LE PATOLOGIE GENETICO - METABOLICHE:
IL NEONATOLOGO E IL PEDIATRA
Bari 27 febbraio 2010

LABORATORIO, DIAGNOSI, TERAPIA

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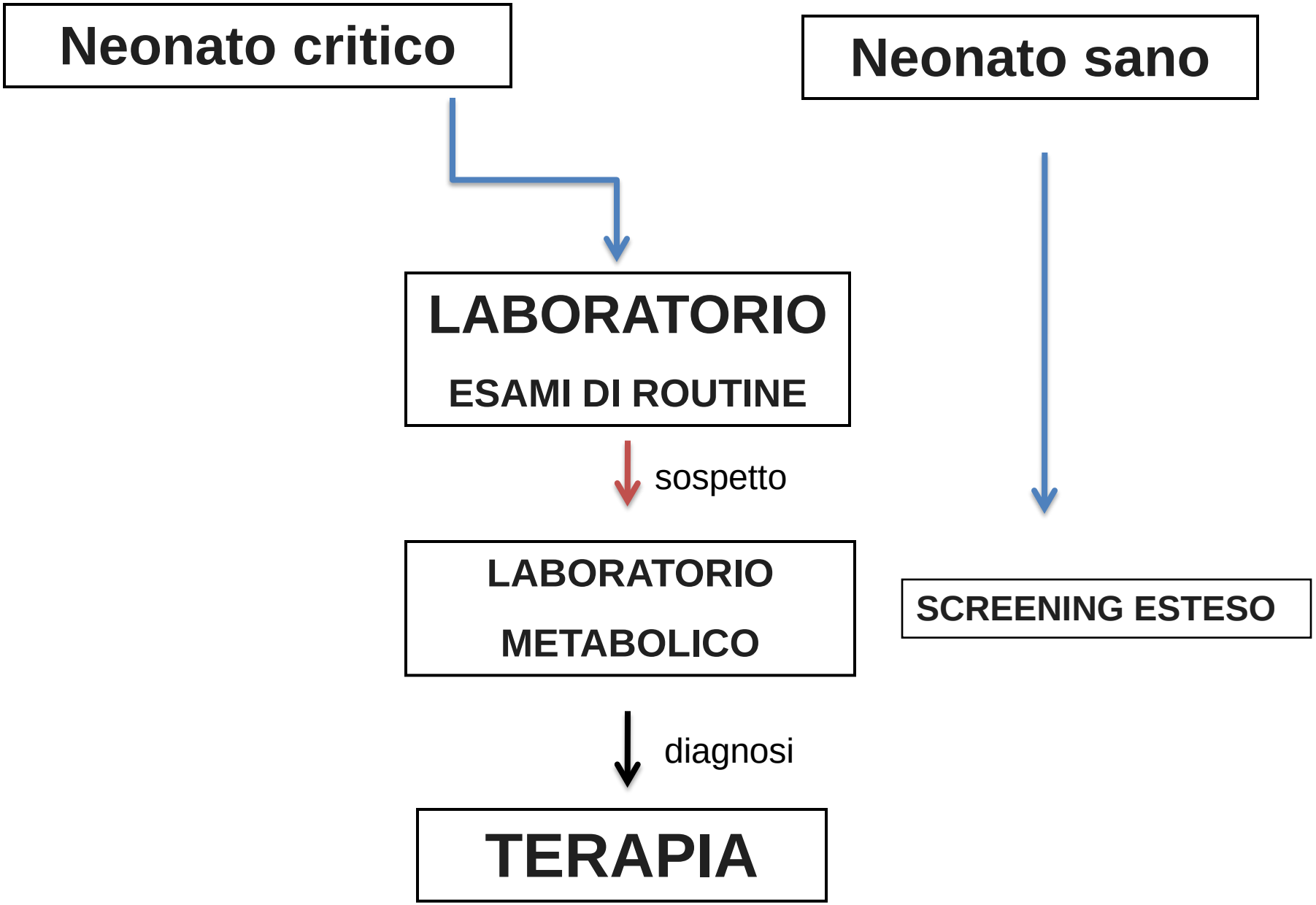
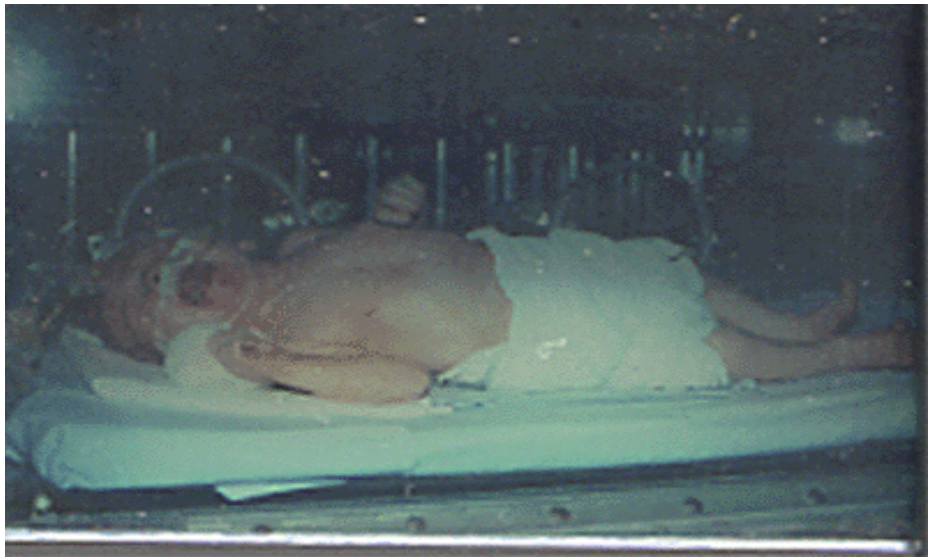
Padova


Neonato critico
Neonato sano
LABORATORIO
ESAMI DI ROUTINE
sospetto
LABORATORIO
METABOLICO
SCREENING ESTESO
diagnosi
TERAPIA

FRIDAY NIGHT PATIENT



1

EQUILIBRIO ACIDO BASE ED ELETTROLITI : anion –gap>20

2

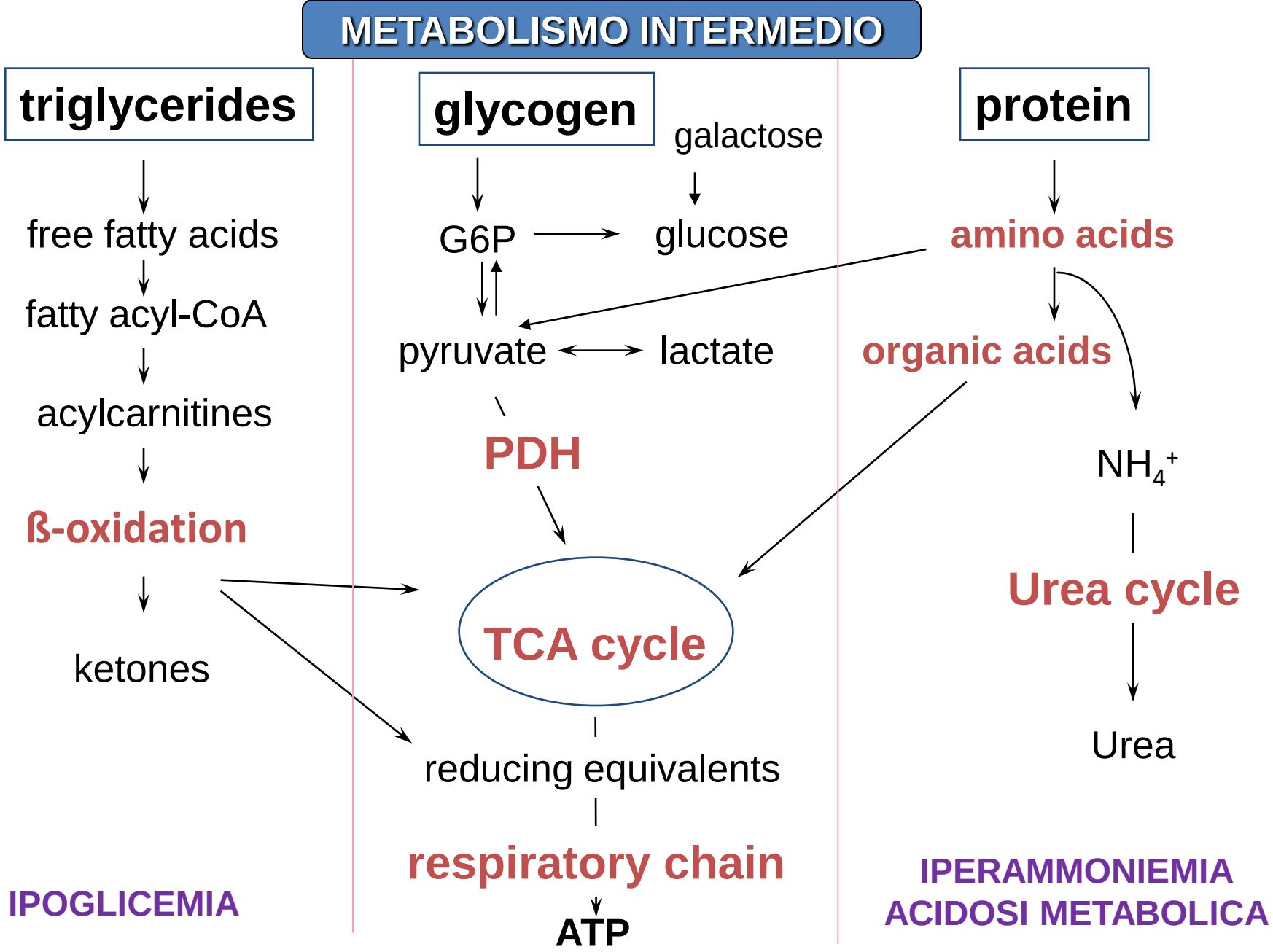
GLICEMIA : < 40 mg/dl e >200 mg/dl

3

CHETONI (++++)

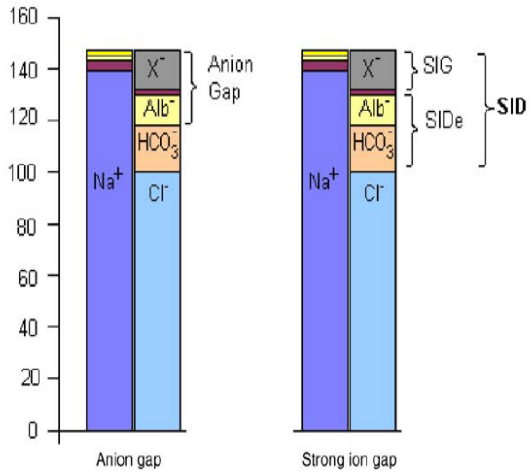
4

AMMONIEMIA > 250 ug/dl


METABOLISMO INTERMEDIO
triglycerides
glycogen
galactose
protein
free fatty acids
G6P
glucose
amino acids
fatty acyl-CoA
pyruvate
lactate
organic acids
acylcarnitines
PDH
NH_4^+
ß-oxidation
Urea cycle
ketones
TCA cycle
Urea
reducing equivalents
respiratory chain
IPOGLICEMIA
IPERAMMONIEMIA
ACIDOSI METABOLICA
ATP
LATTICO ACIDOSI - IPOGLICEMIA

ACIDOSI METABOLICA

- ANION GAP



$$AG = [NA^+] + [K^+] - [Cl^-] - [HCO_3^-]$$

$$AG = [protein] = 8-16 \ mmol/l$$

$$AG = [protein] + [lactate^-]$$

$$\text{Albumin corrected AG} = \text{AG} + (0.25 \times [40 - \text{measured albumin g/l}])$$

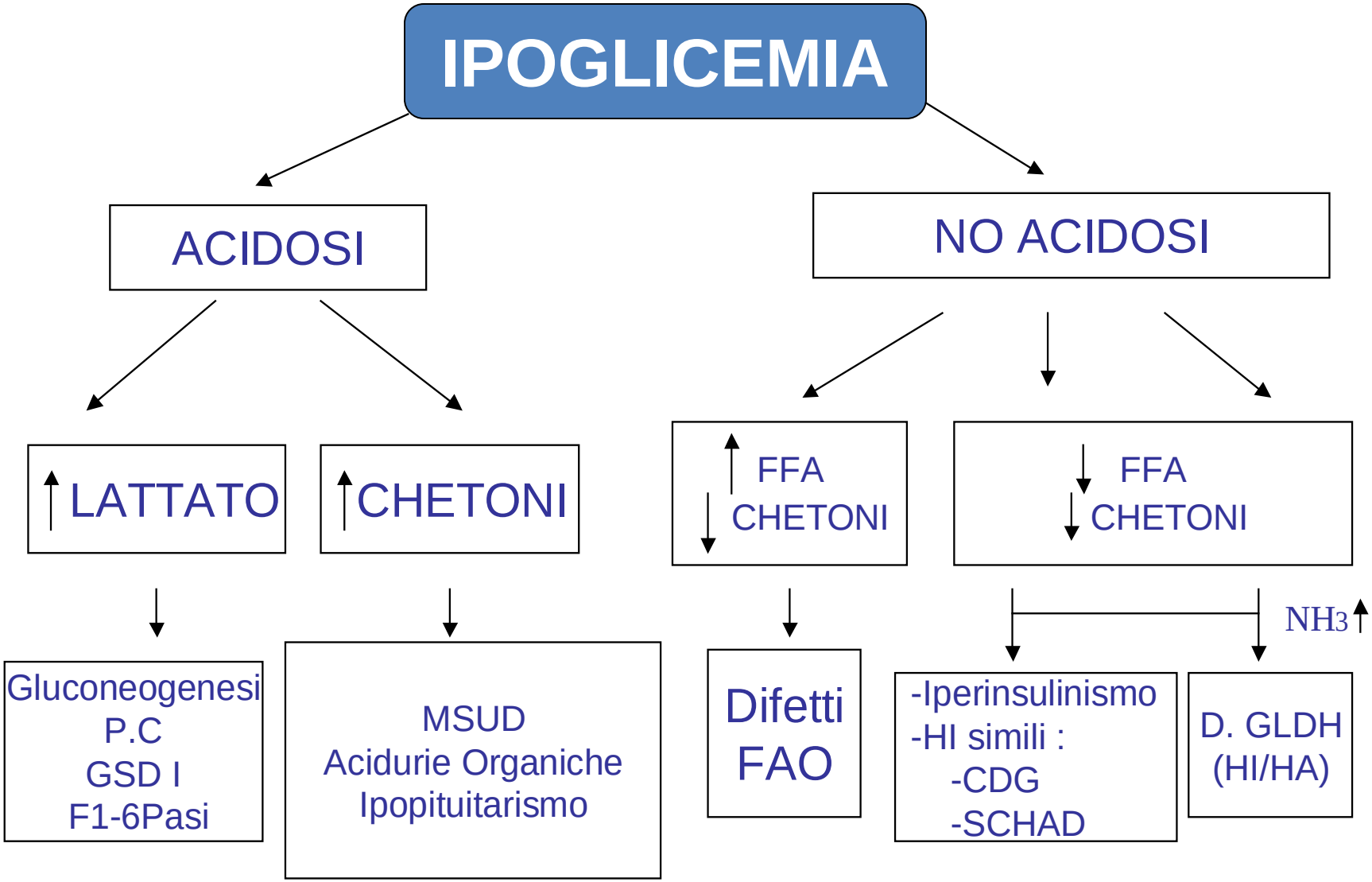
- LATTATO > 2.5 MMOL/l (ESCLUDERE MALATTIE CARDIACHE ED IPOSSIA)
- PIRUVATO : NO (L/P CAVE)

KETOSIS

✓ ***ketonuria*** ***should always be considered*** ***abnormal in neonates*****, it is a physiological result of catabolism in late infancy, childhood, and even adolescence.**

✓**however as a general rule, *hyperketosis* at a level *that produces* METABOLIC ACIDOSIS is not physiological ”**

Saudubray, Inborn Metabolic Diseases,2006


IPOGLICEMIA
ACIDOSI
NO ACIDOSI
↑LATTATO
↑CHETONI
↑FFA
↓CHETONI
↓FFA
↓CHETONI
NH3↑
Gluconeogenesi
P.C
GSD I
F1-6Pasi
MSUD
Acidurie Organiche
Ipopituitarismo
Difetti
FAO
-Iperinsulinismo
-HI simili :
-CDG
-SCHAD
D. GLDH
(HI/HA)

AMMONIEMIA

VALORI NORMALI : < 80 μ MOL/l

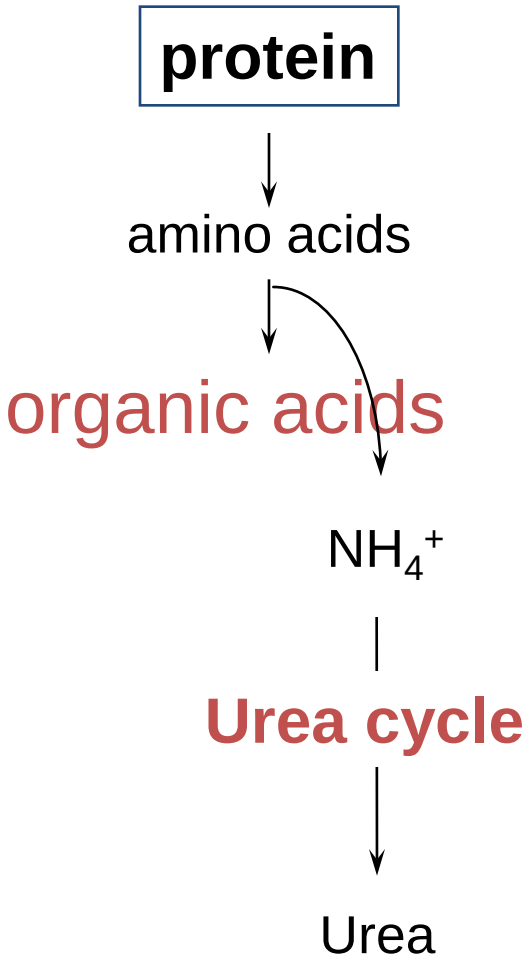
VALORI DUBBI (< 200 μ mol/l)

Acido valproico e tossine

Herpes simplex (neonato)

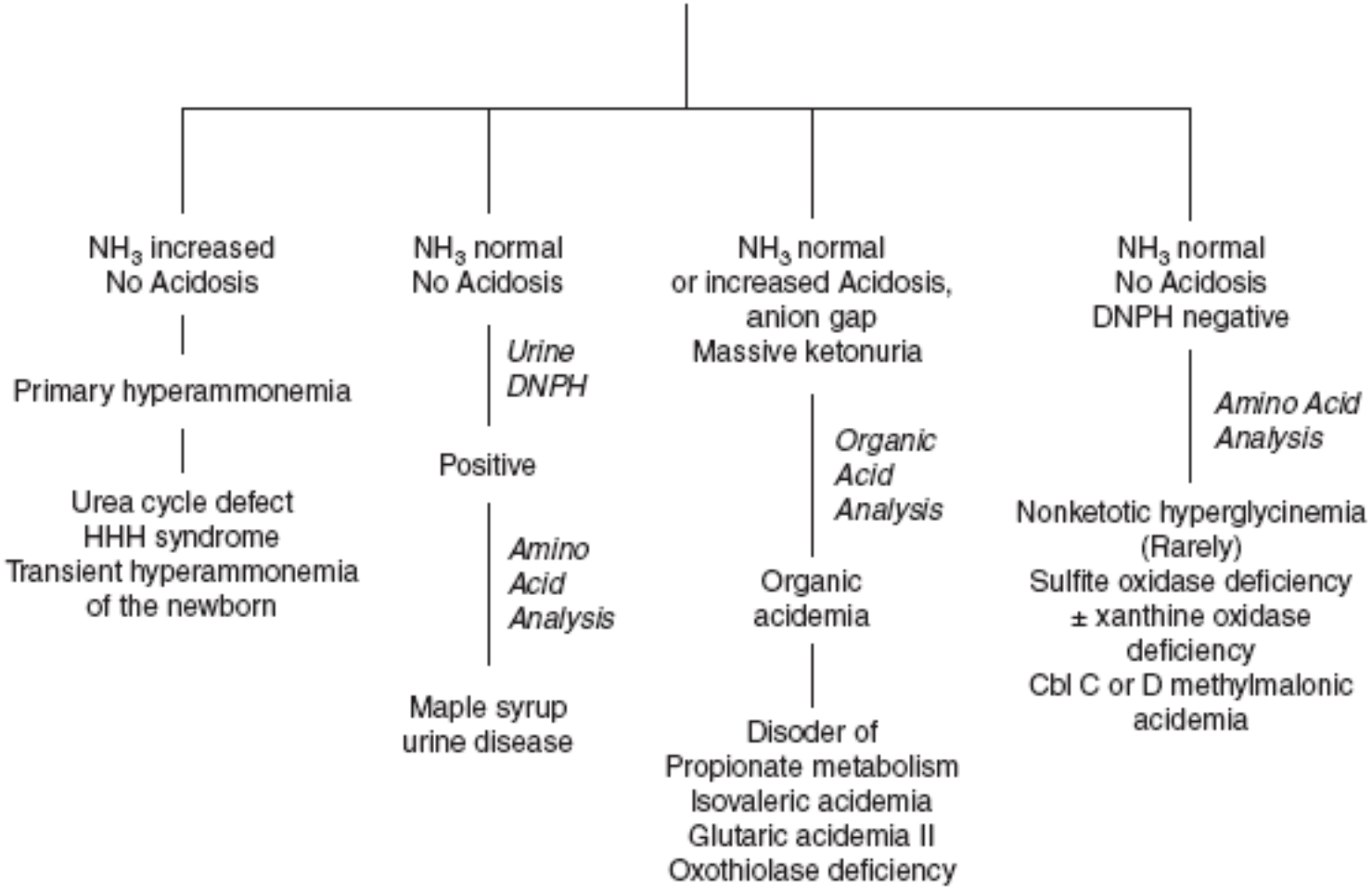
insuff epatica

VALORI METABOLICI: > 250 μg/dl



- Difetti del ciclo dell'urea
- ACIDURIE ORGANICHE

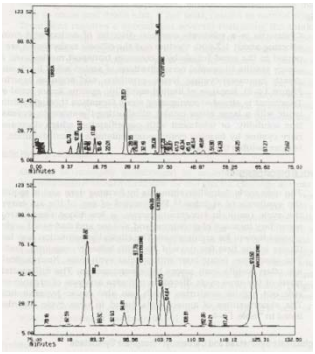
AMMONIO, pH, ELETTROLITI CHETONI



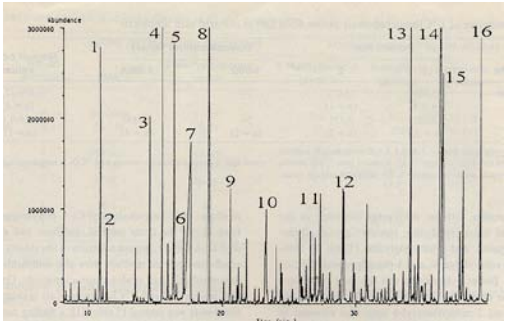
TEST METABOLICI

- Aminoacidopatie
- Difetti ciclo dell' urea
- Acidurie organiche
- Disordini energetici
  - β-ossidazione
  - difetti carboidrati
  - (mitocondriopatie)

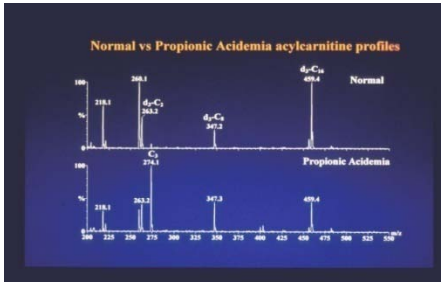
AMINOACIDI

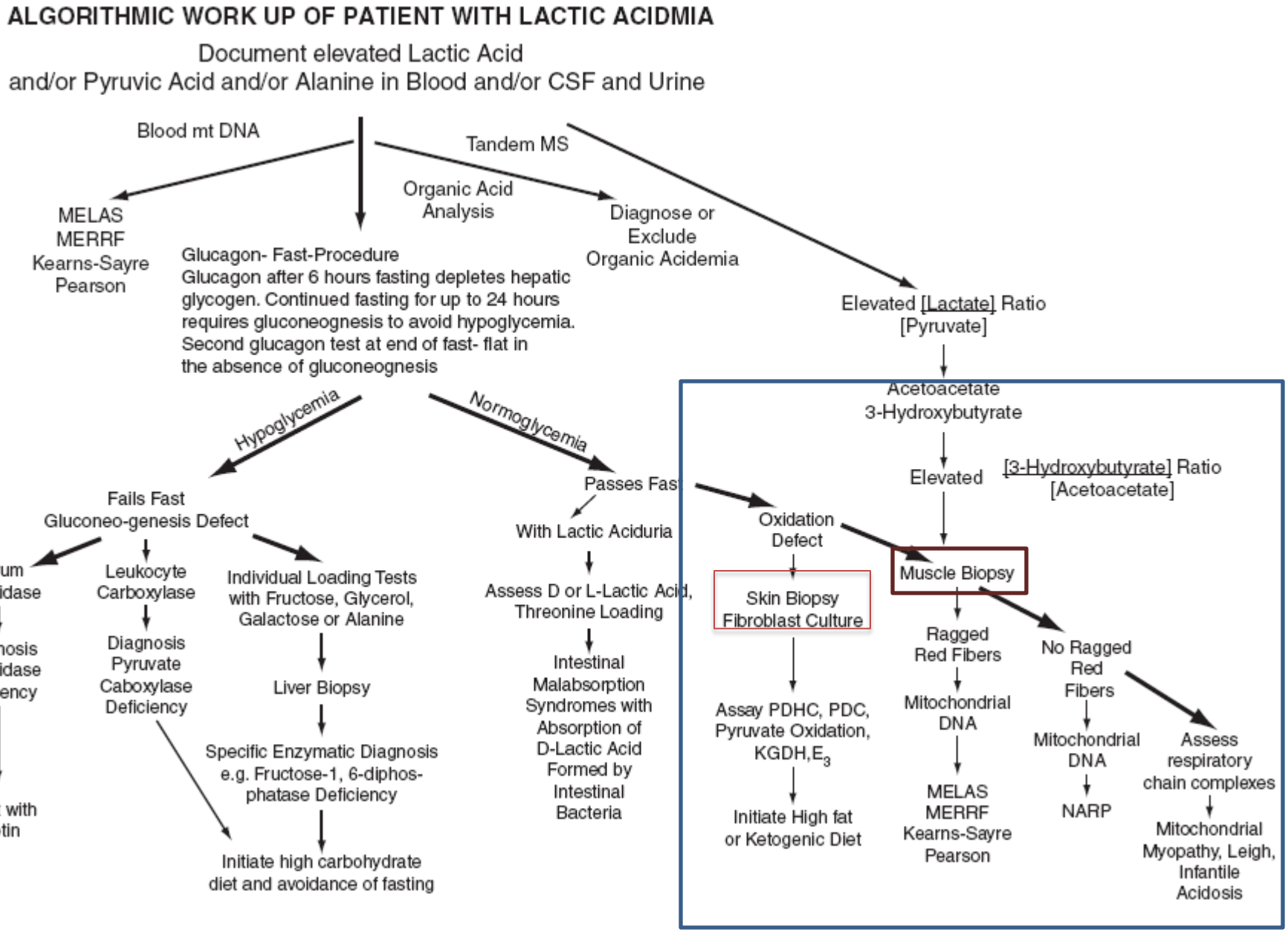


ACIDI ORGANICI



ACILCARNITINE

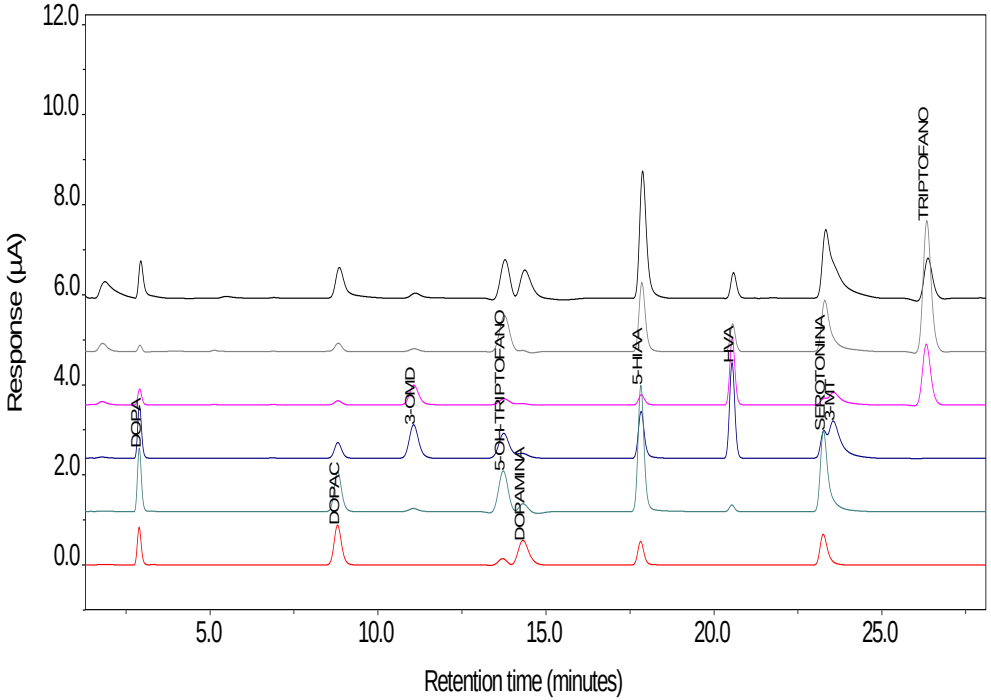


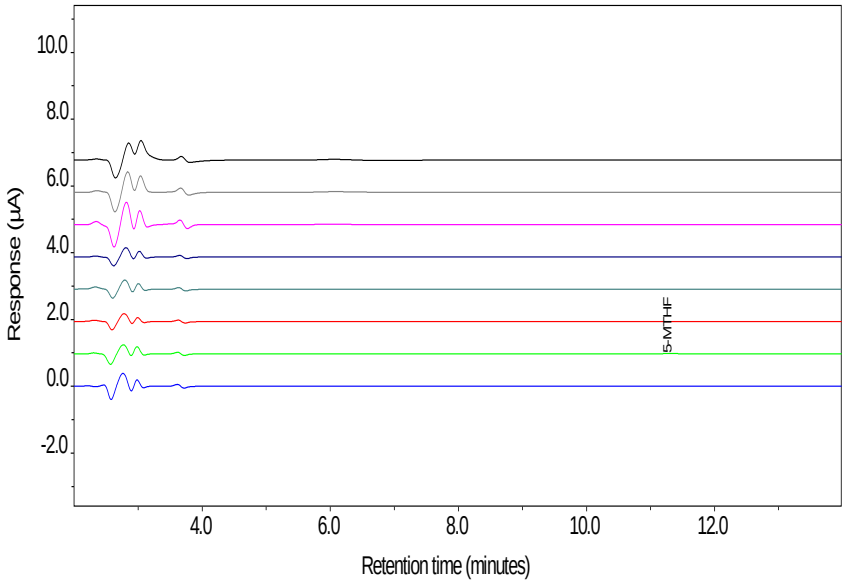

ALGORITHMIC WORK UP OF PATIENT WITH LACTIC ACIDMIA
Document elevated Lactic Acid
and/or Pyruvic Acid and/or Alanine in Blood and/or CSF and Urine
Blood mt DNA
Tandem MS
Organic Acid Analysis
MELAS
MERRF
Kearns-Sayre
Pearson
Diagnose or Exclude Organic Acidemia
Glucagon- Fast-Procedure
Glucagon after 6 hours fasting depletes hepatic glycogen. Continued fasting for up to 24 hours requires gluconeognesis to avoid hypoglycemia. Second glucagon test at end of fast- flat in the absence of gluconeognesis
Elevated [Lactate] Ratio [Pyruvate]
Hypoglycemia
Normoglycemia
Acetoacetate
3-Hydroxybutyrate
Elevated
[3-Hydroxybutyrate] Ratio [Acetoacetate]
Fails Fast
Gluconeo-genesis Defect
Passes Fas
With Lactic Aciduria
Oxidation Defect
Muscle Biopsy
Serum Biotinidase
Leukocyte Carboxylase
Individual Loading Tests with Fructose, Glycerol, Galactose or Alanine
Assess D or L-Lactic Acid, Threonine Loading
Skin Biopsy
Fibroblast Culture
Ragged Red Fibers
No Ragged Red Fibers
Diagnosis Biotinidase Deficency
Diagnosis Pyruvate Caboxylase Deficiency
Liver Biopsy
Intestinal Malabsorption Syndromes with Absorption of D-Lactic Acid Formed by Intestinal Bacteria
Assay PDHC, PDC, Pyruvate Oxidation, KGDH,E3
Mitochondrial DNA
Mitochondrial DNA
Assess respiratory chain complexes
Treat with Biotin
Specific Enzymatic Diagnosis e.g. Fructose-1, 6-diphos-phatase Deficiency
Initiate High fat or Ketogenic Diet
MELAS
MERRF
Kearns-Sayre
Pearson
NARP
Mitochondrial Myopathy, Leigh, Infantile Acidosis
Initiate high carbohydrate diet and avoidance of fasting

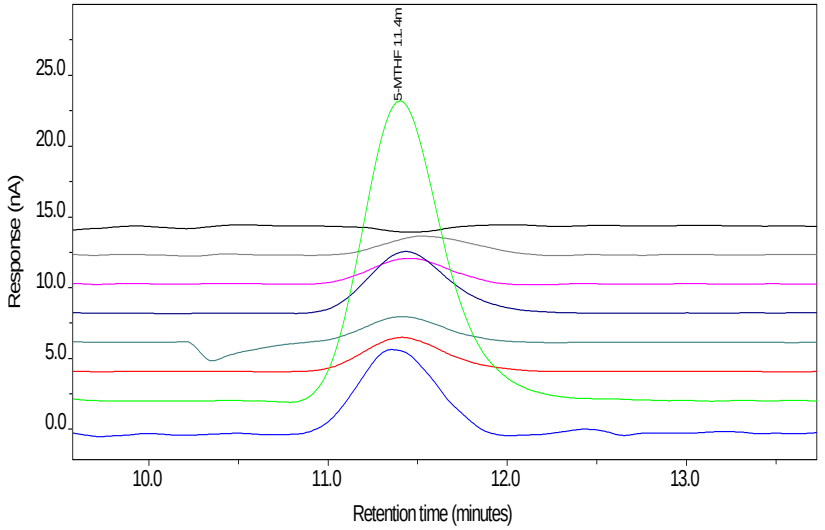
ENCEFALOPATIA METABOLICA

- **Difetti degli aminoacidi glicina e serina**
- **Difetti del metabolismo delle pterine e delle amine biogeniche**
- **Difetti del metabolismo GABA**
- **Difetti del metabolismo pirodossina e folati**
- **Difetti del metabolismo della creatina**

ESAME DEL LIQUOR per AMINOACIDI e NEUROTRAMSMETTITORI


Response (µA)
Retention time (minutes)
DOPA
DOPAC
3-OMD
5-OH-TRIPTOFANO
DOPAMINA
5-HIAA
HVA
SEROTONINA
3-MT
TRIPTOFANO


Response (µA)
Retention time (minutes)
5-MTHF


Response (nA)
Retention time (minutes)
5-MTHF 11.4m

ENCEFALOPATIA EPILETTICA: DIAGNOSI DI LABORATORIO

Treatable disorders	Diagnostic test
PYRIDOXINE DEPENDENCY	Response to pyridoxine; elevated pipecolic acid (CSF, plasma, urine) and a-aminoadipic semialdehyde (urine)
FOLINIC ACID DEPENDENCY	Response to folinic acid; elevated pipecolic acid (CSF, plasma, urine) and a-aminoadipic semialdehyde (urine); unknown peak in CSF HPLC
PNPO DEFICIENCY	Response to pyridoxal 5-phosphate; elevated glycine, threonine, 3-orthomethyldopa, lactate
BIOGENIC AMINES DEFICIENCY	HVA and 5-HIAA in CSF
PHOSPHOSERINE AMINOTRANSFERASE DEFICIENCY	Low CSF and plasma serine and glycine

(J Pediatr 2010;156:420-6).

Prevalence of Developmental Disabilities and Receipt of Special Education Services among Children with an Inborn Error of Metabolism

Kimberly Powell, PhD, RD, Kim Van Naarden Braun, PhD, Rani Singh, PhD, RD, Stuart K. Shapira, MD, PhD,

Table I. Characteristics of children who were diagnosed with a metabolic disorder identified by newborn screening or clinical symptoms and with a developmental disability or receiving special education services

Case number*	Type of metabolic disorder	Age at initiation of treatment	Metabolic control
Identified by newborn screening†			
MADDSP			
CP			
1‡	MSUD	15 days	Excellent
Mental retardation/ID			
1‡	MSUD	15 days	Excellent
2	MSUD	6 days	Excellent
SEDMA§			
Behavior disorder			
3	DG	29 days	Unknown
Learning disorder			
4	DG	17 days	Fair
5	DG	19 days	Excellent
6	PKU	12 days	Poor
Mild ID			
7¶	GAL	10 days	Fair
Moderate ID			
8	GAL	7 days	Fair
Other health impairment			
9	MSUD	12 days	Excellent
Significant developmental delay			
7¶	GAL	10 days	Fair
Speech/language disorder			
10	DG	34 days	Excellent
11	DG	16 days	Excellent
Identified by clinical symptoms**			
MADDSP			
Autism			
12‡	ASA	3 days	Excellent
ID			
12‡	ASA	3 days	Excellent
13	Citrullinemia	Birth	Good
14	Methylmalonic acidemia	Infancy	Unknown
15	Mevalonic aciduria	2 years	Unknown
16	Propionic acidemia	3 days	Excellent
SEDMA			
Other health impairment			
17	Homocystinuria	9 years	Poor
Significant developmental delay			
18	ASA	7.5 years	Excellent

SCREENING BY MS/MS
(MULTIPLEX TESTING)

- Molte malattie
- un test
- Molti metaboliti
- ampio cut-off

$(MME)_n$

↓

MS/MS

↓

$(AA,AC)_n$

↓

0.1-1,000 µM

Table 1 (continued)

Disease	Methods	Relevance ranking	Screening programs	Test[a] available	Therapy available	Benefit from early detection	References	Remarks
Galactosemia	Substrate and/or enzyme assay	++	a	y	y	y	[119]	Long-term outcome not as favorable as initially thought in the 1970s
Glucose-6-phosphate dehydrogenase deficiency	Enzyme assay	?	e	?	y	y	[120]	High genetic variability
Disorders of creatine metabolism	TMS	?	p	?	y	?	[121–123]	Feasibility has been demonstrated, results of pilots not available so far
Lysosomal storage disorders	TMS	?	p	?	y	?	[124, 125]	Enzyme replacement therapy is available for M. Fabry, M. Gaucher, M. Krabbe, M. Niemann-Pick A/B, and M. Pompe
Cystic fibrosis	IRT/DNA	++	m	y	y	y	[126–129]	
Diabetes mellitus type I	DNA	?	p	?	?	?	[130]	"Genetic risk" screening
Other diseases								
Hearing deficiency	Otoacoustic	++	m	y	y	y	[131]	Decentralized
Congenital CMV infection	CMV viral load	+	m	y	y	y	[132–138]	Late-onset hearing loss is not detectable by the otoacoustic method in newborns
Congenital toxoplasmosis infection	Toxoplasmosis viral load	- -	mat	- -	- -	- -	[139]	Not recommended, (prenatal care)
Congenital syphilis infection	Nontreponemal antibodies	- -	mat/epd	- -	- -	- -	[140]	Not recommended, (prenatal care)
Neuroblastoma screening[b]	HPLC	- -	d	- -	- -	n	[141–143]	Not recommended
Duchenne muscular dystrophy	Creatine kinase activity	- -	p	y	n	n	[144, 145]	Disposition-screening; no effect on outcome
SCID	T-cell lymphopenia	?	pro	- -	- -	- -	[146]	Not recommended
HIV	Immunoassays	?	epd	- -	- -	- -	[147]	Not recommended
Hepatitis C	Immunoassays	?	epd	- -	- -	- -	[148]	Not recommended, (prenatal care)
Hepatitis B	HBsAg	?	epd	- -	- -	- -	[149]	Not recommended, (prenatal care)

CPT-I carnitine palmitoyl transferase I, *CPT-II* carnitine palmitoyl transferase II, *HBsAg* hepatitis B surface antigen, *HHH* hyperornithinemia–hyperammonemia–homocitrullinuria, *HPLC* high-performance liquid chromatography, *IEF* isoelectric focusing, *IRT* immunoreactive trypsin, *LCHAD* long-chain hydroxyacyl-CoA dehydrogenase, *MCAD* medium-chain acyl-CoA dehydrogenase, *3-MCC* 3-methylcrotonyl-CoA carboxylase, *NBS* newborn screening, *OAT* ornithine amino transferase, *SCAD* short-chain acyl-CoA dehydrogenase, *SCID* severe combined immunodeficiency, *TFP* trifunctional protein, *TLC* thin-layer chromatography, *TMS* tandem mass spectrometry, *VLCAD* very long chain acyl-CoA dehydrogenase, *a* all, *d* discontinued, *e* ethnic, *epd* epidemiologic, *m* most, *mat* recommended as a prenatal screening test, *n* no, *p* pilot, *pro* proposed, *y* yes, + + unquestioned, + favorable, ? questionable, - unfavorable, - - not recommended

[a] With sufficient sensitivity and specificity, economically justifiable

[b] Specimen for screening is urine dried on filter paper

Table 1 (continued)

Disease	Methods	Relevance ranking	Screening programs	Test[a] available	Therapy available	Benefit from early detection	References	Remarks
3-MCC deficiency	TMS	-	m	?	y	?	[95]	Low clinical expressivity and penetrance
3-Hydroxymethylglutaryl-CoA lyase deficiency	TMS	?	m	y	y	y	[96]	Reliable discrimination from 3-MCC not possible
Holocarboxylase synthase deficiency	TMS	?	m	y	y	y	[97]	Very rare, but easily treatable with biotin; reliable discrimination from 3-MCC not possible
β-Ketothiolase deficiency	TMS	+	m	?	y	y	[98]	Sensitivity and specificity presumably low
Disorders of glutathione metabolism	TMS	?	m	?	?	?	[99]	No prospective data
β-Oxidation defects/disorders of carnitine metabolism								
SCAD deficiency	TMS	- -	m	?	?	?	[100]	Causality between enzyme defect and clinical presentation is not proven
MCAD deficiency	TMS	+ +	a	y	y	y	[101, 102]	Positive effect unquestioned, however, patients that might never become symptomatic are also detected
VLCAD deficiency	TMS	+ +	m	y	y	y	[103]	Mild variants might be missed when the samples are taken under anabolic conditions
LCHAD/TFP deficiency	TMS	+	m	y	y	y/?	[104–106]	Information on long-term outcome is still pending; prognosis for TFP is rather bad
Carnitine transporter deficiency	TMS	+	m	?	y	y	[107]	Sensitivity unclear; free carnitine level can be normal postpartum, depending on maternal supply and renal loss
CPT-I deficiency	TMS	+ +	m	y	y	y	[108]	Ratio of free carnitine to the sum of palmitoylcarnitine and stearoylcarnitine is sensitive and highly specific
CPT-II deficiency	TMS	+	m	?	?	?	[109]	Neonatal onset form with bad prognosis despite early diagnosis; in the late-onset form mainly skeletal muscle is involved, seems to have normal levels of acylcarnitines in the neonatal period
Translocase deficiency	TMS	+	m	y	?	?	[110]	Bad prognosis despite early diagnosis
Endocrinopathies								
Congenital hypothyroidism	ELISA	+ +	a	y	y	y	[111]	
Congenital adrenal hyperplasia	ELISA	+ +	a	y	y	y	[112, 113]	Sensitivity for the salt-wasting form is good, for simple virilizing congenital adrenal hyperplasia approximately 50%
Hemoglobinopathies								
Sickle cell anemia	IEF	+ +	e	y	y	y	[114–116]	
Hemoglobin S/β-thalassemia	IEF	+ +	e	y	y	y		
Hemoglobin SC disease	IEF	+ +	e	y	y	y		
Hemoglobin H	IEF	+ +	e	y	y	y		
Other inborn errors of metabolism								
Biotinidase deficiency	Enzyme assay	+ +	a	y	y	y	[117, 118]	

Table 1 Target diseases for newborn screening

Disease	Methods	Relevance ranking	Screening programs	Test[a] available	Therapy available	Benefit from early detection	References	Remarks
Amino acidopathies								
Phenylketonuria	TMS	+ +	a	y	y	y	[54–58]	Alternative therapies for mild phenylketonuria have been introduced recently
Maple syrup urine disease	TMS	+ +	m	y	y	y	[59–62]	Early blood collection is mandatory
Homocystinuria	TMS	+	m	n	y	y	[13, 63, 64]	Sensitivity and specificity low with methionine as a primary marker; determination of homocysteine could improve NBS
Tyrosinemia type I	TMS	+	m	y/?	y	y	[65–69]	Low sensitivity and low specificity with tyrosine as primary marker; determination of succinyl acetone could improve NBS
Citrullinemia	TMS	+	m	?	?	?	[70]	No positive effect on outcome; patients with a mild biochemical phenotype might never develop symptoms
Argininosuccinic acidemia	TMS	+	m	?	?	?	[71]	No positive effect on outcome
Arginase deficiency	TMS	+	m	?	?	y	[72–74]	Very rare; the first results of NBS and early treatment seem promising
Hyperornithinemia (OAT deficiency and HHH syndrome)	TMS	?	m	n	?	?	[75]	Normal ornithine levels during the first weeks of life
Nonketotic hyperglycinemia	TMS	- -	m	n	n	n	[76]	No therapy available
Histidinemia	TLC	- -	d	- -	- -	n	[77–81]	Benign, does not require treatment
Hydroxyprolinemia	TLC	- -	d	- -	- -	n	[82–84]	Benign, does not require treatment
Organic acidurias								
Glutaric aciduria type I	TMS	+ +	m	y	y	y	[85, 86]	
Isovaleric acidemia	TMS	+ +	m	y	y	y	[87, 88]	Screening also detects unaffected patients with mild variants
Propionic acidemia	TMS	+	m	y	y	?	[89, 90]	Acylcarnitine profile indistinguishable from methylmalonic acidemia profile in newborns
Methylmalonic acidemia (mutase)	TMS	+	m	y	y	?	[90]	Acylcarnitine profile indistinguishable from propionic acidemia profile in newborns
Methylmalonic acidemia (disorders of cobalamine metabolism types A–D, F)	TMS	+	m	y	y	?	[91]	Sensitivity unclear; propionylcarnitine level is often only slightly elevated
Cobalamin EG defect	TMS	- -	?	?	?	?	[92]	Low methionine level is the only marker; sensitivity and specificity unknown, but presumably low; determination of homocysteine in dried blood spots could improve NBS
Malonyl-CoA decarboxylase deficiency	TMS	+	m	y	y	y	[93, 94]	Very rare; no prospective data

POSSIBILI 60 PATOLOGIE METABOLICHE EREDITARIE

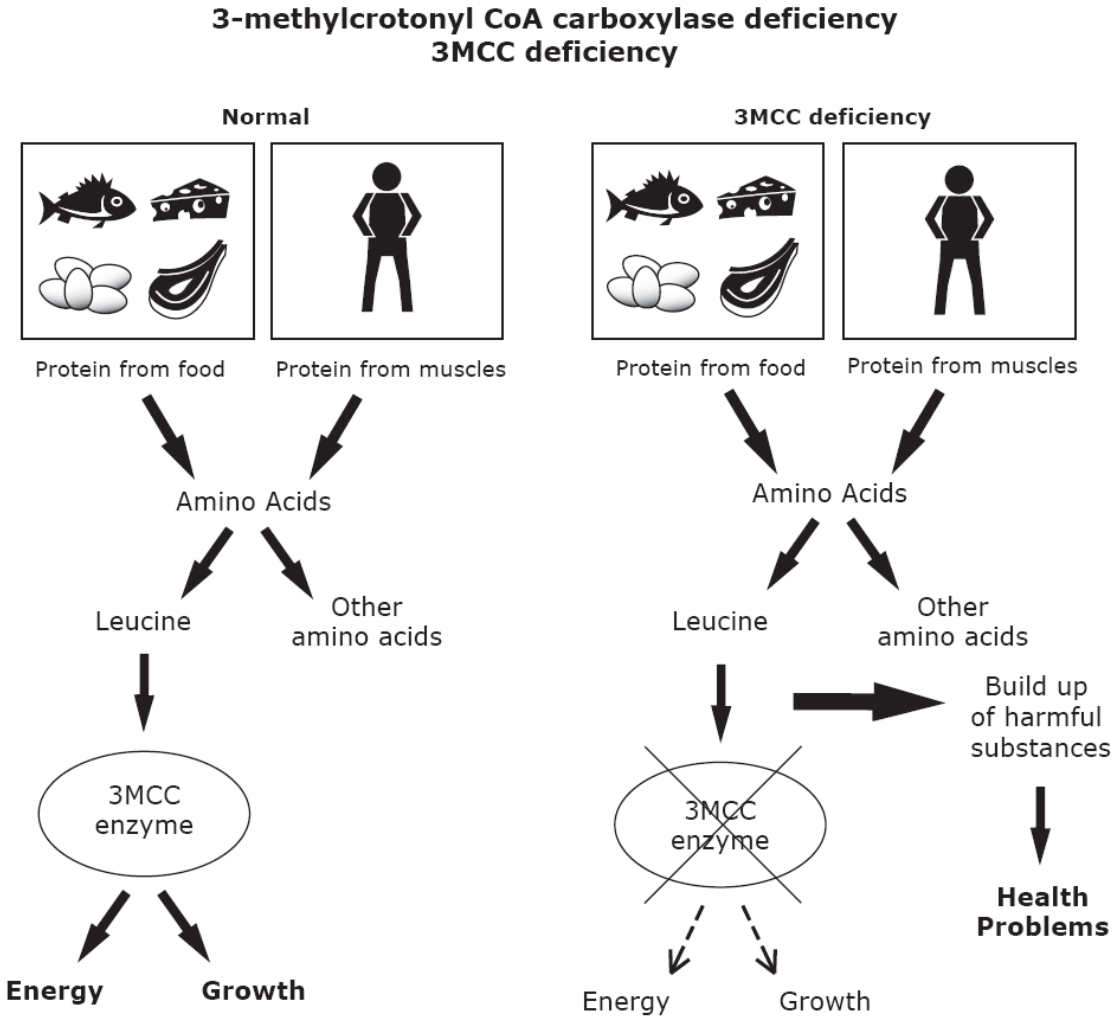
QUALI MALATTIE INCLUDERE ?

- Malattie con morbidità e mortalità significative
- Trattamanto presente ed efficace
- Tempo necessario per intervenire prima che si sviluppino i sintomi
- Validità clinica e utilità clinica dello screening
- Economicamente fattibile (costo/beneficio)
- Che la malattia abbia una storia naturale nota
- Che la malattia abbia una incidenza conosciuta e significativa nella popolazione che sarà screenata

Pediatrics,2009;123:451-457

THE BURDEN OF NON DISEASE

ovvero creiamo malati dove non ci sono ?



MS/MS : ESPERIENZA PILOTA VENETO

(2002 – 2009)

	diagnosi	Screening
MSUD	5	0
Tirosinemia	0	0
ASL	4	0
ASS	4	0
NKH	3	0
Isovalerico acidemia	0	1
3MCC	0	1
MMA	5	1(1)
Deficit cobalamina	5	0
PA	4	0
MCAD	3	2
VLCAD	4	1
MADD	9	0
LCHAD	4	0
CPT I	1	0
Total cases	**51**	**7**

SCREENING NEONATALE

VENETO 2010

Fenilchetonuria
Malattia delle urine allo sciroppo d'acero
Isovalerico acidemia
Glutarico aciduria tipo I
Deficit cobalamina C/metilmalonico acidemia
Deficit dell'acil CoA deidrogenasi a catena media (MCAD)
Deficit dell'acil CoA deidrogenasi a catena molto lunga (VLCAD)

II fase
Tirosinemia tipo I
Citrullinemia
Aciduria argininosuccinica
Argininemia
Deficit dell'acil CoA deidrogenasi a catena molto lunga
Deficit dell'acil CoA deidrogenasi a catena lunga
Deficit dell'acil CoA deidrogenasi a catena corta
Deficit delle acil CoA deidrogenasi (aciduria glutarica tipo II)
Deficit della carnitina palmitoliltrasferasi tipo I
Deficit della carnitina palmitoliltrasferasi tipo II
Deficit del trasportatore della Carnitine
Acidemia Propionica

MANAGEMENT IN EMERGENZA

- Terapia di supporto
- Rimuovere i metaboliti tossici
- Rifornire i prodotti depleti
- Prevenire l'accumulo di cataboliti lungo le vie metaboliche interessate

TERAPIA DI SUPPORTO

- **IDRATAZIONE (100-150 mg/kg/die)**
- **ELETTROLITI : evitare iponatriemia**
- **GLUCOSIO (**5-8 mg/kg/min- 15 mg/kg/min)
  - **per os (rischio di diarrea!)**
  - **endovena : glucosio al 10%**
  - **catetere centrale : glucosio fino al 25%**

ACIDOSI METABOLICA

pH < 7.1 (o > 7.1 ma compromissione delle condizioni generali)
Sodio bicarbonato iniziando con metà correzione
(deficit di basi x peso (kg) x 0.3 / 2)

- Infusione lenta
- Controlli frequenti
- Monitorare sodio / potassio
- Considerare THAM se ↑ fluidi o ↓ sodio

pH > 7.1 ottimizzare la perfusione tissutale
idratazione
Iposodiemia

DISORDERS REQUIRING ANABOLISM

(ACUTE INTOXICATION)

Kalories : 80-100kcal/kg/day

Organic acidurias, maple syrup urine disease, urea cycle disorders ⇒

glucose 15-20 g/kg/day + fat 2 g/kg/day

always: + insulin, starting with 0.05 U/kg/h, Adjustments are made dependent on blood glucose (useful combination is 1 U/8 g glucose)

early: central venous catheterisation

DISORDERS REQUIRING GLUCOSE STABILISATION

(reduced fasting tolerance)

Fatty acid oxidation defects, glycogen storage disease type I, disorders of gluconeogenesis, galactosemia, fructose intolerance, tyrosinemia I ⇒

glucose 7-10 mg/kg/min≈ 10 g/kg/day

DISORDERS REQUIRING RESTRICTION OF ENERGY TURN-OVER

(disturbed energy metabolism)

PDHC deficiency ⇒

reduce glucose supply: 2-3 mg/kg/min ≈ 3g/kg/day

Add fat: 2-3 g/kg/day

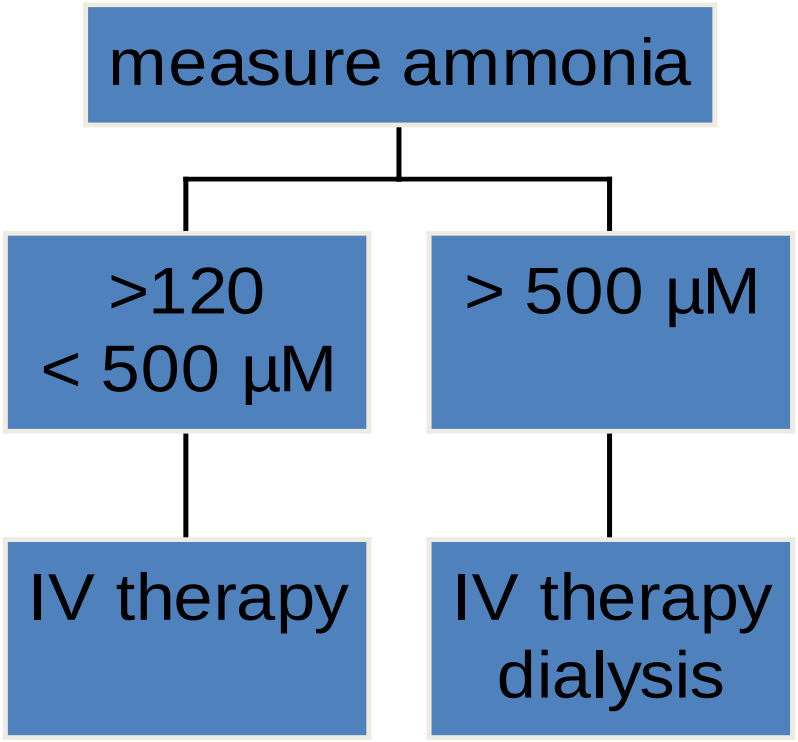
Electron transport chain (RCD) defects: glucose 10-15 g/kg/day

ACIDOSI METABOLICA

Terapia con cofattori :

OH-COBALAMINA (VIT. B12)	**Metilmalonico acidemia**
BIOTINA	**Deficit di olocarbossilasi sintetasi**
	Deficit di biotinidasi
CARNITINA	**Isovalerico acidemia**
	Glutarico aciduria tipo I e II

IPERAMMONIEMIA



Sodio benzoato
Sodio fenilbutirrato
Arginina
Acido Carglumico

ENCEFALOPATIA EPILETTICA: TRATTAMENTO

Treatable disorders	Treatment
PYRIDOXINE DEPENDENCY	Pyridoxine (or pyridoxal-5-phosphate) 30 mg/kg (usually 100 mg) as starting dose, then 30 mg/kg/day
FOLINIC ACID DEPENDENCY	Folinic acid 2-3 mg/kg/day in 3 doses
PNPO DEFICIENCY	Pytidoxal-5-phosphate 30-40 mg/kg/day in 3-4 single doses
PHOSPHOSERINE AMINOTRANSFERASE DEFICIENCY	Serine supplementation

TAKE HOME MESSAGE

- **Evitare di non diagnosticare una malattia metabolica ereditaria trattabile**
- **Prima il paziente (emergency treatment) e poi la famiglia (genetic counselling)**
- **Discutere il caso con il Centro di riferimento**

Saudubray, Inborn Metabolic Diseases,2006