BMI with amniotic fluid amino acids
2. Second trimester amniotic fluid glucose, uric acid, phosphate, potassium, and sodium concentrations in relation to maternal pre-pregnancy BMI and birth weight centiles
3. Dietary patterns reflecting 2nd trimester amniotic metabolomic profile

Second trimester amniotic fluid amino acid levels in relation to maternal pre-pregnancy Body Mass Index

Maria Fotiou[1]; Gianna Georganta[1]; Foteini Tsakoumaki[1]; Alexandra-Maria Michaelidou[1]; Apostolos P. Athanasiadis[2]; George Menexes[3]; Maria Ganidou[2]; Georgios Theodoridis4 ; Costas G. Biliaderis[1]; Basilis Tarlatzis[2]

(1) Aristotle University of Thessaloniki, School of Agriculture, Department of Food Science & Technology; (2) Aristotle University of Thessaloniki, 1st Department of Obstetrics & Gynecology; (3) Aristotle University of Thessaloniki, School of Agriculture, Department of Field Crops and Ecology; (4) Aristotle University of Thessaloniki, Department of Chemistry, Laboratory of Analytical Chemistry

Aim of the study

2nd trimester amniotic fluid amino acid composition

↓

maternal pre-pregnancy Body Mass Index (BMI)

2013 Moscow WORLD CONGRESS XI WORLD CONGRESS OF PERINATAL MEDICINE

Patients - Method

Subjects :

54 singleton pregnant women undergoing amniocentesis for prenatal diagnosis, between 17 and 24 weeks of gestation.

Somatometric parameters :

Data collection from questionnaires

According to the World Health Organization BMI criteria, women were divided into three groups:

- ✓ **the normal group (pre-pregnancy BMI 18.5-24.9 kg/ m², n= 25),**
- ✓ **the overweight group (pre-pregnancy BMI 25-29.9 kg/ m², n= 19),**
- ✓ **the obese group (pre-pregnancy BMI >30 kg/ m², n=10)**

Amniotic fluid analysis:

RP-HPLC / dabsyl chloride derivatives

Krause et al., 1995. Journal of Chromatography A , 715: 67–79

Correlation between amniotic fluid amino acids and maternal pre-pregnancy BMI

		ala	val	ile	leu	phe
maternal pre-pregnancy BMI (kg/m^2)	*Pearson's r*	0.253	0.422	0.306	0.363	0.329
	P	0.065	<0.05	<0.05	<0.05	<0.05

BMI, body mass index

Correlation between amniotic fluid amino acids and maternal pre-pregnancy BMI

Multivariate analysis of variance (MANOVA):

It has long been recognized that several amino acids, including val, leu, ile, are elevated in the blood of obese subjects relative to healthy individuals

Wang & Guo, 2013. Chin Sci Bull, 58: 1228-1235

↑ Amino acid concentrations
↑↑ Placental transport systems and
Paolini CL, et al. 2001. American Journal of Physiology

↓

that are required by fetal development

Gestational age and maternal pre-pregnancy body mass index: potential determinants of human amniotic fluid metabolic profile

Apostolos Athanasiadis[1]; Panagiotis Zoumpoulakis[2]; Alexandra-Maria Michaelidou[3]; Charalambos Fotakis[2]; Maria Fotiou[3]; Foteini Tsakoumaki[3]; Gianna Georganta[3]; George Menexes[4]; Maria Ganidou[1]; Georgios Theodoridis[5]; Costas G. Biliaderis[3]; Basilis Tarlatzis[1]

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Aim of the study

Second trimester amniotic fluid profile

gestational age

maternal pre-pregnancy body mass index (BMI)

using
a conventional analytical research protocol and
a holistic NMR metabolomics approach

Method

Subjects:
44 singleton pregnant women, undergoing amniocentesis for prenatal diagnosis between 17 and 24 weeks of gestation.

Somatometric parameters:

Data collection from questionnaires

According to the World Health Organization BMI criteria, women were divided into two groups:

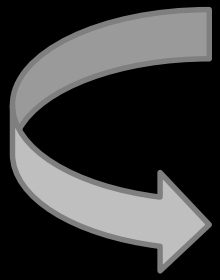
- ✓ **the normal group (pre-pregnancy BMI 18.5-24.9 kg/ m², n= 25),**
- ✓ **the overweight group (pre-pregnancy BMI 25-29.9 kg/ m², n= 19)**

Amniotic fluid analysis:

RP-HPLC / dabsyl chloride derivatives
Krause et al., 1995. Journal of Chromatography A , 715: 67–79

Varian-600MHz NMR spectrometer with a 1H {13C – 15N} 5mm PFG Autox ID probe - National Hellenic Research Foundation

Effect of maternal pre-pregnancy BMI on amniotic fluid by the conventional analytical research protocol (HPLC)



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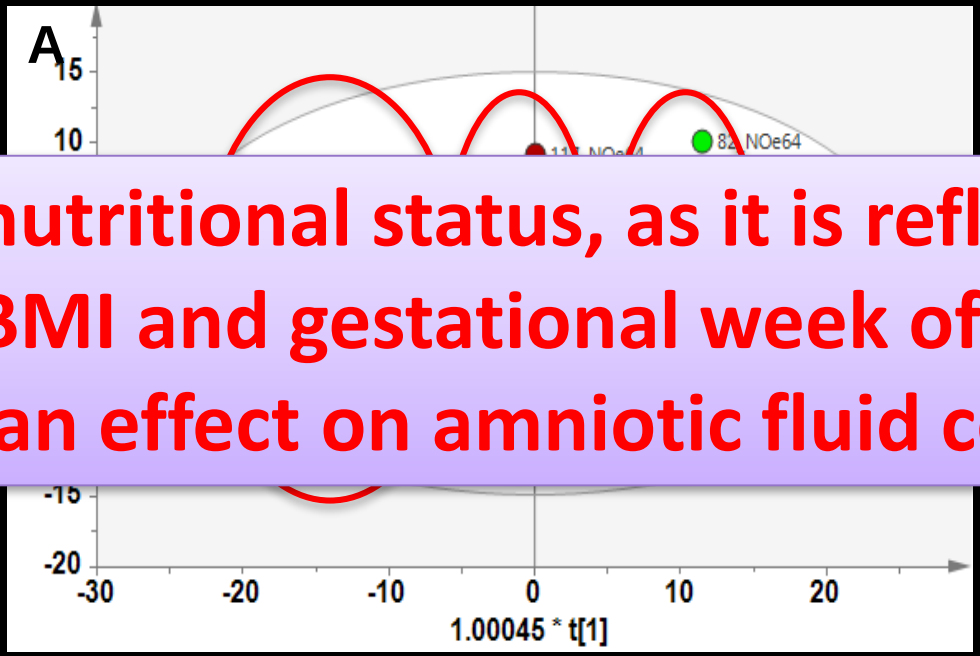
XI WORLD CONGRESS OF
World Association of Perinatal Medi
WAP

Gestational age and maternal pre-pregnancy body mass index: potential determinants of human amniotic fluid metabolic profile

NMR metabolomics



Maternal nutritional status, as it is reflected in pre-pregnancy BMI and gestational week of 2nd trimester may have an effect on amniotic fluid composition.

Figure 1: **OPLS-DA, Par, A=3+2, N=19, R^2= 0.92, Q^2= 0.33**

Group A: BMI=NL,Test Week 18-20. Group B: BMI=NL, Test Week >20.
Group C: BMI=OW Test Week < 20. Group D: BMI=NL, Test Week < 18.

2013 Moscow WORLD CONGRESS XI WORLD CONGRESS OF PERINATAL MEDICINE World Association of Perinatal Medicine WAPM

Influence of maternal nutrition in pregnancy

1. Relation of maternal pre-BMI with amniotic fluid amino acids
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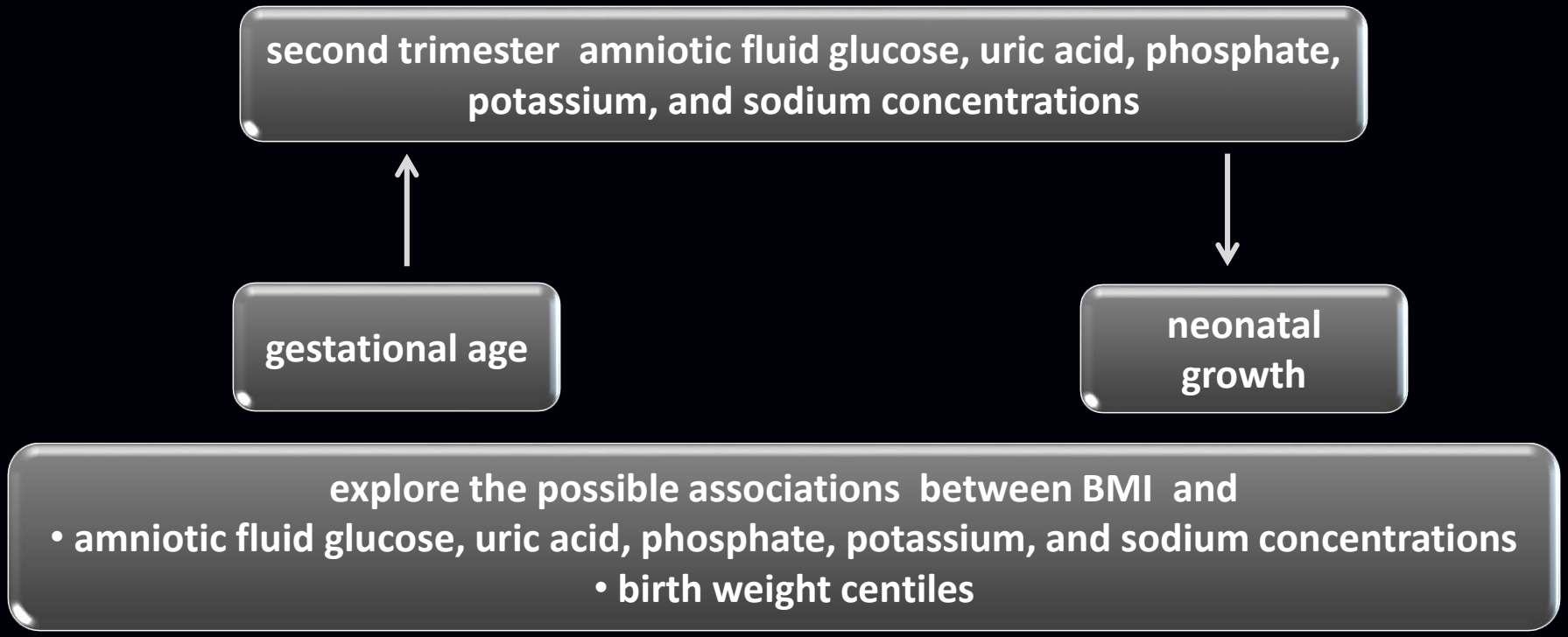
Aim of the study



Fotiou M: JMNFM, 2014

Second trimester amniotic fluid glucose, uric acid, phosphate, potassium, and sodium concentrations in relation to maternal pre-pregnancy BMI and birth weight centiles

Method

Subjects:

52 pregnant women, undergoing amniocentesis for prenatal diagnosis in the second trimester of pregnancy.

Amniotic fluid analysis

automatic multichannel analyzer (Abbott Diagnostics—ARCHITECT c8000)

Department of Biochemistry-Microbiology-Virology, Papageorgiou General Hospital of Thessaloniki, Thessaloniki, Greece

Neonatal somatometric parameters:

Information from individuals' medical records.

Fotiou M: JMNFM, 2014

2nd trimester amniotic fluid glucose, uric acid, phosphate, potassium, and sodium concentrations in relation to maternal pre-pregnancy BMI and birth weight centiles

Pearson's *r* correlation coefficients between amniotic fluid glucose, uric acid, phosphate, potassium, and sodium concentrations and maternal pre-pregnancy BMI

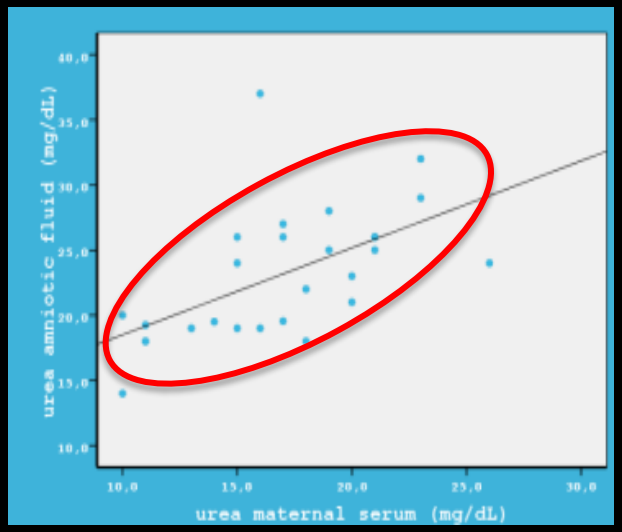
Characteristic		glucose	uric acid	phosphate	potassium	sodium
maternal pre-pregnancy BMI (kg/m²)	Pearson's *r*	0.274	0.460	0.159	0.161	0.254
	p	0.052	<0.01	0.261	0.254	0.070

BMI, body mass index

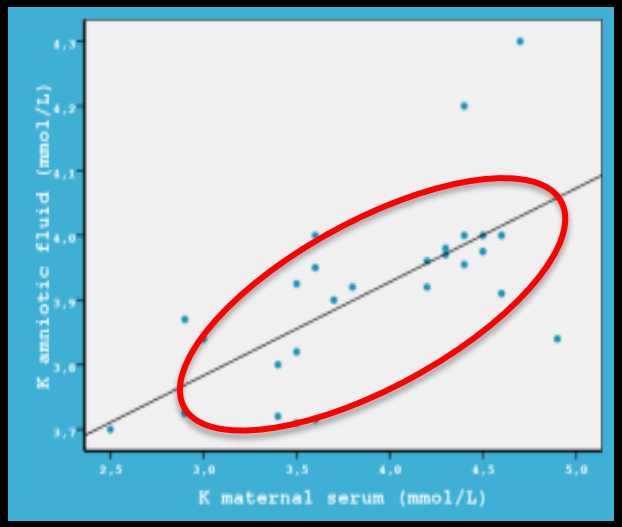
Fotiou M: JMNFM, 2014

Correlations between urea, uric acid and potassium in maternal serum and amniotic fluid

Urea



Potassium



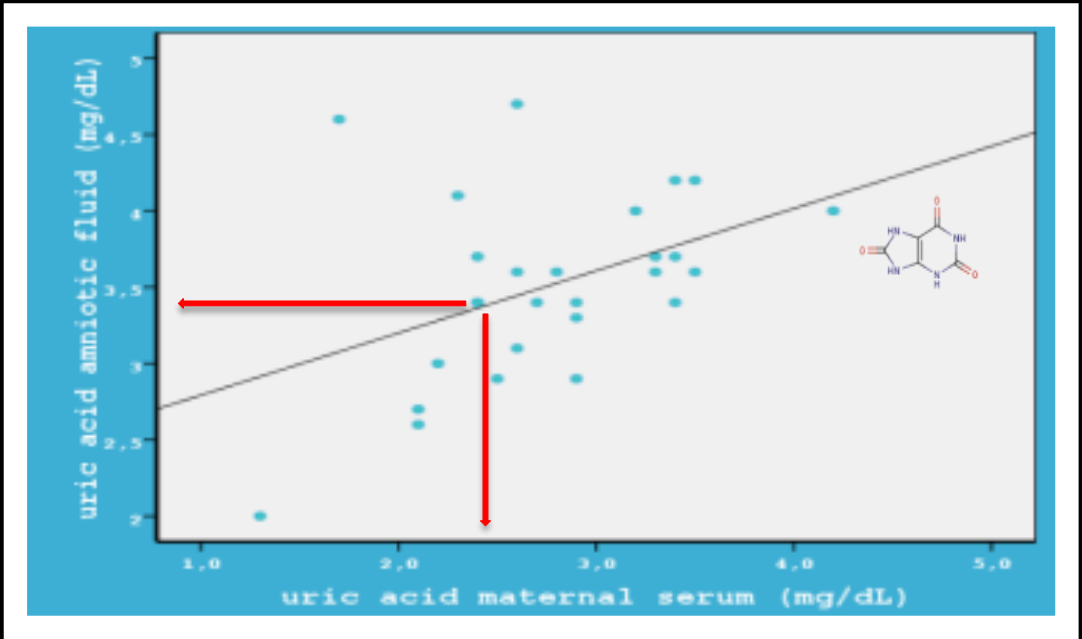
Biochemical markers	*r'*	*p*
Glucose	0.233	0.263
Urea	0.568	0.002
Uric acid	0.426	0.027
Creatinine	-0.060	0.769
Sodium	0.016	0.941
Potassium	0.635	<0.001
Phosphate	0.227	0.254
Calcium	0.318	0.106
Magnesium	0.266	0.180

Urea and potassium levels demonstrate strong positive correlations between maternal serum and amniotic fluid,

Fotiou M: Fetus as Patient, 2014

Correlation between biochemical markers in maternal serum and amniotic fluid in the 2nd trimester of pregnancy

- n=27
- Uric acid in AF and maternal serum were positively associated



Uric acid serum (mg/dl): 2.76 ± 0.63
Uric acid AF (mg/dl): 3.51 ± 0.61
r=0,426 *p*=0,027

Fotiou M: Fetus as Patient, 2014

Possible mechanism for the distribution of URIC ACID in maternal blood and amniotic fluid

NUTRITION

These studies could provide useful information towards lifestyle and dietary interventions

Chang FM, et al. 1987. Biological Research in Pregnancy and Perinatology, 8:35–39.

Koski, GK & Fergusson AM. 1992. The Journal of Nutrition, 122:385–92.

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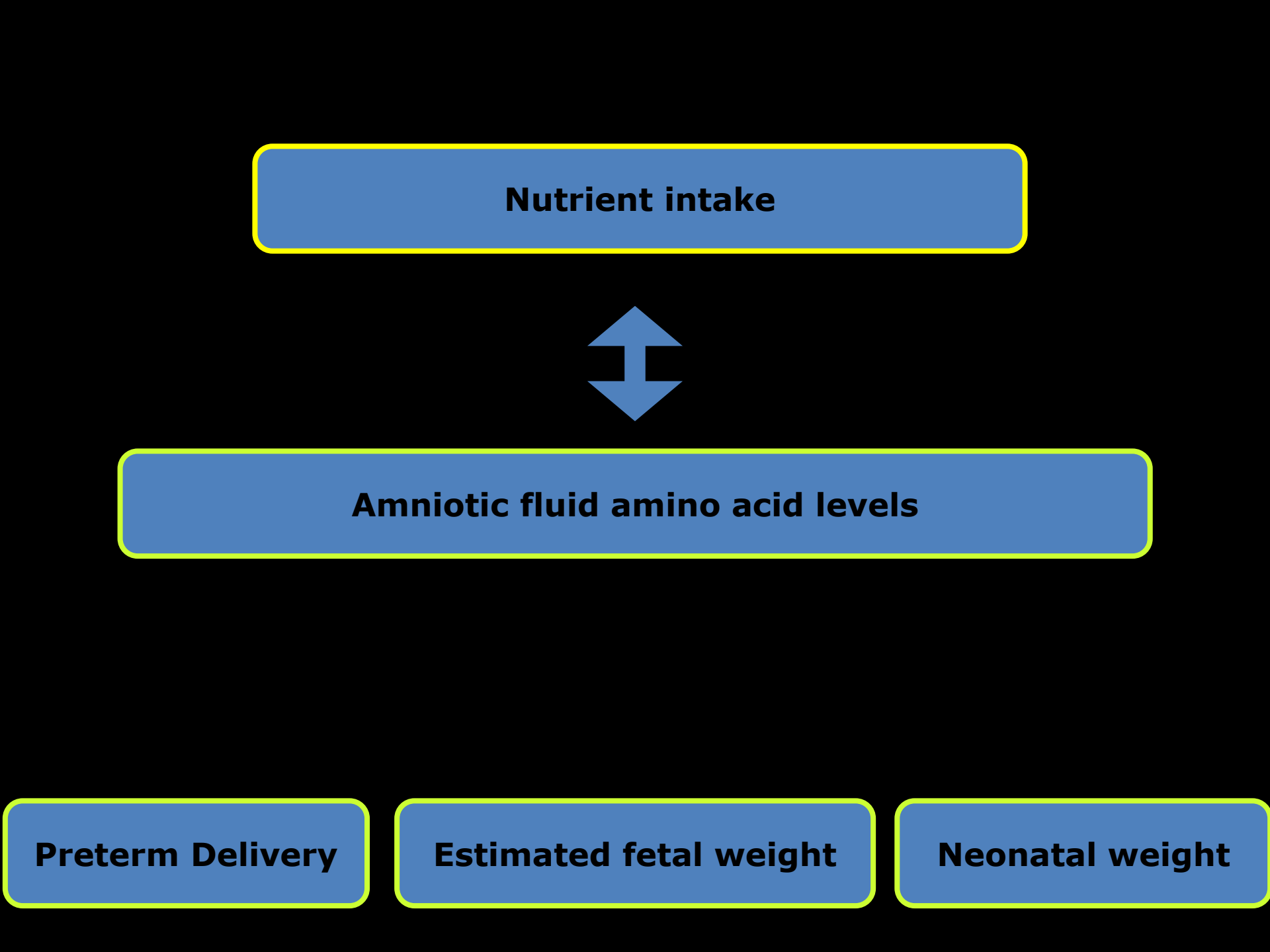

Nutrient intake
Amniotic fluid amino acid levels
Preterm Delivery
Estimated fetal weight
Neonatal weight

Impact of maternal nutrition on amniotic fluid amino acid concentrations in humans

	Amniotic fluid		
	Fed‡	Fasted§	P‖
Taurine	77.2 ±5.4¶	98.9 ±7.8	<0.05
Threonine	197.7 ±28.2	173.7 ±14.2	NS
Serine	33.5 ±3.4	42.9 ±2.6	<0.05
Proline	175.2 ±12.9	159.9 ±9.7	NS
Citrulline	12.0 ±0.5	8.1 ±1.5	<0.02
Glycine	169.8 ±18.2	151.9 ±11.6	NS
Alanine	353.0 ±21.5	264.8 ±17.1	<0.005
α-NH_2 butyrate	13.4 ±2.7	52.7 ±2.3	<0.001
Valine	136.2 ±9.4	255.3 ±18.2	<0.001
Cystine	77.8 ±8.0	72.8 ±4.1	NS
Methionine	19.2 ±2.0	20.3 ±1.4	NS
Isoleucine	23.8 ±3.1	68.3 ±5.3	<0.001
Leucine	51.7 ±5.1	133.4 ±11.0	<0.001
Tyrosine	43.7 ±1.8	46.6 ±3.6	NS
Phenylalanine	48.5 ±1.9	52.2 ±3.4	NS

* The basic amino acids were not measured in the amniotic fluid samples.
‡ Amniotic fluid and maternal plasma were obtained 3–6 hr after the previous meal in subjects maintained on a 2200–2400 kcal diet (n = 6).
§ Amniotic fluid and maternal plasma were obtained after 84–90 hr of fasting (n = 12).
‖ *P* = significance of differences between mean values in fed and fasted groups (unpaired *t* test).
¶ Mean ±SE, μmoles/liter.

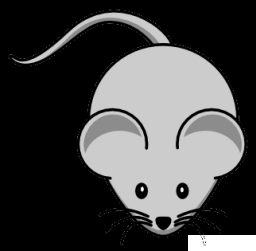
Felig et al: The Journal of Clinical Investigation, 1972

Impact of maternal nutrition on amniotic fluid amino acid concentrations in ewes

Amino acid	Amniotic fluid		
	Control†	Restricted‡	Realimented§
Ala	194 ± 8[a]	71 ± 2[c]	114 ± 11[b]
β-Ala	162 ± 11	149 ± 8	144 ± 13
Arg	186 ± 8[a]	39 ± 4[c]	95 ± 13[b]
Asn	37 ± 2[a]	17 ± 2[b]	32 ± 3[a]
Asp	22 ± 1[a]	15 ± 2[b]	17 ± 1[b]
Cit	178 ± 8[a]	22 ± 3[c]	43 ± 5[b]
Cys	127 ± 4[a]	50 ± 4[c]	87 ± 5[b]
Gln	386 ± 14[a]	86 ± 8[c]	121 ± 8[b]
Glu	69 ± 4[a]	47 ± 3[b]	50 ± 4[b]
Gly	326 ± 14[a]	184 ± 6[c]	240 ± 18[b]
His	26 ± 2[a]	12 ± 1[c]	18 ± 1[b]
Ile	29 ± 2[a]	10 ± 1[c]	14 ± 1[b]
Leu	50 ± 2[a]	17 ± 1[c]	31 ± 2[b]
Lys	55 ± 2[a]	19 ± 2[c]	28 ± 2[b]
Met	22 ± 2[a]	16 ± 1[b]	17 ± 1[b]
Orn	25 ± 1[a]	13 ± 1[b]	14 ± 1[b]
Phe	27 ± 2[a]	9 ± 1[c]	13 ± 1[b]
Pro	141 ± 5[a]	54 ± 3[c]	82 ± 5[b]
Ser	771 ± 43[a]	205 ± 4[c]	365 ± 31[b]
Taurine	127 ± 10	102 ± 8	103 ± 10
Thr	201 ± 13[a]	64 ± 4[c]	99 ± 6[b]
Trp	15 ± 1[a]	9 ± 1[b]	13 ± 1[a]
Tyr	44 ± 2[a]	13 ± 1[c]	20 ± 1[b]
Val	78 ± 4[a]	28 ± 2[c]	50 ± 4[b]
Total α-AA	2988 ± 86[a]	998 ± 22[c]	1620 ± 70[b]

* Data are means ± SEM. AA, amino acids.
† Ewes were fed 100% of NRC nutrient requirements between Days 28 and 135 of gestation (n = 8).
‡ Ewes were fed 50% of NRC nutrient requirements between Days 28 and 135 of gestation (n = 5).
§ Ewes were fed 50% of NRC nutrient requirements between Days 28 and 78 of gestation and realimented to 100% of NRC nutrient requirements between Days 78 and 135 of gestation (n = 8).
[a,b,c] Means with different superscripts are different ($P < 0.05$).

Kwon et al: Biology of reproduction 2004



Impact of maternal nutrition on amniotic fluid amino acid concentrations in rats

Dietary and developmentally induced changes in AF amino acid concentrations during the last 4 d of gestation in rats whose dams were fed varying levels of carbohydrate

	Dietary glucose,[1] %		
Individual amino acids	≤12%	≥24%	Main effect[2]
	μmol/L		
Methionine	72 ± 12[a]	135 ± 20[b]	0.0281
Phenylalanine	436 ± 24[a]	565 ± 40[b]	0.0297
	Gestational age,[3] *d*		
	18/19	20/21	Main effect[4]

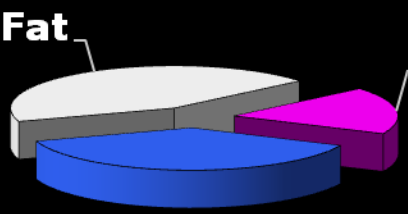
Gurekian & Koski,.
Journal of Nutrition 2005

Maternal dietary intake in our sample (n=240)

Macronutrient distribution

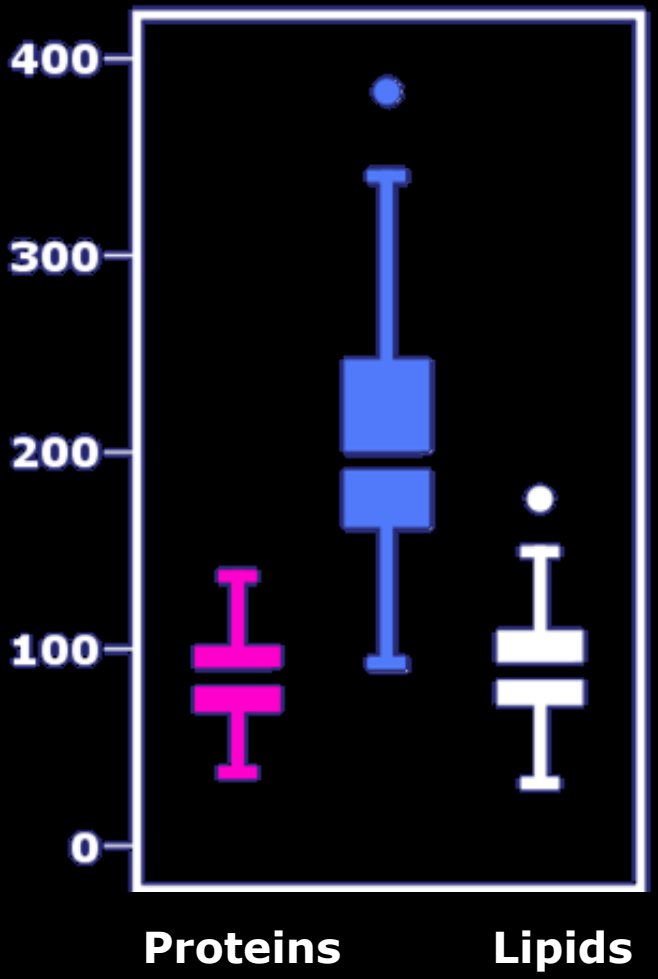
39.8% (RDI 20-35%)

17.2% (RDI 10-35%)



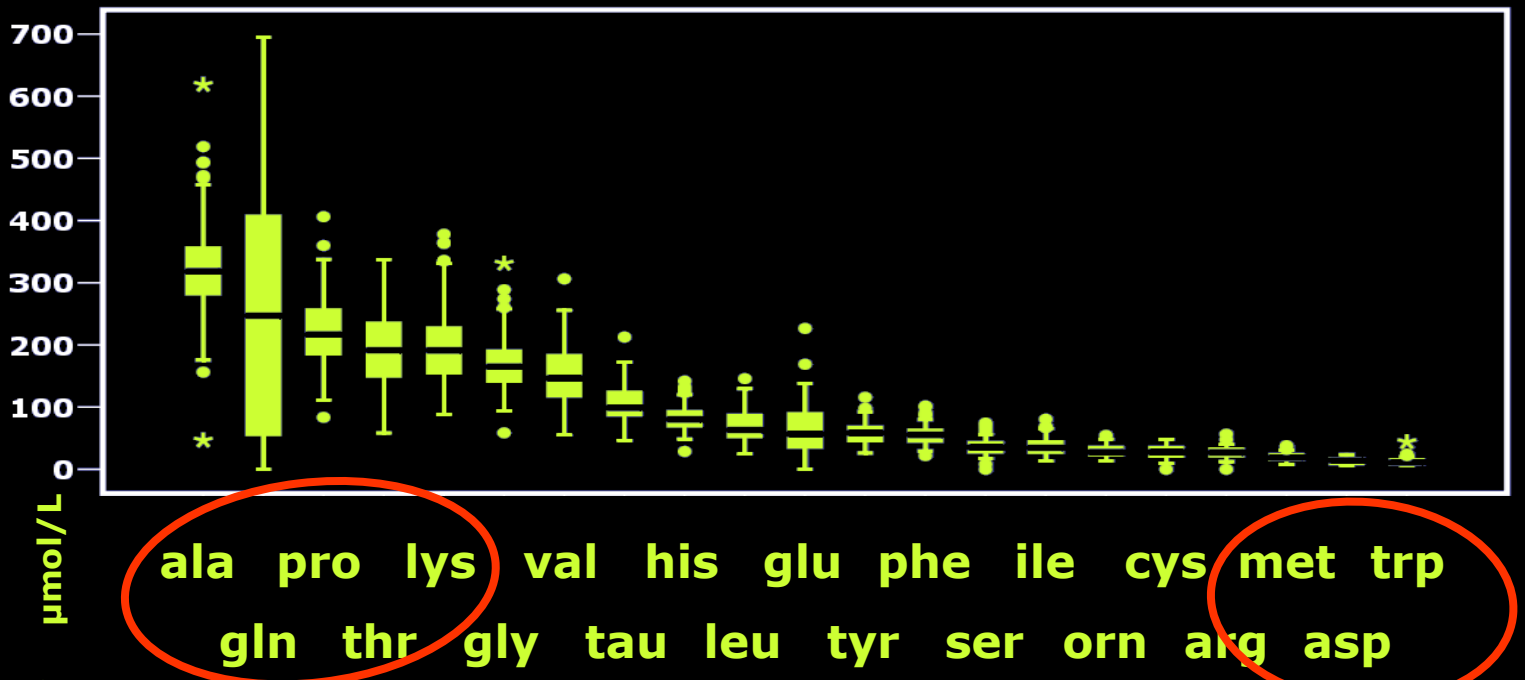
Carbohydrates

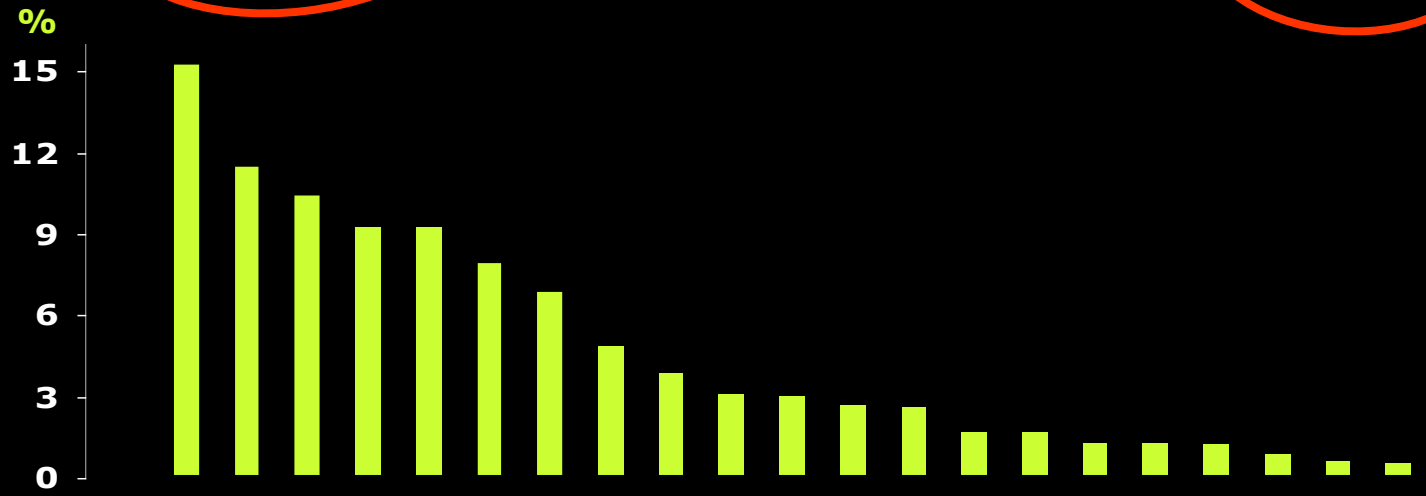
43% (RDI 45-65%)



Fotiou M, et al: Int J F Sci Nutr, (under publication)

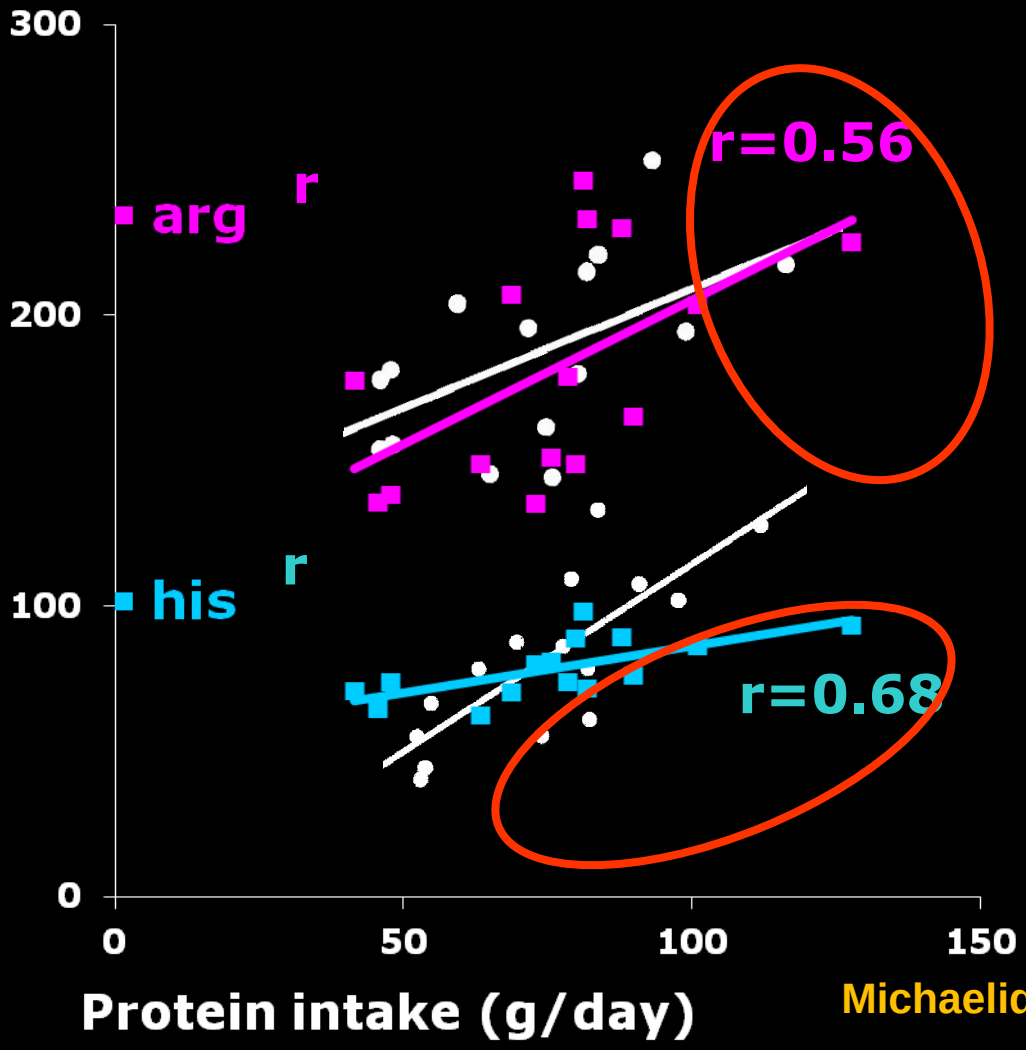
Box plots for amniotic fluid amino acids concentrations (µmol/L) (n=78)





Bar chart of relative amino acid concentrations

Amniotic fluid amino acid concentration in relation to protein intake at 18th gestational week



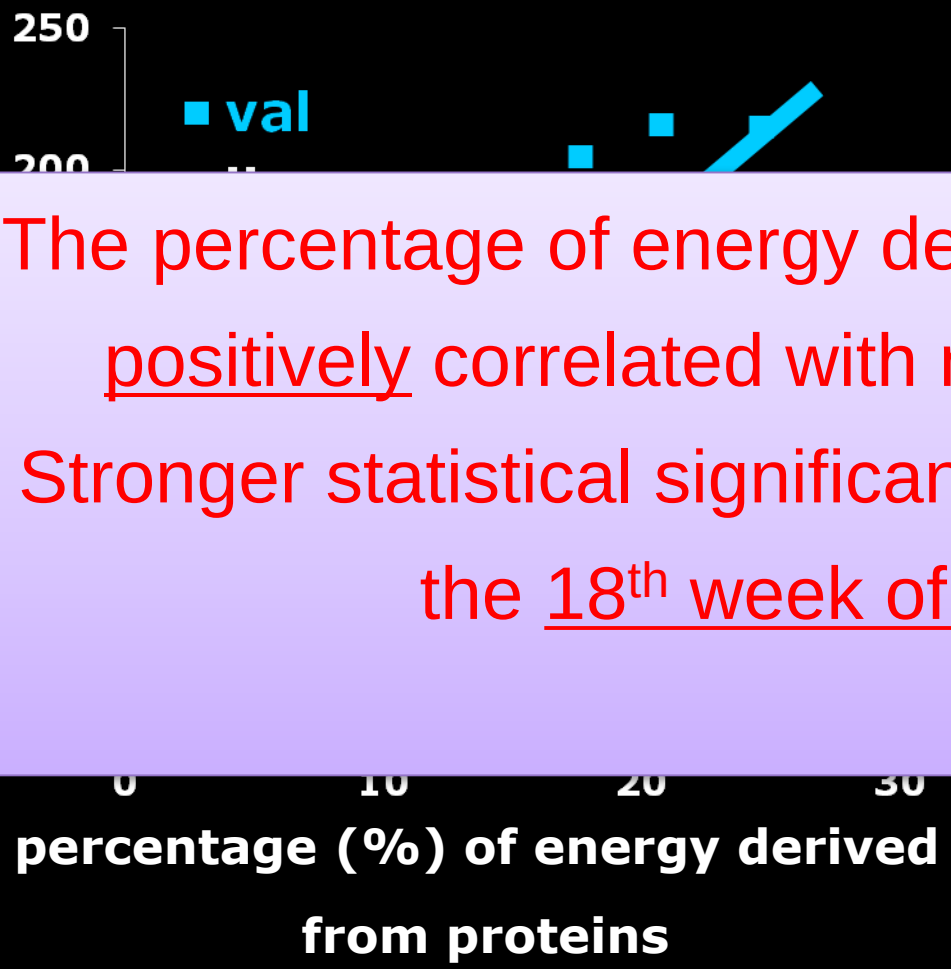
Spearman correlation coefficient (r)

& **Partial correlation coefficient (r')**

adjusted for BMI

Michaelidou AM, et al: AJOG (sup), 2008

Amniotic fluid amino acid concentration in relation to percentage of energy derived from proteins at 18th gestational week



Spearman correlation coefficient

- The percentage of energy derived from proteins was positively correlated with most AF amino acids
- Stronger statistical significance were noticed during the 18th week of pregnancy

Michaelidou AM, et al: AJOG (sup), 2008

Dietary patterns reflection in the metabolic profiles of second trimester maternal blood and amniotic fluid

Subjects:

Serum and amniotic fluid samples were obtained from 27 women that underwent amniocentesis for prenatal diagnosis - between the 18th and 24th gestational week.

Dietary assessment :

Dietary assessment was carried out by registered dietician using a food frequency questionnaire and 24-h recall.

Maternal serum and amniotic fluid analysis:

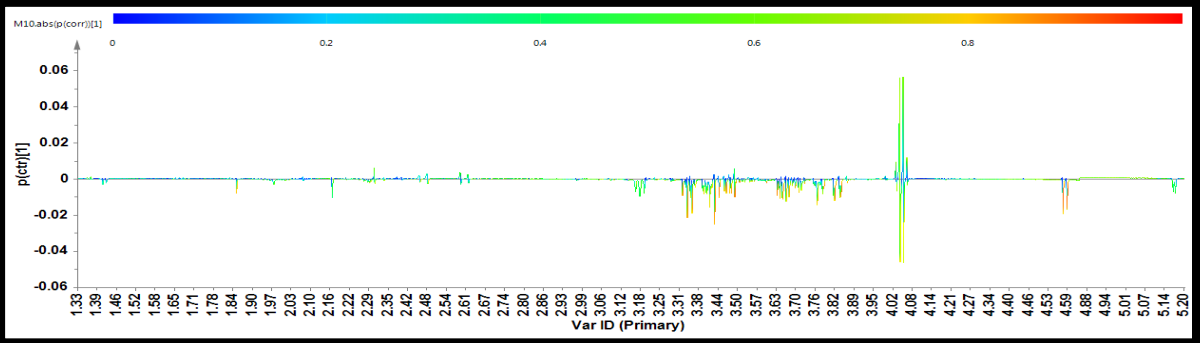
National Hellenic Research Foundation

Holistic metabolomics approach on Varian-600MHz NMR spectrometer and

CPMG pulse sequence. **MestReNova software.**

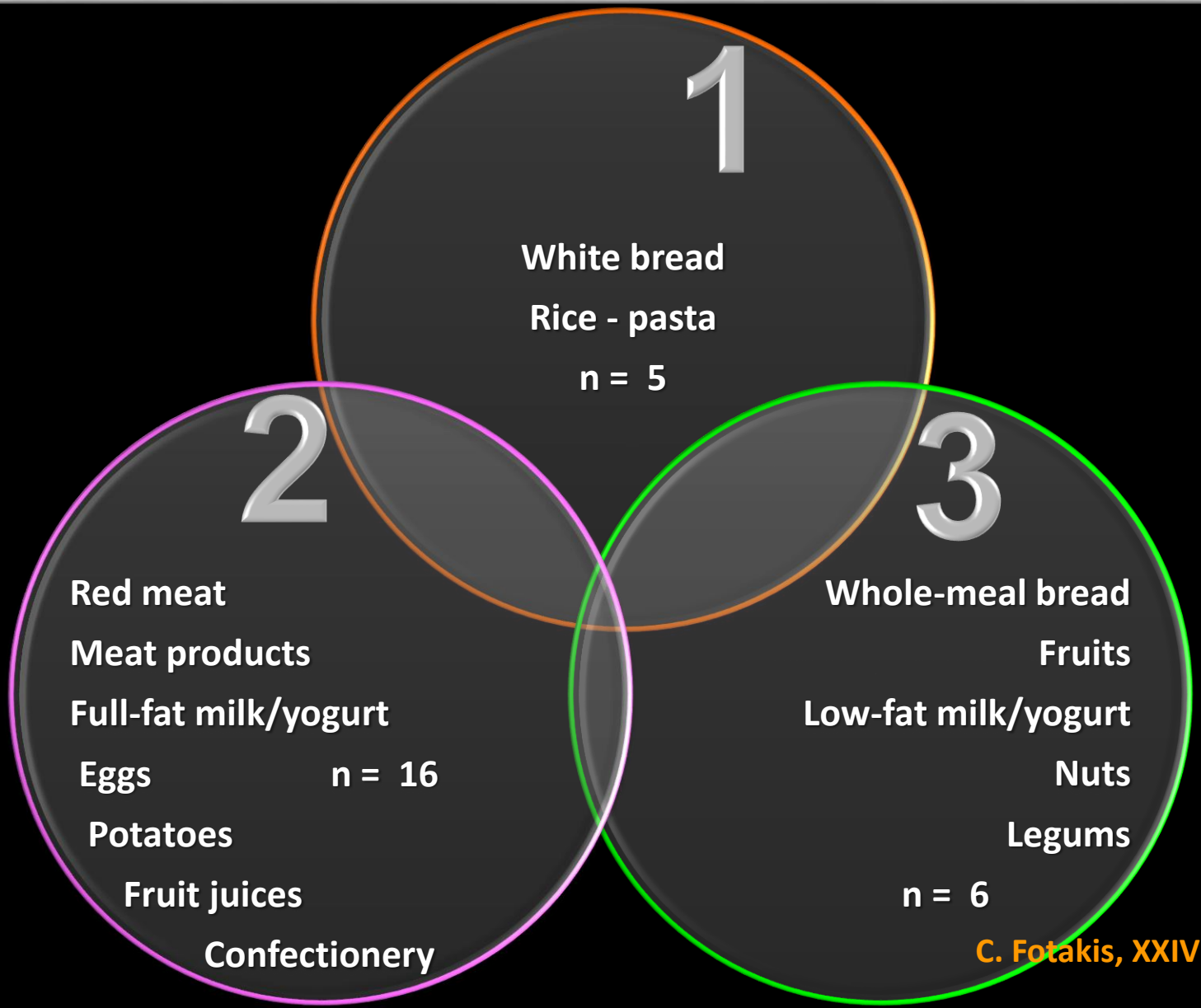
Phase and baseline correction/ Bucketing of 0.001 ppm

Spectral alignment / The SIMCA-P 13.0 (PCA) & (OPLS-DA).



C. Fotakis, XXIV ECPM,2014

Cluster analysis identified 3 dietary patterns (clusters)

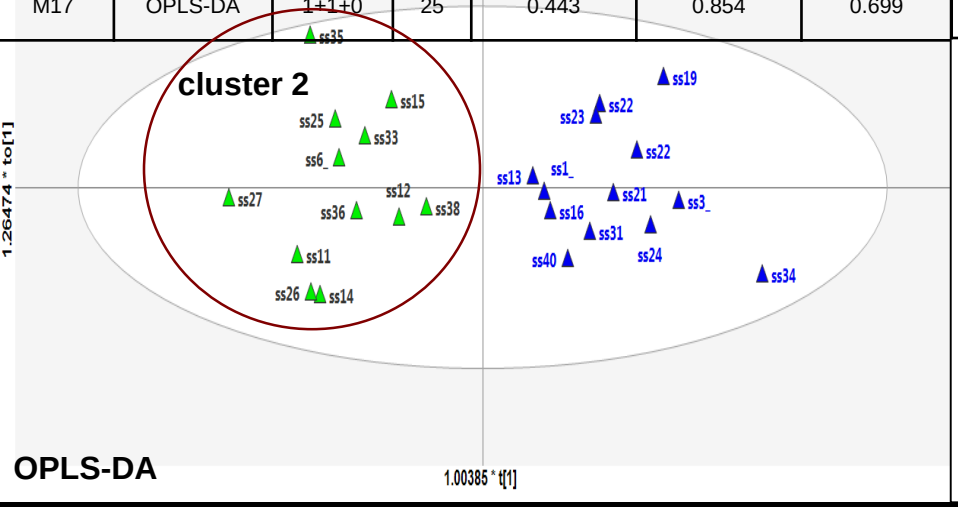


C. Fotakis, XXIV ECPM,2014

Dietary patterns reflection in the metabolic profiles of second trimester maternal blood and amniotic fluid

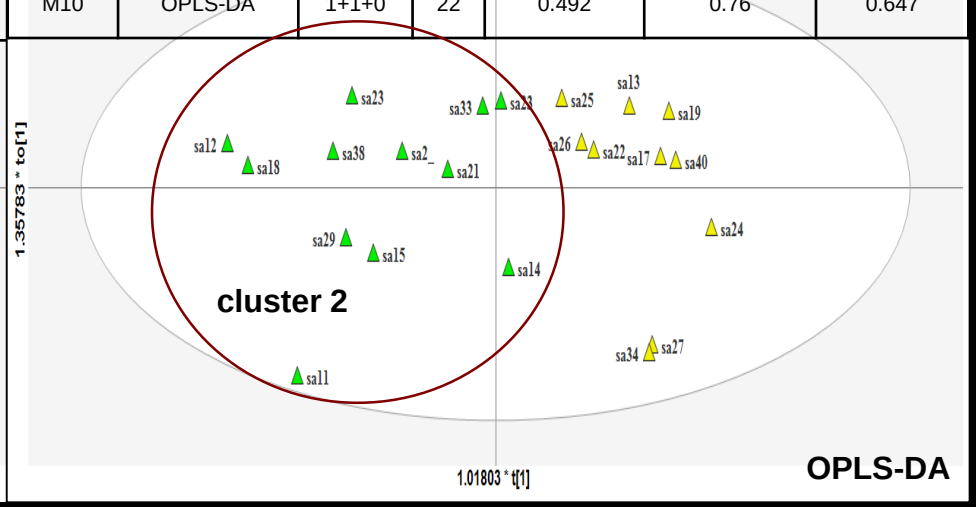
maternal serum

Model	Type	A	N	R2X(cum)	R2Y(cum)	Q2(cum)
M17	OPLS-DA	1+1+0	25	0.443	0.854	0.699



amniotic fluid

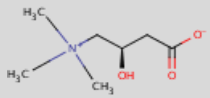
Model	Type	A	N	R2X(cum)	R2Y(cum)	Q2(cum)
M10	OPLS-DA	1+1+0	22	0.492	0.76	0.647

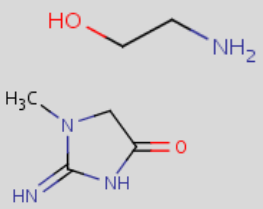


dietary pattern 2

Higher concentration:

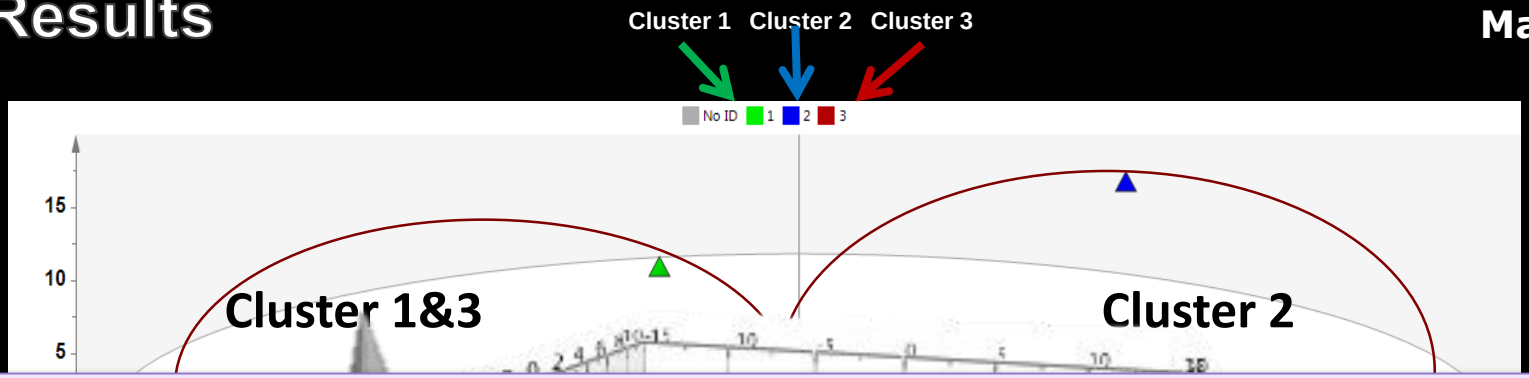
- specific amino acids
- carnitine
- creatinine
- ethanolamine





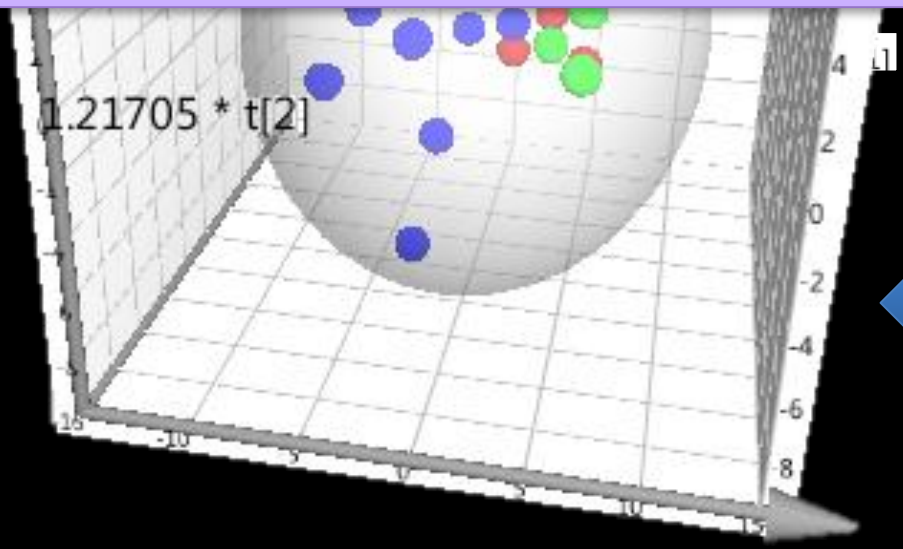
Dietary patterns reflection in the metabolic profiles of second trimester maternal blood and amniotic fluid

Results



Maternal serum

The present research effort suggests that the metabolic profile of second trimester amniotic fluid is influenced by maternal dietary pattern



Amniotic fluid

C. Fotakis, XXIV ECPM,2014

Conclusions

- **The results of these studies show that there is an association between maternal nutritional status and amniotic metabolic profile**
- **Further investigating in “metabolomics” is needed to unravel the biochemical pathways underlying fetal development and gain insight on the potential impact of maternal nutritional management on fetal growth regulation.**

Thank you very much!!!