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By

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LIST OF ABBREVIATIONS

ABCD1 – Gene coding for adrenoleukodystrophy protein
ACADM – Gene coding for medium chain acyl-CoA dehydrogenase
ACADVL – Gene coding for very long chain acyl-CoA dehydrogenase
ACAT1 – Gene coding for acetyl-CoA acetyltransferase 1
ACTH – Adrenocorticotrophic hormone or adrenocorticotrophin or corticotrophin
ADH – Antidiuretic hormone
AFP – α-1-Foetoprotein
AIDS – Acquired immune deficiency syndrome
ALDP – Adrenoleukodystrophy protein
AMH – Anti-Mullerian hormone
AMP – Adenosine monophosphate
β-APP – β-Amyloid precursor protein
APRTase – Adenine phosphoribosyl transferase
ASL – Gene coding for arginosuccinate lyase
ASS1 – Gene coding for arginosuccinate synthetase
AST – Aspartate transaminase
ATP – Adenosine triphosphate
BCKDHA – Gene coding for the $E_1\alpha$ subunit of the branched chain α-ketoacid dehydrogenase complex
BCKDHB – Gene coding for the $E_1\beta$ subunit of the branched chain α-ketoacid dehydrogenase complex
BMD – Bone mineral density
BMI – Body mass index
BPA – Bisphenol A
BTD – Gene coding for biotinidase
BTK – Gene coding for Bruton tyrosine kinase
C – Cytosine
CA – Carbohydrate antigen
CBAVD – Congenital bilateral absence of the vas deferens
CBS – Gene coding for cystathionine β-synthase
Cer – Ceramide
cfDNA – Cell-free deoxyribonucleic acid
CFTR – Gene coding for the cystic fibrosis transmembrane regulator protein

CGH – Comparative genomic hybridisation
CMA – Chromosomal microarray analysis
CO_2 – Carbon dioxide
CoA – Coenzyme A
COMT – Catechol-O-methyltransferase
COX-2 – Cyclo-oxygenase-2
CRH – Corticotrophin releasing hormone
DBT – Gene coding for the E_2 subunit of the branched chain α-ketoacid dehydrogenase complex
DHEA – Dehydroepiandrosterone
DHEAS – Dehydroepiandrosterone sulphate
DHT – Dihydrotestosterone
DLD – Gene coding for the E_3 subunit of the branched chain α-ketoacid dehydrogenase complex
DNA – Deoxyribonucleic acid
DTT – Dichlorodiphenyltrichloroethane
DUOXA1 – Gene coding for the dual oxidase maturation factor 1 protein
DUOXA2 – Gene coding for the dual oxidase maturation factor 2 protein
EGFR – Epidermal growth factor receptor
Et-1 – Endothelin
EU – European Union
FAH – Gene coding for fumarylacetoacetate hydrolase
$FADH_2$ – Flavin adenine dinucleotide
FISH – Fluorescence *in situ* hybridisation
FMR1 – Gene coding for the fragile X mental retardation 1 protein
FMRP – Fragile X mental retardation 1 protein
FSH – Follicle stimulating hormone
G – Guanine
Gal – Galactose
GalNAc – N-Acetylgalactosamine
GCDH – Gene coding for glutaryl-CoA dehydrogenase
GFR – Glomerular filtration rate
GH – Growth hormone
GHRH – Growth hormone regulating hormone
GHRHR – Growth hormone regulating hormone receptor
GIP – Gastric inhibitory polypeptide
GLA – Galactosidase alpha
GLP-1 – Glucagon-like peptide-1
Glu – Glucose
GLUT1 – Glucose transporter 1
GMP – Guanosine monophosphate

GNAS1 – Gene coding for the α-subunit of G protein
GnRH – Gonadotrophin releasing hormone
HADHA – Gene coding for long chain L-3-hydroxyacyl-CoA dehydrogenase (α-subunit of the trifunctional protein)
HADHB – Gene coding for the β-subunit of the trifunctional protein
HBB – Gene coding for the β-globin protein
hCG – Human chorionic gonadotrophin
HDL – High density lipoprotein
HELLP – Haemolysis, elevated liver enzymes and low platelet count
HESX1 – HESX homeobox 1 gene
HGPRTase – Hypoxanthine-guanine phosphoribosyl transferase
HIV – Human immunodeficiency virus
HLCS – Gene coding for holocarboxylase synthetase
HMGCL – Gene coding for 3-hydroxy-3-methylglutaryl-Coenzyme A lyase
HMG-CoA – 3-Hydroxy-3-methylglutaryl-Coenzyme A
hPL – Human placental lactogen
HPV – Human papillomavirus
HRT – Hormone replacement therapy
3-β-HSD – 3-β-Hydroxysteroid dehydrogenase
11β-HSD1 – 11β-Hydroxysteroid dehydrogenase type 1
11β-HSD2 – 11β-Hydroxysteroid dehydrogenase type 2
17-β-HSD – 17-β-Hydroxysteroid dehydrogenase
Ig – Immunoglobulin
IgA – Immunoglobulin A
IGF-1 – Insulin-like growth factor 1
IGF-2 – Insulin-like growth factor 2
IgG – Immunoglobulin G
IgM – Immunoglobulin M
IL-1 – Interleukin-1
IL-6 – Interleukin-6
IMP – Inosine monophosphate
LDL – Low density lipoprotein
L-DOPA – L-Dihydroxyphenylalanine
LH – Luteinizing hormone
LHX4 – LIM homeobox 4 gene
MAO – Monoamine oxidase
MCA – Mucin-like carcinoma associated antigen
MCCC1 – Gene coding for the α-subunit of 3-methylcrotonyl-CoA carboxylase

MCCC2 – Gene coding for the β-subunit of 3-methylcrotonyl-CoA carboxylase
MCEE – Gene coding for methylmalonyl-CoA epimerase (also called methylmalonyl-CoA racemase)
MMAA – Gene coding for methylmalonic aciduria type A protein
MMAB – Gene coding for the methylmalonic aciduria type B protein
MMADHC – Gene coding for the methylmalonic aciduria type D protein
MRI – Magnetic resonance imaging
mRNA – Messenger ribonucleic acid
MTHFR – Gene coding for methylenetetrahydrofolate reductase
MTR – Gene coding for methionine synthase
MTRR – Gene coding for methionine synthase reductase
Mut – Gene coding for methylmalonyl-CoA mutase
NADH – Nicotinamide adenine dinucleotide
NADPH – Nicotinamide adenine dinucleotide phosphate
NANA – N-Acetylneuraminic acid or sialic acid
NIDDM – Non-insulin dependent diabetes mellitus
OCTN2 – Organic cation / carnitine transporter 2 protein
OGTT – Oral glucose tolerance test
1,25-$(OH)_2$D – 1,25-Dihydroxycholecalciferol or 1,25-dihydroxyvitamin D or calcitriol
25-(OH)D –25-Hydroxycholecalciferol or 25-hydroxyvitamin D or calcifediol
17-OHP – 17-Hydroxyprogesterone
PAH – Gene coding for phenylalanine hydroxylase
PAI-1 – Plasminogen activator inhibitor-1
Pap – Papanicolaou
PAPP-A – Pregnancy-associated plasma protein-A
PAX-8 – Paired box 8 gene
PCBs – Polychlorinated biphenyls
PCCA – Gene coding for the α-subunit of propionyl-CoA carboxylase
PCCB – Gene coding for the β-subunit of propionyl-CoA carboxylase
P_{CO_2} – Carbon dioxide pressure
PCR – Polymerase chain reaction
PIGF – Placental growth factor
PIT-1 – Gene coding for the pituitary-specific transcription factor 1 protein
P_{O_2} – Oxygen pressure
PPi – Pyrophosphate
PROP1 – Gene coding for the PROP paired-like homeobox 1 protein
PRPP – Phosphoribosylpyrophosphate

PSA – Prostate specific antigen
PTH – Parathyroid hormone or parathormone
PTHrP – Parathyroid hormone-related protein
Rh – Rhesus
RNA – Ribonucleic acid
rRT-PCR – Real-time reverse transcription-polymerase chain reaction
SD – Standard deviation
sENG – Soluble endoglin
sFLT1 – Soluble fms-like tyrosine kinase-1
SH – Sulfhydryl group
SHBG – Sex hormone binding globulin
SLC5A5 – Gene coding for the iodine-sodium transporter
SLC22A5 – Solute carrier family 22 member 5 gene
SNP – Single nucleotide polymorphism
T_3 – Triiodothyronine
T_4 – Tetraiodothyronine or thyroxine
TBG – Thyroxine binding globulin
TNFα – Tumour necrosis factor α
TPO – Gene coding for thyroperoxidase
TRH – Thyrotrophin releasing hormone
TRT – Testosterone replacement therapy
TSH – Thyroid stimulating hormone or thyrotrophin
TTF-1 – Gene coding for the transcription terminator factor 1
TTF-2 – Gene coding for the transcription terminator factor 2
UDP – Uridine diphosphate
UDPGA – UDP-glucuronic acid
uE3 – Unconjugated oestriol
UK – United Kingdom
USA – United States of America
VLDL – Very low density lipoprotein
WHO – World Health Organisation
XMP – Xanthosine monophosphate

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PREFACE

Throughout life, human beings undergo several hormonal changes responsible for growth and maturation. These alterations in hormone secretion include enhanced or decreased production, the latter of which is mainly observed during ageing. These processes are intrinsic to human development but may vary from individual to individual. Thus, experienced metabolic changes can modify the state of health and even trigger the occurrence of certain pathologies.

The main metabolic differences observed in newborns and children when compared to adults result from the fact that the organism is not yet fully developed. During adolescence, changes in hormone secretion occur that lead to sexual maturation. In the same way, during pregnancy women suffer alterations in the secretion of certain hormones that allow the adaptation of their bodies to that physiological state and the normal development of the foetus. Regarding the elderly, a general decline of health is observed during ageing, and hormonal dysfunctions, such as development of insulin resistance and thyroid dysfunction, frequently occur.

This book focuses on metabolic and hormonal changes during the human lifetime. Screenings best suited for each life stage, the reasons for doing them, and the diseases they allow to diagnose are also presented.

Special thanks for the precious help of Rui Pedro Chaves in the elaboration of Figures and Tables of Chapters 1 and 2.

CHAPTER 1

BIOCHEMICAL CHARACTERISATION

What is going on in our body right now? This is something we think about. If it would be possible to see inside of a cell, an intense activity would be visible. For this reason, understanding metabolism is crucial to understand the behaviour of an organism, since metabolism is in the genesis of the health state and its disturbance can lead to diseases.

The word metabolism comes from the Greek term *metabole*, which means change, and can be described, in a very simple way, as a set of biochemical and physical processes that take place in an organism. Metabolism is divided into two groups: catabolism and anabolism. Catabolism refers to the degradation of molecules yielding simpler ones to produce energy. On the other hand, anabolism is the synthesis of new molecules from simpler ones, with the spending of energy.

Energy is indispensable for the maintenance of vital functions like respiration or heartbeat. These functions spend 70% of the available energy in the organism. This expenditure of energy corresponds to the basal metabolic rate or basal metabolism, which is affected by several factors that can slow it down or make it faster. Genetics, age, sex, lifestyle, and body composition are some of these factors.

The life cycle of human beings has suffered considerable changes over time as a result of the increase in average life expectancy. In a poor world, life expectancy was around 30 years in all regions. However, in the early 19th century, life expectancy in the industrialized countries started to increase while remaining low in the rest of the world. This led to big health divergences between rich and poor countries. Over the last decades, these differences suffered a decrease. Since the beginning of the 20th century the global average life expectancy has doubled and is now reaching 70 years of age. For instance, in the United Kingdom (UK) life expectancy before the 19th century fluctuated between 30 and 40 years. Over the last 200 years it has doubled and is now higher than 80 years. In Japan, the increase in life expectancy started later, but surpassed the UK in the late 1960s.

Demographic changes at world level are a reality and it is worth thinking about it. Therefore, a lot of studies relating age with physiological and

metabolic changes have been showing up. Concerning age, it is important to refer that as age increases metabolism slows down, with a decrease of 5% per decade after the age of 30 years.

The growth process of a human being is affected by internal and external factors. Concerning the internal level, there is a big group of reactions that occur naturally. Nevertheless, some of these reactions can be affected by environmental factors, like food diet, physical activity, and other lifestyle habits of the individual.

The biochemical and biological functions of human beings keep on changing during life due to the hormonal changes occurring in the organism. Some of these are related with specific alterations that occur in puberty or menopause, while others keep on taking place in a gradual fashion. All these changes must be considered when determining the state of health and must be in accordance with the reference values established for each age range, since some diseases are more frequent in certain stages of life. In the elderly, for instance, pathologies like osteomalacia, thyroid dysfunctions or diabetes mellitus are more common.

However, besides the normal changes occurring in the human body throughout life, standard values are used to evaluate specific blood parameters that allow to determine the absence/presence of disorders. Tables 1-1 and 1-2 list the main standard values considered normal for an adult human being, which are used in routine blood tests.

It should be emphasised that a significant variability can exist in the values of biochemical parameters assessed by different laboratories, depending on the method and assay used and on population variations. Reference ranges specific for each laboratory should be used by clinicians whenever possible.

Table 1-1. Standard biochemical parameter values for adults.

Biochemical parameters	Plasma levels
Albumin	35-50 g/L
Alkaline phosphatase	30-150 IU/L
Ammonia	5-69 µg/dL
Aspartate transaminase (AST)	10-50 IU/L
Bicarbonate (total; CO_2)	18-30 mEq/L
Bilirubin (total)	0.2-1.3 mg/dL
Calcium	8.6-10 mg/dL
Carbon dioxide (P_{CO_2} in arterial blood)	4.5-6.0 kPa (35-46 mmHg)
Cholesterol (total; fasting)	< 200 mg/dL
Conjugated bilirubin	0-0.3 mg/dL
Copper	10-15 µg/dL
Creatine kinase (total)	< 90 IU/L
Creatinine	0.6-1.2 mg/dL
α-Foetoprotein	< 10 µg/L
Glucose (fasting)	72-99 mg/dL
γ-Glutamyl transferase (γ-GT)	< 60 IU/L
Haemoglobin	Males: 13-18 g/dL Females: 12-16 g/dL
High density lipoprotein (HDL; fasting)	Males: > 45 mg/dL Females: > 65 mg/dL
Hydrogen ion (arterial blood)	35-46 nmol/L (pH 7.34-7.46)
Low density lipoprotein (LDL; fasting)	< 100 mg/dL
Magnesium	1.3-2.1 mg/dL
Osmolality	285-293 mOsm/kg
Oxygen (P_{O_2} in arterial blood)	11-15 kPa (85-105 mmHg)
Phosphate	3-4.5 mg/dL
Potassium	3.7-5.2 mEq/L
Sodium	135-145 mEq/L
Total protein	60-80 g/L
Triglyceride (fasting)	< 149 mg/dL
Urea	8-23 mg/dL
Uric acid	2-7 mg/dL
Zinc	70-125 µg/dL

Table 1-2. Standard hormonal parameter values for adults.

Hormonal parameters	Plasma levels
Adrenocorticotrophic hormone (ACTH)	9-52 pg/mL
Aldosterone	after 7 h rest: 3-9 ng/dL after 9 h standing: 4-30 ng/dL
Antidiuretic hormone (ADH)	< 2 pg/mL
Calcitonin	Males: < 8 ng/L Females: < 4 ng/L
Corticotrophin releasing hormone (CRH)	24-40 pg/mL
Cortisol (total)	at 9 am: 3-20 µg/dL at midnight: 1.5-10 µg/dL
Cortisol (free)	at 9 am: 0.6-1.6 µg/dL at midnight: 0.2-0.9 µg/dL
Dopamine	0-30 pg/mL
Epinephrine	10-200 pg/mL
Follicle stimulating hormone (FSH)	Males: 1.5-14.3 IU/L Females (follicular phase): 1.4-9.9 IU/L
Gastrin	< 42 ng/L
Glucagon	20-100 pg/mL
Insulin-like growth factor 1 (IGF-1) (25-39 years of age)	114-492 µg/L
Insulin-like growth factor 2 (IGF-2)	210-750 ng/mL
Growth hormone (GH)	1-5 ng/mL
Growth hormone attachment protein	66-306 pmol/L
Growth hormone releasing hormone (GHRH)	< 50 pg/mL
Insulin (in hypoglycaemia)	0.2-0.8 ng/mL
Luteinizing hormone (LH)	Males: 2.0-10 IU/L Females (follicular phase): 1.7-15 IU/L
Norepinephrine	80-520 pg/mL
Oestradiol	Males: 10-50 pg/mL Females (luteinic phase): 100-350 pg/mL
Parathyroid hormone (PTH)	17-73 pg/mL
Prolactin	Males: 1.6-18.8 ng/mL Females: 1.4-24.2 ng/mL
Renin (plasma renin activity)	after 8 h rest: 0.3-3 µg/L/h after 12 h standing: 0.4-8.8 µg/L/h
Secretin	in starvation: 3-15 pg/mL after meals: 30 pg/mL
Testosterone	Males: 260-1000 ng/dL Females: 15-70 ng/dL
Thyroid stimulating hormone (TSH)	0.5-4.7 µIU/mL
Thyroxine (total; T_4)	5-12 µg/dL
T_4 (free)	0.7-1.9 ng/dL

Triiodothyronine (total; T_3)	70-132 ng/dL
T_3 (free)	2.3-4.2 pg/mL
Vitamin D (1,25-dihydroxy)	15-60 ng/L
Vitamin D (25-hydroxy)	9-52 µg/L

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CHAPTER 2

NEWBORN AND CHILDHOOD

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Familial hypercholesterolaemia

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2.1. Hormonal and metabolic changes

During growth, the human body experiences diverse metabolic and biochemical changes. For instance, the rate of resting metabolism suffers a reduction of 1.5-2 times with age, from childhood to adult life. This results from the decrease in heat production as the organism gains body mass, while the ratio of metabolic rate to surface area usually remains constant.

For quite some time, it was believed that the high metabolic rate in infants resulted from metabolic expenditure on growth. However, the data did not confirm this. The most intense period of growth is during the first 6 months after birth, when the growth coefficient is 4.0. One year after birthing this coefficient reduces sharply (by more than 10 times) to 0.3. At this stage, basal metabolism rate reaches its peak and only after 3 years this rate starts to gradually decrease, reaching the level of an adult (25 kcal/kg per day) only during puberty.

Some authors relate the increase in basal metabolic rate during the first year of life of a child with a decrease in volume of intracellular space in most tissues. Moreover, the observed reduction in basal metabolism at 3 years of age is attributed to changes in body composition during growth, like the increase in tissue relative mass with a low resting metabolism rate, due to the uneven growth of organs.

Except for the first year after birth, the higher the relative growth velocity, the greater the reduction in resting metabolism rate. The inhibition of growth processes at 1.5-2 years of age is accompanied by the highest values of resting metabolism, and the period of 6-7 years of age shows an increase in growth velocity that coincides with a considerable decrease in metabolism. The same negative correlation happens in other stages of development.

Moreover, the intensity of body functions in children is much higher than in adults. The rate of children's basal metabolism is 1.5-2 times higher, but the maximum activity level is much lower when compared with adults. As a result, children experience a smaller functional range, making their body existence more stressful.

The transition from intrauterine life to independent existence involves many changes in the organism of a child. Maturation of all systems takes weeks, months or even a few years after birth until they reach complete development.

Blood gases are quite different during pregnancy, and in the outside world, therefore, the capacity of the baby to adapt to the extrauterine environment is crucial for his/her survival. All body systems suffer physiological changes after birth, but the most critical one is probably the

respiratory system. In the uterus, the foetus receives oxygen and nutrients through the placenta and excretes carbon dioxide into the mother's circulatory system. Just after birth, the newborn fills its respiratory tract and the first gas exchanges start to occur, with a decrease in lung vascular pressure to allow the increase of blood flow to the lungs.

The upper respiratory tract of a baby continues suffering changes and only reaches maturity around the age of 13 years. It is due to this immaturity that the baby's respiratory system is different from the adult's one. Moreover, the diameter of nasal cavities is also smaller, and any obstruction can lead to breathing problems, directly influencing food intake and sleep.

Renal glomeruli are completely formed at 36 weeks of gestation, however, due to the low blood flow in the kidneys, the glomerular filtration rate (GFR) at birth is low. In the last weeks of pregnancy, the GFR increases in parallel with gestation age until the 36^{th} week and, after that, goes on increasing but at a lower rate. At birth, the GFR is 20 mL/min/1,73m^2 and is measured through inulin clearance.

Tubular function is still immature, and bicarbonate and glucose reabsorptions are low, leading to low serum levels of bicarbonate. Children's capacity to concentrate urine is rather low, and they can reach as much as 600 mmol/kg of osmolarity. In newborns, kidney excretion of water and electrolytes is also limited, and the intravenous administration of liquids might be needed.

The liver has an important role in bilirubin metabolism since it is responsible for its uptake, conjugation and excretion. Newborns can have difficulty in bilirubin conjugation, leading to jaundice, which can develop in the first days of life.

The standard values for certain biochemical parameters in the newborn are different from the observed in adults and can even change through childhood. For this reason, blood analysis should always be interpreted in accordance with the age of the individual. These differences are observed in the mean and upper limit of plasma potassium levels in newborns (Table 2-1) that are higher than in adults (Table 1-1 in Chapter 1. Biochemical Characterisation). Also, plasma calcium levels are always higher at birth and reach normal adult values within 72 hours. Moreover, plasma phosphate is also higher at birth, but then declines, remaining higher (Table 2-1) than in adults during childhood. Blood phosphate levels rise at puberty and then fall to adult concentrations (Table 1-1 in Chapter 1. Biochemical Characterisation).

Alkaline phosphatase also shows changes in plasma levels during growth. The activity of this plasma enzyme is high at birth but falls rapidly, remaining 2-3 times higher (Table 2-1) than the adult level (Table 1-1). Its concentration rises again during adolescence due to bone growth (existence

of bone isoenzyme), falling to adult levels when bone growth ceases. This enzyme is secreted by osteoblasts and plays an important role in the production of hydroxyapatite required for bone formation. Alkaline phosphatase is probably involved in the release of phosphate from pyrophosphate, which will later combine with calcium ions to produce hydroxyapatite for osteoid synthesis. For this reason, bones are good reservoirs of calcium and phosphate.

Another group of proteins showing fluctuations during childhood is constituted by the immunoglobulins (Ig). At birth, immunoglobulin A (IgA) and immunoglobulin M (IgM) have low plasma levels but rise steadily thereafter. IgA may only reach adult levels at the end of the first decade of life. On the other hand, regarding immunoglobulin G (IgG), since it is transported across the placenta during the last trimester of pregnancy, its plasma levels are high at birth, except in premature babies. IgG levels start to decrease when maternal IgG is cleared from the body of the newborn. After that, it starts to be replaced by the IgG of the newborn. Hypogammaglobulinaemia observed in children is one of the reasons for their high susceptibility to infections.

Table 2-1. Standard biochemical and hormonal parameters for newborns and children.

Biochemical/hormonal parameter	Plasma levels
Alkaline phosphatase	0-6 years old: 82-350 IU/L 6-12 years old: 49-446 IU/L
Dehydroepiandrosterone (DHEA)	< 6 years old: 20-130 ng/dL 6-8 years old: 20-275 ng/dL 8-10 years old: 31-345 ng/dL
Dihydrotestosterone (DHT)	Premature babies: 2-13 ng/dL Newborns: < 2-15 ng/dL 30-60 days: < 3 ng/dL
Deoxycortisol	Premature babies: 48-579 ng/dL Newborns until 3 days: 13-147 ng/dL 1-12 months: < 156 ng/dL 1-10 years old: 20-155 ng/dL
Deoxycorticosterone	1 week to 12 months: 7-49 ng/dL 1-10 years old: 2-34 ng/dL
Dopamine	3-8 years old: 80-378 µg/24h 9-12 years old: 51-474 µg/24h
Epinephrine	3-8 years old: 1-7 µg/24h 9-12 years old: < 8 µg/24h

Estradiol	Boys: 1-5 years old: 3-10 pg/mL 6-9 years old: 3-10 pg/mL 10-11 years old: 5-10 pg/mL Girls: 1-5 years old: 5-10 pg/mL 6-9 years old: 5-60 pg/mL 10-11 years old: 5-300 pg/mL
FSH	Boys: 2 weeks: 1.2-5.2 IU/L 1-18 months old: 0.19-3.0 IU/L 19 months-10 years old: 0.25-1.9 IU/L 10-12 years old: 0.2-5.8 IU/L Girls: 2 weeks: 2.1-30.4 IU/L 1-18 months old: 1.1-14.4 IU/L 19 months-10 years old: 0.70-5.6 IU/L 10-12 years old: 0.68-7.3 IU/L
GH	< 10 ng/mL
GH attachment protein	Boys: 3-5 years old: 57-282 pmol/L 6-9 years old: 60-619 pmol/L Girls: 3-5 years old: 62-519 pmol/L 6-9 years old: 58-572 pmol/L
IGF-1	Boys: 2 months-5 years old: 17-248 µg/L 6-8 years old: 88-474 µg/L 9-11 years old: 110-565 µg/L Girls: 2 months-5 years old: 17-248 µg/L 6-8 years old: 88-474 µg/L 9-11 years old: 117-771 µg/L
IGF-2	2 months-5 years old: 300-860 ng/mL 6-9 years old: 520-1050 ng/mL 10-17 years old: 530-1140 ng/mL
Gastrin	Newborn: 69-109 ng/L Infants: 55-186 ng/L Children: 3-4 h fasting: 2-168 ng/L 5-6 h fasting: 3-117 ng/L >8 h fasting: 1-125 ng/L
17-Hydroxy-corticoids	1.1-7.5 mg/24h
17-Hydroxy-pregnenolone	Premature babies: 64-2380 ng/dL Newborns: 3 days: 10-829 ng/dL

	1-6 months: 36-763 ng/dL 6-12 months: 42-540 ng/dL Children (1-10 years old): 15-221 ng/dL
Hydroxyprogesterone	Boys: 1-5 days: 80-420 ng/dL 1-5 months: 15-135 ng/dL 6-11 months: 25-145 ng/dL 1-5 years old: 20-80 ng/dL 6-9 years old: 15-65 ng/dL 10-11 years old: 15-45 ng/dL Girls: 1-5 days: 82-400 ng/dL 1-5 months: 20-190 ng/dL 6-11 months: 25-155 ng/dL 1-5 years old: 20-50 ng/dL 6-9 years old: 20-40 ng/dL 10-11 years old: 20-70 ng/dL
LH	Boys: 2 weeks: 4.8-10.0 IU/L 1-18 months: 0.04-3.0 IU/L 19 months-7.9 years old: 0.02-1.0 IU/L 8-9.9 years old: 0.01-0.78 IU/L 10-11.9 years old: 0.03-4.4 IU/L Girls: 2 weeks: 0.29-7.9 IU/L 1-18 months: 0.02-1.8 IU/L 19 months-7.9 years old: 0.03-0.55 IU/L 8-9.9 years old: 0.02-0.24 IU/L 10-11.9 years old: 0.02-4.1 IU/L
Norepinephrine	3-8 years old: 5-41 µg/24h 9-12 years old: 5-50 µg/24h
Phosphate	4-7 mg/dL
Potassium	3.4-4.7 mEq/L
Testosterone (free)	Boys: Newborn: 3-19 ng/L 5-7 months: 0.4-4.8 ng/L 6-9 years old: 0.1-3.2 ng/L 10-11 years old: 0.6-5.7 ng/L Girls: Newborn: 2-4 ng/L 5-7 months: 0.2-0.6 ng/L 6-9 years old: 0.1-0.9 ng/L 10-11 years old: 1-5.2 ng/L

Testosterone (total)	Boys: Newborn: 17-61 ng/dL 1-5 months: 1-177 ng/dL 6-11 months: 2-7 ng/dL 1-5 years old: 2-25 ng/dL 6-9 years old: 3-30 ng/dL 10-11 years old: 5-50 ng/dL Girls: Newborn: 16-44 ng/dL 1-5 months: 1-5 ng/dL 6-11 months: 2-5 ng/dL 1-5 years old: 2-10 ng/dL 6-9 years old: 2-20 ng/dL 10-11 years old: 5-25 ng/dL
Thyroglobulin	2-16 years old: 2.3-39.6 ng/mL
T_4	1-2 days old: 11.8-23.2 µg/dL 3-9 days old: 9.9-21.9 µg/dL 10-44 days old: 8.4-16.2 µg/dL 45-89 days old: 6.4-14 µg/dL 3-11 months: 7.8-16.5 µg/dL 1-4 years old: 7.3-15 µg/dL 5-9 years old: 6.4-13.3 µg/dL 10-14 years old: 5.6-11.7 µg/dL
T_3	1-2 days old: 32-216 ng/dL 3-9 days old: 50-250 ng/dL 1-11 months: 105-280 ng/dL 1-4 years old: 105-269 ng/dL 5-9 years old: 94-241 ng/dL 10-14 years old: 83-213 ng/dL
Vitamin D (1,25-dihydroxi)	3-17 years old: 27-71 ng/L
Vitamin D (25-hydroxi)	3-17 years old: 13-67 µg/L

2.2. Screening for diseases

Children may present various conditions at birth (congenital) that can affect their health and wellness. Most of these diseases are clinically difficult to detect in the beginning of life and, during development, can lead to severe disturbances that can range from problems in processing particular nutrients (metabolic), to hormonal changes (endocrine), or to the production of abnormal forms of specific proteins like haemoglobin. Most of these conditions are rare, and some are more frequent in certain families or ethnic groups. Most of them cannot be cured, but in many cases a treatment strategy can be applied so that the child can grow and live a relatively normal life.

Screening tests in newborns are essential for the identification of congenital disorders within days of birth, before the development of symptoms. In this way, life-threatening health problems and serious intellectual disabilities can be avoided or minimised.

Several criteria are used to consider a disorder for a neonatal screening test. First, it must be a condition that is fatal or leads to severe disability if not treated. The condition must be relatively common, treatable, and a reliable (no false negatives, some false positives are acceptable), cheap and non-invasive or almost non-invase screening test must be available.

Nowadays, it is possible, through blood tests performed between the 3rd and the 6th day of life, to diagnose a group of congenital diseases even before the appearance of clinical signs and immediately start the corresponding treatment. Newborns are routinely screened for these disorders before leaving the hospital, using a few drops of blood. Sample collection is usually done through a foot prick, usually known as "foot test", or from cord blood at birth. These screenings started to be developed in the beginning of the 1960s with the detection of high levels of phenylalanine that are indicative of the disorder phenylketonuria.

Newborn screening tests are organized into different broad categories: metabolic disorders, for instance phenylketonuria; endocrine disorders, such as congenital hypothyroidism and congenital adrenal hyperplasia; haemoglobin disorders, for instance sickle cell anaemia; and other disorders, such as cystic fibrosis and severe combined immunodeficiencies.

In 2009, within the European Union (EU) Program of Community Action in Public Health of the European Commission, the action "Evaluation of population newborn screening practices for rare disorders in Member States of the EU" was launched. This project aimed the adoption of national plans and strategies for rare diseases from 2013 to establish lines for the cooperation and coordination of the Member States for a better use of national resources and expertise in this field and reduce inequalities in the accessibility to high quality care.

All the European countries have a newborn screening program except Albania. However, the adopted programs are very heterogenous, and there is no consensus concerning the number of diseases and which of these to screen for or even the methodology used in the tests. It seems that every EU country has done a selection on its own through an analysis of literature. In these countries, newborn screening programmes are usually covered by the government.

Among the EU countries, the number of diseases covered by the program varies between one and fifteen. Moldova and Armenia screen for only one disease, Malta for two, Luxemburg screens five diseases, the UK