



Double Blind Placebo Control Trial in PKU with NeoPhe

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Large Neutral Amino Acids (LNAA)

- Phenylalanine (Phe)
- Leucine
- Tyrosine
- Tryptophan
- Methionine
- Histidine
- Isoleucine
- Valine
- Threonine

Transport of LNAA to the Brain

	K_m mmol/L	K_m app
● Phenylalanine (Phe)	0.12	0.45
● Leucine	0.15	0.53
● Tyrosine	0.16	0.58
● Tryptophan	0.19	0.71
● Methionine	0.19	0.77
● Histidine	0.28	1.10
● Isoleucine	0.33	1.30
● Valine	0.63	2.50
● Threonine	0.73	3.00

Pardridge, Inborn Errors of Metabolism in Humans.
MTP Press, 1980.

Andersen AE, Avins L

- **LNAA injected to rat pups**
- **Phenylalanine hydroxylase was inhibited by parachlorophenylalanine**
- **Brain phenylalanine decreased**

1976 Arch Neurology 33:684

Tyrosine in The Treatment of PKU

Lou et al used Tyr 160 mg/kg in treated patients with PKU

- Increased attention span
- Increased dopamine synthesis

1987 Acta Paediatr Scand 76:560

Tyrosine in Treatment of PKU

- Pietz et al. used high dose tyrosine in adults with PKU and high blood Phe
- No difference in treated group vs placebo

1995 J Pediatr 127:936

Tryptophan in Treated PKU

- Nielsen et al used tryptophan 4.5 gm/day to treated PKU for 3 weeks
- Showed a 3 fold increase in 5-HIAA in CSF despite high blood Phe

**1988 Dietary Phenylalanine and Brain Function.
Birkhauser**

VIL in Treatment of PKU

- **1990 Berry et al used VIL (valine 150 mg/kg, isoleucine 150 mg/kg and leucine 200 mg/kg) to reduce phe entry to brain**
- **Substantial lowering of CSF Phe found**
- **Tyrosine also was also substantially reduced in CSF**
- **Am J Dis Child 144:539**

LNAA Supplementation in PKU

- **Dotremont et al. used LNAA and a low protein diet 0.6 gm/kg on 4 patients with PKU**
- **After 1 month subjects found with negative nitrogen balance**
- **Lysine was limiting amino acid**

1995 J Inherit Metab Dis 18:127

LNAA Supplementation in PKU

- Pietz et al. 6 males with PKU given a load (100 mg/kg Phe) with and without LNAA
- Limited brain Phe entry by MRS
- EEG normal with LNAA
- EEG slowing without LNAA
- Radiology 1996, 201:413

$$K_m (\text{app}) = K_m \left(1 + \frac{\sum[\text{aa}]}{K_m} \right)$$

This predicts that, if the plasma level of an LNAA is much less than its value of K_m , then that amino acid will not compete effectively for the carrier protein

Absolute and apparent Km values of neutral amino acids for the neutral amino acid transporter in the BBB (Partridge, 1980)

Amino acid	Typical plasma level (mM)	Km (mM)	App Km (mM)
LNAA's			
Phe	0.05	0.12	0.45
Leu	0.10	0.15	0.53
Tyr	0.09	0.16	0.58
Trp	0.10	0.16	0.71
Met	0.04	0.19	0.77
Isoleu	0.07	0.33	1.3
Val	0.14	0.63	2.5
Thr	0.19	0.73	3.0

Absolute and apparent Km values of neutral amino acids for the neutral amino acid transporter in the BBB (Partridge, 1980)

Amino acid	Typical plasma level (mM)	Km (mM)	App Km (mM)
Basic aa's			
His	0.05	0.28	1.1
Arg	0.10	0.09	0.40
Lys	0.30	0.10	0.25

LNAA Transport in Intestinal Mucosa K_m mmol/L

- **Phenylalanine 1.0**
- **Leucine 2.0**
- **Valine 3.0**
- **Methionine 5.0**
- **Histidine 6.0**
- **Competition effect is not likely to occur in tissue other than brain unless high concentration of amino acids is used**

Pardridge, Inborn Errors of Metabolism in Humans. MTP Press, 1980.

Amino acid inhibition of Phe transport in Caco-2-cells – 10uM Phe in buffer applied to monolayers in presence of 1 mM concentration of each amino acid

Inhibitor	% inhibition
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LNAAs

Leu	55%
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Tyr	45%
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Trp	36%
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Basis aa's

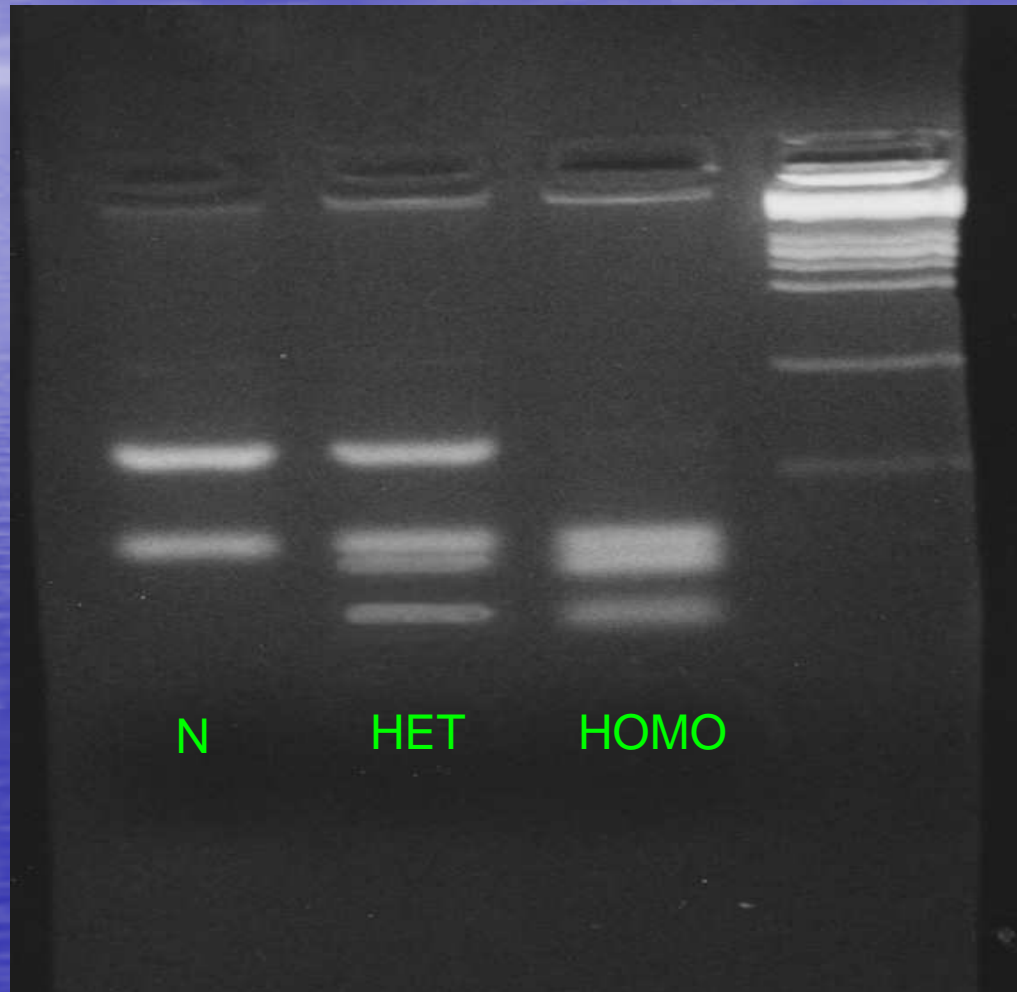
Lys	50%
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His	33%
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Hidalgo Biochem Biophys. Acta 1008: 5-30a (1990)

Genotype of ENU2 Mice

(F263S)



LNAA in Treatment of Mice with PKU

- ENU2/ENU2 mice with PKU
- Before treatment blood phe and tyr
- LNAA given in form of PreKUnil
- Dose 0.5 g/kg or 1.0 g/kg
- Blood phe and tyr up to 6 weeks post treatment

PKU Mice on NeoPhe
phe mg/dl

Mice	Control				NeoPhe	
	78-	80-	83-	86-	159-	162-
F 577	23.8	21.4	19.4	24.9	18	11
F 579	23.7	25.1	28.4	33.1	8.3	14.8
F 582	28.8	21.9	22.2	20.9	10.3	8.3
F 584	23.8	30	25.3	30.7	7.8	12.4





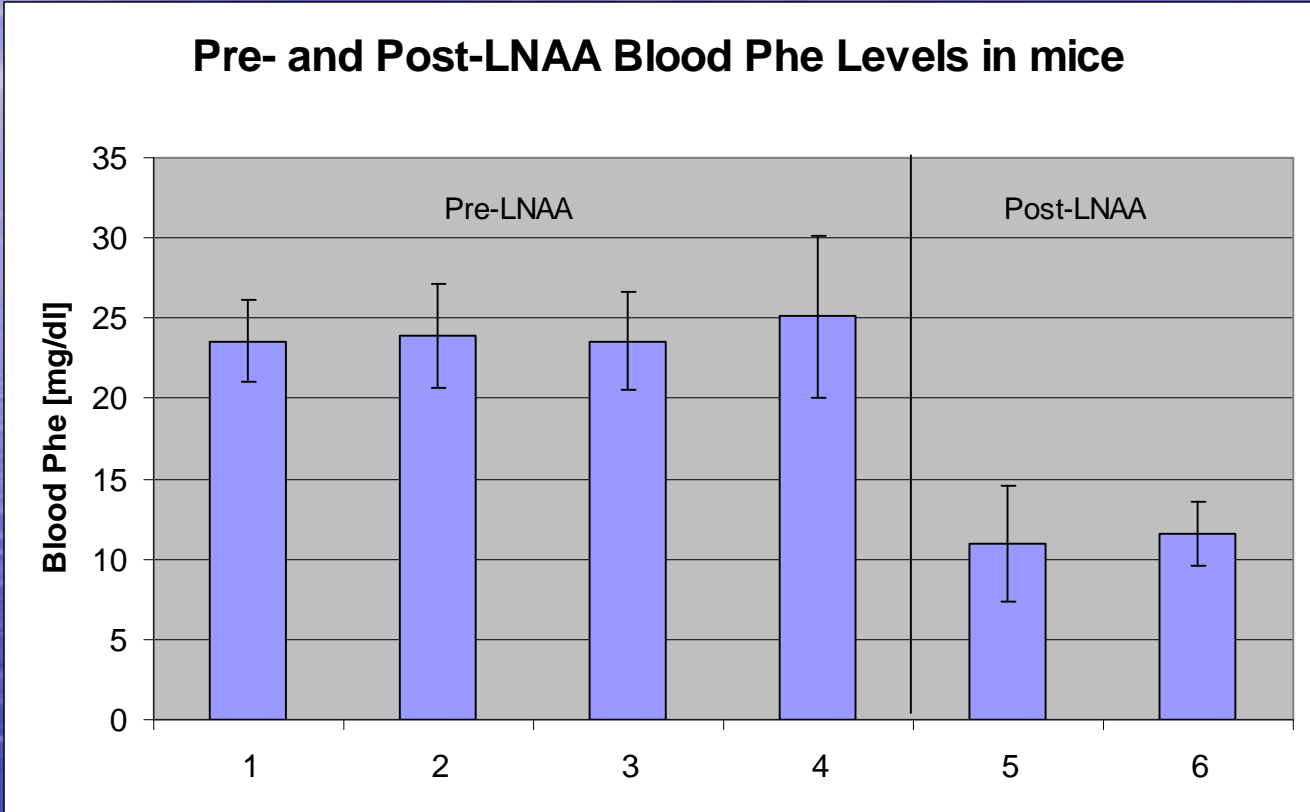




PKU Mice on NeoPhe

Mice	phe mg/dl					
	Control			NeoPhe		
	78-	80-	83-	86-	159-	162-
F 585	20.6	21.7	24.4	19.8	8.5	12.3
F 586	23.2	25.7	21.2	21.9	13.5	11.3
F 588	21.6	21.7	24.2	24.4	10.9	10.9
Avg each time pt	23.6	23.9	23.6	25.1	11	11.6
Avg all Pre-LNAA	24.1					
Avg all Post-LNAA	11.3					

Pre- and Post-LNAA Blood Phe Levels in mice





Double Blind Placebo Control
Trial in PKU with NeoPhe in US

Figure 1. Blood Phe Response to 0.5g/kg NeoPhe in Patients with PKU

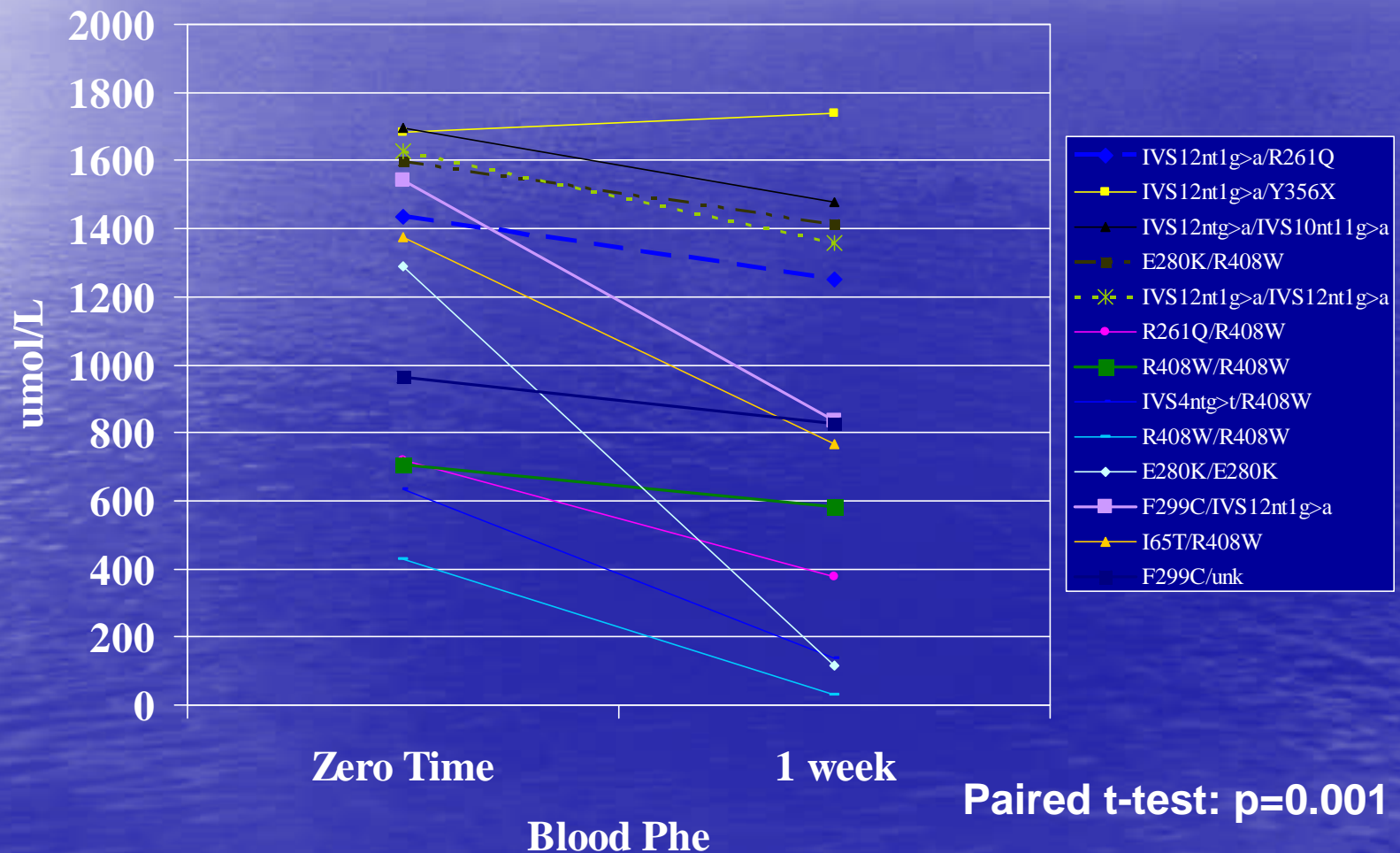
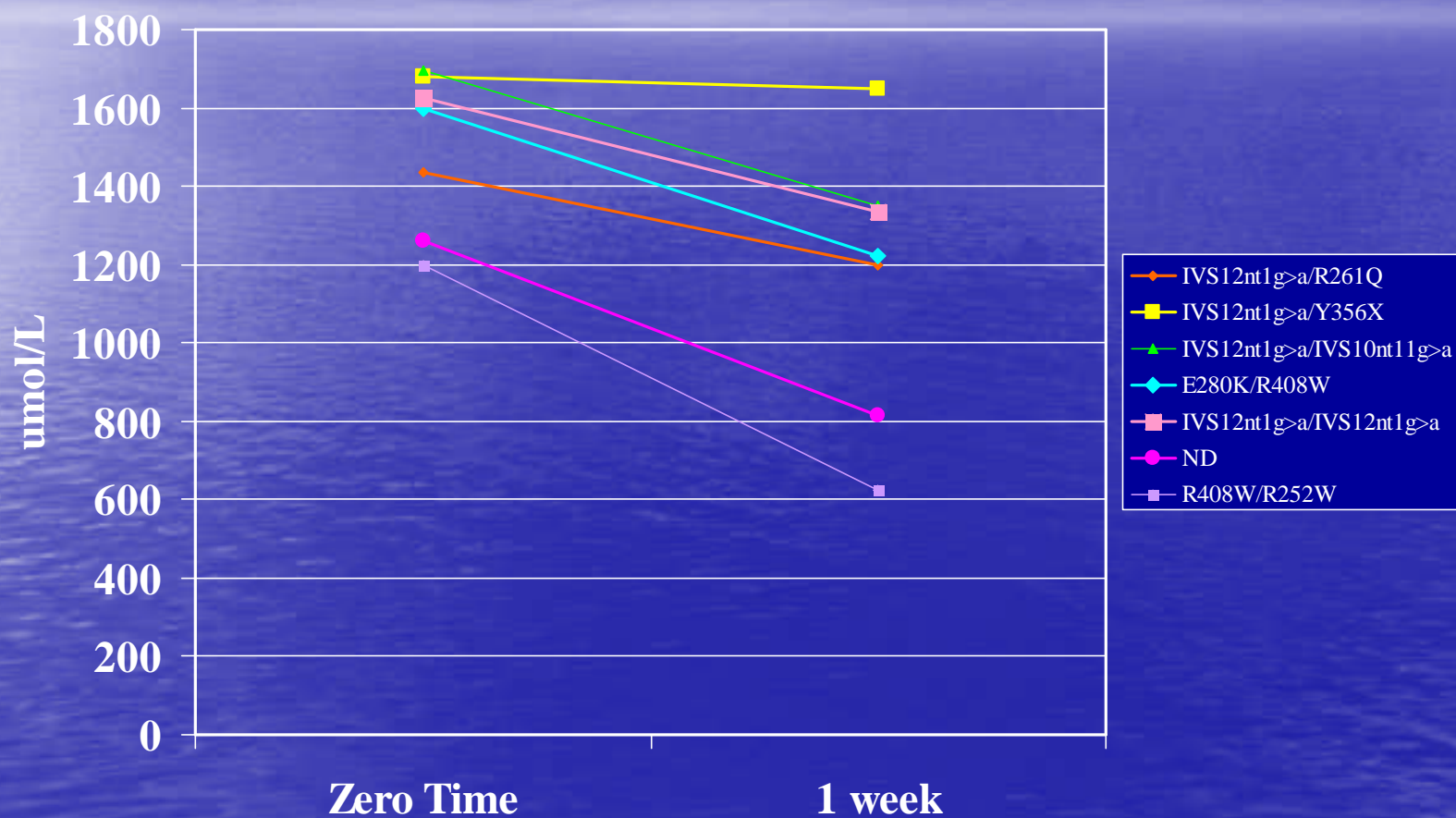


Figure 2. Blood Phe Response to 1.0 g/kg NeoPhe in Patients with PKU



Blood Phe

Paired t-test: p=0.006

Double-Blind Placebo Control on patients in the US

- **Patients were genotyped**
- **Baseline Phenylalanine was determined 3 times**
- **Placebo or Neophe was administered for one week, 1tablet/kg/day**
- **Blood Phe was determined 3 times**

E280K/E280K

mg/dl

μmol/L

Before Neophe

zero 1

26.1

1566

zero 2

29.36

1761

zero 3

27.2

1632

Neophe

24 hrs

16.04

962

72 hrs

16

960

96 hrs

8.4

504

1 week

8.8

528

Placebo

24 hrs

21.2

1272

72 hrs

24.1

1446

96 hrs

23.6

1416

1 week

22.5

1350

299C/IVS12 nt1 g>a

mg/dl

μmol/L

Before Neophe

zero 1

25.6

1536

zero 2

32.9

1974

zero 3

26.8

1608

Neophe

24 hrs

14.3

858

72 hrs

16.8

1008

96 hrs

18.1

1086

1 week

12.2

732

Placebo

24 hrs

19.2

1152

72 hrs

20.6

1236

96 hrs

24.8

1488

1 week

23.7

1422

F299C/-

mg/dl

μmol/L

Before Neophe

zero 1

16.09

965.4

zero 2

18.1

1086

zero 3

17.2

1032

NeoPhe

24 hrs

12

720

72 hrs

14.1

846

96 hrs

10.1

606

1 week

11.4

684

Placebo

24 hrs

16.2

972

72 hrs

18.2

1092

96 hrs

17.1

1026

1 week

16.12

967.2

I65T/R408W

mg/dl

μmol/L

Before Neophe

zero 1

24.1

1446

zero 2

23.0

1380

zero 3

21.1

1266

Neophe

24 hrs

12.8

768

72 hrs

11.2

672

96 hrs

12.9

774

1 week

13.5

810

Placebo

24 hrs

18.2

1092

72 hrs

22.2

1332

96 hrs

19.1

1146

1 week

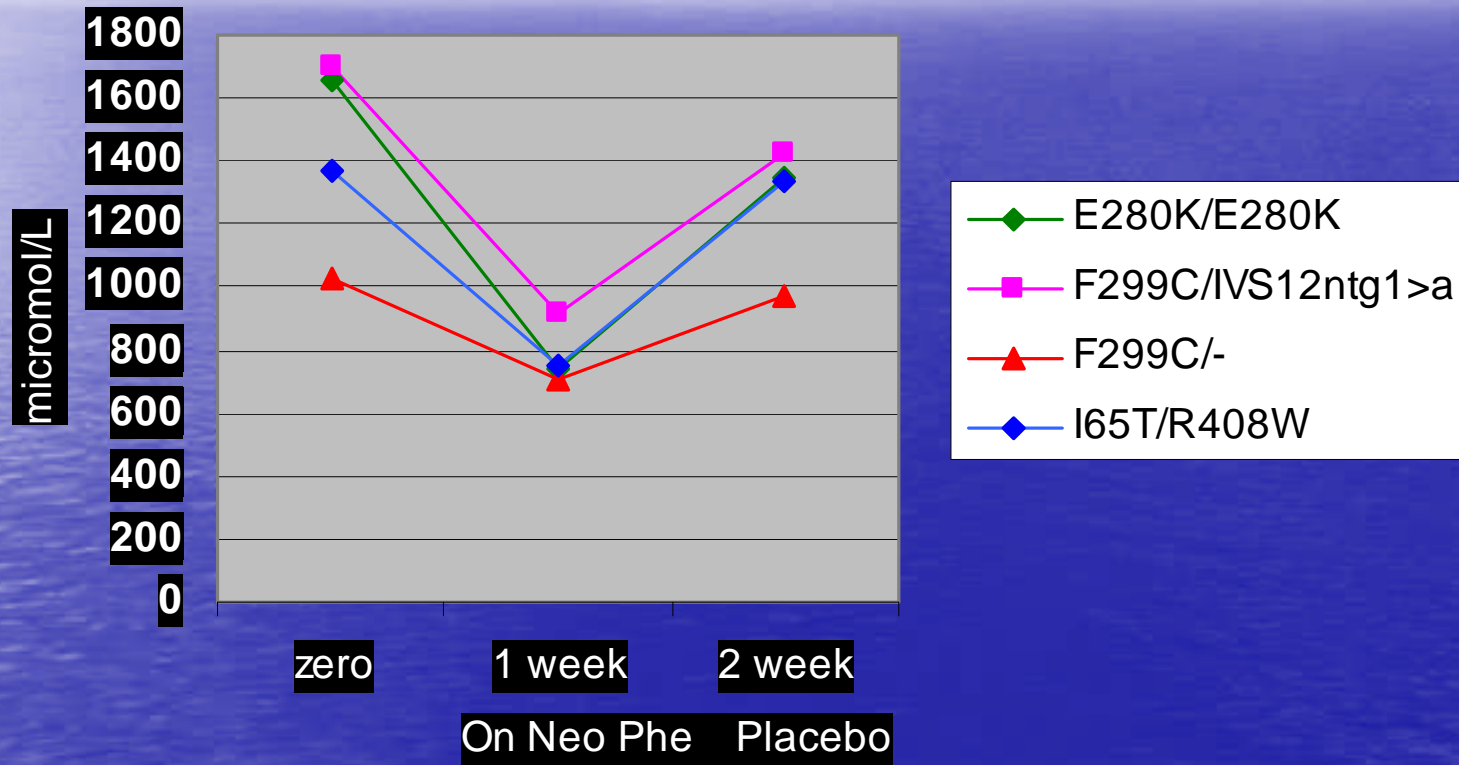
22.3

1339

Summary of Average Blood Phe

	Zero $\mu\text{mol/l}$	NeoPhe $\mu\text{mol/l}$	Placebo $\mu\text{mol/l}$
E280K/E280K	1653	738.5	1350
F299C/IVS12ntgl>a	1706	921	1422
F299C/-	1027.8	712	967.2
I65T/R408W	1364	752	1339.2

Summary of Double Blind Study in US



Conclusion

- LNAA can reduce blood Phe levels when given with meals
- Longer term double-blind placebo control studies are needed
- Establishing the efficacy and safety of LNAA can improve treatment of PKU

Double-Blind Study in Other Centers

- Brazil
- Russia
- Ukraine
- Italy



EXPERIENCE IN BRAZIL

Apae-Rio

WITH LARGE NEUTRAL AMINO ACIDS

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Diagnósticos Laboratoriais Especializados (DLE)²

Rio de Janeiro, Brasil

Methods

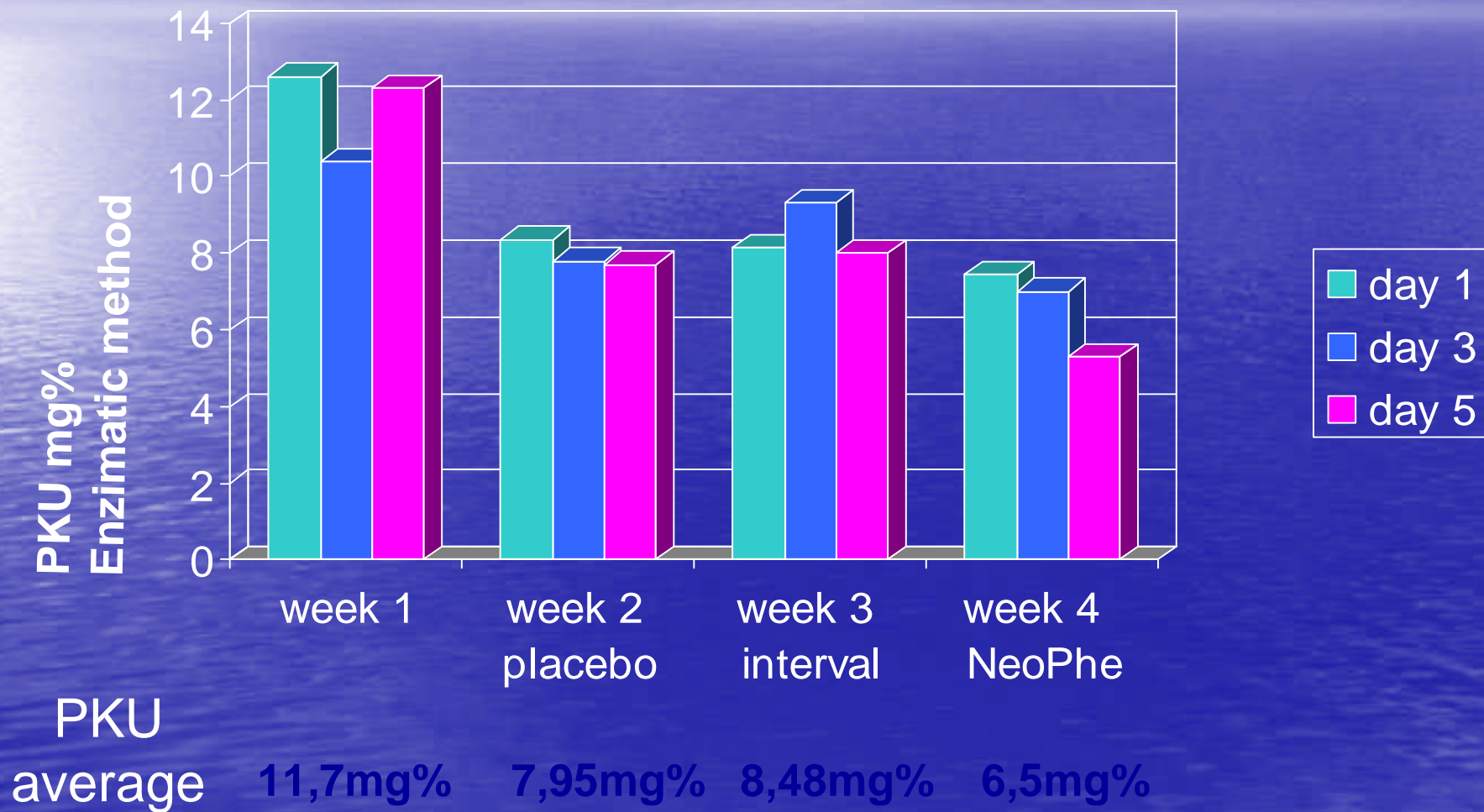
We studied three patients (1male, 2 females), aged 13-14y, in a double blind study, in four weeks as the protocol:

- Week 1: specific diet (aminoacids formula)
- Week 2: specific diet and placebo or NeoPhe (1 pill/Kg)
- Week 3: specific diet
- Week 3: specific diet and placebo or NeoPhe

PKU concentrations were determined three times a week during all the weeks by an enzymatic colorimetric method (NeoLISA PKU Kit-Intercientífica) in duplicate. All the aminoacids were determined also by tandem mass spectrometry in day five of every week.

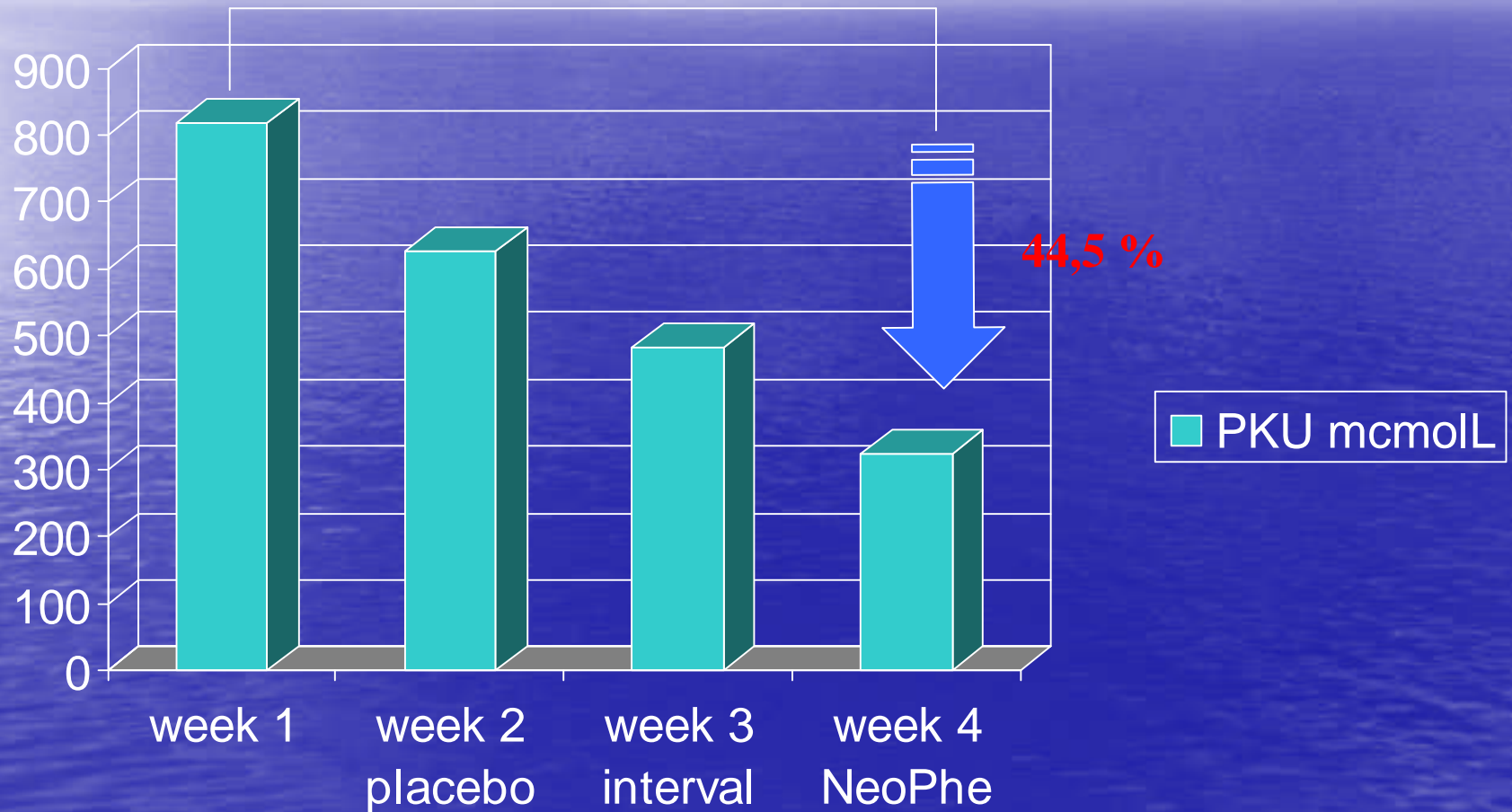
One patient (case 3) presented vomits in the fourth week (NeoPhe)

Case 1. RSL



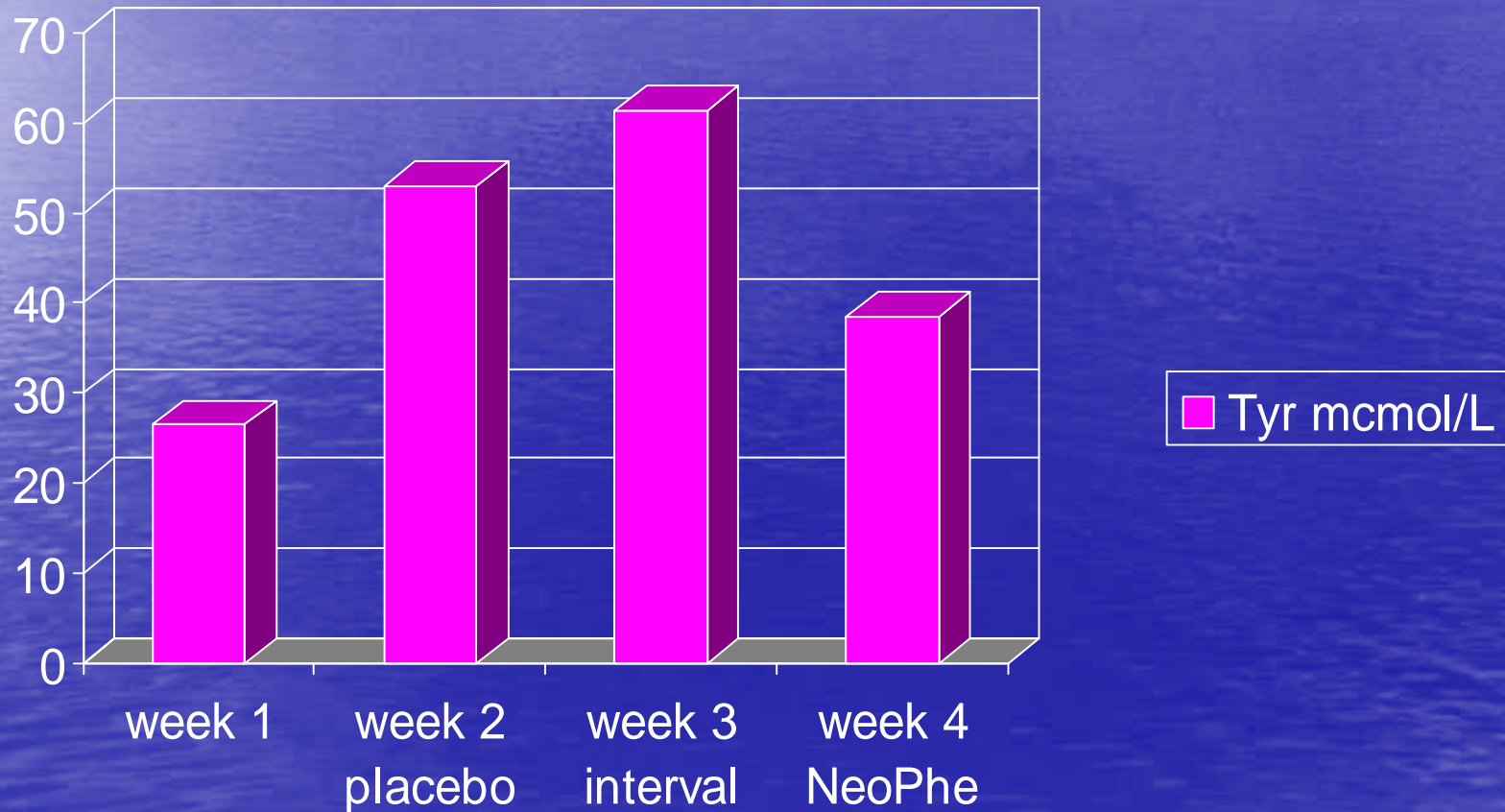
Case 1. Phenylalanine

Tandem mass spectrometry

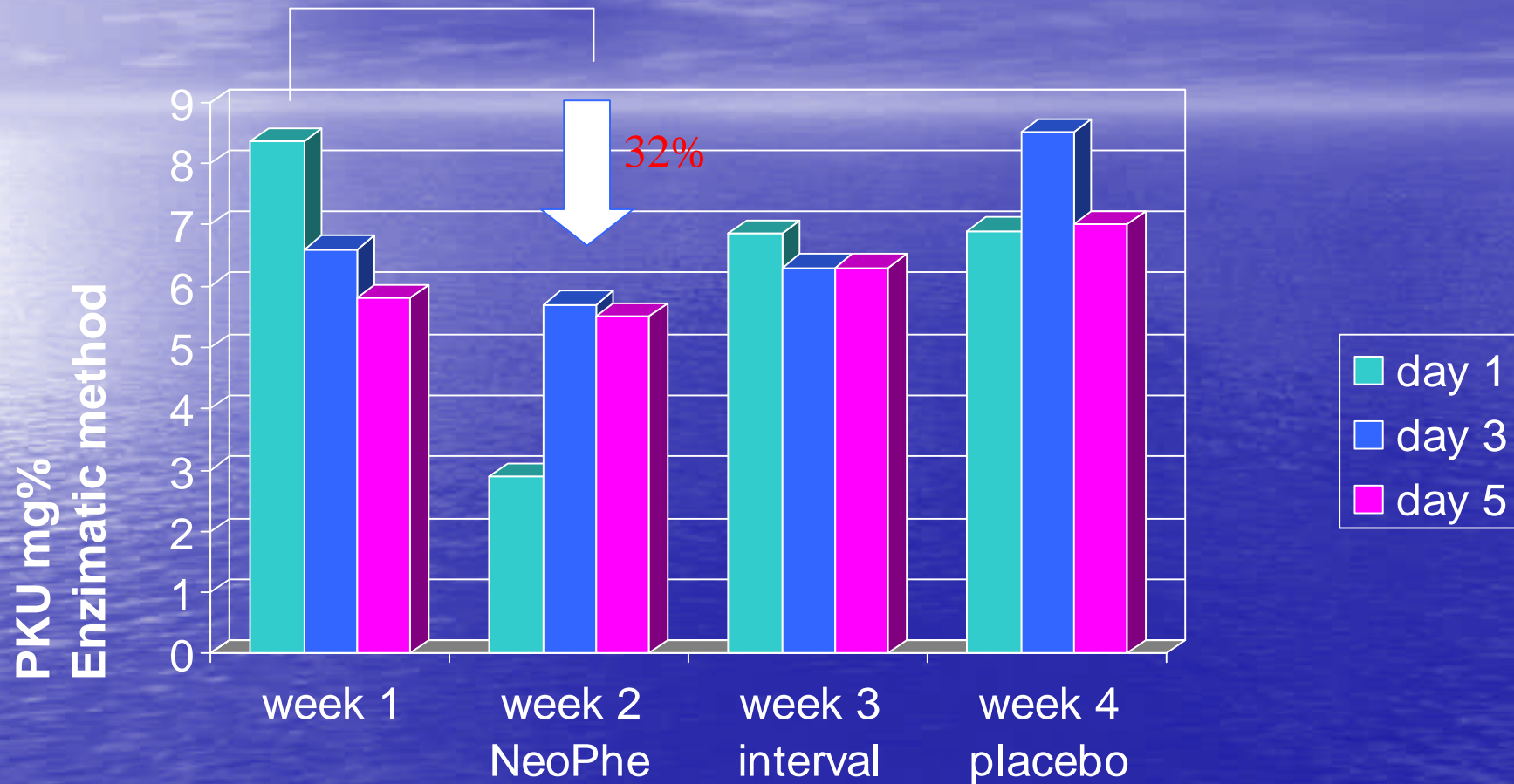


Case 1. Tyrosine

Tandem mass spectrometry



Case 2. TSA

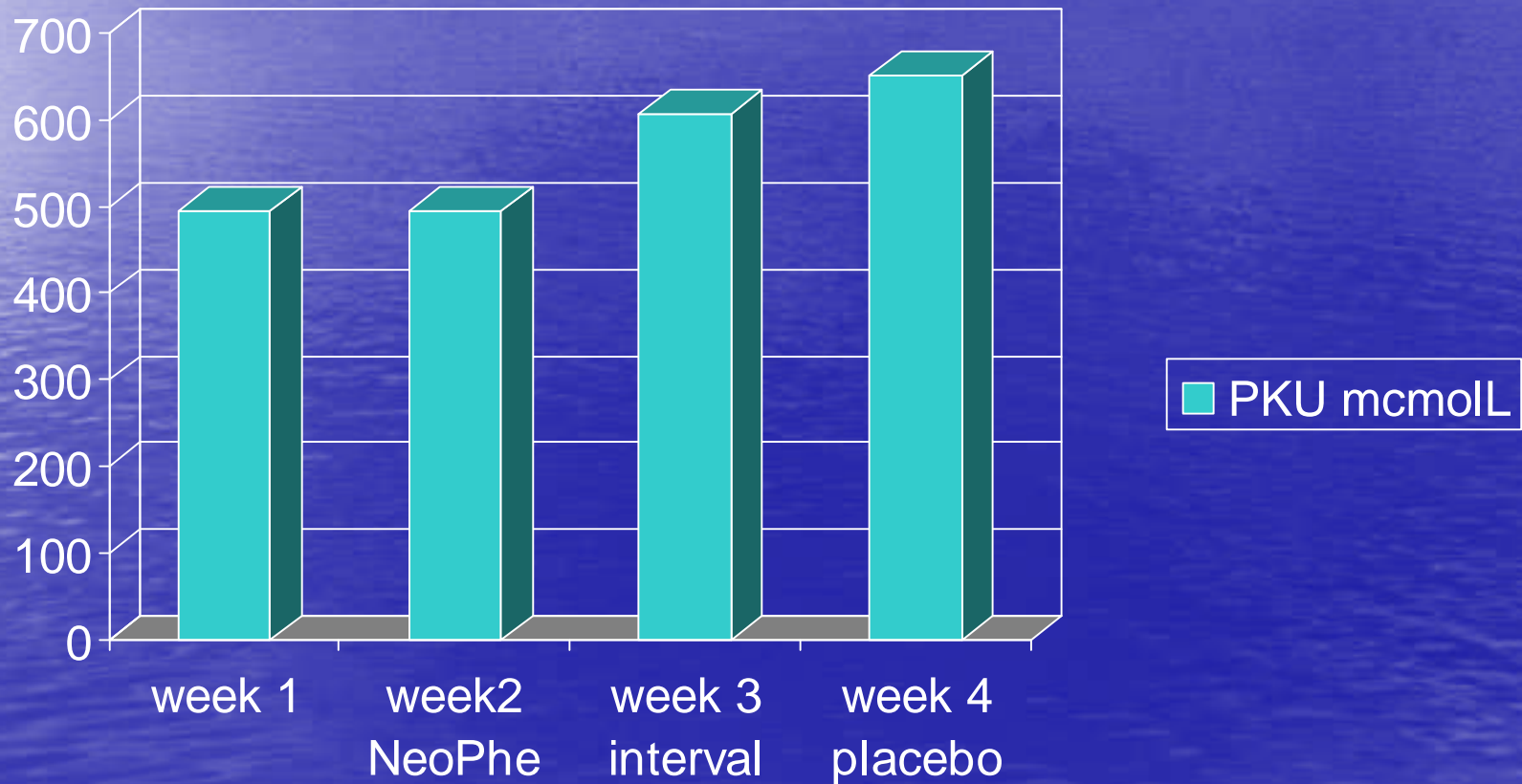


PKU

average 6,9 mg% 4,7 mg% 6,5 mg% 7,46mg%

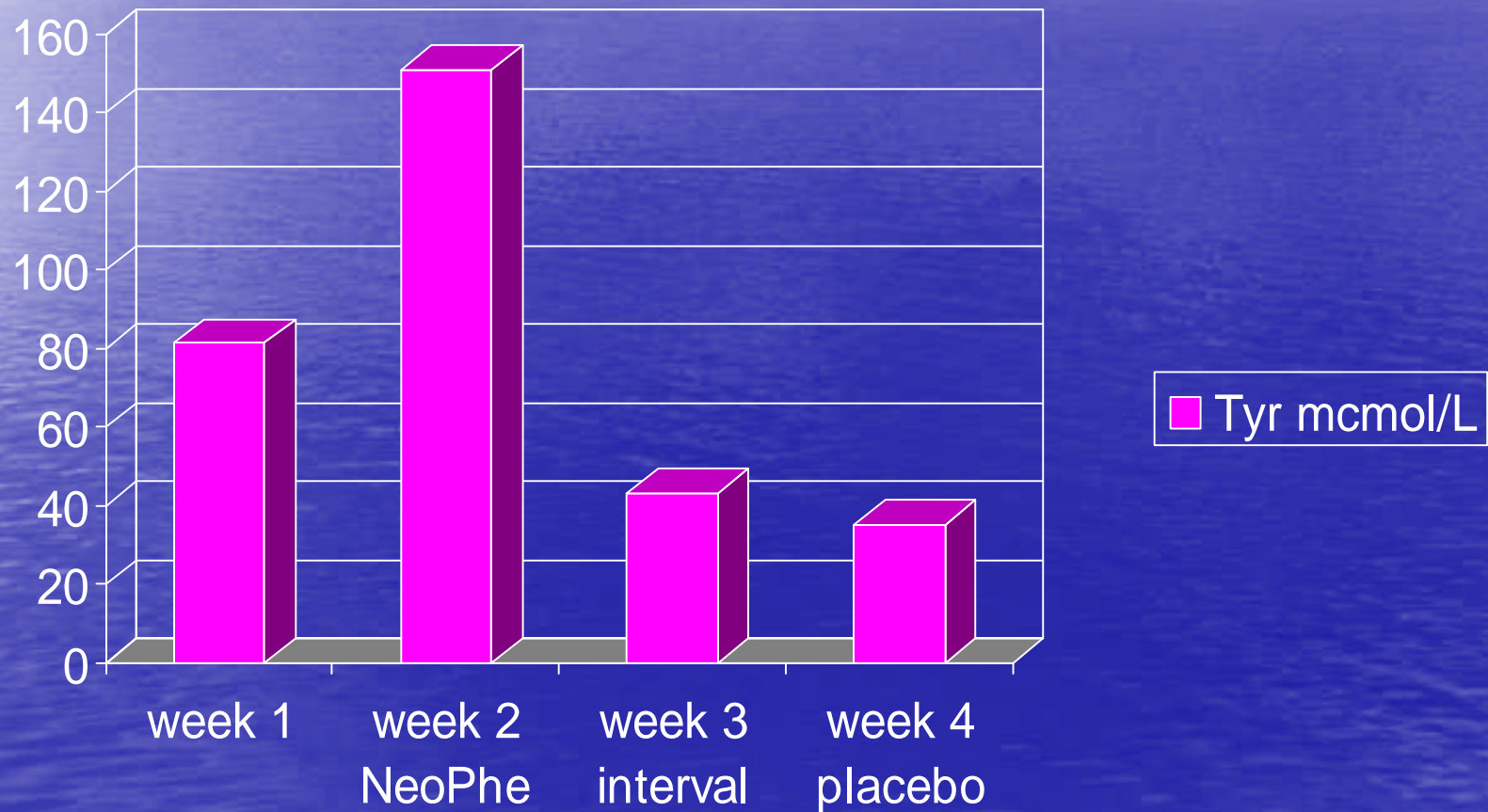
Case 2. Phenylalanine

Tandem mass spectrometry



Case 2. Tyrosine

Tandem mass spectrometry



**“Experience in Russia with Large
Neutral Amino Acids”**

Peter Novikov

**Moscow Research Institute of
Pediatrics and Child Surgery,
Moscow, Russia**

Russia LNAA Study

Time	Phe		Tyr	
<i>KH</i>	$\mu\text{mol/l}$	mg/dl	$\mu\text{mol/l}$	mg/dl
0'	635.4	10.59	33.0	0.60
3 days	554.4	9.24	242.0	4.40
3 days	322.2	5.37	94.6	1.72
3 days	136.2	2.27	110.0	2.00
3 days	102.6	1.71	94.0	1.71

Russia LNAA STUDY

Time	Phe		Tyr		
	<i>KN</i>	$\mu\text{mol/l}$	mg/dl	$\mu\text{mol/l}$	mg/dl
0'		707.4	11.79	42.9	0.78
3 days		607.2	10.12	126.5	2.30
3 days		572.4	9.54	159.5	2.91
3 days		585.6	9.76	83.6	1.52

Russia LNAA STUDY

Time	Phe		Tyr	
<i>KA</i>	$\mu\text{mol/l}$	mg/dl	$\mu\text{mol/l}$	mg/dl
0'	718.8	11.98	53.9	0.98
3 days	668.4	11.14	91.3	1.66
3 days	523.2	8.72	103.4	1.88
3 days	376.2	6.27	108.3	1.97

Response Phe levels in PKU patients with NeoPhe administration (Russia)

Patient	Age	Gender	Genotype	Mean Phe levels (μmol/l) pre NeoPhe	Phe levels (μmol/l) post NeoPhe	Decrease of Phe blood levels (%)	Phe levels (μmol/l) post Placebo
1	22	F	R408W/ R252W	1261,19	782,6	37,9	-
2	12	M	Not detected	1264,2	626,08	50,5	-
3	11	F	R408W / R408W	1023,4	692,3	32,4	-
4	12	F	R408W / R408W	1023,4	662,2	35,3	903
5	19	F	R261X/R408W	1143,8	933,1 *	18,4	-
Means	15,2			1143,19	739,29	35,3	903

* - The patient stopped to receipt NeoPhe because girl has complications

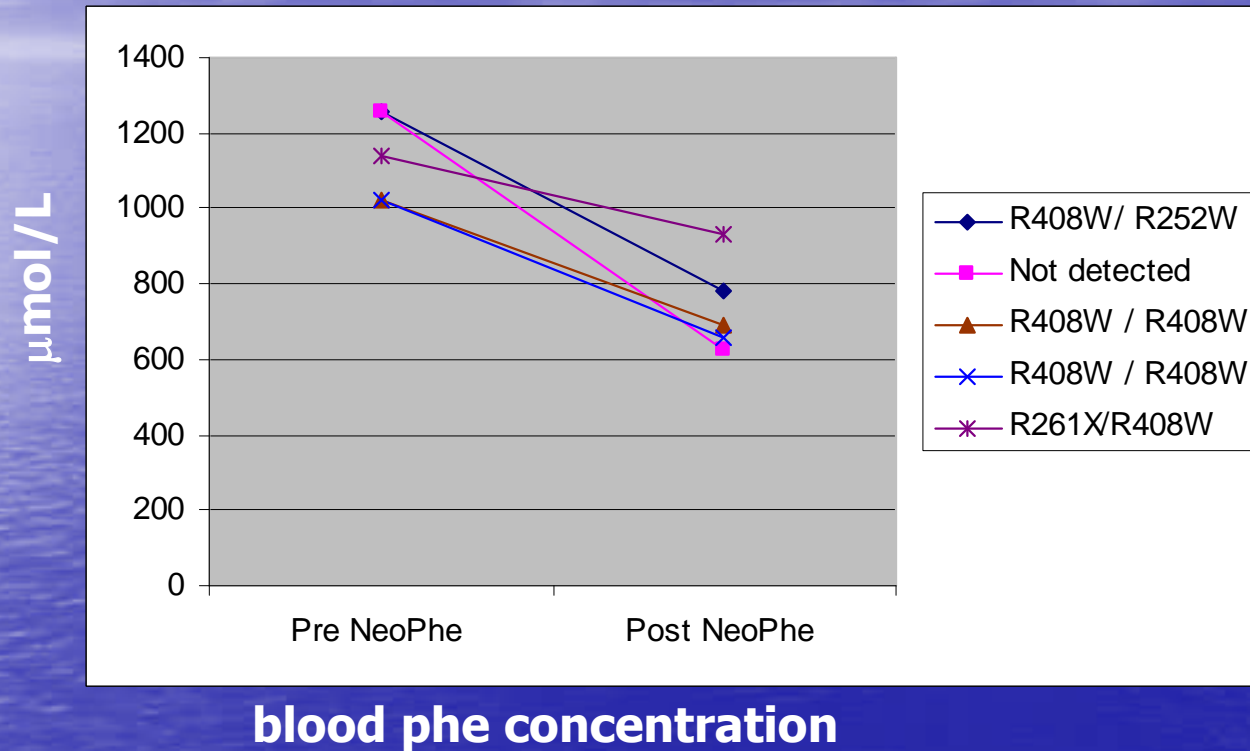
Conclusions

- LNAA can decrease blood Phe levels in patients with classical PKU
- Decreasing of Blood Phe levels is accompanied by increased of blood Tyr levels
- The grade of the decrease of Phe levels can be higher after the short period without LNAA
- It will be necessary to check the different hypotheses of LNAA effects

Double-Blind Placebo study in Ukraine

Elena Grechanina and Irene Novikova
University of Kharkiv, Ukraine

Response to NeoPhe : Double-Blind Study, Ukraine



Blood Phe response to 0.5 g/kg NeoPhe in 5 PKU patients

Large Neutral Amino Acids – a novel treatment for PKU

Marcello Giovannini, Laura Fiori

Department of Pediatrics, San Paolo
Hospital, University of Milan, Italy

LNAA

	Week 1, on caps (phe mg% - mM/L)	Week 2, (no caps)	Week 3, on caps (phe mg% - mM/L)
Basal	16.7 - 984	-	18.3 - 1098
	16.4 - 984	-	14.9 - 894
	14.4 - 864	-	11.9 - 714
	12.9 - 774	-	13.4 - 804
End of week	12.7 - 762	-	15 - 900
Pl. phe decrease %	22%		18% (max 34%)

F, age 29

BW: 49 kg.

**LNAA: 1/kg
BW**

R158Q / -

NeoPhe-Placebo study in Italy- Padova

Alessandra Burlina and Alberto Burlina

Response to NeoPhe: Double-Blind Study

Patient	Age	Baseline	NeoPhe	Placebo	% decrease
MC	26	1004	787.3	937.5	21.58
NV	15	597	157	554	73.70
BL	21	926		1225	
AL	20	1067		1375	

Acknowledgement

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- Generous supply of NeoPhe was given by PreKulab, Denmark
- To all Centers in Brazil, Italy, Ukraine and Russia for their participation in the study

