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Evaluation of Body Composition and Nutritional Status in Patients with Inborn Errors of Metabolism

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RESUMO

Os erros innatos do metabolismo (EIM) son entidades de base xenética caracterizadas xeralmente por deficiencia de encimas que dan lugar a acúmulo de compostos tóxicos no organismo. Os nenos con EIM teñen que seguir unha dieta especial restrinxida en nutrientes esenciais, o que pode supoñer risco de alteracións nutricionais. O obxectivo deste estudo é avaliar en pacientes con EIM e controis, a composición corporal e o estado nutricional, así como a súa relación cos estilos de vida, inxesta alimentaria e actividade física.

Noventa e nove pacientes con EIM de entre 5 e 19 anos e 98 controis pareados por idade e sexo foron analizados para determinar as súas características antropométricas e composición corporal mediante DEXA. Recolléronse datos de antecedentes persoais e familiares, socioeconómicos, de inxesta alimentaria e de actividade física mediante cuestionarios validados, e se analizaron biomarcadores nutricionais en todos eles.

A z-score da talla reduciuse significativamente nos pacientes (-0,28 vs. 0.15, p = 0.008), afectando máis aos que presentan defectos do metabolismo de carbohidratos seguidos daqueles con desordenes do metabolismo dos aminoácidos. A adiposidade non presenta diferenzas significativas entre pacientes e controis (z-score do índice de masa corporal 0.56 vs. 0.42, p = 0.279). Con todo, a z-score de circunferencia de cintura é superior nos pacientes (-0,08 vs. -0.58, p = 0.005). Con respecto á mineralización ósea, os pacientes presentan unha densidade mineral ósea (DMO) significativamente menor (0.89 vs.1.6, p = 0.001), supoñendo un maior risco de osteopenia (z-score < -2: 33.3% vs. 20.4%) e de osteoporose (z- score < -2.5: 7.1% vs. 0%), sen que ningún presentase fracturas. Houbo unha correlación positiva significativa entre a inxesta de proteínas naturais e a DMO. Destaca entre os estudos analíticos os baixos niveis de selenio e altos niveis de folato, particularmente nos desórdenes do metabolismo de aminoácidos.

Os nosos resultados indican que os pacientes con EIM en tratamento dietético, especialmente aqueles con desordes do metabolismo dos aminoácidos, presentan alteracións na composición corporal, como baixa talla, unha tendencia ao sobrepeso e a obesidade e unha diminución da densidade mineral ósea.

Palabras chave: Biomarcadores nutricionais, densidade mineral ósea, inxesta dietética, risco nutricional, trastornos xenéticos

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RESUMEN

Los errores innatos del metabolismo (EIM) son entidades de base genética caracterizadas generalmente por deficiencia de enzimas que dan lugar a acumuló de compuestos tóxicos en el organismo. Los niños con EIM tienen que seguir una dieta especial restringida en nutrientes esenciales, lo que puede suponer riesgo de alteraciones nutricionales. El objetivo de este estudio es evaluar en pacientes con EIM y en controles, la composición corporal y el estado nutricional, así como su relación con los estilos de vida, ingesta alimentaria y actividad fisica.

Noventa y nueve pacientes con EIM de entre 5 y 19 años y 98 controles pareados por edad y sexo fueron analizados para determinar sus características antropométricas y composición corporal mediante DEXA. Se recogieron datos de antecedentes personales y familiares, socioeconómicos, de ingesta alimentaria y de actividad física mediante cuestionarios validados, y se analizaron biomarcadores nutricionales en todos ellos.

La z-score de la talla se redujo significativamente en los pacientes (-0,28 vs. 0.15, $p = 0.008$), afectando más a los que presentan defectos del metabolismo de carbohidratos seguidos de aquellos con desordenes del metabolismo de los aminoácidos. La adiposidad no presenta diferencias significativas entre pacientes y controles (z-score del indice de masa corporal 0.56 vs. 0.42, $p = 0.279$). Sin embargo, la z-score de circunferencia de cintura es superior en los pacientes (-0,08 vs. -0.58, $p = 0.005$). Con respecto a la mineralización ósea, los pacientes presentan una densidad mineral ósea (DMO) significativamente menor (0.89 vs. 1.6, $p = 0.001$), suponiendo un mayor riesgo de osteopenia (z-score < -2: 33.3% vs. 20.4%) y de osteoporosis (z-score < -2.5: 7.1% vs. 0%), sin que ninguno haya presentado fracturas. Hubo una correlación positiva significativa entre la ingesta de proteínas naturales y la DMO. Destaca entre los estudios analíticos los bajos niveis de selenio y altos niveis de folato, particularmente en los desordenes del metabolismo de aminoácidos.

Nuestros resultados indican que los pacientes con EIM en tratamiento dietético, especialmente aquellos con desórdenes del metabolismo de los aminoácidos, presentan alteraciónes en la composición corporal, como baja talla, una tendencia al sobrepeso y la obesidad y una disminución de la densidad mineral ósea.

Palabras clave: Biomarcadores nutricionales, densidad mineral ósea, ingesta dietética, riesgo nutricional, trastornos genéticos.

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SUMMARY

Inborn errors of metabolism (IEM) are genetically based entities generally characterized by enzyme deficiencies that result in the accumulation of toxic compounds in the body. Children with IEM must follow a special diet restricted in essential nutrients, which can put them at risk for nutritional disorders. This study's objective is to evaluate body composition and nutritional status in patients with IEM and controls, as well as their relationship to lifestyles, food intake, and physical activity.

Ninety-nine patients with IEM between the ages of 5 and 19 years and 98 controls by age and sex were analyzed to determine their anthropometric characteristics and body composition using DEXA. Data on personal and family history, socioeconomic status, food intake, and physical activity were collected through validated questionnaires, and nutritional biomarkers were analyzed in all of them.

Height z-score was significantly reduced in IEM patients (-0.28 vs. 0.15, $p = 0.008$), affecting more those with carbohydrate metabolism defects followed by those with amino acid metabolism disorders. Adiposity does not present significant differences in these patients and controls (z-score of the body mass index 0.56 vs. 0.42, $p = 0.279$). The z-score of waist circumference is higher in patients (-0.08 vs. -0.58, $p = 0.005$). With regard to bone mineralization, patients present a significantly lower bone mineral density (BMD) (0.89 vs. 1.6, $p = 0.001$), assuming a higher risk of osteopenia (z-score < -2: 33.3% vs. 20.4%) and osteoporosis (z-score < -2.5: 7.1% vs. 0%), none of which has presented fractures. There was a significant positive correlation between natural protein intake and BMD. Among the analytical studies, the low levels of selenium and high levels of folate stand out, particularly in amino acids metabolism disorders.

Our results indicate that patients with IEM in dietary treatment, especially those with disorders of amino acid metabolism, present alterations in body composition, such as low height, a tendency to overweight and obesity, and a decrease in bone mineral density.

Keywords: Bone mineral density, dietary intake, genetic disorders, nutritional biomarkers, nutritional risk.

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ABBREVIATIONS

AAF	Amino Acid Formula
AAs	Amino Acids
AD	Autosomal Dominant
AR	Autosomal Recessive
BF	Body Fat
BH4	Tetrahydrobiopterin
BIA	Bioelectric Impedance Analysis
BMD	Bone Mineral Density
BMI	Body Mass Index
BMT	Bone Marrow Transplantation
CG	Classic Galactosemia
CK	Creatinine Kinase
CPT2	Carnitine Palmitoyltransferase deficiency2
DEXA	Dual-energy X-ray absorptiometry
EAA	Essential Amino Acid
ECF	Extracellular Fluids
FAOD	Fatty Acid Oxidation Defects.
FFM	Fat Free Mass
FM	Fat Mass.
GALT	Galactose-1-phosphate uridyltransferase.
GSD	Glycogen Storage Disorders.
HELLP	Haemolysis, Elevated Liver Enzymes, Low Platelets.
HFI	Hereditary Fructose Intolerance.
IEIPM	Inborn Errors of Intermediary Protein Metabolism.
IEM	Inborn Errors of Metabolism.
LC-FAOD	Long Chain Fatty Acid Oxidation Disorders.
LCHADD	Long chain HydroxyacylCoA Dehydrogenase deficiency
LSDs	Lysosome storage disorders.
MADD	Multiple acyl-CoAdehydrogenase deficiency.
MBD	Mineral bone disease.
MCADD	Medium chain acyl-CoA dehydrogenase.

MMA	Methylmalonic Acidemia.
MPS	Mucopolysaccharidoses.
MS/MS	Tandem Mass Spectrometry
MSUD	Maple Syrup Urine Disease
NBS	Newborn Screening.
NTBC	2-(2-Nitro-4-trifluoromethylbenzoyl)-1,3 -cyclohexanedione.
PA	Propionic Acidemia.
PAH	Phenylalanine Hydroxylase.
Phe	Phenylalanine.
PKU	Phenylketonuria.
RDI	Recommended Dietary Intake.
SRT	Substrate reduction therapy.
SSIEM	Society for the Study of Inborn Errors of Metabolism.
TBW	Total Body Water.
UCDs	Urea Cycle Defects.
UNICEF	The United Nations International Children's Emergency Fund.
VLCAD	Very long chain acyl-CoA dehydrogenase deficiency.
WHO	World Health Organization.

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1. INTRODUCTION

1.1 Inborn errors of metabolism (IEM)

1.1.1 Definition

Metabolism is the regulated and coordinated set of chemical reactions that take place in a living organism; for these chemical reactions to be carried out, the correct functioning of proteins called enzymes is necessary. These enzymes can act at the level of the general metabolism or of a particular cycle, as a cellular receptor, a membrane transporter, or as part of a cellular organelle (Patel *et al*., 2011).

Inborn errors of metabolism (IEM), also known as hereditary metabolic disorders, are genetic alterations with a deficiency or abnormality of an enzyme or its cofactor that is responsible for a clinically significant point in a metabolic pathway. As a result, an abnormal build-up of a substrate or deficiency of the product is recognized. In the majority, this is due to a single gene defect that encodes a particular enzyme important in the metabolic pathway (El-Hattab, 2015). IEM are rare diseases from an individual point of view, but, as a whole, they are responsible for significant pediatric morbidity and mortality (Nassogne *et al.,* 2005; McBryde *et al.,* 2006). The European Union (EU) defines rare diseases when there is a risk of death or chronic disability and prevalence of fewer than 5 cases per 10,000 inhabitants. This translates into an estimate of 29 million affected in the EU and 3 million in Spain (Palau, 2009).

The first studies on IEM were in 1908 when Archivald E. Garrod, a professor of Medicine at Oxford, coined this term and described four inherited metabolic diseases (cystinuria, alkaptonuria, pentosuria and albinism) (Galton, 2008). In 1934, Ivar Asbjorn Folling described the phenylketonuria (PKU) correlated with mental retardation. Bickel in 1953 published the results of dietary therapy in PKU (Bickel, 1953).

Later appear the test of the wet diaper of Centerwall in 1957 and the test of Guthrie in 1963. Thus, at present, the early diagnosis and treatment of metabolic diseases allow us to predict in most cases, a substantial reduction in morbidity and an improvement in the chronic conditions.

1.1.2 Epidemiology

The incidence and prevalence of IEM are varying among countries. The frequency of IEM is increased in the countries or regions where the rate of consanguineous marriages is high. Up to now, more than 1200 IEM have been detected, examples of some IEM and their enzyme defects are listed in Table 1.

The incidence of overall IEM is high and varies dramatically in different countries and regions (Campeau *et al.,* 2008). For example, the incidence of IEM was reported 1/667 in Saudi Arabia. (Moammar *et al.,* 2010), 1/784 in United Kingdom (Sanderson *et al.,* 2006), 1:2,500 in Canada (Applegarth *et al.,* 2000), 1:2,900 in Germany (Lindner *et al.,* 2011), 1:1,944 in Egypt (Hassan *et al.,* 2016), 1:2,916 in Malaysia (Yunus *et al.,* 2016), 1: 2,800 in South Korea (Yoon *et al.,* 2005), and 1:3,165 in Singapore (Lim *et al.,* 2014). Newborn Screening (NBS) programs in the United State revealed an incidence of 1:67,766 for biotinidase deficiency, 1:53,554 for classic galactosemia, 1:197,714 for maple syrup urine disease (MSUD), and 1:23,080 for phenylketonuria (PKU) (Therrell Jr *et al*., 2014). In Turkey reported incidence of PKU by NBS was 1:5,049 (Tezel *et al*., 2014), and in Spanish population is 1: 9,201 (Marín Soria *et al*., 2011). In Galicia, the NBS program applied in 99.9% of all newborns helps in the early diagnosis of many IEM.The most detected disorder is hyperphenylalaninaemias (benign HPA, 1:6,005; PKU, 1:12,363), followed by medium chain acyl-CoA dehydrogenase deficiency (MCADD), cystinuria and galactosemia with (1:19,106 in each case).Methionine S-adenosyltransferase (MAT I/III) deficiency with a prevalence of (1:26,271 newborns). The most frequently diagnosed organic aciduria was glutaric aciduria type 1 (GA-1) with 1:35,027. Urea cycle disorders (UCD) had prevalence of 1:210,165 (Couce *et al*., 2011).

Table 1: Examples of classified inborn errors of metabolism and their defect enzymes.

Disorder	Enzyme Defect
Amino Acid Metabolism Disorders	
Phenylketonuria	Phenylalanine hydroxylase
Maple syrup urine disease	Branched chain α-keto acid dehydrogenase complex
Tyrosinemia type I	Fumarylacetoacetate hydrolase
Tyrosinemia type II	Tyrosine aminotransferase
Homocystinuria	Cystathionine β –synthase
Organic Aciduria	
Methylmalonica cidemia (MMA)	Methylmalonyl-CoA mutase
Propionic aciduria (PA)	Propionyl-CoA carboxylase
Isovaleric aciduria	Isovaleryl-CoA dehydrogenase
Glutaric aciduria type I	Glutaryl-CoA dehydrogenase
Urea Cycle Defect (UCD)	
Ornithine transcarbamylase deficiency	Ornithine transcarbamylase
Carbohydrate Metabolism Disorders	
Classic galactosemia	Galactose-1-phosphate uridyl transferase
GSD Type I (Von Gierke's disease)	Glucose-6-phosphatase
GSD Type II (Pompe's disease)	Acid α –glucosidase
GSD Type V (McArdle disease)	Muscle glycogen phosphorylase
Hereditary fructose intolerance	Aldolase B
Fatty Acid Oxidation Disorders	
MCAD deficiency	Medium chain acyl coenzyme A dehydrogenase (MCAD)
SCAD deficiency	Short chain acyl-CoA dehydrogenase (SCAD)
LCHAD deficiency	Long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD)
VLCAD deficiency	Very long chain acyl-CoA dehydrogenase (VLCAD)
CPT-I deficiency	Carnitine palmitoyl transferase type I (CPT-I)
CPT-II deficiency	Carnitine palmitoyl transferase type II (CPT-II)
CACT deficiency	Carnitine acylcarnitine translocase (CACT)
Mitochondrial Disorders	
Kearn Sayre syndrome (KSS)	Mutation of mtDNA
Leigh syndrome	Mutation of mtDNA
Peroxisomal Disorders	
Zellweger syndrome	Peroxisome membrane protein
Lysosomal Storage Disorders	
Hunter syndrome	Iduronate 2-sulphatase deficiency
Hurler syndrome	α -iduronidase deficiency
Gaucher's disease	Glucocerebrosidase deficiency
Tay-Sachs disease	Hexosaminidase A deficiency
Fabry's disease	α -galactosidase deficiency
Niemann Pick disease Type A and B	Sphingomyelinase deficiency
Purine or Pyrimidine Metabolic Disorders	
Lesch-Nyhan syndrome	Hypoxanthine guanine phosphoribosyl transferase
Orotic aciduria type I	Uridine monophosphate synthase deficiency

1.1.3 Inheritance and pathophysiology

Most IEM are monogenic disease. In 95% of cases, the inheritance pattern is autosomal recessive (AR) when both parents are carriers of the gene, in this case they have a 25% chance of transmitting it to their offspring, this probability is repeated in each pregnancy (Alfadhel *et al.*, 2013).

Some IEMs are transmitted as X-linked alleles, and therefore have higher prevalence rates in males. A few of IEM are inherited in an autosoma dominant (AD) manner (Ezgu, 2016; Wertheim-Tysarowska *et al.*,2015).The pathophysiology behind most IEM disorders is a defect in a specific enzyme that results in incomplete conversion of substrates into their direct products. That defect leads to the accumulation of substances, which induce toxic effects and abnormal alternative substrate metabolism, in addition to reduced downstream essential products (Vernon, 2015). Figure1.

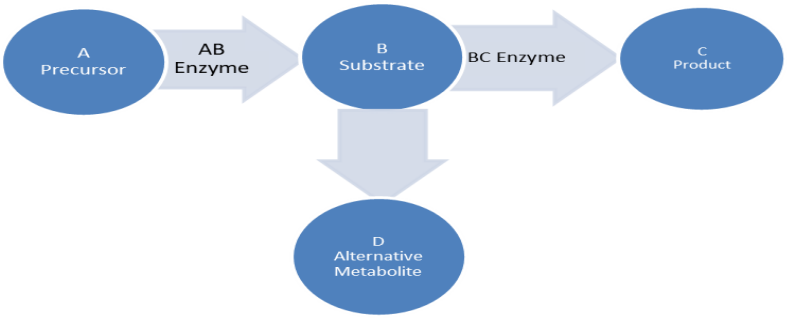


Figure1: Mechanism of IEM. Enzyme AB converts metabolite A to product B.A defect in enzyme BC leads to an accumulation of metabolite B that may cause an activation of the pathway BD. This causes an abnormal concentration of metabolite D and a deficiency in the product C. Alterations in these compounds are called the metabolic signature of the "ABCD disease" which can be used for diagnosis if all compounds are detected.

The classification of IEM is a challenge. Due to its high genetic and clinical heterogeneity, IEM include a series of diseases that are difficult to classify in many cases. However, from a practical point of view, it is useful to consider a classification according to symptoms onset time and clinical manifestations presentation.

There are different ways for classification of IEM; based on pathophysiology, IEM are divided into three sub groups (Fernandes *et al.*, 2006). However, more complex classification systems have recently been proposed. A very useful classification from the pathophysiological point of view is that which divides metabolic disorders into three large groups (Fredrickson *et al*.1995).

– Group I: Intoxication causing disorders.

It is corresponding to intermediate metabolism disorders that lead to either acute (vomiting, coma, liver failure) or chronic intoxication (developmental delay, cardiomyopathy, failure to thrive). Belonging to this group: amino acids (AAs) catabolism disorders (PKU, MSUD, homocystinuria, tyrosinemia type 1), AAs synthesis errors (serine, glutamine, and proline/ornithin metal intoxication's (Wilson, Menkes), and carbohydrate intolerance; also included the synthesis and catabolism of neurotransmitters congenital errors (mono-amines, and glycine) and organic acidurias (propionic aciduria (PA)), MMA and isovaleric aciduria). This group of disorders has a variable period free of symptoms. Diagnosis of such disorders based on the determination and quantification of AAs, organic acids, and acylcarnitine in plasma and urine. Most of them are treatable and require urgent elimination of toxin through special diets, or chelating drugs (carnitine, sodium benzoate, sodium phenyl butyrate).

– Group II: Disorders involving energy metabolism.

They are usually related to energy production or utilization deficiency in the liver, muscle, myocardium, brain, or other tissues. These disorders can be subdivided into mitochondrial and cytoplasmic energy deficiencies.

Mitochondrial defects lead to Krebs cycle and mitochondrial respiratory chain disorders are the most severe and untreatable disorders. Cytoplasmic disorders are less severe and are treatable such as defects in glycolysis, glycogen metabolism, gluconeogenesis and hyperinsulinism. Symptoms include hypoglycemia, hyperlactatemia, severe hypotonia, hepatomegaly, cardiomyopathy, failure to thrive, myopathy, sudden death, and impaired brain.

– Group III: Disorders involving complex molecules.

The lysosomal and peroxisomal diseases, congenital disorders of glycosylation (CDG), and cholesterol synthesis errors are mainly included. This group of disorders usually presents a chronic route, slowly progressive and without acute metabolic crises. Lysosomal disorders (LSD) are marked by accumulation of lysosomal pathways substrate inside the lysosome. Peroxisomal disorders are characterized by enzymatic defects in peroxisomal pathways leading to metabolic changes, especially lipids.

According to Sedel classification, these metabolic disorders can be divided into five groups (Sedel, 2013):

- Energy metabolism defects: include disorders of respiratory chain, pyruvate dehydrogenase, GLUT1, fatty acid β-oxidation (FAO), and key cofactors such as electron transfer flavoprotein, thiamine, biotin, riboflavin, vitamin E and coenzyme Q10.

- Intoxication syndromes: these include porphyria's, UCD, organic acidurias and AAs disorders.

- Lipid metabolism disorders: the LSD (Krabbe disease, metachromatic leukodystrophy, Niemann-Pick disease type C, Fabry disease and Gaucher's disease), peroxisomal disorders (adrenoleukodystrophy, peroxiso biogenesis), Tangier disease and cerebrotendinous xanthomatosis.

- Metal storage disorders: include Wilson's disease, neuroferritinopathy, *PLA2G6* mutations and a recently identified disorder of manganese metabolism. The hallmark of these diseases is the metal deposits that occur in the basal ganglia and that are visible on brain MRI.

- Neurotransmitter metabolism disorders**:** mostly represented by defects in the synthesis of serotonin, dopamine and dopamine.

A recent proposal of IEM classification combines elements from a clinical diagnostic perspective and a pathophysiological approach (Saudubray *et al*., 2018):

Category 1 includes disorders that either involve only one functional system (such as the endocrine system, immune system, or coagulation factors) or affect only 1 organ or anatomic system (such as the intestine, renal tubules, erythrocytes, or connective tissue). Presenting symptoms are uniform (eg, bleeding tendency in coagulation factor defects or haemolytic anaemia in defects of glycolysis), and the correct diagnosis is usually easy to predict.

Category 2 includes diseases in which the basic biochemical lesion either affects 1 metabolic pathway common to a large number of cells or organs or is restricted to one organ but gives rise to humoral and systemic consequences (eg, hyperammonemia in UCD, hypoglycemia in hepatic glycogenesis). The diseases in this category have a great

diversity of presenting symptoms but can be divided into 3 diagnostically useful groups:

– Group 1: Disorders of Intermediary Metabolism affecting Small Molecules

IEM in this group have plasma and/or urine metabolic marker (i.e., small diffusible water-soluble molecules) that can be easily and rapidly measured. These markers are also useful for therapy monitoring since most of these IEM are responsive to treatment. This group includes IEM that lead to acute or progressive intoxication from the accumulation of normal or unusual compounds proximal to the metabolic block. They encompass classical inborn errors of amino acid catabolism (PKU, MSUD, homocystinuria, tyrosinemia type 1), most organic acidurias (MMA, PA, isovaleric, biotin responsive multiple carboxylase deficiency (MCD), galactose (classic galactosemia), and fructose (hereditary fructose intolerance (HFI)) metabolism defects.

Metal disorders can behave in two different ways, like intoxication in cases of accumulation such as in (Wilson disease, hemochromatosis, neuroferritinopathies) and as neurodegeneration with brain iron accumulation syndromes

In case of deficiency of small molecules symptoms result primarily from the defective synthesis of compounds that are distal to the block or from the defective transport of an essential molecule through cellular or organelle membranes. Clinical signs are, at least in theory, treatable by providing the missing compound. Most of these defects affect neurodevelopment, have a congenital presentation (antenatal), and may present with birth defects.

Inborn errors of neurotransmitter and brain amino acid synthesis are also included in this group, because they share many biochemical characteristics: their diagnosis relies on plasma, urine, and cerebrospinal fluid investigations (amino acid, organic acid analyses, etc); and some are amenable to treatment even when the disorder is present in utero.

– Group 2: Disorders involving primarily energy metabolism

Consist of IEM with symptoms due to a deficiency in energy production or utilization within the liver, myocardium, muscle, brain, and other tissues. Diagnosis can be done by functional tests measuring glucose, lactate, ketones, and other energetic molecules (AA and acylcarnitines) in blood and urine, confirmed by enzyme assays and molecular testings.

Membrane carriers of energetic molecules (glucose, lactate, and pyruvate) are the most important molecules involved in energetic carrier defects. These disorders are treatable or partially treatable.

Cytoplasmic energy defects are generally less severe. They include glycogen metabolism, gluconeogenesis, hyperinsulinism, which are all treatable, creatine metabolism disorders, which are partially treatable, and pentose phosphate pathways, untreatable with a phenotype mostly linked to defective NADP/NADPH production.

Mitochondrial defects are the most severe and are generally untreatable. They encompass aerobic glucose oxidation defects with congenital lactic acidemia (pyruvate transporter, pyruvate carboxylase, pyruvate dehydrogenase system, and Krebs cycle defects), mitochondrial respiratory-chain disorders, mitochondrial transporters of energetic and other indispensable molecules, coenzyme Q biosynthesis, FAO, and ketone body defects. Mitochondrial diseases are clinically diverse and can present at any age. They can manifest in a specific tissue or multisystem manner, most often organs with the highest energy demands such as brain, skeletal muscle, eyes, and heart.

- Group 3: Disorders Involving Complex Molecules

Include diseases that interrupt the synthesis, processing, and catabolism of complex molecules that take place in mitochondria, lysosomes, peroxisomes, and Golgi apparatus. In this group, clinical symptoms are permanent, very often progressive, and independent of intercurrent events, unrelated to food intake.

This group includesLSD, peroxisomal disorders, CDG syndrome, inborn errors of purine and pyrimidine, inborn errors of cholesterol and bile acid synthesis, inborn errors of intracellular triglycerides, phospholipids and glycosphingolipids synthesis and remodelling.

It also includes glycogen storage depletion, Phospholipids (PL), glycosphingolipids (GSL). Mainly related to the synthesis and recycling of these molecules, which take place in organelles. They may interfere with fetal development. Most present as neurodevelopmental or neurodegenerative disorders unrelated to food intake.

Inborn errors of cholesterol and bile acid synthesis present either with multiple malformation syndromes, neonatal cholestasis, or with late onset neurodegenerative disorders.

Many other defects affecting systems involved in intracellular vesiculation trafficking, and processing of complex molecules can be anticipated. For example, the CEDNIK neurocutaneous syndrome owing to mutation of SNAP29 implicated in intracellular vesiculation, as well as by mutations in AP5Z1, the cellular phenotype of which bears striking resemblance to features described in a number of lysosomal storage disorders. Due to the great diversity of IEM and their low individual prevalence, determine that both the diagnosis of suspicion and the biochemical-molecular analysis for diagnostic confirmation and treatment are complex.

1.1.4 Clinical Presentation of IEM

IEM may present at various ages in different ways. Clinical presentation of the disease can occur even before birth, at birth, or during the first days of life as deterioration after normal birth and delivery (Saudubray *et al*., 1999) or in later stages.

Errors in fetal metabolism may be correlated with developing maternal complications most frequent during pregnancy, such as HELLP syndrome (hemolysis, elevated liver enzymes, low platelets), and the fatty liver (Wilcken *et al*., 2003). At birth, IEM can manifest as

perinatal asphyxia, or later as nonspecific chronic manifestations such as delays in childhood development. Acute metabolic decompensation in the neonatal age may also present as acidosis, or hyperammonemia (Waters *et al*., 2018). Most IEM babies born at term seem to be well but then worsen quickly, at the beginning of food intake. Also, in moderate forms, they can manifest symptoms in late childhood, adolescence or even in adulthood.

Symptoms usually appear due to changes in catabolism, resulting in an accumulation of metabolites that cause toxic signs. The rate of deterioration is variable according to disease type, the extent of the disease, and what is the affected organ. For example, symptoms and signs of debut may be neurological, unexplained hypoglycemia, cardiomyopathy, hepatic failure, or sudden death. Other diseases have more complex presentations, such as odor that is not generally detected (Leonard and Morris, 2000). In general, IEM can affect more than one system or organ, or they have a localized effect (Colonetti *et al*., 2018).

IEM are responsible for a significant part of childhood disability and deaths (Ferreira *et al*., 2019). The United Nations International Children's Emergency Fund (UNICEF), in the statistics of mortality of children mentioned that 9% corresponds to congenital anomalies[1].

Mainly the signs and symptoms resulting from IEM can be divided into the early-onset and late-onset forms (Vassili and Tien, 2013).

1.1.4.1 **Early-Onset Signs and Symptoms**

Disorders such as non-ketotic hyperglycinemia, multiple deficiency of Acyl-CoA dehydrogenase (MADD), cobalamin defects, and sometimes UCDs are known to begin during the prenatal period (Illsinger and Das, 2010; Mouchegh *et al*., 2007).

[1] https://www.unicef.org/media/47626/file/UN-IGME-Child-Mortality-Rep

The neonate has a limited repertoire of responses to serious diseases and usually manifests itself with nonspecific symptoms that could be easily attributed to infection or some other common cause. However, there are more common forms of presentation alert symptomatology that help us to guide the diagnosis (Couce *et al.*, 2008; Raghuveer *et al.*, 2006; Burton, 1998). Figure 2.

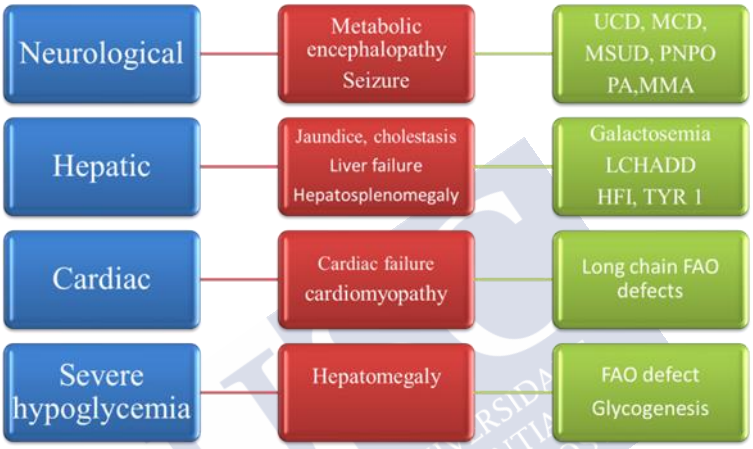


Figure 2: Early onset (Neonatal Period to Infancy) signs and symptom.

1.1.4.1.1 *Neurological distress*

Most of IEM that result in intoxication or energy deficiency is brought to medical attention because of neurological deterioration. In the case of intoxication, the initial symptom free interval varies in duration depending on the condition.

Metabolic encephalopathy: present in a newborn after a few hours or healthy days begins without apparent cause with digestive and neurological symptoms, presenting weak suction, vomiting, lethargy and quickly enters a coma, with changes in muscle tone and involuntary movements (movements of boxing and pedaling in MSUD), and dehydration. There may be abnormal smell of urine and skin. Tests

routinely performed on sick infants, such as chest x-ray, cerebrospinal fluid examination, bacteriological studies, brain ultrasound are usually normal. The unexpected and the "mysterious" deterioration after a normal initial period is the most important indicator. It can manifest the disorders of protein metabolism (UCDs, organic acidurias, leucinosis).

Seizures: are the most distinctive sign of neurological disease in the newborn period and can be caused by a broad range of systemic and central nervous system disorders. Among the metabolic defects, several treatable metabolic disorders can present in the neonatal period or early in infancy predominantly with 'intractable' seizures: pyridoxine responsive seizures, pyridoxine-5'-phosphate oxidase deficiency (responsive to pyridoxal phosphate but not to pyridoxine), 3-phosphoglycerate dehydrogenase deficiency and other inborn error of serine synthesis (Hart *et al*., 2007) (responsive to serine supplementation) and persistent hyperinsulinaemic hypoglycaemia. Folinic acid responsive epilepsy is probably not a true entity, but rather corresponds to undiagnosed B6- responsive seizures (Gallagher *et al*., 2009). Biotin responsive holocarboxylase synthetase deficiency can also, albeit rarely, present predominantly with neonatal seizures. GLUT1 deficiency (brain glucose transporter), which can be treated with hyperketotic diet, and biotinidase deficiency can also present in the first months of life as epileptic encephalopathies. Biotin sensitive holocarboxylase synthetase deficiency occurs very rarely with neonatal seizures. There are other recently described disorders that have severe early seizures in which a possible treatment has been suggested (Van Hove and Lohr, 2011; Parisi *et al*., 2015).

Neurological distress with lactic acidosis: infants with lactic acidosis present a diagnostic problem. A high plasma lactate can be secondary to hypoxia, cardiac disease, infection, or convulsions, whereas primary lactic acidosis may be caused by disorders of pyruvate metabolism and respiratory chain defects. Some IEM (FAO, organic acidaemias, and UCD) may also be associated with a secondary lactic acidosis (Poggi-Travert *et al*., 1996). In a neonate who was not asphyxiated and who has no evidence of other organ failure, the persistent increase of plasma lactate above 3 mmol/L should lead to

further investigations for an IEM. The most likely causes are abnormalities of pyruvate metabolism (pyruvate carboxylase or pyruvate dehydrogenase deficiency) or a respiratory chain deficit. The presence of cerebral pseudo cysts or myelination abnormalities may suggest a pyruvate carboxylase deficiency. It may also be due to the multiple carboxylase deficiency.

Hypo and hypertonia: hypotonia are more generally observed in severe non-metabolic neuromuscular disorders. Metabolic hypotonias are observed in congenital hyperlactacidemias, respiratory chain disorders, UCD, non-ketotic hyperglycinemia, sulphite oxidase deficiency, peroxisomal disorders, Lowe syndrome and tri-functional protein deficiency. Prader-Willi syndrome, one of the most frequent causes of neonatal hypotonia, can simulate hypotonia-cystinuria syndrome. Serious forms of Pompe disease can mimic respiratory chain disorders or tri-functional enzyme deficiency when generalized hypotonia is associated with cardiomyopathy, but it does not start strictly many times in the neonatal period (Saudubray, 2012).

Neonatal hypertonia is very common in sulphite oxidase deficiency and in hyperplexia due to abnormal glycerinergic transmission (mutations in receptors and transporters). Severe Ponto cerebellar hypoplasia's, neonatal forms of Krabbe and gangliosidosis can also produce great hypertonia with signs of hyper excitability.

1.1.4.1.2 *Hepatic and gastrointestinal presentation*

The involvement distinguished in 6 predominant patterns:

- Hepatocellular insufficiency: liver failure with vomiting, jaundice, coagulation disorder, may be due to classic galactosemia, fructosemia (in rare cases if sucrose is administered). Another cause is tyrosinemia type 1, this usually manifests after the 3rd week of life. Transaldolase deficiency and respiratory chain disorders are also possible (Kelly *et al*., 1993). Severe fetal growth delay, lactic

acidosis, hyperaminoaciduria, very high concentrations of ferritin, hepatic hemosiderosis and early death alert to Gracile syndrome (Visapä *et al*., 2002).

- Hepatomegaly with hypoglycemia and seizures: this sign give alert to gluconeogenesis defects; type I or III glycogenesis. Severe hyperinsulinism may present with moderate hepatomegaly.

- Cholestasis: genetic disorders are an important cause of neonatal cholestasis, and α-1antitrypsin deficiency accounts for a significant portion of these cases. Prolonged neonatal cholestasis jaundice associated with progressive hepatosplenomegaly is the most common sign in Niemann-Pick disease type C. Spontaneously resolves by 2–4 months of age in most patients, it may lead to liver failure in about 10 % of cases (Brunetti-Pierri *et al*., 2012).

- Hepatosplenomegaly: in the neonatal period is rare but can be seen with signs of storage (coarse faces, macroglossia, fetal hydrops, ascites, oedema, multiple dysostosis, and vacuolated lymphocytes) in lysosomal disorders (GM1 gangliosidosis, sialidosis type II, Niemann-Pick A, MPS type VII, galactosialidosis), and congenital erythropoietic porphyria (Schwarz *et al*., 2004).

- Hepatic steatosis: hepatic presentations of inherited FAO disorders and UCD consist in acute steatosis or Reye syndrome with normal bilirubin rather than true liver failure. Long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency is an exception, which may present early in infancy (but not strictly in the neonatal period) as cholestasis jaundice, liver failure and hepatic fibrosis (Saudubray, 2012).

- Congenital diarrhea disorders: can be caused by mutations in genes related to disacaridase deficiency, like defect of transport of ions or nutrients by mutations in SLC26A3 causing congenital hydrochloride, pancreatic insufficiency. Affected newborns have vomiting, colic pain and non-bloody watery diarrhea, protein losing enteropathy, hypoalbuminemia, and hyperlipidaemia.

1.1.4.1.3 *Cardiac involvement*

Some disorders can present predominantly with cardiac disease. Cardiac failure and a dilated hypertrophic cardiomyopathy (pure dilated cardiomyopathy is infrequent), most often correlated with hypotonia, muscle weakness and failure to thrive, suggests FAO, respiratory chain disorders (with severe lactic acidosis), Pompe disease or fatal congenital heart glycogenesis due to mutations in the gene that makes an enzyme called acid α-glucosidase (GAA), (Burwinkel *et al.*, 2005).

1.1.4.1.4 *Antenatal manifestations: malformations, fetal hydrops.*

Antenatal manifestations can be classified into three main clinical categories (Van Spronsen *et al.,* 2005):

- True major malformations: such as skeletal malformations, congenital heart disease, visceral aplasia's, and neural tube defects.

- Dysplasia's, for example: cortical heterotopias, cortical cysts, polycystickidneys, and liver cysts.

- Functional manifestations (intrauterine growth retardation, hydrops fetalis, hepatosplenomegaly and microcephaly).

According to this classification, irreversible major malformations are only observed in O-glycosylation disorders or secondary to *SLC39A8* mutations in the manganese transporter; in defects of cholesterol synthesis; in disorders of amino acid synthesis, such as glutamine and asparagine synthetase deficiency (lissencephaly), and rarely in severe energy defects such as MADD; some respiratory chain disorders; and in the defect of mitochondrial thiamine pyrophosphate carrier *SLC25A19* responsible for Amish lethal microcephaly.

The defect of the ω−3 fatty acid transporter (MFSD2A) causes significant abnormalities in brain convolutions and premature death. The vast majority of "true intoxication" disorders (amino acids and organic acid catabolism disorders) do not interfere with embryo-fetal development. Highlight of cholesterol biosynthesis defect, S. Smith-Lemli-Opitz, in which there is a deficiency of 7-dehydrocholesterol reductase, and manifests with microcephaly, cleft palate, cardiac malformations, polydactyly, genitourinary abnormalities and in 2/3 of the cases.

Also, in the disorders of the peroxisome biogenesis, rhizomelic chondrodysplasia punctuates with facial malformations and rhizomelic limb shortening, as well as alterations at the respiratory, ocular, skeletal, and physical and mental development levels. Zellweger syndrome or cerebro-hepato-renal syndrome with deep hypotonia, neonatal seizures, difficulty feeding, delayed psychomotor development and usually presents with malformations in the face and skull, liver disorders, with hepatomegaly and hepatic fibrosis, and renal cysts (Saudubray, 2012).

1.1.4.2 **Late-Onset Signs and Symptoms**

Those with higher residual enzyme activity generally present later in life. The presentation may be seen in childhood, adolescence, or even during adulthood. The clinical representation is usually made up of recurrent attacks during the chronic progressive course (Saudubray *et al.*, 2006; Barends *et al.*, 2014).

Initiation of the attacks may be triggered by fever, excessive intake of the substrate by the diet, fasting and accompanying medications (Vassili and Tien, 2013; Hynynen *et al.*, 2014). The clinical signs and symptoms may include neurologic signs (macrocephaly, microcephaly, attention deficit, mental and motor retardation, hypo/ hypertonia, spasticity, seizures, movement disorders, ataxia, coma, and psychiatric signs), muscular pain, organomegaly (hepatomegaly, splenomegaly), hematologic abnormalities (thrombocytopenia), dysmorphic features (coarse facial features), skeletal abnormalities, liver failure, heart failure (cardiomyopathy), ophthalmologic findings, failure to thrive, sensory problems, and skin lesions (Vassili and Tien, 2013; Sirrs *et al.*, 2013).

1.1.4.3 **General clinical symptoms in intermediary metabolism groups**

1. General Clinical of Amino Acid Metabolism Disorders.

Amino acidopathies are caused by a genetic defect in the metabolic pathways of AAs. Symptoms generally result from the accumulation of toxic substances. The initial signs or symptoms usually present during the newborn period or early infancy, several days after feeding, and protein exposure. The clinical course may progress to encephalopathy, coma, or death if not recognized and treated immediately in disorders such as MSUD. In diseases such as PKU, the clinical course is later and results in mental and motor retardation if left untreated (Leonard and Morris, 2000; Tezel *et al.*, 2014).

2. General Clinical of Organic Acidemias Disorders.

Classic organic acidemias are characterized by the build-up of abnormal and toxic organic acids resulting from the genetic enzyme defects in the degradation of the branched-chain amino acids. They also manifest clinically during the newborn period or early infancy.

Following an initial healthy period, the children develop metabolic decompensating with poor feeding, vomiting, and lethargy, which are usually life-threatening. The clinical picture may proceed to death if not recognized and treated immediately. In older children, developmental delay or regression is generally present. Cytopenia can also be found in some patients resulting from bone marrow suppression (Raghuveer *et al*., 2006).

3. General clinical of Urea Cycle Disorders.

The deficiency of any of the enzymes in the urea cycle results in impaired excretion of urea. UCDs present with severe hyperammonemia encephalopathy after several days of feeding in newborn. In patients who have residual enzyme activity, a later-onset clinical picture is seen (Leonard and Morris, 2000).

4. General clinical of Carbohydrate Metabolism Disorders.

The most common disorders of carbohydrate metabolism disorders are classic galactosemia, HFI, and glycogen storage disorders. They can lead to hypoglycemia, liver dysfunction, and myopathy, depending on the disorder. Refusal fruits are a characteristic feature of HFI. Cataracts and early-onset sepsis are commonly seen in classic galactosemia (Martins, 1999).

5. General clinical of FAOs.

These disorders include defects in the metabolism of short, medium, or long chain fatty acids. Hypoglycemia with encephalopathy is the most common finding in patients with FAOs, especially during fasting. Hepatomegaly with elevated liver function tests can also be observed. Hyperammonemia and metabolic acidosis also may occur. The defects in ketogenesis generally present with hypoglycemia,

acidosis, and hepatomegaly. The defects of ketolysis on the other hand present with recurrent attacks of ketoacidosis (Saudubray *et al.,* 2006; Martins, 1999).

6. General clinical of Mitochondrial Disorder.

Mitochondrial disorders are characterized by a defect in ATP synthesis, mainly producing from the Krebs cycle or electron transport chain. The result is the involvement of single or multiple organs, which require a significant amount of energy for functioning such as skeletal muscle, brain, heart, kidney, and liver. The clinical signs and symptoms commonly consist of myopathy, feeding difficulty, vomiting, cardiomyopathy, liver failure, seizures, central nervous system abnormalities, developmental delay, retinopathy, blindness, deafness, focal neurologic findings, or renal Fanconi syndrome (Kisler *et al*., 2010).

7. General clinical of LSD.

LSD result from the impaired lysosomal degradation of natural substrates that leads to the build-up of glycosaminoglycans, glycoproteins, or glycolipids in tissues and organs. The clinical features in general consist of progressive hepatomegaly, splenomegaly, neurologic regression, coarse facial features, reduced mobility of joints, and peripheral neuropathy depending on the type of the LSD (Saudubray *et al*., 2006).

8. General clinical of Peroxisomal Disorders.

Dysmorphic features, in addition to neurologic and liver involvement form the clinical picture of peroxisomal disorders. In adrenoleukodystrophy, adrenal glands are also affected (Saudubray *et al*., 2006).

9. General clinical of Purine and Pyrimidine Metabolism Disorders.

Disorders of purine and pyrimidine metabolism can result in an array of clinical manifestations including neurologic manifestations. Multiple systems are affected by disorders of purine and pyrimidine metabolism. The defects in the metabolism of purines and pyrimidine's may include renal calculi, neurologic dysfunction, mental and motor retardation, self-mutilation, haemolytic anaemia, and immune deficiency (Martins, 1999).

10. General clinical of Porphyrias.

The porphyrias are inherited defects of the haem biosynthetic pathway. As a result, body porphyrins or porphyrin precursors accumulate in tissues. The nervous system and/or the skin are the most affected systems (Martins, 1999).

11. General clinical of CDG.

CDGs result from the inherited deficiency of the enzymes required for the post-translational glycosylation of proteins. CDGs are multisystem disorders with a wide range of clinical signs and symptoms, depending on the type of CDG. Manifestations include central nervous system involvement, developmental delay, failure to thrive, hypoglycemia, abnormal distribution of subcutaneous fat, liver function abnormalities, protein-losing enteropathy, and short stature (Leonard and Morris, 2000; Raghuveer *et al*., 2006).

1.1.5 Diagnosis

The clinical characteristics of IEM are overlapping, therefore, clinical strategies for diagnosing IEM are challenging as manifesting symptoms in infants can have a wide variety of possible causes.

1.1.5.1 Family history

Investigating family history is of the utmost importance since IEM are generally of autosomal recessive inheritance. Also, it will investigate unexplained deaths, especially in siblings and firstborn. It has been shown that for example, mothers with a fetus affected by a defect in the oxidation of fatty acids, mainly LCHADD or carnitine palmitoyltransferase (CPT2) deficiency, run more risk of developing acute fatty liver and HELLP syndrome, so inquiring about obstetric history is of the utmost importance (Couce *et al*., 2008).

1.1.5.2 Biochemical tests

Diagnosis of IEM based on biochemical tests are divided into two paths:

►Screening tests to detect possible abnormal levels of metabolic biomarkers in blood and/ or urine before the disease manifests.

►Tests to identify specific biomarkers.

Although biochemical genetic and molecular genetic tests are required to confirm a diagnosis, basic laboratory tests are still important and often provide the first clues to a possible underlying IEM.

Table 2 showed routine laboratory tests that ordered when an IEM is suspected, results of the basic analysis tests can help indicate the underlying pathophysiology and narrow the focus of additional testing to identify a metabolic disorder or category of disorders. Further complementary biochemical or molecular tests may be required to confirm the initial findings, or to make a definitive diagnosis.

Biochemical diagnosis procedures depend on the identification of abnormally high levels of the principal substrates or of by-products that arise from alternative pathways of the enzymatic blockage. These compounds can be identified together with lower levels of the product

of that enzyme or any of its metabolites (Cleary and Green, 2005; Vernon, 2015). Metabolic investigations are the primary knowledge about diagnosis and are necessary for adequate treatment monitoring that could be therapeutic, prevent disease progression, or limit disability (van Karnebeek *et al*., 2014; van Karnebeek and Stockler, 2012).

Table 2: Laboratory studies in acute phase when IEM suspected

<table>
<tr><th colspan="2">Basic Analysis</th><th colspan="2">Biochemical Diagnosis</th></tr>
<tr><td>Blood</td><td>- Hemogram
- Blod gas, electrolyte, anino gap
- Glucose, urea, uric acid, transaminase, phosphokinase.
- Ammonia, lactate
- Basic coagulation (protrompine time ((PT), activiated partial thromboclastin time (aPTT)</td><td>Blood</td><td>- Pyruvate
- Ketones bodies
- Free fatty acids
- Amino acids
- Acylcarnitine (Guthrie card)</td></tr>
<tr><td>Urine</td><td>- Odor
- Ketone bodies, glucose, pH
- Ketacidosis (DNPH test), dinitrophenylhydrazine with maple syrup disease
- Sufite test (sulfite oxidase deficiency)</td><td>Urine</td><td>- Amino acids
- Organic acids
- Orotic acid
- Sugars</td></tr>
<tr><td colspan="2" rowspan="2">It is very important that the sample collection is carried out in parallel and before starting the treatment. Keep sample frozen at -20C of urine (5-10cc), plasma and serum (3-4cc), and if a lumbar puncture is performed, save cerebrospinal fluid for more specific later studies.</td><td>LCR</td><td>- Glucose
- Protein
- Ammonia
- Lactate pyruvate
- Amino acids
- Organic acids</td></tr>
<tr><td colspan="2">To evaluate ketone body in blood and used for analysis of ß-hydroxybutyrate of extracted capillary blood.</td></tr>
</table>

1.1.5.3 Newborn screening (NBS)

NBS is applied at presymptomatic identification of specific genetic, metabolic, or infectious states by using tests that can be applied to the entire population of newborns. Screening programs are considered as an essential activity in the context of preventive actions in public health. NBS objective is the early identification and treatment of affected individuals so that timely medical intervention avoids neurological damage, reduces morbidity, mortality, and possible disabilities associated with these diseases.

The origin of the NBS is located in the United States, when Guthrie, in the sixties of the last century, launched an analytical procedure for the measurement of Phe in which he used as a biological sample capillary blood obtained from the heel of the newborn and impregnated in filter paper.

In 1961 Bickel introduced the Guthrie method to Europe. Helped by Guthrie himself, he set up the first European newborn screening laboratory in Marburg, Germany, which began receiving samples in 1962. Spain was one of the few European countries where Guthrie's influence was noticeable (Couce *et al*., 2008; Yuan *et al*., 2015).

A widely accepted definition of NBS stated that screening is the application of a test for the identification of individuals at risk of suffering a specific disease to benefit from additional research or treatments among people who have not attempted medical attention because of the symptoms of that disease by (Wald, 1994). NBS is, therefore, the set of actions aimed at the systematic detection of congenital diseases of metabolism in neonatal age (Cocho de Juan *et al*., 2006).

Therefore, NBS has proven effective and efficient, both from diagnosis and from that of public health and economic profitability. An important step in recent years has been the application of tandem mass spectrometry (MS-MS) to the systematic analysis of the dried blood sample collected on the filter paper card to assess metabolism or IEM. This advance allows the detection of most amino acid metabolism

disorders, organic acidurias, and fatty acid oxidation defects. It is a very sensitive and specific technique, with few false-positive results, which has been incorporated as part of the NBS programs (Dulín-Íñiguez *et al*., 2006).

1.1.5.4 Tandem mass spectrometry (MS/MS)

It is a very useful technique for neonatal screening, late diagnosis, and for disease progression. The development of the technique of MS/MS for the diagnosis of congenital metabolic diseases begins in the 70s, but it is in the 90s when this technology begins to be applied for NBS. The basic methodology for the neonatal screening of metabolic diseases by MS / MS was developed by Millington (Millington *et al*., 1990). MS/MS is a technique of multiple separation and identification of analysts based on the specific pattern of ionic fragmentation that each compound produces under certain conditions of analysis, and on the separation-detection of each ionic species according to its mass / charge ratio.

It is thus possible to separate, detect and quantify in the same test, without the need for an additional chromatographic system and from a single disc of dried blood on filter paper, the amino acids and acylcarnitines that are used as biomarkers, which makes detection possible of congenital metabolic diseases that affect the urea cycle, amino acids, organic acids and β-oxidation of fatty acids with high sensitivity and specificity (Bodamer *et al*., 2007; Waisbren, 2006).The analysis is very fast (2-3 minutes) and only requires limited sample preparation (Chace *et al*., 2003).

The application of this technology has a very important advance in neonatal screening and has also made it possible to expand biomedical knowledge about different metabolic diseases, in terms of their genetic and phenotypic heterogeneity, since it has allowed us to discover metabolic deficiencies in parents asymptomatic when studying their children (Walter *et al.*, 2009; Wilcken, 2008).

Mass spectrometry is an analytical technique that provides information on previously analysed molecules converted into ions. The molecules of interest are generally part of a heterogeneous mixture that does not necessarily require a previous separation and are first subjected to an ionization source where they are ionized acquiring negative or positive charge. The ions pass through the mass analyser until they reach different parts of the detector according to their mass / charge ratio (m / z). Once in contact with the detector, signals are generated that are registered in the computer system and represented in a mass spectrum that shows the relative abundance of the signals based on their m / z ratio. Mass spectrometry analysis basically comprises four processes:

- ►Ionization of the sample.
- ►Acceleration of ions by an electric field.
- ►Dispersion of the ions according to their mass / charge.
- ►Ion detection and production of the corresponding electrical signal.

The ionization of the sample can be carried out under different conditions, depending on the nature of the sample itself and what is intended to be detected in the analysis. It can be done in high vacuum conditions, by electronic impact, or at atmospheric pressure.

In a liquid phase analysis, an electrospray ionization (ESI) will be performed. In this case, the analyte is introduced into the solution source by an injection pump or from the eluate of a liquid chromatography system. The analyte passes through a stainless steel or silica quartz capillary tube, to which a high potential difference is usually applied in the range of 2.5 to 6 kV. This forces the fogging of the charged drops in the capillary, with a surface charge of the same polarity as the capillary itself. The drops are repelled from the capillary to the cone of the sampling electrode sampling source. With the help of a high temperature in the source of ESI and / or nitrogen gas, as the drops cross the space between the capillary and the cone, they are

continuously reducing their size, by evaporation of the solvent, which implies an increase of the surface charge density as the radius of the drops decreases. Finally, the force of the electric field in the charged drops reaches a critical point at which the passage to the gas phase for the surface ions is kinetically and energetically possible. At that time, the surface tension is not able to maintain the load (Rayleigh limit), so there is a "Coulombic explosion" and the drop breaks into smaller drops. The process is repeated until only ions remain. Figure 3.

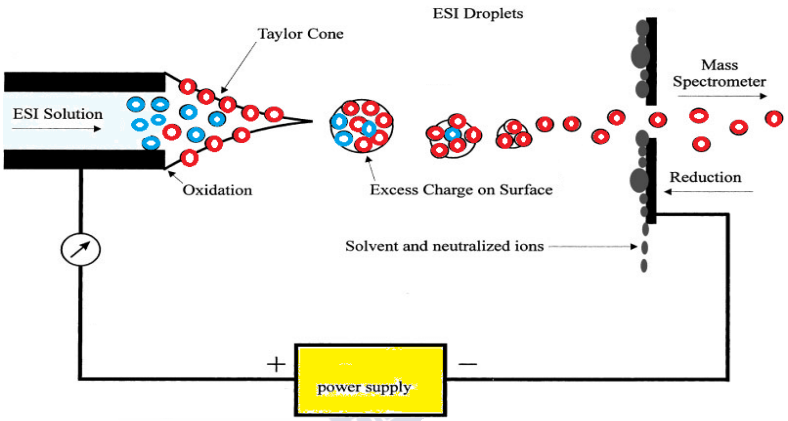


Figure 3: Schematic representation of electrospray ionization (ESI) process

The main disadvantage presented by this technique is that it produces very little or no fragmentation, so for studies in which a structural assessment is necessary, the use of tandem mass spectrometer is necessary to achieve such fragmentation.

In the gas phase, a chemical ionization or a photoionization will be performed. It is a process that uses a large injection flow (0.2-2.0 mL/min) and is applied to non-polar molecules, thermally stable compounds, normally weighing less than 1300 uma. Nebulizer gas and current discharge are required to produce ionization. The process consists mainly of three steps: ion ionozation by nebulizing gas, forming of reactive ions, and reaction with analyte molecules. Figure 4.

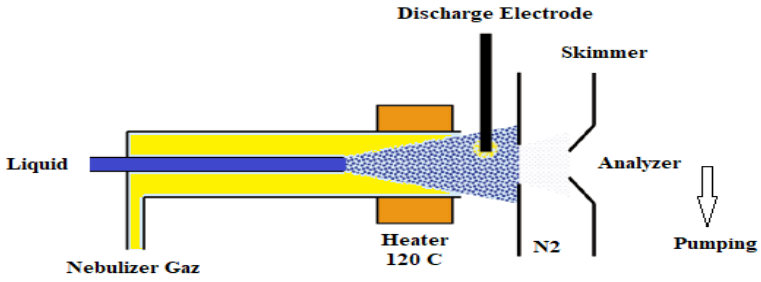


Figure 4: Atmospheric Pressure Chemical Ionization (APCI). Through a high voltage, the nebulizing gas (air or nitrogen) is ionized forming the first ions. These first ions react immediately with solvent molecules forming reactive ions. Reactive ions react with analyte molecules forming in positive or negative mode.

1.1.5.5 Genetic diagnosis

Genetic investigations play a significant role in IEM diagnosis. There are three methods employed for molecular genetic testing: a traditional, well established, and more precise method of Sanger sequencing, and a more advanced method known as Next generation sequencing (NGS). Both traditional and Sanger methods allow for sequencing of genes, determining the sequence of base pairs in the DNA of the exons/exon-intron boundaries or coding regions of a gene. The NGS utilizes the multiplexing of all the sequencing fragments and thus can sequence any number of DNA fragments simultaneously. The NGS genetics has allowed a very considerable advance in the diagnosis of IEM. NGS is now becoming the standard diagnostic methodology in most of the genetic laboratories, including metabolic disorders. (Ghosh., *et al*., 2017).

1.1.6 Treatment

Significant development has been achieved for the treatment of IEM, especially during the past ten years. Many studies are still being carried for better therapies aiming to cure nearly all IEM. Early diagnosis is essential to initiate early treatment in IEM, which can help to prevent morbidity and mortality. The initial treatment of IEM is aimed to avoid as possible the clinical and biochemical manifestations of the disease and establish irreversible complications while trying to maintain adequate growth and development in the child. See Figure 5.

Even today, the treatment poses big problems, since restoring total normality is still an illusion in the IEM (Fernández, 1999). The treatment approaches in IEM can be investigated in two main topics: acute and chronic treatment. Acute treatment includes all the measures to be taken to approach and treat a patient with a severe attack resulting from a suspected IEM (Treat hypoglycemia, hyperammonemia, and acidosis) (Baumgartner *et al*., 2014).

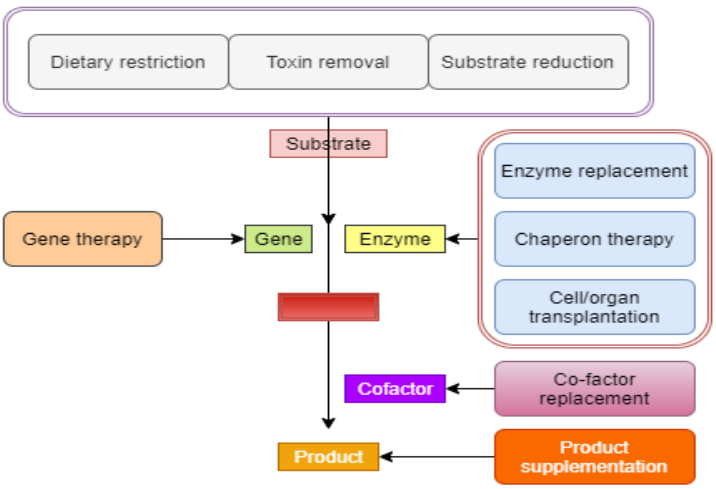


Figure 5: Treatment strategies of IEM disorders.

According to Ezgu the chronic therapeutic approaches for IEM can be categorized into four groups (Ezgu, 2016):

1.1.6.1 Substrate reduction therapy (SRT)

SRT has recently gained broad interest. In addition to directly correcting the enzyme defect, SRT aims to reduce the bioavailability of the compound that cannot be fully metabolized by the defective enzyme ('substrate reduction'), thereby recovering a steady-state balance of the pathway by lowering the accumulating substrate. Dietary treatment of PKU is often considered the first original application of SRT (Schiffmann, 2015).

SRT has gained broad appeal because of the possible administration using small molecule inhibitors that can be taken orally. SRT would particularly apply to the intoxication type of IEM, where toxic metabolite accumulation leads to acute clinical symptoms (Matalonga *et al*., 2017). Reducing substrate accumulation applied by manipulation of diet, chelation, or biosynthesis inhibition.

Gaucher and Niemann-Pick type C diseases are treated by an iminosugar called Miglustat, which inhibits the enzyme glucosylceramide synthase (Cox *et al*., 2015; Patterson *et al*., 2007).

In some disorders, the abnormal metabolic pathway can be blocked at a different level to prevent toxic substrate accumulation. In tyrosinemia type 1, the deficiency leads to an accumulation of succinylacetone, which is toxic especially for the kidney and the liver. To prevent its accumulation, the pathway is blocked successfully by the inhibitor 2-(2-nitro-4-trifluoromethylbenzoyl)-1.3 cyclohexanedione (NTBC) which can the prevent toxic substrate accumulation (Masurel-Paulet *et al*., 2008). Genistein instead has been shown to reduce the accumulation of glycosaminoglycans in mucopolysaccharidoses (MPS) (Malinová *et al*., 2011).

1.1.6.2 Stimulation of the residual enzyme

Biotin replacement in biotinidase and multiple carboxylase deficiencies; hydroxycobalamine in defects of cobalamin metabolism and thiamine in thiamine-responsive MSUD deficiency are such examples of cofactor therapy (Barends *et al.,* 2014). Some disorders result from a defect in enzyme protein folding and lead to conformational change. Pharmacological chaperones are small molecules which can recover the misfolded proteins by stimulating them to be folded. This fact has been used for the treatment of a group of patients with PKU by using BH4. BH4 acts as a chaperone for the same enzyme (Underhaug *et al.,* 2012)

1.1.6.3 Replacement of the missing enzyme

Enzyme replacement therapy (ERT) is an approach to treating specific IEM, including LSDs, that replaces a deficient or absent enzyme (Parenti *et al.,* 2015). ERT is based on the ability of most cells to uptake the exogenous enzyme through the mannose or mannose-6-phosphate receptors present on their cell surface and pass it to lysosomes (Rome *et al.,* 1979). ERT is available for Gaucher disease, Fabry disease, Pompe disease, and MPS types I, II, IV, and VI and is under development in other disorders (Desnick and Schuchman, 2012).

The main disadvantage of the existing enzyme preparations that they do not cross the blood-brain barrier and, as a result, do not affect the central nervous system findings (Kishnani and Beckemeyer, 2014; Desnick and Schuchman, 2012). Transplantation of the cells, organs that have the potential to replace the missing enzyme, has been used as an authorized treatment for some IEM. Bone marrow transplantation (BMT) has successfully been used for MPS type I. BMT has the advantage of providing the missing enzyme to CNS as well as the other tissues. Liver transplantation is a choice of treatment of some cases of MMA and PA, UCDs, and some glycogen storage disorders (Boelens *et al.,* 2009; Mazariegos *et al.,* 2014).

1.1.6.4 Removal of the toxic substrate

Branched-chain amino acids (leucine, isoleucine, and valine) accumulation in aminoacidopathies, as the levels of these toxic compounds, do not decrease rapidly in response to diet, it is necessary to force their removal to avoid/minimize associated neurological damage. To achieve this, the toxin can be removed directly (dialysis, for example), or medication can be administered to divert the metabolic pathway or provoke rapid excretion in the urine. However, hemodialysis or hemofiltration continues to be the fastest and effective method for toxic metabolites removing such as ammonia. Some medications are also being used orally or with intravenous infusions, to help with the excretion of the toxic substrate. (Brusilow *et al*., 1979), introduced the idea of treating UCD by using alternative biochemical pathways to eliminate excess nitrogen. This led to the use of oral sodium phenyl butyrate as a maintenance medication for disposal of excess nitrogen, intravenous sodium phenyl acetate (the active, metabolic product of sodium phenyl butyrate), and sodium benzoate, used in conjunction with arginine hydrochloride for emergency management of hyperammonemia (Enns *et al*., 2007; Brusilow, 1991). In situations where ammonia levels cannot be controlled by the above interventions, hemodialysis is used (Alfadhel *et al*., 2013). L–carnitine has proven its efficacy for the treatment of certain organic acidemias and diseases that affect mitochondrial metabolism. L–carnitine administration main functions are: first, to promote formation of organic acylcarnitines to re-establish the coenzyme, second is to bind to free organic acids so that they can be filtered and excreted effectively by the kidneys (Clarke, 2005).

1.1.6.5 Dietary therapy in IEM disorders.

Diet therapy is the mainstay of treatment for several type of IEM, especially small molecule metabolic diseases, such as AA, organic acids, UCDs, carbohydrate metabolism defects, such as galactosemia and HFI, and energy metabolism defects, such as GSD and FAO defects as MCAD. The principal strategies of dietary therapy in IEM include restricting the offending substrates or metabolites and providing deficient products or alternative energy sources to by-pass the defective pathway. The main goal is to maintain healthy growth and development (Gambello and Li, 2018). Dietary recommendations for patients with IEM are based on or extrapolated from estimated requirements for healthy populations, including recommendations from the WHO/FAO. However, as IEM diets often differ in natural protein and energy intake from these recommendations, their impact on long term growth and body composition needs on-going assessment. Effective treatment requires an understanding of both the biochemistry of such defects and the individual nutritional requirements, to provide an adequate intake and maintain metabolic balance (Collins and Leonard, 1985). The approach to dietary therapy is specific to each metabolic disorder, but the principles are identical. Examples of IEM required nutritional intervention are listed in Table 3.

Table 3: Selected Inborn errors disorders require nutritional therapy.

Disease	Required adjustment
Amino acids disorders	
Phenylketonuria (PKU)	Restriction of phenylalanine, supplementation of tyrosine
Maple syrup urine disease (MSUD)	Restriction of valine, leucine, isoleucine
Homocystinuria	Restriction of methionine.
Organic acid disorder	
Methylmalonic aciduria (MMA)	Restriction of valine, threonine, methionine, isoleucine
Propionic acidemia	Restriction of valine, threonine, methionine, isoleucine
Urea cycle disorders	
Ornithine trans carbamylase deficiency (OTC)	Low protein, supplement essential amino acids
Citrullinemia	Low protein, supplement essential amino acids
Arginine succinic aciduria	Low protein, supplement essential amino acids
Arginase deficiency	Restriction of arginine
Carbohydrate disorders	
Galactosemia	Low lactose, galactose
Hereditary fructose intolerance (HFI)	Restriction of fructose, sucrose, and sorbitol
Glycogen storage diseases (Type Ia)	Restriction of lactose and fructose
Fatty acid oxidation disorders	
Long chain acyl-CoA dehydrogenase deficiency (LCHADD)	Low fat, low long chain fatty acids, avoid fasting
Medium chain acyl-CoA dehydrogenase deficiency (MCADD)	Low fat, low medium chain fatty acids, avoid fasting

In inborn errors of intermediary protein metabolism (IEIPM) the dietary plan is to restrict the quantity of natural protein to 'tolerance,' that is, the amount that maintains metabolic stability with or without the use of precursor free amino acid formulas (AAF) to provide additional protein (Fernandes *et al*.,1988). Adequate energy is also essential to promote anabolism and prevent catabolism of body tissue that can increase the toxic metabolite. For IEIPM including organic acidaemias such as MMA, and PA, the routine use of AAF or essential amino acid supplements, is considered critical.

In some, IEM protein is not directly restricted or modified, but thedietary approach required to maintaining metabolic stability may

cause an indirect effect on protein intake. In some GSD, there may be lacticaemia associated with hypoglycemia, and treatment focuses on adequate carbohydrates for energy.

In Long Chain Fatty Acid Oxidation disorders (LC-FAOD), there is an accumulation of toxic acyl-carnitines of long chain fatty acids and ketone production is limited and treatment requires adequate carbohydrates with medium chain triglyceride supplementation to supply sufficient energy. In both groups of disorders, it is important to avoid decompensation due to catabolism, and overtreatment with carbohydrates may occur and result in inadequate dietary intake due to an indirect reduction in protein intake (Kishnani *et al.,* 2010).

Because of these manipulations, these dietary regimens are oftenextremely restrictive with significant variations of protein and total energy intake from that of the healthy population. Protein intake may be considerably altered in quantity or quality, and the diet may be high in carbohydrate and/or fat. Micronutrient intake is often affected. Moreover, during periods of physical stress such as intercurrent illness, an acute change in diet is regularly required to prevent metabolic decompensation, particularly in disorders that result in severe intoxication. This may result in an exaggeration of the original diet, with periods of minimal protein intake and high energy intake, thus increasing periods of nutritional imbalance.

In many IEM, particularly those of intermediary metabolism, a precise understanding of the disorder and the pathogenesis of the harmful effects is needed. The various approaches indicated may require substrate restriction, removal of toxic metabolites, replacement of deficient products, or stimulation of residual enzymes. More current therapies include enzyme replacement and gene therapy. Often, the cornerstone of treatment is dietary. Substrate restriction not only a diet low in the indicated substrate but also strict calorie support is necessary to avoid catabolism. Recommended levels of substrate restriction may require the use of supplements of "medical foods," for example, amino acid mixtures. The introduction of deficient products is essential in disorders affecting energy metabolism.

In MSUD, treatment with a strict low-protein diet, supplemented by a branched-chain-free amino acid mixture is successful, regimen for sick days is vital, and lifelong therapy is needed. UCDs are dietary challenges because while a very low-protein diet is required, no specific AA needs to be avoided, and there is a critical line between adequate protein intake and chronic catabolism. For LC-FAOD long chain fats must be avoided and medium chain fats must be substituted while strictly preventing catabolism. GSD require strict care to providing carbohydrates, always, including throughout the night. Many patients with IEM do not need any specific dietary therapy (e.g., those with storage or neurodegenerative disorders (Wilcken, 2004).

Dietary restrictions required to manage IEM are necessary for metabolic control; however, it may result in an increased risk to both short and long-term nutritional status. Dietary factors commonly likely to influence nutritional status are energy intake, protein quality and quantity, micronutrient intake, and the frequency and length to which the diet must be altered during periods of increased physical or metabolic stress. Patients on restrictive diets, especially those with intakes of low levels of a natural protein, have the highest nutritional risk.

Due to the difficulties in determining specific requirements, dietary intake recommendations, and nutritional monitoring tools used in patients with IEM are the same as, or derived from, those used in healthy populations and include Recommended Dietary Intake (RDI) for energy, protein and other macro and micronutrients, and reference growth standards for weight and height. Consequently, the evidence is lacking for the most reliable dietary prescriptions required to manage these patients long term because tolerance to dietary therapy generally described in terms of metabolic stability rather than long term health and nutritional outcomes. While the most frequent therapeutic dietary manipulation in IEM is dietary protein alteration, protein status is critically dependent on adequate energy, the use of a protein to energy ratio (P: E ratio) as an additional tool will better define the relationship between these critical components. P: E ratio could accurately identify

dietary quality and ensure that not only adequate but also safe and balanced intake is provided (Humphrey *et al*., 2014).

Dietary factors that contribute to nutritional outcomes are multifactorial, including the quality and quantity of protein tolerated, the frequency of protein restriction, and high nonprotein energy intake during metabolic decompensation (Frazier *et al.*, 2014; Gardeitchik *et al.*, 2012; Häberle *et al.*, 2012; Prietsch *et al.*, 2002; Baumgartner *et al.*, 2014) and the abnormal feeding behaviours and food dislike observed in patients with these disorders (Touati *et al.*, 2006).

Taken together, these protein-restricted regimens may result in short and long-term nutritional risks. It should be borne in mind that treatment must be maintained for life long, even after the stabilization of symptoms. The requirement for such food stimulated the development of food designed for prolonged treatment of patients with metabolic diseases. The cost of this food is elevated, and their flavours, despite continuous developments, remain different from a regular diet (Walter and MacDonald, 2006).

Examples of diet therapy intervention among different IEMs

1. Disorders of AAs metabolism

Except for UCD, the dietary management is similar for all AAs disorders (MacDonald *et al*., 2004), although the precise method of managing dietary management will vary between countries, and even between centers in the same country. General recommendations for AAs treatments include AAs restriction to maintain normal blood levels, this can be achieved by restricted intake of high protein sources such as meat, eggs, and fish. Consuming moderate/low protein food to provide minimum requirements of AAs by using exchange system and providing free substrate AAs is necessary. It is also recommended to maintain normal energy intake by encourage consuming natural low protein food and specially processed low protein food as pasta, and biscuits, and finally provision of supplement of vitamins and minerals

if necessary. Protein metabolism disorders pose unique challenges. The potentially harmful long term effect of natural protein restriction used in the treatment is underscored by the emerging evidence for longer term functional benefits of protein intake for some groups above that required for growth (Dewey *et al*., 1996), maintenance (Millward, 2004), or above RDI (Hoffman and Falvo, 2004; Millward *et al*., 2008; Fulgoni III, 2008; Heaney and Layman, 2008). Since the discovery that PKU could be treated by restricting phenylalanine in diet, there have been significant advances in treatment for other amino acid and organic acid metabolism diseases. MSUD and homocystinuria are examples of amino acid defects in which the level of toxic products can be restricted and controlled by a special diet. In other diseases such as organic acidemia and certain UCD, although restricting protein intake can reduce the accumulation of toxic compounds, neither the biochemical and clinical results always favourable as an accumulation of intermediate substrates continue to be produced even after the establishment of diet (Touati *et al*., 2006). Moreover, restricted protein diets may consist of reduced quality proteins resulting in inadequate essential amino acid and micronutrient intake.

The dietary prescription may be worsened by severe modifications to nutritional intake, which may frequently occur in some patients. Most often, this happens during the management of an intercurrent disease when the risk of metabolic decompensation is high. In this instance, as shown in Table 1.4 the substrate that cannot be efficiently metabolized must be reduced, and an increase in dietary energy is provided to induce anabolism (Prietsch *et al.*, 2002).

►Deficiency of phenylalanine hydroxylase.

The goal of nutritional management for those with PKU is to maintain blood Phe within normal range (children ≤ 12 years old between 120-360 μmol/L) and maintain tyrosine (Tyr) within normal limits for optimal outcomes that support optimal growth, development, and mental functioning while providing a nutritionally complete diet. PKU treatment consists of aggressive restriction of Phe from the diet.

Since the consumption of Phe, an essential amino acid is limited, Tyr becomes a conditionally essential amino acid, since the endogenous synthesis of tyrosine via phenylalanine hydroxylase (PAH) is severely limited.

Tyr must be supplemented in the diet to prevent deficiency and maintain blood concentrations within the normal range. Restricting dietary phenylalanine by restricting natural protein sources in the diet, without adding an alternative low Phe protein source can cause protein malnutrition and nutrient insufficiencies. Therefore the PKU diet requires supplementation of sufficient nitrogen, vitamins, minerals, and micronutrients in the form of a Phe-free AA formula (Singh *et al.*, 2014).

► Tyrosinemia.

The dietary restriction of Tyr and Phe with low protein diet may be useful to control clinical symptoms of all three types of tyrosinemias. The drug nitisinone known as NTBC has shown to be effective for the treatment of Tyrosinemia type 1 (Couce *et al.*, 2019)

► Urea cycle defect.

In UCD, natural protein intake is severely restricted and most cases provide routine supplementation with essential AA (Acosta *et al.*, 2005; Singh, 2007). Foods of high biological value are severely limited, and foods moderate in protein content, such as potatoes and cereals, are given in small and controlled portions; the amount being determined on an individual basis according to the condition severity, age, growth rate and metabolic control. Nutritional modification with low protein diet may help in controlling the level of ammonia in the body. Sodium phenyl butyrate is the primary medication used to treat the high levels of ammonia by UCD. This drug allows an alternative pathway to disposal of nitrogen from the body. If medicine and nutritional treatment failed, liver transplantation becomes an option for UCD's patient (Peña-Quintana *et al.*, 2017).

► Classic Homocystinuria.

Homocystinuria patients in Spain are not responsive to vitamin pyridoxine (B6). Methionine restricted diet with betaine, folic acid, vitamin B6, and B12 supplementations are used to control the biochemical abnormalities, especially to manage the plasma homocysteine concentrations and prevent complications, in particular, thromboembolism (Kaur *et al*., 1995).

► Maple syrup urine disease.

Dietary leucine restriction, supplementation with isoleucine and valine, and frequent clinical and biochemical monitoring may help to manage MSUD patients (Chuang *et al.*, 2006).

2. Disorders of carbohydrate metabolism

Carbohydrate disorders show a wide range of clinical symptoms because of metabolism abnormalities. Carbohydrates are the main component of our diet, which are made up of long chains of simple sugar molecules. Normally cellular enzymes metabolize carbohydrates into glucose or simpler molecules. If an enzyme needs to metabolize specific sugar is missing, the sugar can accumulate in the body, causing health problems. Carbohydrate metabolic disorders result due to the defect in one or more enzymes involved in carbohydrate metabolism.

► Classic galactosemia.

Classic galactosemia is caused by enzyme galactose-1-phosphate uridyltransferase (GALT) deficiency, manifests in the neonatal period, triggered by galactose intake, generally from breast milk, leading to cholestasis jaundice, liver failure, renal tubular dysfunction, sepsis, and cataracts.

The clinical symptoms of galactosemia can be controlled with nutritional therapy, mainly by galactose and lactose free diet. Infants should be fed a formula (e.g., soy formula) that contains trace levels of galactose or lactose. Continued restriction of dairy products in older children is recommended for galactosemia. However, studies have described that dietary restriction does not affect the long-term complications as psychomotor development delays, and motor abnormalities, possibly because of endogenous galactose production, which is independent of restricted dietary intake (Bosch, 2006).

► Hereditary fructose intolerance.

HFI is caused by deficiency of the enzyme aldolase B which splits fructose-1-phosphate into dihydroxyacetone phosphate and glyceraldehyde and converts the triphosphates into glucose and lactate. Accumulation of fructose-1-phosphate inhibits both hepatic glycogenolysis and gluconeogenesis, hence inducing hypoglycemia, and results in depletion of adenosine triphosphate.

This results in a number of disturbances, e.g. inhibition of protein synthesis and ultra-structural lesions, causing hepatic and renal dysfunction (Rake *et al.,* 2006).

Fructose is a natural monosaccharide found in many fruits, vegetables, and honey. The deficiency of enzyme fructose-1,6-bisphosphate aldolase allows the accumulation of fructose-1-phosphate in the liver, kidney and small intestine resulting in metabolic inhibition of glycogen and glucose, thereby causing severe hypoglycemic condition (low sugar in the blood) in the human body. Nutritional treatment along with a fructose-free diet is effective to manage symptoms of HFI (Yasawy *et al.,* 2009).

► Glycogen storage diseases (GSD)

A genetic defect in catabolic pathway of glycogen lead to develop GSD. Deficiency of enzymes involved in the glycogen metabolism result in progressive accumulation of glycogen in the liver and muscles. The most affected organ is the live (Adeva-Andany *et al*., 2016). Glycogen storage disease type I (GSD-I) also known as Von Gierke disease is an inherited defect due to glucose-6-phosphatase enzyme deficiency, it causes metabolic abnormalities in glycogen metabolism. Nutritional therapy may help to maintain blood glucose levels, to control hypoglycemia, and to provide optimal nutrition for growth and development.

Dietary therapy improved survival of patients with GSD-I. Prognosis and occurrence of complications depend on the long-term metabolic control regarding life-threatening hypoglycaemia or lactic acidosis. GSD-I patients may be neurologically normal under adequate dietary treatment. However, because of recurrent severe hypoglycaemia they may also be mentally handicapped. Normal growth and pubertal development can be expected today (Mayatepek *et al*., 2010).

The nutritional interventions include frequent daytime feedings, night-time intragastric continuous glucose infusion and oral uncooked corn starch may be necessary for the management of this disease. Liver function test must be monitored for the efficacy of dietary treatments (Froissart *et al.*, 2011).

In GSD III, a high protein diet is used to treated infants and children. Corn starch may already be introduced in the first year of life. Although fructose and galactose can be metabolized, the restrictions of so called simple/fast carbohydrates are a matter of debate. These simple sugars include glucose, galactose, lactose (galactose + glucose), fructose, sucrose (fructose + glucose), and maltodextrin. Fasting should be avoided, and for the overnight, a combination of snacks, frequent feeds, corn starch may be needed. Adolescents and adults have lower basic carbohydrate requirements. The recommended daily amount of protein is 25 % of the total caloric intake. A bedtime snack or an

overnight high protein formula may be prescribed for patients with myopathy (Kishnani *et al.*, 2010).

3. Disorders of fatty acids oxidation metabolism.

Nutrition management of all FAODs includes avoidance of fasting, aggressive treatment during illness, and supplementation of carnitine, if necessary.

► Dietary treatment of asymptomatic/symptomatic Very long chain acyl– CoA dehydrogenase deficiency (VLCADD), MCADD, and LCHADD.

Asymptomatic newborns without elevated creatinine kinase (CK) and transaminase, continuation of breast milk is suggested. In MCADD, treatment comprises regular meals and avoidance of prolonged fasting periods (Spiekerkoetter *et al*., 2009b). In case of MCADD children and adults require regular meals and snacks during the day and before bed to prevent hypoglycemia and fatigue (Frazier, 2008)[2]. In LCHADD should be added a product with medium chain triglycerides (MCT)

The primary goal of nutrition management of LC-FAOD is to limit long chain fat as a substrate for energy production both by preventing β-oxidation and catabolism and by limiting the amount of dietary long chain fat while still providing adequate nutrients for normal growth and development. Avoiding essential fatty acid (linoleic acid C18:2n6 and α-linolenic acid C18:3n3) deficiency is important, and the majority of long chain fat consumption should come from oils rich in essential fatty acids such instead of saturated long chain fatty acids as butter, fatty meats, etc. (Rohr and Calcar, 2008)[3]. In symptomatic patients with elevated CK or transaminase, the diet should contain greater amounts

[2] http://gmdi.org/Resources/Nutrition-Guidelines/MCAD

[3] http://gmdi.org/Resources/Nutrition-Guidelines/VLCAD

of MCT formula and less or no breast milk (Spiekerkoetter *et al*., 2003; II *et al*., 2018).

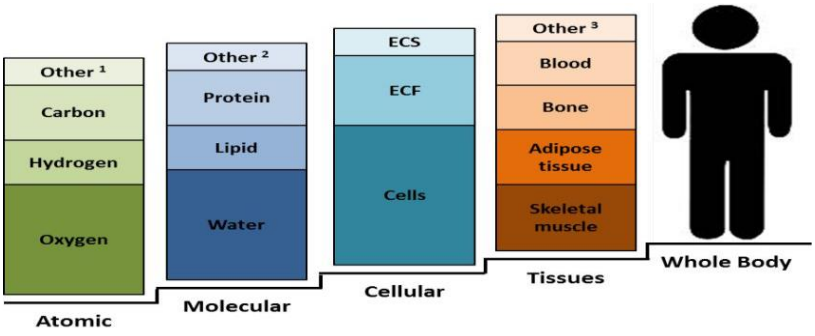
Supplementation with specific oils such as walnut or flaxseed oil may be necessary to meet essential fatty acid requirements. Patients are at higher risk for becoming deficient in fat soluble vitamins and may require supplementation with a fat restricted diet, patients with LC-FAODs are potentially at risk for inadvertently lowering their protein intake unless they specifically consume low fat, high protein foods (e.g., nonfat dairy, lean meats).

An investigation of a higher protein diet in LCHADD patients showed a higher protein diet with less carbohydrates does not improve metabolic control, but may be beneficial for body composition and liver lipid content (Gillingham, 2015; II *et al*., 2018). Nutrition management of MADD is complex and requires a low fat (20–25% energy) and low protein diet to decrease excess intake of isoleucine, leucine, lysine, tryptophan, and valine (Angle and Burton, 2008). Patients even need to avoid MCT oil because β-oxidation of all fatty acid chain lengths is compromised (El-Gharbawy and Vockley, 2018). Additionally, patients require avoidance of fasting, adequate energy to prevent catabolism as well as supplementation with riboflavin and carnitine (Olsen *et al*., 2007; II *et al*., 2018) L-carnitine supplementation in FAOD was started because of low carnitine plasma concentrations found in many patients, suggesting secondary carnitine deficiency (Winter, 2003; II *et al*., 2018).

1.2 Body Composition Measurements

The study of body composition allows us to know the proportions of the different principal components of the human body; in this way, its variation with age, growth, sports practice, and the various physiological and pathological conditions can be evaluated. Body composition can include estimates of percent body fat (BF) or fat mass (FM), fat free mass (FFM), lean body mass (LBM), bone mineral content (BMC), and total body water (TBW) (B. Heymsfield *et al*., 1997). There are different methods to measure body composition.

Anthropometrics could include measures of height, weight, and circumferences. In contrast, BF, FFM, and muscle mass are often predicted using methods such as a sum of skinfolds, Bioelectric Impedance Analysis (BIA) and dual-energy X-ray absorptiometry (DEXA). The human body can be assessed at five levels, depending on clinical concerns. Figure 6 shows the multi-compartmental or five level model of body composition.



ECS: Extracellular solids, ECF: Extracellular fluids

Figure 6: Multi-compartmental or Five levels body composition assessment

1.2.1 Indirect measurement

Indirect methods, including anthropometry and BIA, provide estimates or indices of body composition.

1.2.1.1 Anthropometric.

Anthropometric assessment is the set of measurements of the body (weight, height, circumferences, and skinfolds) at different ages and determines the different levels and degrees of nutritional status of an individual or a group. The measures are relatively simple, fast, and

economical, the anthropometric data are capable of reflecting changes in the nutritional intake produced in the long term and the results obtained should be evaluated compared with standard references according to the age and sex of the individual (Frisard *et al*., 2005).

► Weight establishes an indicator of body mass and volume, which is the most used and useful measure in pediatric practice. A variety of scales is available for weight measuring and should be calibrated regularly for accurate assessments of weight.

► Height measured easily with a variety of wall mounted equipment (Figure 7). Additional methods have been developed for predicting height when it cannot be measured directly, e.g., for the disabled or mobility impaired. Stature reflects skeletal growth; this has efficacy in the comparison of population groups or the long-term monitoring individual (Chumlea *et al.*, 1994, 1998).

Figure 7: Height stadiometer.

► Body mass index (BMI) is a descriptive index of the body that expressed as weight divided by height squared (kg/m2). A significant advantage of BMI is the availability of an extensive national reference data and its established relationships with levels of body fatness, morbidity in children and adults, and besides mortality in latest adults (Organization; 2000; Chumlea; Guo,2000).

High BMI percentage levels based on Centers for Disease Control and Prevention (CDC) BMI growth charts and changes in parameters of BMI curves in children are linked to significant levels of risk for comorbidity in pediatrics age and adult obesity (Guo *et al*., 2002, 2000). BMI is widely used to estimate body fat because it is inexpensive and straightforward. (Organization, 2000; Cole *et al*., 2000).

► Measurements of the circumferences (wrist, arm, waist, hip, thigh, and leg) give us complete information about body composition and BF distribution specially waist and hip measurement (Figure 8). Abdominal circumference, a centralized fat pattern is associated with the deposition of intra-abdominal and adipose tissue (Smith *et al*., 2001).

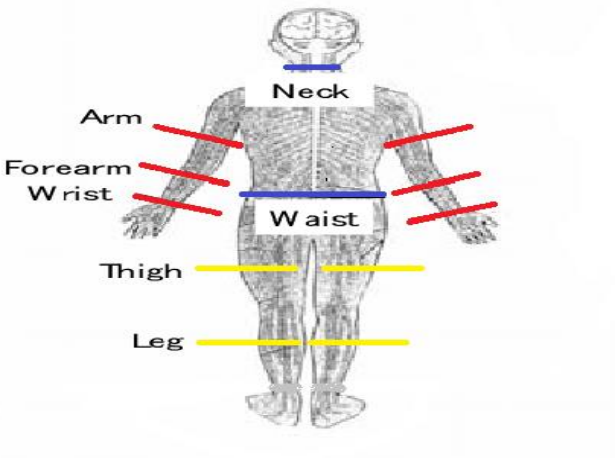


Figure 8: Circumferences common body sites

► Skin-fold measurements describe subcutaneous fat thickness in different body regions (Figure 9). Most national reference data available are for skinfolds at the triceps and subscapular locations. Skinfolds are particularly useful in monitoring changes in fatness in children because of their small body size, and most of the fat is subcutaneous. With the measurement of the four biceps, triceps, scapular, and suprailiac skinfolds, the greater or lesser degree of adiposity can be predicted (Brambilla *et al.*, 1994; Bartrina *et al.*, 2006).

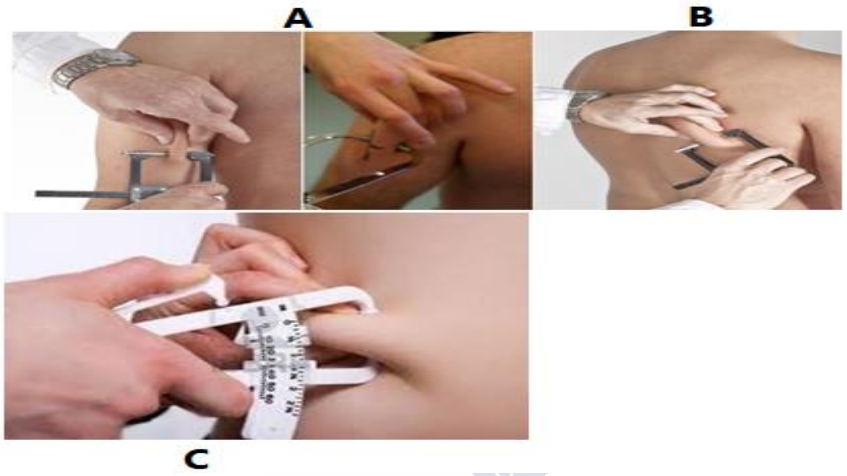


Figure 9: Skin folds measurements A: biceps and triceps, B: subscapular, C: suprailiac skin folds.

1.2.1.2 Bio-electric Impedance Analysis (BIA).

BIA is a commonly used method for estimating body composition FFM and FM. BIA measures the impedance or resistance to a small electrical current as it travels through the body's water pool. BIA's advantages include its portability and ease of use, relatively low cost, minimal participation required, and safety (not recommended for participants with a pacemaker), thus making it attractive for large-scale studies (Rush *et al*., 2006; Chumlea and Guo, 1994).

1.2.2 Direct measurements.

Are methods for analysis from the atomic view through the cellular levels, such as neutron activation, isotope dilution, and total body counting.

1.2.2.1 Total Body Water (TBW).

TBW is easy to measure as it does not require undressing or any real physical participation. Water is the most abundant component in the body, and TBW volume is measured by isotope dilution. Water maintains a relatively stable relationship to FFM; therefore, measured water/isotope dilution volumes allow prediction of FFM and fat in weight individuals (Chumlea *et al.*, 2002; Armstrong *et al.*, 1997).

1.2.2.2 Total Body Counting and Neutron Activation.

Total body counting (also called whole-body counting) measures the amount of naturally radioactive potassium 40. As potassium is found almost entirely within cell bodies, measuring potassium can provide an estimate of body cell mass. FFM can then be estimated once total body potassium is known (Ellis, 2000). Neutron Activation involves high levels of neutron radiation exposure and has not been used in large-scale population research.

1.2.2.3 Imaging method.

Three major techniques are used for imaging of the body: computer tomography (CT), magnetic resonance imaging (MRI), and DEXA. Imaging methods measure a property of the body, such as its density, or describe amounts and distributions of skeletal, muscle, and adipose tissues via x-ray or magnetic imaging techniques.

► Dual-energy X-ray Absorptiometry (DEXA).

DEXA provides whole body and regional estimates of three main components: FM, muscle mass, and BMC. These compartments are estimated based on the tissue attenuation of two different x-ray energies (Williams *et al*., 2006). DEXA has been considered a standard gold method for assessing body composition because of its validity and reliability in previous research. It is quick, safe, can be used on almost all populations and requires little pretesting preparations for the individual, (Figure 10). Participants are exposed to tiny amounts of radiation and are safely available for repeated use. Some limitations are that the DEXA is costly, long duration, non-portable (Lohman *et al*., 2005).

Figure 10: Dual-energy X-ray Absorptiometry (DEXA)

The most common purpose of a DEXA scan is to assess whether a person's bones are weak and or at risk of fracture. It also helps a doctor diagnose osteoporosis. Osteoporosis causes the bones to lose density or become thin. When the bones get thin, they also become fragile, which makes them more susceptible to breaks. The bone mineral can be measured using DEXA in three different sites in the human body, scanning for the total body, column scan, and the scan of the left femur leg. Figures 11 and 12.

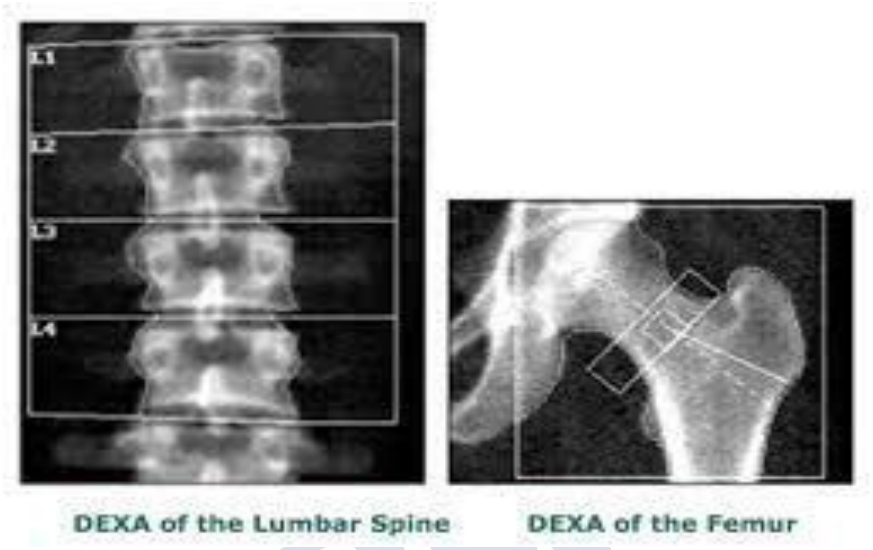


Figure 11: Lumbar spine and femur DEXA scan

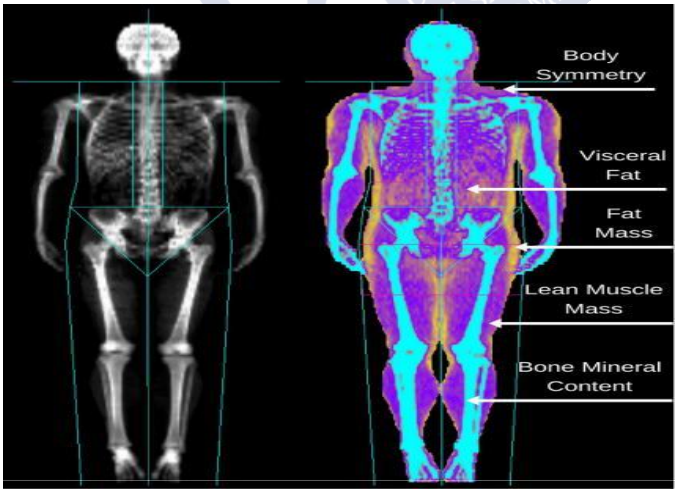


Figure 12: Total body DEXA scan

The results of a DEXA scan for bone density use a system called T-score. The T-score involves comparing scan results with the bone density of a healthy matched reference. A DEXA scan may also report results using a z-score, which indicates the amount of bone a person has compared with other people of the same size, age, and sex. It can help determine whether something uncommon is leading to bone loss.

The WHO provides the following definitions of bone density levels for adults: A T-score of -1.0 or higher is normal bone density. A T-score of -1.1 to -2.4 indicates osteopenia or low bone density. A T-score of -2.5 or lower means a risk of osteoporosis.

The interpretation of bone densitometry results in children differes from that in adult. The term “osteopenia” and “osteoporosis” based on bone densitomety findings alone should not be used in younger patients; instead, bone mineral density that falls > 2 SDs is labled “low for age”. Pediatric osteoporosis is defined with using of the following criteria: occurrence of ≥1 vertebral fracture, or low bone density for age and a fracture history (defined as occurrence of ≥ 2 long bone fracture before 10 years of age or ≥ 3 times long bone fractures before 19 years of age). According to the International Society for Clinical Densitometry (ISCD), z-score that is over -2.0 is considered normal for age. In pediatric a z-score below -2.0 is what doctors interpret as below the normal range with a risk of osteopenia (Ramos *et al.*, 2012).

In addition to evaluating bone mineral density, a whole-body scan can also measure total body composition and fat content with a high degree of accuracy. DEXA can determine total body fat percentage, total fat mass, and fat-free mass. Most importantly, DEXA provides regional body fat results (trunk, arms, legs, pelvis, and android/gynoid regions). DEXA scan can also measure visceral fat / abdominal fat, which is often associated with increased risk for cardiovascular disease and type 2 diabetes.

1.2.3 The validity of DEXA to measure body composition in IEM

The chosen techniques for estimating body composition must be valid and reliable (yield the same results with repeated applications). The assumptions underlying a given method influence its validity, whereas the equipment and skills of the individual using the technique can influence reliability. Generally, a comparison of body composition assessment by DEXA and BIA according to the BMI has been poorly documented. Few studies have shown good accord between the two methods (Stewart *et al.*, 1993; Thomson *et al*., 2007).

Many other studies have shown weak accord between the two methods. These conflicting results may probably due to some limiting factors, including the use of different BIA devices with different equations from the manufacturer, small population size, and the differences in age, ethnicity, and body weights in the studied sample (Gába *et al.*, 2015; Leahy *et al.*, 2012; Marra *et al*., 2018; Mattar *et al*., 2011; Pateyjohns *et al*., 2006; Neovius *et al.*, 2006;).

The usability of skin-fold equations versus DEXA has yet to be evaluated for those affected by IEM. For that, no available research studies concerning DEXA validity in the evaluation of body composition in IEM. A unique study determines the precision of six anthropometric skinfold equations versus air displacement plethysmography (ADP) for predicting BF percentage in female adolescents with PKU (Douglas *et al.*, 2012).

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2. JUSTIFICATION

Nutritional status is an important indicator of health in children and affects BMD. For many purposes, anthropometric measurements as the weight for age, weight for height, and BMI provide adequate information about the nutritional status of children. However, diseases or treatment may influence body composition, which is not reflected in anthropometry. Changes in body fat and lean body mass occur in many IEM.

IEM involves a broad spectrum of disorders, frequently affecting body composition (fat mass, lean mass, and bone). Some disorders fundamentally affect bone and present with noticeable skeletal features. In other diseases, alterations, such as decreased bone mass, may be secondary to nutritional deficiencies because of a strict diet. As a wide range of pathophysiological mechanisms involved, therapeutic approaches will be different for each disorder. However, some generalizations can be made in all conditions, physicians should be aware of general measures to optimize body components bone health, such as adequate intake of calcium/phosphate and vitamin D and optimal physical activity.

For all these reasons, it is of interest to know the nutritional status of patients with IEM and its relationship with the pathological condition and the prescribed diet, in order to be able to make recommendations for reducing the adverse effects of the treatment on nutritional and metabolic health in these patients.

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3. OBJECTIVES

The fundamental objectives of this work have been to study the body composition and the nutritional status of IEM patients followed in the Unit of Diagnosis and Treatment of Congenital Metabolic Diseases of the University Hospital Complex of Santiago de Compostela.

3.1 Main outcomes

1) Study of growth and development of the IEM Children by anthropometric and body composition evaluation.

2) Evaluate nutritional status in the IEM children by dietary intake record and nutritional biochemical biomarkers.

3.2 Secondary

1) Determine the risk of osteopenia and/or osteoporosis by bone mineral density measurement with DEXA in different body zone.

2) Analyse the effects of prolonged dietary restrictions on the state of micronutrients.

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4. MATERIAL AND METHODS

4.1 Patients

4.1.1 Study Design

This is a cross-sectional observational study that uses cases (patients with IEM) after the start of a dietary and/or medical treatment) carried out in the Unit of Diagnosis and Treatment of Congenital Metabolic Diseases during the period 2017-2019. A similar matched group by age and sex of 98 healthy children was collected during the same period.

4.1.2 Population

A total of 99 metabolic patients with age range 5 to 19 years old were studied. The collected data of the patients included: type of diagnosis, diagnosis time, sex, age (years), birth weight(kg) and height (cm), socioeconomic status and educational level of parents, anthropometric measurements (weight, height, BMI, circumferences (mid-upper arm, wrist, waist, hip, thigh, and calf circumferences), skinfolds (biceps, triceps, subscapular, and suprailiac), puberty stage, FM, FFM, bone densitometry of total body, lumbar spine and proximal femur by DEXA were performed. Each child underwent biochemical, nutritional evaluation. Dietary and pharmacological treatment and treatment compliance data were also included. Physical activity questionnaires were evaluated. The same measurements applied in sex and age-matched healthy controls.

The project was explained to participating children and adolescents who met the inclusion criteria and none of the exclusion and to their parents or legal guardians. They were also provided with an information sheet related to the study. Once their participation was accepted, an informed consent approved by the Research Ethics Committee (Galicia CEIC Registration Code: 2017/310) was signed by the parents or legal

guardians and by children over 12 years of age. Appendix B: informed consent.

4.1.3 Inclusion and Exclusion criteria

In order to participate in the study, they had to meet the following criteria.

Inclusion criteria

- Children 5-19 years of age diagnosed with inherited metabolic disorders.
- Informed consent to participate in the study will be signed by the parents or guardians of the children. The data will be collected in an encrypted manner in a database. Appendix B: informed consent.

Exclusion criteria

- Do not approve informed consent.
- Have been diagnosed in the last year.
- Have another associated disease that affects growth.
- Be participating in a research project or less than three months that have participated.

4.1.4 Ethical aspects

This study followed the Organic Law 3/2018, of December 5, on the Protection of Personal Data and guarantee of digital rights, and follow the fundamental principles established in the Declaration of Helsinki, in the Council of Europe Convention on Human Rights and Biomedicine, in the UNESCO Universal Declaration on Human Rights, and the requirements established in the Spanish legislation in the field of medical research, and will follow the rules of Law 14/2007 on Biomedical Research and Organic Law 15/1999, RD 1720/7007 for the protection of personal data, Biomedical Research, and adjusts to what is established in Law 31/1995, of November 8, on the Prevention of Occupational Risks, and in the Royal Decrees that develop it regarding risks related to exposure to biological agents. Appendix A.

4.2 Method

4.2.1 Anthropometric assessment

The following parameters are collected in the study. These measurements were performed by the same person (the researcher), which were repeated three times each to minimize both inter-observer and intra-observer bias.

►**Weight** was measured to the nearest 0.1kg while the child was wearing light clothing and no shoes, with the use of SECA 701 electronic medical scales with a class III digital display. They placed on the scale in a standard anatomical position without using any support point that could vary the measure. The measured values are converted to z-score according to the international reference values of the Centers for Disease Control and Prevention (CDC) (Kuczmarski, 2000).

►**Height** was measured by using the Harpenden stadiometer, measuring 600-2100 mm, approved by the University of London Institute of Child Health.

Height is measured while the child is standing without shoes, heavy outer garments, and hair ornaments. The back of the head, shoulder blades, buttocks and heels are touching the stadiometer. The head of the stadiometer is lowered so that the hair is pressed. Reading of the height should be done from eye level. The measured values were converted to z-score according to the international reference values of the World Health Organization (WHO)[4].

►**BMI:** the Quetelet index (weight / size^2) was calculated as kilogram per square metre (kg/m^2), along with standardised scores and centiles, which was scored using the the international reference values of the World Health Organization (WHO)[5].

►**Body circumferences:** measured with a flexible, not extensible Seca 201 tape, allows measuring circumferences with millimeter precision. For measuring, the tape is held at a right angle to the limb or body segment being measured, and the tape tension must be constant. This constant tension is achieved by ensuring that there are no gaps between the skin and the tape and maintains its place at the specified reference. Hold the tape box with the right hand and the end of the tape with the left. Passes the end of the tape around and takes the tip of the tape with the right hand, then holds both the end and the box. Apply enough tension to the tape with your right hand to keep it in position, while your left-hand goes under the box to retake the end. Now the tape outlines the segment to be measured; the middle fingers of both hands are free to precisely position the tape at the mark and orient it so that the zero is easily read. When recording the reading, the tester's eyes

4 https://www.who.int/growthref/who2007height for age

5http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi

should be at the same level as the tape to avoid any error of parallelism between tape and limb or segment. All measures were converted to z-score according to the Spanish reference value for children's and adolescents /enkid study among the Spanish population (Serra-Majem *et al*., 2002).

The following sites have been measured:

- **Mid-Upper Arm Circumference (MUAC):** measured on the left arm left upper arm at the mid-point between the tip of the shoulder and the tip of the elbow (olecranon process and the acromion). At this level and with the arm relaxed, surrounded the tape without compressing the tissues. MUAC values were converted to z-score according to the Spanish reference value for children and adolescents /enkid study among the Spanish population (Serra-Majem *et al*., 2002).
- **Wrist circumference:** place the tape around the wrist above the ball joint of the ulna to determine wrist's circumference in cm. Body frame size can be determined measuring your wrist and comparing it to your height.
- **Waist circumference:** measurement is taken around the abdomen at the level of the umbilicus (belly button). Waist circumferences were converted to z-score according to the Spanish reference value for children's and adolescents /enkid study among the Spanish population (Serra-Majem *et al.*, 2002).
- **Hip circumference:** the circumference was taken in the horizontal plane of the maximum level of the gluteal muscles, the individual remained standing and with the gluteal muscles relaxed. Hip circumferences were converted to z-score according to the Spanish reference value for children's and adolescents /enkid study among the Spanish population (*Serra-Majem et al*., 2002).

- **Thigh circumference:** the circumference of the thigh was taken with the individual in a biped position, distributing the weight on both feet, placing the tape 1cm under the buttock fold perpendicular to the longitudinal axis of the thigh.
- **Calf circumference:** was measured using inelastic tape with participants in the upright position at the maximum circumference at the plane perpendicular to the calf's longitudinal line.

►**Skinfolds measurements:** they were measured using Harpenden Analog Skin-Fold Caliper, Model Harpenden Skinfold Caliper, for Medical Research, which reports a compression of 10 gms/sq.mm, with a measurement range of up to 80 mm, in divisions of 0.2, being able to interpolate accurately up to 0.1 mm. The exact point where the skinfold is taken must be carefully located using anatomical marks before evaluation. In this study, measurements were made on the left side of the body.

For the correct taking of the fold, it must be "pinched" in the previously marked place. Once the double portion of skin plus the underlying subcutaneous adipose tissue is "pinched," it is held under pressure between the thumb and index finger. Hold the caliper with your right hand, pressing to separate the branches and then applying them at a right angle to the direction of the fold and approximately one centimeter from the fingers. Later the pressure exerted on the equipment is released, wait two seconds, and proceed to take the reading in millimeters. Finally, the instrument is removed, and the fold is released.

Four skinfolds have been measured and all converted to z-score according to Serra Spanish reference of enkid study (Serra-Majem *et al*., 2002):

- **Triceps skinfold**: pinch the subcutaneous fat at the triceps level on the back of the arm, 1 cm above the mark made for the brachial perimeter. Check that the skin-fold separates from the muscle. The caliper is applied horizontally over the mark with the right hand, holding the pinch with the left hand, for about 3

seconds before recording it. Triceps values converted to z-score according to Serra Spanish reference of enkid study (Serra-Majem *et al*., 2002).

- **Biceps skinfold: i**t is taken at the same level as the triceps fold but on the front of the arm. A fold of skin and subcutaneous fatty tissue should be made along the longitudinal axis of the arm. Biceps values converted to z-score according to Serra Spanish reference of enkid study (Serra-Majem *et al*., 2002).
- **Subscapular skinfold:** locate the left scapular angle and make a mark. Pinch the subcutaneous fat 1 cm above and medial to the mark made, forming an angle of 45o concerning the vertebral column (the fold points to the contralateral elbow). The same procedure described for the triceps fold measurement will be followed. Subscapular values converted to z-score according to Serra Spanish reference of enkid study (Serra-Majem *et al*., 2002).
- **Suprailiac skinfold:** pinch the subcutaneous fat at the level of the intersection of the mid-axillary line with the iliac crest. Oblique fold forward and down. Suprailiac values converted to z-score according to Serra Spanish reference of enkid study (Serra-Majem *et al*., 2002).

4.2.2 Puberty stage

The pubertal stage only in the IEM patients was assessed by a study physician. TheTanner scale describes the physical changes observed in the genitals, breast and pubic hair in both sexes. This internationally accepted scale classifies and divides pubertal changes into five stages ranging from child (I) to adult (V). For our study, we divided children and adolescents into two groups, prepubertal (Tanner I) and pubertal (Tanner II, III, IV and V).

4.2.3 Body composition assessment

Body composition (FM, muscle mass, and BMC) in addition to BMD was determined through dual-beam X-ray absorptiometry (lunar

DEXA DPX, General Electric), measurements at the lumbar level (L2, L3, and L4) and proximal femur. We used a z-score for comparing BMD with a population of the same sex and age, using the database published by (Zanchetta *et al*., 1995) as reference values. Bone mineral density z-score that falls ≤ -2 is labled as osteopenia risk. A z-sccore ≤ -2.5 is labled as osteoporosis risk.

Body components of FM and FFM reported by DEXA were converted to z-score reference values for the Spanish population (Zanchetta *et al.*, 1995).

4.2.4 Biochemical study

Blood collection was performed by venous puncture under the following conditions: fasting for at least 12 hours, except for FAO and some CHD, which was 6 hours. No intense physical activity in the hour before the extraction. The determinations of the following serum parameters were made using the techniques:

Advia 2400 Chemistry System (Siemens Healthcare Diagnostics, Erlangen, Germany) is used in the following measured values: total Cholesterol (120.0 - 255.0 mg/dL), Triglycerides (27.0 - 150.0 mg/dL), total proteins (6.4 - 8.5 g/dL), Albumin (4.4 - 5.6 g/dL), Urea (14.0 - 43.0 mg/dL), Creatinin (0.4 - 1.1 mg/dL), Calcium (9.0 - 10.5 mg/dL), Sodium (134.0-145.0 mmol/L) and Glucose (74.0 - 105.0 mg/dL).

SAS-3 Cholesterol Profile kit from Helena Biosciences Europe (Tyne and Wear, UK). Reference values: HDL-c (34.0 - 91.0 mg/dL), LDL-c (55.0 - 125.0 mg/dL).

Vitamin D: 25(OH)D and 1-25(OH)2D were performed through a fully automated electrochemiluminescent system (Rocha Diagnostico GmbH, Mannheim, Germany). Reference values: deficiency: <10 ng/mL, insufficiency: 10-20 ng/mL, recommended: > 20 ng/mL.

Vitamin A: High Performance Liquid Chromatography (HPLC)-Ultraviolet. Reference values: both sex (1- 6) years 0.20 - 0.43 mg/L (7-12) years 0.26 - 0.49 mg/L, (13-19) years 0.26 - 0.72 mg/L, > 19 years 0.30 - 0.80 mg/L.

Vitamin E: High Performance Liquid Chromatography (HPLC) - Ultraviolet. Reference values: both sex (1 – 12) years 0.30 - 0.90 mg/dl, both sex (13 – 19) years 0.60 - 1.00 mg/dL, both sex > 19 years 0.50 - 1.81 mg/dL.

Vitamin K1: High Performance Liquid Chromatography (HPLC)-Fluorimetry. Reference value: 0.10 - 2.10 ng/ml

Zinc: atomic absorption spectrometry (flame exciter), Reference values: zinc (65-140 μg/dL). Selenium: Inductively coupled plasma with mass detector. (45.81 - 143.76 ug/L, 60 – 150 μg/L).

Plasma amino acids: high performance liquid chromatography (HPLC). Tyrosine (40-92 umol/L); Phenylalanine (38-78 umol/L); Glutamine (396-740 umol/L); Arginine 45-125umol/L; Methionine: 16-36 umol/L; Citrulline: 18-50 umol/L; Isoleucin 38-94 umol/L, Leucine: 80-200 umol/L; Valine 70-320 umol/L.

Acylcarnitines: electrospray ionization-tandem mass spectrometry. Reference values: Octanoylcarnitine (C8) <0.26 umol/L; Decanoylcarnitine (C10): <0.33umol/L; C8/C10: <2.25; Butyrylcarnitine (C4): <0.95umol/L; Methylmalonylcarnitine (C4DC): <0.57 umol/L. Free carnitine: 6-55 umol/L; Glutarylcarnitine (C5DC): <0,13; 3-OH-isovalerylcarnitine (C5OH): <0,35 umol/L; 3-Methylglutarylcarnitine (C6DC): <0,13 umol/L.

4.2.5 Assessment of parents' educational level

Data from the parents' educational level were collected and adapted to the International Standard Classification of Education. Appendix C.

They were categorized into a low, medium, and high level of education.

- **Low level of education**: secondary in both parents or secondary for one of them and primary in the other, secondary in one of them and without studies in the other or primary education in both or primary in one of them.
- **Medium level of education**: bachelor's degree in both parents or bachelor's degree in one of them and secondary education in the other.
- **High level of education**: university degree in both parents or university degree in one of them and a bachelor in the other.

4.2.6 Assessment of parents' socioeconomic status

For this work, the data collected on socioeconomic background was used through CON-94 (National Occupational Classification Questionnaire). (Appendix C). Socioeconomic and educational level of the parents). Parents' socioeconomic level, the occupation level variables, and the education level of parents were noticed and whether they are currently working. Obtaining three socioeconomic levels:

- **Low socioeconomic level:** low educational level and low occupation level in both parents.
- **Average socioeconomic level:** parents with average educational and occupational levels.
- **High socioeconomic level:** both parents with high educational and high occupation levels.

4.2.7 Physical activity and feeding questionnaires

A valid standard international questionnaire was used on the study sample and data about lifestyle were collected. (Appendix C). Questionnaire on frequency of food consumption and physical activity).

- **Physical activity questionnaires**

Regarding the physical activity habits and degree of this, the International Physical Activity Questionnaire (IPAQ) was used, from which we collected information about different physical activity patterns, both programmed and spontaneous. For this, we ask a series of questions to obtain the variables. We gathered information about how they travel to school (in transport or walking), in case of walking we observed the time they spent and classified them into three groups the first group includes those who walk more than ten minutes, the second group those who walked between 5 and 10 minutes, and the third one those who walked less than 5 minutes. To obtain data on regulated sports, we asked if they belonged to any sports club, data were also collected on the type of sport they practice in the sports club.

Also, we obtained data about moderate and vigorous/intense activity, by which we created the variables referring to < 7 hours per week or ≥7 hours per week of moderate and intense physical activity; data collected for days of performing vigorous exercise per week.

IPAQ responses converted to Metabolic Equivalent Task minutes per week (MET-min/wk.) according to the IPAQ scoring protocol: total minutes over last seven days spent on the vigorous activity, moderate-intensity activity, and walking were multiplied by 8.0, 4.0, and 3.3, respectively, to create MET scores for each activity level. MET scores

across the three sub-components were summed to indicate overall physical activity.[6]

- **Three-Days Food Intake Record**

  A 3-days survey of food intake was collected from patients for thetotal amount of intake during the day, then was analysed using the software to calculate the dietary intake average for the recorded 3 days[7]. Appendix C.

4.2.8 Control group

A control group was established with 98 healthy people of similar characteristics in age and sex for patients. Control includes children visited the consult of nutrition and hepatic metabolism suffering from abdominal pain; those children are directed to do lactose test from the gastrointestinal consult after complete analysis and appear completely healthy without any related health problems. Before the inclusion of the participant in the control group, they and/or their legal guardians gave their informed consent to sign it for participation.

4.2.9 Statistical analysis

The analysis of the results was carried out using the statistical package IBM SPSS Statistics 22.

[6] (www.ipaq.ki.se)
[7] (www.odimet.es)

A descriptive statistical study of the sample was performed, analyzing the parameters of centralization (mean and median) and those of dispersion (standard deviation, maximum and minimum, quartiles and range) and position parameters (percentages 5, 10, 25, 50, 75, 85, 90, 95 and 99). Dummy variables were used for categorical variables that had more than two categories. Univariate (frequencies and proportions) and bivariate (contingency tables) methods were used.

The Chi-Square test was used to see if there were statistically significant differences. Pearson and Spearman's bivariate correlations were also made. We used descriptive statistics to report the mean and standard deviation (SD) as measures of central tendency and variance for all primary outcome variables: height z-score, weight z-score, BMI z-score, % FM, calories, protein (g/kg and % of calories), fat (% of calories), and carbohydrate (% of calories).

Paired t-test used to determine if there is a difference in body composition variables between the patient's population and the control group. We used 95% confidence intervals for the mean outcomes of the primary variables. $P < 0.05$ is considered statistically significant. Pearson correlation used to measure the strength of a linear association between variables. Binary logistic regression was used to predict the odds of being a patient or control based on the independent variables (predictors). We used correlation analysis and binary logistic regression analysis to examine the relationship between status (patients and controls) with age, sex, body composition, and dietary intake.

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5. RESULTS

5.1 General characteristics of sample

5.1.1 Total sample: IEM patients and controls, general characteristics

197 subjects (including 99 IEM patients and 98 healthy matching controls) participated in this stuy. The females represented 57.6% and 51.1% in IEM patients and controls group respectively with no significant differences between the two groups. No significant difference in age stage between IEM patients and the healthy matched controls with age range between 5 and 19 years (median age= 11.66 years) Tables 4 and 5.

Table 4: Sample distribution by sex

	Patients		Controls		
Sex	N	%	N	%	*P*
Females	57	57.6	50	51.1	0.082
Males	42	42.4	48	48.9	0.074
Total	99	100	98	100	

P significant < 0.05

Table 5: Sample distribution by age stages

	Patients		Controls		
Age	N	%	N	%	*P*
5 to 9	38	38.4	33	33.7	0.062
10 to 14	25	25.3	32	32.7	0.067
15 to 19	36	36.3	31	31.6	0.063
Total	99	100	98	100	

P significant < 0.05

In both groups of IEM patients and healthy controls, the presence of pregnancy-related diseases, hypertension, and diabetes in mothers during pregnancy, occurs only in 2% and 1% respectively, without significant differences. Chronic diseaes such as celiac disease, IEM, etc., are presented in 8% of IEM patient mothers and 4% in control mothers. Among these diseases, 5 of IEM patient mothers were diagnosed with IEM 3HPA and 2 hypermethioninemia MAT I/III mothers. Tables 6, 7 and 8.

Table 6: Mothers diseases during pregnancy for IEM patients and controls

		Patients		Controls		
	Presence of disease	N	%	N	%	P
Pregnancy related diseases	Yes	2	2	1	1	0.919
	No	96	98	97	99	0.941
	Total	98	100	98	100	
Cronic disease	Yes	8	8	4	4	**0.024**
	No	90	92	94	96	**0.039**
	Total	98	100	98	100	

P significant < 0.05

Table 7: Frequency of disease in mothers during pregnancy for IEM patients and controls

	Patients		Controls		
Disease	N	%	N	%	*p*
Diabetes	1	1	1	1	0.945
Hypertension	1	1	0	0	0.921
Non	96	98	97	99	
Total	98	100	98	100	

P significant < 0.05

Table 8: Frequency of cronic diseases in mothers of IEM patients and controls

	Patients		Controls		
Disease	N	%	N	%	*p*
Celiac disease	1	1	1	1	0.931
Hypercholesterolemia	0	0	2	2	0.840
Hepatitis	0	0	1	1	0.838
Thyroid disease	2	2	0	0	0.645
IEM disease	5	5	0	0	**0.042**
Non	90	92	94	96	0.457
Total	98	100	98	100	

P significant < 0.05

5.1.2 Total sample, IEM patients, general characteristics

Most of the IEM patients of this study, 94% have been diagnosed early by NBS, patients diagnosed later represent only 6% of the total sample. Table 9.

Table 9: Frequency of diagnosis time in IEM patients

Diagnosis time in patients	N	%
Early diagnosis (newborn screening)	92	94
Late diagnosis	6	6
Total	98	100

Thirteen IEM patients, 8 females and 5 males, had affected relatives, some of them with the presence of more than member in the same family. In total 21% (n=21) members in families of the 13 IEM patients are diagnosed with IEM.The highest percent of the disease observed in the brothers of the patients followed by mothers and sisters with 9% and 5% respectively. Tables 10, 11, 12, 13 and 14.

Table 10: Frequency of family history of IEM in patietns

	N of patients	%
Patients with no family history of IEM	85	86.7
Patients with Family history of IEM	13	13.3
Total	98	100

Table 11: Frequency of IEM among patient's family members

Presence of IEM in patient's family	N	%
No IEM in family members	78	79
Family members with IEM	21	21
Total	99	100

Table 12: Who in the family have IEM

Family member	N	%
No one	78	79
Father	1	1
Mother	5	5
Brother	9	9
Sister	5	5
Others (cousins.)	1	1
Total	99	100

Table 13: Frequency of IEM among patients' families for sex

	Patients				
	Females		Males		
Presence of IEM in patient's family	N	%	N	%	*P*
No	49	86	37	88.1	**0.000**
Yes	8	14	5	11.9	**0.003**
Total	57	100	42	100	

Table 14: Frequency of incidence of IEM in members of IEM patient family for sex

	Patients				
	Females		Males		
Family member	N	%	N	%	*P*
No one	44	77	34	81	**0.000**
Father	1	2	0	0	0.062
Mother	2	4	3	7	**0.003**
Brother	7	12	2	5	**0.000**
Sister	3	5	2	5	**0.005**
Others (cousins.)	0	0	1	2	0.063
Total	57	100	42	100	

N: number of subjects, P significant at <0.05

According to the socio-economic level, in the IEM patient's, only 16% reach a high level, while 21% have a low level, and the rest represent 63% with a moderate level. Table 15.

Table 15: Frequency of the socioeconomic status in IEM patients' parents

Socioeconomic Status level	N	%
Low level	20	21
Moderate level	62	63
High level	16	16
Total	98	100

Regarding the level of education, 40% of the patient's parents have a medium level, while 51% represented those with low education levels, 9% of the patient's parents have a higher level of education. Table 16.

Table 16: Frequency according to the level of education in IEM patients' parents

Education level	N	%
Low level	50	51
Moderate level	39	40
High level	9	9
Total	98	100

Binary logistic regression analysis, with the dependent variable as a group (patients versus controls) and the independent variables sex, birth weight, study level of parents, and family history of the presence of IEM disorders, was conducted and the identified model (Likelihood Ratio: statistics= 90.787, p =0.001). In parents, at the study level, the positive coefficient indicated that an increase of one degree in the study level of parents increases the presence of healthy children. More family members with IEM recorded in patients' group than controls.

The logistic regression analysis identified two risk factors for this pathology: family history of IEM and parent study level. According to the results presented in the next table, family history IEM is expected to be almost five times more frequent in the IEM group compared with the controls. According to the parent study level, a high level of education is more prevalent in the control group than patients, and its 1.6 times higher in healthy controls group. Table 17.

Table 17: Results of binary logistic regression analysis of IEM patients and controls

	Coefficient	S.E.	Wald	*P**	OR
Sex	-0.196	0.314	0.390	0.533	0.822
Birth weight (kg)	0.283	0.316	0.800	0.371	1.327
Parents study level	0.467	0.255	3.354	**0.017**	1.595
Family history of IEM	-3.000	1.040	8.317	**0.004**	5.089

R^2 =0.597
SE: standard error, OR: odds ratio, *significant at p<0.05.
Dependent variable :(0: patients, 1: controls).
Independent variables: sex (1: female;2: male), Birth weight (kg),
Parent study level (low, moderate, high), family history of IEM (1: yes,2: no).

5.1.3 General characteristics of intermediary metabolism disorders

AA metabolism disorders were the most prevalent in IEM patients, representing 77.8% (n=77) followed by carbohydrate metabolism disorders (CHD) with 12.1% (n=12) of the total IEM patient's sample, while fat metabolism disorders 10.1% (n=10). Table 18 and Figure 13.

Table 18: Frequency of intermediary metabolism disorders in IEM patients

	Patients	
	N	%
Amino acids disorders	77	77.8
Fat disorders	10	10.1
Carbohydrate disorders	12	12.1
Total	99	100

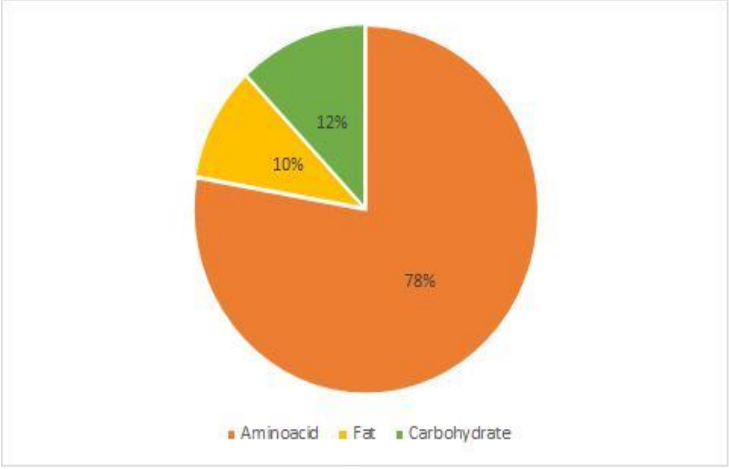

Figure 13: Disease category for all IEM patients

For the AAs meabolism disorders, the most incidence was HPA (12 females and nine males), and PKU (14 females to 11 males), five males and four females have hypermethioninemia (14.7% vs. 9.3%, respectively), four MSUD comparing to two males and two MMA females. In fat metabolism disorders, patients with SCADD were all females. In CHD, three males and two females were presented with HFI disease, two females, and one male with classic galactosemia. Table 19.

Table 19: Specific disorders diagnoses in IEM patients enrolled in the study for sex

	Patients			
	Females		Males	
	N	%	N	%
Amino acids disorders				
HPA	12	27.9	9	26.5
PKU	14	32.5	11	32.4
Hypermethioninemia MAT I/III	4	9.3	5	14.7
MSUD	4	9.3	2	5.8
Tyrosinaemia type 1	2	4.7	2	5.8
Glutaric aciduria type 1	3	7	3	8.8
Citrulinemia type I	1	2.3	0	0
OTC deficiency	0	0	1	2.9
3-Hydroxy-3-methylglutaric aciduria	0	0	1	2.9
Methylmalonic aciduria	2	4.7	0	0
Nonketotic hyperglycinaemia	1	2.3	0	0
Carbohydrate disorders and defects transport of carbohydrates				
Classic galactosaemia	2	28.6	1	16.7
Hereditary fructose intolerance	2	28.6	3	50
Glycogen storage disease	2	28.6	2	33.3
Glucose transporter 1 deficiency	1	14.2	0	0
Fat oxidation disorders				
MCADD	3	42.9	3	100
SCADD	4	57.1	0	0

HPA: Hyperphenylalaninemia, MCADD: Medium chain acyl-CoA dehydrogenase deficiency, MSUD: Maple syrup urine disease, OTC: Ornithine transcarbamylase, PKU: Phenylketonuria, SCADD: Short chain acyl-CoA dehydrogenase deficiency.

5.2 Anthropometric characteristics

5.2.1 Total sample: IEM Patients and Controls Anthropometric characteristics.

56.6% of the IEM pateints and 65.3% of the controls were having normal weight (BMI 5-85th percentile n=56 vs. n=64), 35.4% of patients and 30.6% of the controls were overweight /obesity (BMI >85th percentage n=35 vs. n=30) among this 25 IEM patients showed BMI > 95th percentage while only six controls in the same percentile. 8.1% of IEM patients and 4.1% of the controls were underweight (BMI < 5th percentile, n=8 vs. n=4)[8]. Table 20.

Table 20: Distribution of BMI percentage according to WHO International standards in patients and controls.

	Patients		Controls		
BMI	N	%	N	%	*p*
Underweight (P<5th)	8	8.1	4	4.1	**0.039**
Normal weight (p 5-85th)	56	56.6	64	65.3	**0.000**
Overweight and obesity (p>85th)	35	35.4	30	30.6	**0.000**
Total	99	100	98	100	

BMI: body mass index, N: number of subjects, *p* significant at <0.05.

Table 5.18 shows that IEM patients are more frequent with high BMI z-score, nine IEM patients, having z-score between 1.036 to1.64, in the z-score 1.645 to3.09 we founded 24 IEM patients in this category, while 2 of the IEM patients recorded a z-score ≥ 3.090, eight IEM patients have underweight with z-score < -1.645. In the controls group, only 4 of them recorded a low z-score < -1.645, among the highest BMI

[8] http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi

z-score only five controls presented with BMI z-score >1.645 and no one with the highest z-score ≥ 3.090. Table 21.

Table 21: Distribution of BMI z-score in IEM patients and controls

Category (z-score)	Patients		Controls		
	N	%	N	%	*p*
- 3.090 to ≤ -1.645	8	8.08	4	4.1	**0.044**
-1.645 ≤ 1.030	56	56.6	64	65.3	**0.037**
1.036 to ≤ 1.645	9	9.09	25	25.5	**0.017**
1.645 to 3.090	24	24.2	5	5.1	**0.044**
≥3.090	2	2.02	0	0	0.614
Total	99	100	98	100	

P significant at <0.05

Females IEM patients have the highest frequency of underweight than males (8.8% vs. 7.1%), seventeen females, and eighteen male IEM patients having overweight with p> 85th. In the control group, eleven females and nineteen males were having overweight [9]. Table 22.

Table 22: Prevalence of the degree of adiposity according to WHO International standards by sex

	Patients					Controls				
	Females		Males			Females		Males		
BMI categories	N	%	N	%	*p*	N	%	N	%	*p*
Underweight (p<5th)	5	8.8	3	7.1	**0.032**	2	4	2	4.2	0.683
Normal weight (p 5-85th)	35	61.4	21	50	**0.000**	37	74	27	56.3	**0.000**
Overweight /obesity (p>85th)	17	29.8	18	42.9	**0.049**	11	22	19	39.6	**0.000**
Total	57	100	42	100		50	100	48	100	

BMI: body mass index, N: number of subjects, *P* significant at <0.05

[9] http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi

We observed that no significant difference recorded in BMI mean z-score between IEM patients and controls. This could be explained as more patients tend to have higher and lower BMI z-score (underweight <1.65, and overweight > 1.645) comparing to weak control participants presented with the same z-score distribution.

Figure 14 shows the frequency of BMI category in IEM patients and controls in total and for sex.

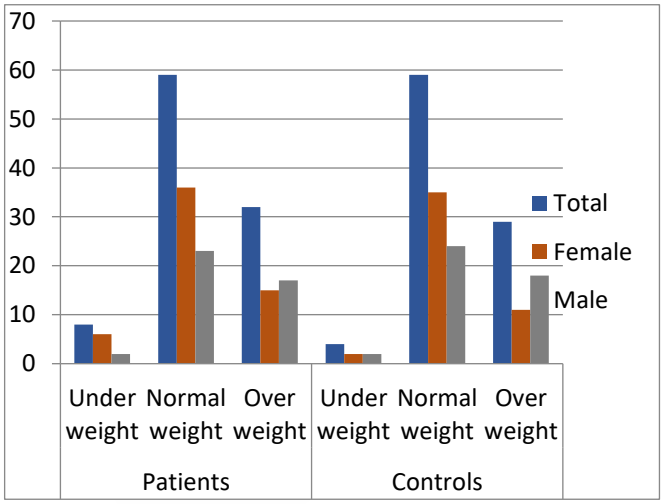

Figure 14: BMI categories in total sample and sex for patients and controls

IEM patients had significantly lower height z-scores mean (-0.28±1.2 vs. 0.16±0.95; p =0.008) than controls. Also, significant differences between IEM patients and controls recorded in waist circumference mean z-score in which IEM patients tend to have higher waist circumference mean z-score than in controls (-0.08±1.3 vs. -0.58±1.1, p =0.005). For the other anthropometric measurements, no significant differences between IEM patients and controls have been observed. Table 23.

Table 23: Means of body composition z-score measurements in patients and controls

Anthropometric measurements	Patients mean±SD	Controls mean±SD	*P*
Weight z-score	0.42±1.2	0.49±0.86	0. 631
Height z-score	-0.28±1.2	0.16±0.95	**0.008**
BMI z-score	0.56±1.3	0.42±0.89	0.279
Mid-arm circumference z-score	-0.31±1.2	-0.37±0.95	0.745
Waist circumferences z-score	-0.06±1.3	-0.56±1.1	**0.005**
Hip circumferences z-score	-0.66±1.1	-0.82±0.88	0.287
Biceps skin-fold z-score	0.68±1.7	0.29±1.4	0.101
Triceps skin-fold z-score	0.07±1.3	0.02±1.2	0.831
Subscapular skin-fold z-score	1.02±2.4	0.58±1.8	0.177
Suprailiac skin-fold z-score	1.4±1.9	0.58±1.9	0.197

BMI: body mass index N: number of subjects, *P* significant at <0.05, SD: standard deviation.

We distributed the height percentage of the sample according to WHO reference values. 11.1 % of patients are having a short stature (p<5th), while no controls presented with this percentage. On the other hand, seven patients presented with p>95th comparing to five controls at the same percentage with no significant differences. Table 24.

Table 24: Distribution of height percentage according to WHO International standards in patients and controls.

	Patients		Controls		
Height percentage	N	%	N	%	*p*
P <5th	11	11.1	0	0	**0.001**
P ≥th5 to <95th	81	81.8	93	94.9	**0.007**
P >95th	7	7.1	5	5.1	0.812
Total	99	100	98	100	

P significant at < 0.05

As shown in Table 25, eight of IEM patients with short stature were having a z-score ≤ -2 with the lowest values recorded was equal to -2.94, more controls were presented within the z-score 1-2 with 17 controls versus 10 IEM patients.

Table 25: Distribution of height z-score according to WHO International standards in IEM patients and controls.

	Patients		Controls		
Height z-score	N	%	N	%	*P*
≤-2	8	8.1	0	0	**0.001**
-2 to -1	15	15.2	12	12.2	0.511
-1 to 1	61	61.6	64	65.4	0.322
1 to 2	10	10.1	17	17.3	0.056
≥2	5	5	5	5.1	0.849
Total	99	100	98	100	

P significant at < 0.05

Waist circumferences (WC) z-score mean was higher in IEM patients than controls, so we search the distribution of the WC percentiles and z-scores (Serra-Majem *et al.*, 2002). More patients are presented with a high waist percentage (>95th), while no controls present with the same percentage. Among the percentiles of 5-10th more controls than patients (29 vs. 16, respectively), within the percentage 85-95th, more patients are presented than controls (11 vs. 5, respectively). Table 26.

Table 26: Distribution of waist percentage reference in IEM patients and controls (according to Serra Majem *et al.*).

	Patients		Controls		
	N	%	N	%	*p*
P 5-10th	16	16.2	29	29.6	**0.005**
P 10-85th	62	62.6	64	65.3	0.884
P 85-95th	11	11.1	5	5.1	**0.035**
P>95th	10	10.1	0	0	**0.002**
Total	99	100	98	100	

P significant at < 0.05

IEM patients and controls distributed based on WC z-score in which we founded that IEM patients tend to be more frequent in the highest values of z-score (>1 to > 2) than controls indicating that they have significantly higher WC than controls. Reduced WC in controls

also explained by more control children among the lowest z-score (≤-2). Table 27.

Table 27: Distribution of waist z-score reference in IEM patients and controls (according to Serra Majem *et al.*).

Waist z-score	Patients N	Patients %	Controls N	Controls %	*p*
≤ -2	6	6.1	11	11.2	**0.041**
-2 to -1	23	24.2	23	23.5	0.741
-1 to 1	49	48.5	58	60.2	**0.037**
1 to 2	12	12.1	6	5.1	**0.047**
≥2	9	9.1	0	0	**0.003**
Total	99	100	98	100	

P significant at < 0.05

In the study of anthropometric measurements in IEM patients and control according to sex significant differences observed between females in patients and controls groups in waist circumferences z-score with the highest z-score mean value recorded in patients females than in controls females (-0.12±1.4 vs. -0.56±1.2, $p = 0.041$). In contrast, in patients and control groups, males we founded significantly differ in height with reduced height z-score mean presented in patients males than in controls males (-0.42±1.2 vs. 0.18±0.98, $p = 0.008$).

WC z-score means was higher in males' patients with IEM than in male controls (-0.03±1.3 vs. -0.62±1.1, $p = 0.046$). According to skinfolds, biceps and triceps z-score mean was significantly higher in male patients than in male controls ($p < 0.05$). Table 28.

Table 28: Means of body composition z-score measurements in patients and controls according to sex.

			Patients		Controls	
Anthropometric	Sex	N	Mean ± SD	N	Mean ±SD	*P*
Weight z-score	F	57	0.33±1.09	50	0.44±0.76	0.534
	M	42	0.54±1.3	48	0.53±0.96	0.981
Height z-score	F	57	-0.17±1.3	50	0.12±0.92	0.176
	M	42	-0.42±1.2	48	0.18±0.98	**0.008**
BMI z-score	F	57	0.37±1.15	50	0.35±0.78	0.805
	M	42	0.81±1.5	48	0.48±1.01	0.164
Mid-arm circumference	F	57	-0.37±1.2	50	-0.4±1.02	0.884
z-score	M	42	-0.24±1.2	48	-0.34±0.87	0.712
Waist circumference	F	57	-0.12±1.4	50	-0.56±1.2	**0.041**
z-score	M	42	-0.03±1.3	48	-0.62±1.1	**0.046**
Hip circumference	F	57	-0.78±1.2	50	-0.86±0.8	0.687
z-score	M	42	-0.49±1.2	48	-0.78±0.96	0.231
Biceps z-score	F	57	0.57±1.7	50	0.55±1.3	0.957
	M	42	0.84±1.7	48	0.05±1.6	**0.026**
Triceps z-score	F	57	-0.04±1.3	50	0.28±1.2	0.617
	M	42	0.23±1.2	48	-0.22±1.3	0.094
Subscapular z-score	F	57	0.83±2.3	50	0.82±1.6	0.975
	M	42	1.28±2.5	48	0.37±1.9	0.062
Suprailiac z-score	F	57	1.34±1.9	50	1.5±1.6	0.679
	M	42	1.4±1.7	48	0.54±1.6	**0.021**

F: females, M: males, BMI: body mass index, N: number of subjects, *p* significant at <0.05.

5.2.2 Intermediary metabolism disorders anthropometric characteristics.

Overweight was more prevalence in patients with AA metabolism disorders with 36.4% (n=28) and underweight with 6.5% (n=5) respectively followed by CHD disorders with 33.3% (n=4) overweight patients and 16.7% (n=2) with underweight, while in FAO disorders presented 30% (n=3) patients with overweight and one with underweight.[10] Tables 29.

Table 29: Prevalence of the degree of adiposity according to WHO International standards in intermediary metabolism disorders

BMI category	AA disorders		Fat disorders		CH disorders	
	N	%	N	%	N	%
Underweight (P<5th)	5	6.5	1	10	2	16.7
Normal weight (P 5-58th)	44	57.1	6	60	6	50
Overweight and obesity (p>85th)	28	36.4	3	30	4	33.3
Total	77	100	10	100	12	100

N: number of subjects, AA: Amino acids, CH: Carbohydrate

Twenty-eight patients with AA disorders are reported with z-score > 1.036 in which two of them were with the highest z-score value > 3.090, CHD come next with four patients recording z-score > 1.036, but no one has a z-score > 3.090, only two patients with FAO presented with > 1.036. Table 30.

[10] http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi

Table 30: Distribution of BMI z-score in intermediary metabolism groups.

Category z-score	AA disorders		Fat disorders		CH disorders	
	N	%	N	%	N	%
- 3.090 to ≤ -1.645	5	6.5	1	10	2	16.7
-1.645 to ≤ 1.030	44	57.1	6	60	6	50
1.036 to ≤ 1.28	8	10.4	1	10	0	0
1.645 to 3.09	18	23.4	2	20	4	33.3
>3.090	2	2.6	0	0	0	0
Total	77	100	10	100	12	100

N: number of subjects, AA: Amino acids, CH Carbohydrate.

In assessment of body composition for intermediary metabolism disorders, weight mean z-score was no significant differ for each group. Patients with CH disorders have the lowest height z-score mean (-1.173 ±1.037, $p = 0.002$) followed by AA metabolism disorders with z-score mean value (-0.268 ±1.176, $p = 0.000$). BMI presented a higher z-score with significant differences in AA and FAO metabolism disorders from controls ($p < 0.05$). No significant differences observed in body circumferences except in waist circumferences, which presented significant differences in AA metabolism disorders ($p < 0.05$). In skinfolds measurements, biceps wase higher and significant in AA metabolism disorders ($p = 0.000$). Table 31.

Table 31: Means of body composition z-score measurements among intermediary metabolism disorders.

		Patients		Controls		
Anthropometric	Disorders	N	Mean ± SD	N	Mean ±SD	*P*
Weight z-score	AA	77	0.469 ±1.12	98	0.486 ±0.86	0.913
	FAO	10	0.935 ±0.98	20	0.297 ±0.94	0.136
	CHD	12	-0.349±1.39	20	0.248 ±0.76	0.179
Height z-score	AA	77	-0.267±1.18	98	0.148 ±0.95	**0.012**
	FAO	10	0.711±1.16	20	-0.13 ±0.82	0.061
	CHD	12	-1.173±1.04	20	-0.03 ±0.92	**0.007**
BMI z-score	AA	77	0.662 ±1.22	98	-0.373±0.95	**0.000**
	FAO	10	0.64 ±1.33	20	-0.68 ±0.98	**0.014**
	CHD	12	-0.033±1.73	20	-0.379±0.83	0.447
Mid-arm circumference z-score	AA	77	-0.242±1.14	98	-0.373±0.99	0.417
	FAO	10	-0.15±1.3	20	-0.68±0.98	0.287
	CHD	12	-0.9±1.45	20	-0.379±0.83	0.339
Waist circumference z-score	AA	77	-0.075±1.35	98	-0.588±1.11	**0.008**
	FAO	10	-0.285±1.57	20	-0.794±1.09	0.382
	CHD	12	0.052±1.2	20	-0.363±0.69	0.251
HIP circumference z-score	AA	77	-0.635±1.08	98	-0.821±0.88	0.319
	FAO	10	0.219±1.19	20	-1.014±0.83	**0.080**
	CHD	12	-1.16±1.23	20	-0.778±0.84	0.443
Biceps z-score	AA	77	0.778±1.57	98	0.288±1.44	**0.036**
	FAO	10	0.241±2.44	20	0.109±1.28	0.672
	CHD	12	0.443±1.52	20	0.787±125	0.726
Triceps z-score	AA	77	0.131±1.25	92	0.015±1.4	0.547
	FAO	10	-0.215±1.59	12	0.022±1.33	0.708
	CHD	12	-0.052±1.28	15	0.298±1.03	0.514
Subscapular z-score	AA	77	1.1±2.38	98	0.578±1.79	0.106
	FAO	10	1.293±1.95	20	0.82±1.84	0.811
	CHD	12	0.817±2.15	20	0.792±1.46	0.930
Suprailiac z-score	AA	77	1.38±1.86	98	1.03±1.66	0.201
	FAO	10	1.293±1.95	20	0.82±1.84	0.565
	CHD	12	1.363±2.04	20	1.176±1.12	0.755

AA: amino acids, BMI: body mass index, CHD: carbohydrate disorders, FAO: fatty oxidation disorders, N: number of participants, SD: standard deviation, *p* significant at <0.05.

Binary logistic regression indicates that height, biceps, triceps, and waist are significant predictors of status of IEM patients and controls (Chi-Square= 34.414, df =4 and p =0.000). All the four predictors "explain" 22% of the variability of patients and controls. Height, biceps, triceps, and waist are significant at the 5% level [waist Wald= 8.449, p =0.004 (p <0.05); height (Wald= 9.878, p =0.002); biceps (Wald= 8.240, p =0.004); and triceps (Wald= 11.843, p =0.000). Height showed a positive coefficient indicated that controls were higher than patients. The negative coefficients (-0.941) in biceps indicated that patients have higher biceps skinfold than controls, an indirect indication of fat mass, which is higher in patients. Triceps were higher in controls than IEM patients (coefficient = 1.336), while waist with negative coefficient = -0.605. The odds ratio (OR) for the waist is 0.546, height 1.672, triceps 3.804, and biceps 0.390. The model predicted correctly at 67.5%. Among all these variables both triceps and height consider the most risk factors in predicting patients and controls, in which patients are 1.7 times lower in height than controls and 3.8 times lower in triceps circumference than controls. Table 32.

Table 32: Summary of Logistic Binary Regression Analysis for anthropometric variables in patients and with IEM and controls

	Coefficient	S.E.	Wald	*p*	OR
Height z-score	0.514	0.164	9.878	**0.002**	1.672
Biceps z-score	-0.941	0.328	8.240	**0.004**	0.390
Triceps z-score	1.336	0.388	11.843	**0.000**	3.804
Waist z-score	-0.605	0.208	8.449	**0.004**	0.546

R^2=0.220
S.E.: standard error, OR: odd ratio, p significant at <0.05.
Dependent variable :(0: patients, 1: controls).
Independent variables: height, biceps, triceps, waist.

5.3 Body Composition Assemment.

5.3.1 Total sample: IEM patients and controls body composition assessment.

The study of the DEXA, there were no significant differences in muscle mass and fat mass measurements and in bone density in the lumbar spine L2-L4 between IEM patients and controls, in the other hand significant differences in the total body density between IEM patients and controls with low mean value in IEM patients (0.89±0.95, 1.6±1.5, p =0.001) as shown in Figure 15. The femur in the three sites (neck, trochanter, and ward) was significantly lower in IEM patients (p <0.05). Table 33.

Table 33: Means of body composition z-score measured by DEXA in patients and controls

	Patients mean ± SD	Controls mean ± SD	p
Muscle mass z-score	0.09±1.6	0.29±1.3	0.317
Fat mass z-score	0.26±1.8	-0.07±1.3	0.159
BMD total z-score	0.89±0.95	1.6±1.5	**0.001**
Lumbar spine L2-L4 z-score	0.65±0.69	0.8±0.64	0.117
Femur neck z-score	0.45±0.76	0.67±0.75	**0.044**
Femur trochanter z-score	0.5±0.98	0.84±0.89	**0.012**
Femur ward z-score	-0.21±0.71	0.04±0.8	**0.023**

BMD: bone mineral density, SD: standard deviation, p significant at <0.05.

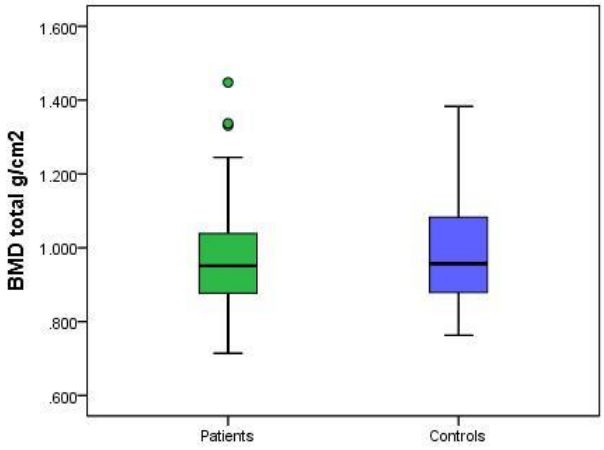

Figure 15: Mean total body bone mineral density in IEM patients and Controls

Our results demonstrated that BMD total body z-score mean was significantly different between IEM patients females and controls females (0.91 ±0.96 vs. 1.4±0.96, p= 0.008, respectively). Reduced BMD total body z-score means were also founded in patients males when compared to controls males (0.88±0.96 vs. 1.9±2, p = 0.003, respectively). Femure BMD z-score mean was lower in IEM patients males than in controls males in three measured sites with significant differences between the two groups (p< 0.05). Femur neck was 0.19±0.61 in patients males versus 0.52±0.62 in controls males, femur trochanter with 0.19±0.64 in patients males and 0.59±0.63 in controls males, femur ward was -0.39±0.73 in patients males versus 0.033±0.62 in controls males. No significant differences regarding muscle mass and fat mass were founded between males and females of the two studied groups. Table 34.

Table 34: Means of body composition z-score measured by DEXA according to sex in patients and controls

			Patients		Controls	
	Sex	N	Mean ±SD	N	Mean ±SD	*p*
Muscle mass z-score	F	57	0.45±1.3	50	0.42±0.91	0.896
	M	42	-0.41±1.8	48	0.16±1.6	0.118
Fat mass z-score	F	57	-0.18±1.5	50	-0.22±0.89	0.871
	M	42	0.86±2.1	48	0.09±1.7	0.063
BMD total z-score	F	57	0.91±0.96	50	1.4±0.96	**0.008**
	M	42	0.88±0.96	48	1.9±2	**0.003**
BMD Lumbar spine L2-L4	F	57	0.75±0.61	50	0.83±0.64	0.522
z-score	M	42	0.51±0.77	48	0.77±0.65	0.096
BMD Femur neck	F	57	0.64±0.81	50	0.81±0.84	0.275
z-score	M	42	0.19±0.61	48	0.52±0.62	**0.017**
BMD Femur trochanter	F	57	0.73±1.1	50	1.08±1.02	0.099
z-score	M	42	0.19±0.64	48	0.59±0.63	**0.004**
BMD Femur ward	F	57	-1.4±0.69	50	0.08±0.95	0.161
z-score	M	42	-0.39±0.73	48	0.033±0.62	**0.045**

BMD: Bone Mineral Density, F: Female, M: Male, N: Number, SD: standard deviation, *p* significant at <0.05,

Table 35 shows the correlation between anthropometric and BMD of all skeletal sites in patients and controls. In patients, Pearson's correlation coefficient (r) was significant (p <0.01) and positive among bone variables (BMD of the spine, femoral trochanter, femoral ward, and femur), with weight. BMI founded to be significant (p <0.01) and positive with BMD total body and BMD of the spine, and significant (p <0.05) and positive correlation with (femoral trochanter, femoral ward, and femur). Total body BMC, BMD spine were positively correlated with age (p <0.01), while a positive significant (p <0.05) founded with femur and trochanter. On average, the weight seemed to have a strong correlation with BMD in IEM patients.

In controls group Pearson's correlation coefficient (r) was significant (p <0.01) and positive among bone variables (BMD total, BMD of the spine, femur trochanter, femur ward, and femur neck), with weight, and BMI. Age founded to be significant (p <0.01) and positive with BMC and with BMD total and femur (p <0.05).

Table 35: Pearson's correlation coefficient between bone mineral density (BMD and anthropometrics variables in IEM patients and controls

Patients							
		BMD total	BMC	BMD L2-L4	BMD Femur	BMD trochanter	BMD Ward
Age in years	r Pearson	-0.153	**0.834****	**-0.261****	**-0.252***	**-0.233***	-0.142
Weight z-score	r Pearson	**0.865****	0.184	**0.784****	**0.723****	**0.477****	**0.409****
BMI z-score	r Pearson	**0.618****	0.110	**0.528****	**0.517***	**0.477***	**0.247***
Controls							
Age in years	r Pearson	**0.209***	**0.547****	-0.192	-0.208*	-0.183	-0.107
Weight z-score	r Pearson	**0.920****	**0.235***	**0.519****	**0.769****	**0.329****	**0.435****
BMI z-score	r Pearson	**0.807****	0.164	**0.483****	**0.439****	**0.379****	**0.422****

**. Correlation is significant at 0.01.
*. Correlation is significant at 0.05.
BMD: bone mineral density, BMC: bone mineral content, BMI: body mass index.

Binary logistic regression analysis, with the dependent variable as a group (patients versus controls) and the independent variables BMD total body, spine, and femur were conducted and the identified model (Chi-square=20.609, $p = 0.000$). The logistic regression analysis identified BMD total as risk factors for this pathology. According to the results presented in Table 5.26, the BMD total is expected to be almost 1.9 times higher in controls than the patients IEM group. The other two variables show no significant relation. Table 36.

Table 36: Results of binary logistic regression analysis of patients with IEM and controls

	Coefficient	S.E.	Wald	*p*	OR
BMD total z-score	0.636	0.167	14.504	**0.000**	1.888
BMD spine L2-L4	-0.386	0.301	1.646	0.200	0.680
BMD femur	0.217	0.203	1.134	0.287	1.242

R^2=0.220
S.E.: standard error, OR: odd ratio, *P* significant at <0.05, BMD: bone mineral density
Dependent variable :(0: patients, 1: controls).
Independent variables: BMD total; BMD spine L2-L4; BMD femur.

According to the determination of BMD by DEXA, osteopenia risk in both groups was detected with significant differences between the patients and the controls group ($p = 0.036$), no history of fracture was recorded in any of the children in patients and controls. In the patient's group, 33 (33.3%) presented osteopenia risk and seven with sever reduced BMD <-2.5 labled as osteoporosis risk (7.1%). Among the controls group, 20 (20.4%) presented osteopenia risk, and no one has a very low bone density (z < -2.5). Table 37.

Table 37: Prevalence of osteopenia and osteoporosis risk for IEM patients and controls.

	BMD L2-L4 z-score groups			
	Normal % (n)	Osteopenia risk % (n)	Osteoporosis risk % (n)	*p*
Patients	58.6 (58)	33.3 (33)	7.1 (7)	**0.036**
Controls	79.6 (78)	20.4 (20)	0	

BMD: Bone Mineral Density, *p* significant at <0.05.

5.3.2 Intermediary metabolism disorders body composition assessment.

According to DEXA, measured variables BMD for the intermediary metabolic disorders showed that the FM z-score was higher among AA disorders than in controls (p <0.05). Total body BMD z-score was lower in AA metabolism disorders patients than in controls (0.833±0.92 vs. 1.66±1.35, p= 0.000). AA metabolism disorders, according to the femur, three sites bone density (femur neck, trochanter, and ward) founded to be significantly lower from reference controls (p <0.05). Table 38.

Table 38: Means of body composition z-score measured by DEXA in intermediary metabolism disorders

		Patients		Controls		
	Diseases category	N	Mean ±SD	N	Mean ±SD	*p*
Muscle mass	AA	77	0.023±1.39	98	0.297±1.26	0.383
z-score	FAO	10	1.515±2.3	20	0.24±1.16	0.110
	CHD	12	-0.687±1.39	20	0.046±1.24	0.254
Fat mass	AA	77	0.315±1.83	98	-0.071±1.35	0.026
z-score	FAO	10	-0.021±2.34	20	-0.35±1.22	0.676
	CHD	12	0.162±1.65	20	0.086±0.73	0.940
BMD total	AA	77	0.833±0.92	98	1.66±1.56	0.000
z-score	FAO	10	1.38±0.76	20	1.493±1.06	0.780
	CHD	12	0.863±1.26	20	1.639±1.63	0.213
BMD Spine L2-L4	AA	77	0.641±0.68	98	0.88±0.64	0.321
z-score	FAO	10	1.024±0.59	20	0.935±0.78	0.818
	CHD	12	0.38±.71	20	0.88±0.76	0.206
BMD femur Trochanter	AA	77	0.491±095	98	0.84±0.89	0.013
z-score	FAO	10	1.06±1.16	20	0.45±1.25	0.753
	CHD	12	0.11±0.85	20	0.49±1.15	0.350
BMD Femur neck	AA	77	0.424±0.79	98	0.67±0.75	0.039
z-score	FAO	10	0.528±1.33	20	0.867±0.76	0.473
	CHD	12	0.26±0.56	20	0.36±0.89	0.733
BMD femur Ward	AA	77	-0.252±0.71	98	0.04±0.8	0.013
z-score	FAO	10	0.283±0.66	20	-0.25±1.2	0.229
	CHD	12	-0.33±0.59	20	-0.33±0.84	0.994

AA: amino acids, BMD: Bone mineral density, CHD: carbohydrate disorders, FAO: fatty acid oxidation, *p* significant at <0.05.

According to disorders category AA metabolism disorders patients, we observed a high prevalence of osteopenia risk with 25 IEM patients of the total patient sample (32.5%). For CH metabolism disorders, six patients having risk of osteopenia and two with sever low BMD < -2.5 having a risk of osteoporosis. The IEM patients with FAO metabolism disorders have recorded two cases with osteopenia risk. Table 39.

Table 39: Prevalence of osteopenia and osteoporosis risk for intermediary metabolism disorders.

	BMD L2-L4 z-score group		
Disorders	Normal	Osteopenia risk	Osteoporosis risk
	% (n)	% (n)	% (n)
AA disorders	61 (47)	32.5 (25)	6.5 (5)
FAO disorders	80 (8)	20 (2)	0
CH disorders	33.3 (4)	50 (6)	1.7 (2)

AA: amino acids, CH: carbohydrate, FAO: fatty acids oxidation, BMD: bone mineral density, n: number of subjects

5.3.3 Spine L2-L4 BMD correlation with age and physical activity in patients and controls.

When studying the correlation between age (years) and physical activity (minutes/week) with BMD spine L2-L4, we found a significant negative relation between age and BMD spine L2-L4 in both IEM patients and control groups indicated that as age increase the density of spine bone decreased. According to physical activity, a significant positive correlation with BMD spine was present in IEM patient's group mean that more physical activity leads to strength and increase bone density. Table 40.

Table 40: Correlation of age and physical activity with BMD spine L2-L4

		Patients		Controls	
		Age years	Physical activity	Age years	Physical activity
BMD spine L2-L4	r pearson	-0.260**	0.272**	0.238*	0.445
	p	**0.008**	**0.007**	**0.023**	0.107

** Correlation is significant at 0.01
*Correlation is significant at 0.05

AA and FAO metabolism disorders were having a negative and significant correlation with age; a one-year increase will result in one degree of decrease in BMD spine L2-L4 ($p<0.01$) while in CHD non-significant negative correlation observed. Physical activity was strongly positivel correlated with the BMD spine in the FAO metabolism disorders (r=0.774, p =0.022), also with the AA metabolism disorders (r=0.237, p = 0.034). At the same time, in CHD, a weak positive non-significant correlation was presented. Table 41.

Table 41: Correlation of age and physical activity with BMD spine L2-L4 in intermediary metabolism groups.

		AA disorders		FAO disorders		CH disorders	
		Age	PA	Age	PA	Age	PA
BMD spine L2 - L4	r pearson	-0.225*	0.237*	-0.567	0.774*	-0.274	0.195
	p	**0.049**	**0.034**	0.087	**0.022**	0.389	0.543

*Correlation is significant at 0.05
BMD sine L2-L4, PA (Physical activity) in minutes/week,
AA: amino acids, CH: carbohydrate, FAO: fatty acid oxidation.

5.4 Patterns of Physical Activity

5.4.1 Total sample: IEM patients and controls patterns of physical activity.

According to the patterns of physical activity, we observe that there is a higher percentage of children and adolescents in IEM patients use transportation to go to school, only 33.3% are walking to school, being only 30% of them, those who employ more than 10 minutes to do it. In the group of controls children and adolescents, we found that 36.7% of them walking to school, with only 28% who employ more than 10 minutes of walking time.Tables 42 and 43.

Table 42: Frequency according to the way of traveling to school.

	Patients		Controls		
	N	%	N	%	*P*
Walking	33	33.3	36	36.7	0.843
Transport	66	66.7	62	63.3	0.821
Total	99	100	98	100	

N: number of participants, *p* significant at <0.05.

Table 43: Frequency according to walking time to school

	Patients		Controls		
	N	%	N	%	*p*
< 5 min	1	3	1	3	0.941
5-10 min	22	67	25	69	0.612
>10 min	10	30	10	28	0.941
Total	33	100	36	100	

N: number of participants, *p* significant at <0.05.

Among the IEM patient's, 89% vs. 65% of the control group do not get vigorous activity three days a week (p = 0.0001). Only 10% and 19% (p = 0.041) of IEM patients vs. control group respectively comply with the WHO recommendations for moderate intense physical activity. Tables 44 and 45.

Table 44: Vigorous physical activity practice (days / week), compliance with the recommendations of physical activity according to the WHO.

Days of Vigorous Physical Activity	Patients		Controls		
	N	%	N	%	*P*
< 3 days/week.	88	89	64	65	**0.002**
≥3 days/week.	11	11	34	35	**0.000**
Total	99	100	98	100	

p significant at < 0.05.

Table 45: Moderate physical activity practice (hours / week), compliance with the recommendations of physical according to the WHO.

Moderate physical activity Recommendations WHO	Patients		Controls		
	N	%	N	%	*p*
< 7 hours/week	89	90	79	81	**0.022**
≥ 7 hours/week	10	10	19	19	**0.041**
Total	99	100	98	100	

p significant at < 0.05.

A positive significant correlation founded between the socioeconomic and eduction level of parents with physical activity, means that as the level of both socieconomic and education levels in parents increased more physical activty can be done by the childs. Table 46.

Table 46: Correlation between parents socieconomic level and physical activity

		Physical ctivity mins/week
Parents study level	r Pearson	**0.259****
	P	0.005
Socieconomic level	r Pearson	**0.427****
	P	0.000

**. Correlation is significant at the 0.01 level (2-tailed).

When correlating moderate and vigorous physical activity measured in minutes/week with the body composition measured by DEXA, in IEM patients, we observed a positive correlation between moderate and vigorous physical exercise and muscle mass (r = 0.314, p = 0.002; r=0.212, p =0.035). In the control group, we did not find these significant differences when we compared moderate activity with the muscle mass, while a significant correlation founded between vigorous exercise and muscle mass. Regarding the fat mass in IEM patients and controls, we recorded a negative non-significant relationship between moderate and vigorous physical activity with fat mass. Table 47.

Regarding the Pearson coefficient correlation of physical activity levels and BMD at different body sites, IEM patients and controls showed no significant correlation between the two types of physical activity with total body BMD. In the spine L2-L4 with a vigorous level of physical activity in the group, patients have significant positive relation (r= 0.227, p =0.024). For the femur significant positive correlation observed in IEM patients at femur neck with moderate and vigorous level (r= 0.0.252, p =0.012), and with vigorous activity too was positivly correlated (r= 0.221, p = 0.028). Table 47.

Table 47: Pearson coefficient correlation with body composition and BMD measured by DEXA

		Patients		Controls	
		Moderate PA	Vigorous PA	Moderate PA	Vigorous PA
Muscle mass	r Pearson	**0.314****	**0.212***	0.022	**0.486***
	p	**0.002**	**0.035**	0.836	**0.006**
Fat mass	r Pearson	-0.151	-0.149	-0.004	-0.033
	p	0.136	0.141	0.968	0.757
BMD total	r Pearson	0.119	0.108	0.077	0.022
	p	0.242	0.287	0.468	0.838
BMD L2-L4	r Pearson	0.132	**0.227***	0.114	0.052
	p	0.191	**0.024**	0.281	0.621
BMD Femur	r Pearson	**0.252***	**0.221***	0.048	0.021
	p	**0.012**	**0.028**	0.647	0.997

**. Correlation is significant at the 0.01 level.
*. Correlation is significant at the 0.05 level.
PA: Physical activity measured in minutes/week, BMD total: Bone mineral density.

5.5 Dietary Contribution and Correlation with Body Composition.

5.5.1 Total sample: IEM patients and controls dietary contribution and correlation with body composition.

Protein intake significantly very low among children with IEM comparing to the control (55.75±21.23, 75.67±4.61, $p = 0.000$), also for protein-energy, protein %, and protein total per kg of body weight ($p < 0.05$). See Figures 16 and 17.

Both groups are no different in dietary intake of fat and energy. Patients consume more carbohydrates with total intake in grams per day (234.57 ±119.76, vs. 201.79±39.26, $p = 0.013$) with a percent of carbohydrate energy from the total calories intake 88.3% compared to healthy with 52.46%. Table 48.

Among the nutrient intake, there was no significant differences in cholesterol intake between groups with low mean recorded in IEM patients than controls (172.97 vs. 179.86). Minerals intake present equal consumption between IEM patients and controls, potassium, calcium, magnesium, phosphorus, iron, selenium, and zinc. A lower significant difference in sodium intake with a mean (1111.54 mg vs. 1397.71 mg, $p = 0.000$) in patients and controls respectively, flour intake was higher in patients (mean=10.96 mg) comparing to controls (mean = 5.21 mg, $p = 0.042$). Vitamins intake of folate is differing significantly with high intake in IEM patients versus controls (283.3±155.3 µg vs. 226.13±165.3, $p = 0.015$), fat-soluble vitamins D, K, and E have no significant differences. In contrast, vitamin A was higher in IEM patient's, with a mean intake of (527.28 vs. 440.7, $p = 0.047$). Table 48.

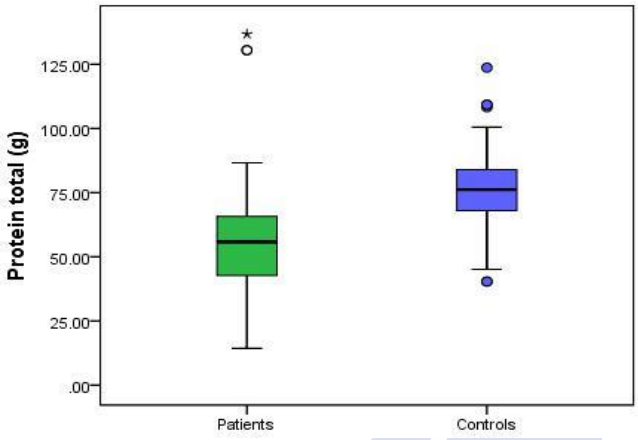

Figure 16: Mean dietary total protein intake in IEM patients and controls.

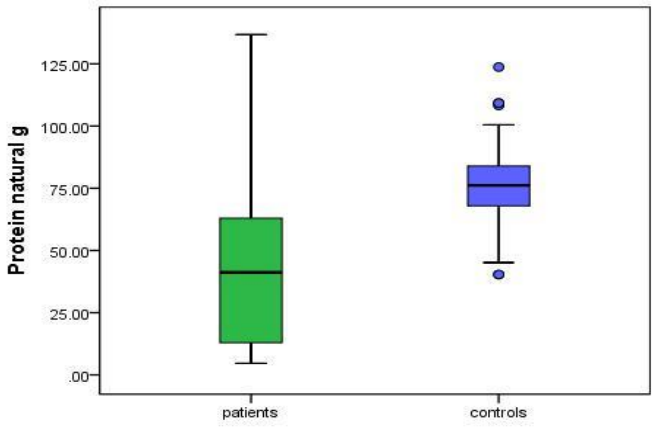

Figure 17: Mean dietary protein natural intake in IEM patients and controls.

Table 48: Means of dietary intake/day in IEM patients and controls

	Patients	Controls	
Dietary intake/day	Mean ±SD	Mean ±SD	*P*
Protein total (g)	55.75±21.23	75.67±14.61	**0.000**
Energy Protein (Kcal)	216.19±83.63	302.59±58.57	**0.000**
Energy Protein %	13.95±4.65	20.06±3.77	**0.000**
Fat Total (g)	47.96±20.21	47.73±10.86	0.802
Energy Fat (Kcal)	418.37±158.69	430.40±97.42	0.628
Energy Fat %	26.91±7.43	28.08±5.22	0.290
CH Total (g)	234.57±119.76	201.79±39.26	**0.013**
Energy CHO (Kcal)	893.84±265.69	808.76±157.07	**0.008**
Energy CHO %	88.34±5.68	52.46±5.81	0.093
Energy total (Kcal)	1566.93±399.72	1540.83±224.77	0.487
Energy (Kcal/kg)	40.99±17.06	43.09±19.49	0.489
Protein total (g/kg)	1.41±0.65	2.08±0.89	**0.000**
Cholesterol (mg)	172.97±104.99	179.86±102.62	0.647
Sodium (mg)	1111.54±346.58	1397.71±593.01	**0.000**
Potassium (mg)	1814.71±503.78	1782.63±598.12	0.688
Calcium (mg)	717.46±231.69	673.46±325.80	0.284
Magnesium (mg)	190.26±74.40	182.71±86.32	0.517
Phosphorus (mg)	879.07±273.92	874.15±323.69	0.117
Iron (mg)	11.84±7.22	10.17±7.35	0.910
Fluor (µg)	10.96±23.01	5.21±14.56	**0.042**
Selenium (µg)	64.57±29.67	66.91±34.97	0.617
Zinc (mg)	7.18±3.42	6.88±3.57	0.557
Folate (µg)	283.30±155.27	226.13±165.30	**0.015**
Vitamin B12 (µg)	5.91±6.78	6.61±15.41	0.685
Vitamin A (µg)	527.28±296.93	440.7±301.52	**0.047**
Vitamin D (µg)	1.49±0.82	1.56±1.23	0.624
Vitamin K (mg)	71.66±63.73	75.77±94.87	0.724
Vitamin E (mg)	4.51±3.52	4.16±3.47	0.491

CH: Carbohydrate, *p* significant at <0.05.

Protein intake shows a non-significant positive correlation with weight and BMI. However, protein intake has a negative correlation with FM. Still, it was non-significant; for muscle mass, a significant positive relation between protein intake and muscle mass (r =0.234) which explain that the increase of protein intake will increase the muscle mass), p <0.05. Intake of natural protein was significant correlated with muscle mass (r= 0.217, p = 0.003). Energy intakes have a significant positive correlation with body weight (r = 0.206, p <0.05). Table 49.

Table 49: Correlation of protein dietary intake with weight, BMI, FM, and muscle mass for IEM patients.

	Weight	BMI	Fat mass	Muscle mass
Protein total (g)	0.079	0.139	-0.073	**0.234***
Protein natural (g)	0.045	0.069	-0.048	**0.217****
Energy total (kcal)	**0.206***	0.130	0.099	0.088

**. Correlation is significant at the 0.01 level
*. Correlation is significant at the 0.05 level.
BMI: body mass index

Table 50 shows the correlation between dietary intake and anthropometric variables in control group. Protein intake shows a non-significant positive relationship with weight and BMI, muscle mass, and a negative correlation with fat mass. For muscle mass, a significant positive association between protein intake and muscle mass (r= 0.187) (the increase of protein intake will increase the muscle mass). Energy intake has a significant positive correlation with BMI (r= 0.235, p <0.05).

Table 50: Correlation of protein dietary intake with weight, BMI, Fat mass, and muscle mass for Controls.

	Weight	BMI	Fat mass	Muscle mass
Protein total (g)	0.121	0.098	-0.129	0.187
Protein natural (g)	0.121	0.098	-0.129	0.187
Energy total(kcal)	0.176	**0.235***	0.138	0.131

*. Correlation is significant at the 0.05 level.
BMI: body mass index

Both total protein and natural protein intake were positively and significantly correlated with total body BMD (r = 0.186, r= 0.254, p <0.01) respectively. A significant positive Pearson coefficient revealed between protein intake with trochanter (r= -0.177, p = 0.014) and ward (r= 0.171, p =0.018). Also, protein natural was positives and significant relation with femur (r = 0.213, p =0.003), trochanter (r= 0.257, p =0.000) and ward (r= 0.233, p =0.001). Both calcium and vitamin D intake have no relation with BMD. Table 51.

Table 51: Pearson correlation of dietary intake and BMD in the total sample

	BMD total	BMD L2-L4	BMD femur	BMD trochanter	BMD Ward
Protein total	**0.186****	0.047	0.130	**0.177***	**0.171***
Protein natural	**0.254****	0.140	**0.213****	**0.257****	**0.233****
Calcium	0.036	0.015	0.051	0.012	0.087
Vitamin D	0.050	0.071	0.015	0.005	0.033

**. Correlation is significant at the 0.01 level.
*. Correlation is significant at the 0.05 level.
BMD: bone mineral density.

When separating the sample, the IEM patients BMC was correlated positively with total protein, total energy, and negatively with the phosphorous intake (p < 0.01). At the same time, BMD has only a positive significant correlation with natural protein intake. BMD of the ward and trochanter had a positive relation with natural protein intake (p < 0.05). Both calcium and vitamin D intake have no relation with bone density in IEM patients. Table 52.

Table 52: Pearson correlation of dietary intake and BMD in IEM patients

		BMD total	BMC Tot g	BMD L2-L4	BMD trochanter	BMD Femur	BMD Ward
Protein total (g)	r Pearson	0.179	**0.454****	0.013	0.147	0.065	**0.198***
Protein natural(g)	r Pearson	**0.264****	0.196	0.156	**0.264****	**0.208***	**0.287****
Calcium (mg)	r Pearson	0.036	0.107	0.015	0.012	0.051	0.087
Phosphorus (mg)	r Pearson	-0.019	**-0.363****	-0.124	-0.097	-0.159	-0.007
Vitamin D (µg)	r Pearson	0.050	0.031	0.071	0.005	0.015	0.033

**. Correlation is significant at the 0.01 level.
*. Correlation is significant at the 0.05 level.
BMD: bone mineral density, BMC: bone mineral content

Among the controls group BMC, BMD trochanter, BMD femur, and BMD ward not correlated significantly with total protein, total energy, and phosphorous intake, protein natural, calcium, and vitamin D intake. BMD has only a positive significant correlation with energy intake. BMD of the spine was having a significant positive relation with vitamin D intake (r =0.263, *p* =0.011). Table 53.

Table 53: Pearson correlation of dietary intake and BMD in controls

		BMD total	BMC total g	BMD L2-L4	BMD trochanter	BMD femure	BMD ward
Protein total (g)	r Pearson	0.046	0.144	0.045	0.080	0.013	0.032
Protein natural (g)	r Pearson	0.046	0.144	0.045	0.080	0.013	0.032
Calcium (mg)	r Pearson	0.076	0.142	0.094	0.080	0.106	0.163
Phosphorus (mg)	r Pearson	-0.068	-0.104	-0.079	-0.017	-0.029	-0.003
Vitamin D (µg)	r Pearson	0.036	0.021	**0.263***	0.082	0.147	0.122

*. Correlation is significant at the 0.05 level.
BMD: bone mineral density, BMC: bone mineral content

Binary logistic regression indicates that protein total, protein natural, total energy intake, and protein /kg body weight are significant predictors of the status of IEM patients and controls [Chi-Square= 113.119, df= 6 and p =0.000 (p <0.05)]. All four predictors explain 59.6% of the variability of patients and controls. Dietary intake of protein and energy are significant at the 5% level [protein total intake, Wald=3.795, p =0.040; protein natural intake, Wald= 3.797, p =0.040]; [energy total, Wald= 5.364, p =0.021]; and [protein total kg, Wald= 5.830, p =0.000]. The odds ratio (OR) for protein total is 0.860, and protein natural is 1.614, energy total 0.997, and protein total/kg 2.666. The model correctly predicted 78.8% of IEM patients and 88% of controls, giving an overall percentage correct prediction rate of 83.2%. Both protein total/kg, and protein natural considers the most important factors in predicting patients and controls, in which IEM patients are 2.7 times lower in protein total g/kg than controls and 1.6 times lower in protein natural than controls, coefficients negative for carbohydrate and energy intake indicate that patients consumed more carbohydrate and energy than controls, but they consumed more fat as the coefficient is positive. Table 54.

Table 54: Results of binary logistic regression analysis of patients with IEM and controls

	Coefficient	S.E.	Wald	*P*	OR
Protein total (g)	0.088	0.286	3.795	**0.040**	0.860
Protein natural (g)	0.015	0.269	3.797	**0.040**	1.614
Fat total (g)	0.022	0.018	1.584	0.208	1.023
CH total (g)	-0.006	0.004	2.250	0.134	0.994
Energy total (kcal)	-0.003	0.001	5.364	**0.021**	0.997
Protein total (g/kg)	0.726	0.300	5.830	**0.016**	2.666

R2=0.220
S.E.: standard error, OR: odd ratio; p significant at <0.05, CH: carbohydrate
Dependent variable :(0: patients, 1: controls).
Independent variables: protein total, protein natural, fat total, CH total, energy total, protein total (g/kg).

5.5.2 Intermediary metabolisn disorders dietary contribution and correlation with BMD.

According to intermediary metabolism disorders, daily dietary intake varies with category. The lowest significant protein intake observed in AA metabolism disorders 50.38 g/d (p =0.000), also significant difference presented with the highest amount of fat consumed by CH metabolism disorders patients (60.48 g/d vs. 44.58 g/d in controls), while FAO metabolism disorders patients consumed less fat than controls (38.09, p =0.033). CH intake was significantly higher in AA metabolism and CHD disorders comparing to controls. Total energy intake per day was not significantly differ between intermediary groups and controls. Table 55.

Table 55: Means of dietary intake/day in intermediary metabolism disorders.

		Patients		Controls		
Dietary intake/day	Diseases category	N	Mean ±SD	N	Mean ±SD	*p*
Protein total (g)	AA	77	50.38±15.9	98	75.67±14.6	**0.000**
	FAO	10	83.29±27.26	20	74.55±14.97	0.753
	CHD	12	67.21±24.9	20	79.17±15.3	0.821
Protein natural (g)	AA	77	32.18±23.57	98	75.67±14.6	**0.000**
	FAO	10	83.29±27.26	20	74.55±14.97	0.658
	CHD	12	67.21±24.9	20	79.17±15.3	0.835
Protein (g/kg)	AA	77	1.23±0.45	98	2.08±0.89	**0.000**
	FAO	10	2.03±0.58	20	2.297±0.95	0.432
	CHD	12	2.08±1.02	20	1.99±0.64	0.421
Fat total (g)	AA	77	47.67±18.11	98	47.73±10.86	0.954
	FAO	10	38.09±13.11	20	49.04±9.27	**0.033**
	CHD	12	60.48±32.24	20	44.58±9.3	**0.002**
CH total(g)	AA	77	237.63±127.95	98	201.79±39.26	**0.012**
	FAO	10	243.73±94.66	20	209.92±41.22	0.654
	CHD	12	216.12±78.46	20	199.31±34.55	**0.004**
Energy total (Kcal)	AA	77	1541.75±386.65	98	1540.83±224.77	0.945
	FAO	10	1695.64±480.22	20	1587.58±237.04	0.854
	CHD	12	1678.12±406.34	20	1516.69±200.7	**0.027**
Energy (Kcal/kg)	AA	77	39.34±16.39	98	43.09v19.49	0.843
	FAO	10	42.4712.89	20	49.12±17.88	0.895
	CHD	12	52.48±22.56	20	38.67±13.37	0.756

AA: amino acid, CHD: carbohydrate disorders, FAO: fatty acid oxidation, *P* significant at <0.05.

When studying the relationships between dietary intake and BMD in patients with IEIPM, daily dietary intake of protein natural founded to be correlated to bone density with positive and significant relation with $p< 0.01$ in BMD total and BMD trochanter, and with $p < 0.05$ with BMC, spine, femur, and ward. Both protein and energy total intake correlated with BMC with positive significant and seemed to be higher in protein (r=0.61 vs. r=0.339), respectively. A negative correlation between BMC and BMD trochanter and phosphorus intake, which means high dietary intake of this mineral, could cause low bone density as high dietary intake of phosphorus will deplete calcium from bones, especially if accompanied with low calcium intake. Table 56.

Table 56: Pearson correlation of dietary intake and BMD in patients with IEIPM

		BMD total	BMC tot g	BMD L2-L4	BMD Femur	BMD Ward	BMD trochanter
Protein total (g)	r Pearson	0.168	**0.610****	0.034	0.036	0.126	0.052
Protein natural (g)	r Pearson	**0.296****	**0.227***	**0.257***	**0.256***	**0.274***	**0.295****
Calcium (mg)	r Pearson	0.117	0.143	0.083	0.162	**0.257***	0.206
Phosphorus (mg)	r Pearson	-0.095	**-0.336****	-0.203	**-0.288***	-0.111	**-0.276***
Vitamin D (µg)	r Pearson	0.008	0.083	0.027	0.001	0.066	0.011

**. Correlation is significant at the 0.01 level.
*. Correlation is significant at the 0.05 level.
IEIPM: Inorn error of intermediary protein metabolism, BMD: bone mineral density, BMC: bone mineral content.

5.5.2.1 Adherence to dietary treatment in PKU patient

The sample consists of 26 patients with PKU (12 females, 14 males) with age range 6-18 years old (12.15 4.35). Several clinical phenotypes are distinguished according to the levels of Phe at the diagnosis: Classical PKU: Phe > 20 mg / dl (> 1,200 μmol / L).

In our sample, we have sixteen patients with classic PKU, ten with mild-moderate PKU. Eleven patients are treated with BH4. Table 57.

Table 57: General characteristics of PKU patients

Mean age	12.15 ± 4.35
Sex (Female/Male)	12 F / 14 M
Phenotype	
Classic	16 (61.5%)
Mild/Moderate	10 (28.5%)
Treatment with BH4	11 (42.3%)
Mean weight (kg)	43.22 ±16.23
Mean height (m)	154.94 ±10.16)
BMI (Kg/m^2)	21.22 ±4.75
Underweight	7 (26.9%)
Normal weight	16 (61.5%)
Overweight	3 (11.5 %)

BMI z-score mean was significantly different in the total sample between females and males, classic PKU patients presented a significant difference among sex with the lowest BMI was recorded in females. Table 58.

Table 58: Mean anthropometric in PKU patients females and males.

	Females	Males	*p*
Age	13.1±4.6	11.7±4.1	0.401
Classic PKU	12.8±5.1	12.3±2.7	0.785
Moderate PKU	13.96±3.4	11.1±5.4	0.376
Phe µmol/L	6.49±4.6	5.33±3.13	0.453
Classic PKU	8.07 ±4.96	6.04±3.8	0.374
Moderate PKU	3.3±0.7	4.4±1.7	0.285
Weight	0.11±1.05	0.47±0.77	0.204
Classic PKU	0.54±0.96	0.88±0.76	0.436
Moderate PKU	-0.76±0.65	0.26±1.1	0.143
Height	-0.55±1.08	-0.58±0.83	0.933
Classic PKU	-0.44±1.15	-0.16±0.93	0.564
Moderate PKU	-0.78±1.07	-1.15±0.66	0.506
BMI	0.06±0.94	0.91±0.95	**0.044**
Classic PKU	-0.09±0.96	0.97±0.9	**0.041**
Moderate PKU	0.36±0.83	0.83±1.2	0.314

P significant at <0.05, BMI: Body mass index.

FM was significantly different in classic PKU patients with the lowest z-score recorded in females -0.77±0.78 versus 1.54±2.67 in males, $p = 0.034$. BMD total was lower in males with classic PKU than in females. In patients with moderate PKU, ward BMD was lower in females than in males. Table 59.

Table 59: Body components and BMD measured by DEXA

	Females	Males	*p*
Muscle mass	-0.21±1.86	0.08±1.11	0.621
Classic PKU	0.26±0.99	0.08±0.96	0.722
Moderate PKU	-1.16±2.93	0.08±1.38	0.385
Fat mass	-0.44±1.03	1.09±2.48	0.058
Classic PKU	-0.77±0.78	1.54±2.67	**0.034**
Moderate PKU	0.22±1.26	0.51±2.29	0.832
BMD total body	0.84±0.59	1.06±0.76	0.422
Classic PKU	0.89±0.59	0.55±0.19	**0.041**
Moderate PKU	0.74±0.65	0.54±0.71	0.667
BMD spine L2-L4	0.56±0.41	0.69±0.41	0.391
Classic PKU	0.49±0.46	0.84±0.43	0.136
Moderate PKU	0.71±0.24	0.52±0.49	0.362
BMD femur	0.34±1.18	0.46±0.43	0.717
Classic PKU	0.36±1.47	0.49±0.42	0.815
Moderate PKU	0.28±0.36	0.42±0.49	0.646
BMD trochanter	0.38±1.73	0.66±0.73	0.577
Classic PKU	0.38±2.15	0.69±0.61	0.702
Moderate PKU	0.37±0.45	0.63±0.92	0.618
BMD ward	-0.34±0.66	0.12±0.42	**0.043**
Classic PKU	-0.26±078	0.11±0.44	0.254
Moderate PKU	-0.49±0.34	0.12±0.45	**0.049**
BMC	1.88±0.49	2.01±0.52	0.424
Classic PKU	1.82±0.48	1.88±0.63	0.829
Moderate PKU	2.01±0.55	2.19±0.28	0.501

BMD: Bone mineral density, BMD: Bone mineral content, *p* significant at < 0.05.

No significant differences were observed in dietary intake of energy, fat %, CH %, and total protein according to sex in classic and moderate PKU patients. Protein natural intake was significantly different between sex in classic PKU with the lowest consumption observed in females (8.94±2.36) than in males (12.45±2.83). Table 60.

Table 60: Mean dietary intake/day in classic and moderate PKU patients

	Females	Males	*p*
Energy total kcal	1910.13±384.06	1580.48±445.8	0.192
Classic PKU	1679.26±288.66	1681.56±568.68	0.771
Moderate PKU	2011.93±623.33	1291.6±333.49	**0.047**
Protein total (g)	52.06±15.27	46.28±13.61	0.335
Classic PKU	45.8±10.3	49.28±11.2	0.529
Moderate PKU	63.33±17.3	41.04±17.6	0.098
Protein total g/kg	1.38±0.46	1.06±0.21	0.189
Classic PKU	1.13±0.3	1.03±0.2	0.487
Moderate PKU	1.71±0.19	1.26±0.68	0.198
Protein natural (g)	34.34±24.06	27.28±21.11	0.449
Classic PKU	8.94±2.36	12.45±2.83	**0.017**
Moderate PKU	63.33±17.04	41.04±17.6	0.098
Fat energy %	26.57±6.63	28.63±5.47	0.274
Classic PKU	25.42±6.74	26.39±6.47	0.775
Moderate PKU	27.56±4.28	34.04±7.19	0.135
CHO energy %	60.07±7.12	61.5±8.04	0.205
Classic PKU	63.72±6.13	66.19±9.9	0.547
Moderate PKU	64.0±7.92	54.66±6.09	0.189
Energy kcal/kg	48.09±18.46	38.38±17.71	0.197
Classic PKU	43.61±18.05	35.41±9.76	0.299
Moderate PKU	56.19±18.14	43.6±28.23	0.442

p significant at < 0.05.

The caloric intake and the total protein intake are also collected, differentiating between natural sources, and that comes from a supplement. The values were compared with the RDA reference values for matched age, (Organization, and University, 2007). These recommendations indicate the daily intake that is considered sufficient to meet the needs of a nutrient.

First, the contribution of carbohydrates is collected as a percentage of the total caloric. The values provided by the patients range between 46-78% of CH. Regarding fat intake, it ranges between 16-39%. These ranges are far from the data that diets usually follow for the general population, which are often 45-65 % of CH and 25-35% of lipids over the total caloric intake. PKU patients consumed more CH than fat, in addition to their protein modified diet. Finally, the protein intake is collected, we founded that the total protein intake ranges from 0.75 to 1.93 g / kg/day, depending on the Phe tolerance of each patient. Table 61.

Bad tolerance is seen in 4 patients with classic PKU, despite that the amount of dietary intake protein is low, the median level of Phe in blood was > 600 μmol/L (717.4, 700.8, 1143.6, 676.5 μmol/L, respectively), all the patients are adolescent with age (15,15,17,18 years old). Generally, protein consumption is higher in most patients, especially in those PKU patients with BH4 treatment, low protein consumption founded to be lower recommended in four patients only. Table 61.

Table 61: Nutritional characteristics of PKU patients

Sex	Age years	PKU phenotype	Treatment with BH4	Energy kcal (RDA)	Prot total/ kg/day (RDA)	Protein natural /kg/day	Energy Fat %	Energy CH %	Median Phe µmol/l (r.v))
F	11	Mild	Yes	2624.41 (1650-3330)	1.9 (0.9)	0.88	32.2	45.9	172 (<480)
F	11	Mild	Yes	2642.4 (1650-3330)	1.93 (0.9)	0.87	36.8	52.98	178.6 (<480)
F	7	Classic	No	1547.87 (1300-2300)	1.75 (0.87)	0.38	34	60.5	311.8 (<480)
F	7	Classic	No	1540 (1300-2300)	1.9 (0.87)	0.36	33	61.2	333 (<480)
F	8	Classic	No	1537.18 (1300-2300)	1.1 (0.87)	0.26	18.52	70.53	280 (<480)
M	13	Classic	No	1272.81 (1650-3330)	0.99 (0.92)	0.29	19.38	78.08	206.4 (<600)
M	13	Classic	No	1102.55 (1650-3330)	0.83 (0.92)	0.25	23.76	72.44	293.6 (<600)
M	15	Classic	No	2267.24 (1400-2500)	0.91 (0.88)	0.25	37.51	53.44	717.4 (<600)
F	17	Classic	No	1553.49 (1400-2500)	0.79 (0.83)	0.08	22.57	63.3	700.8 (<600)
F	18	Classic	No	2077.7 (1400-2500)	0.75 (0.84)	0.17	32.65	55.9	1143.6(<600)
M	7	Classic	No	1313.02 (1300-2300)	1.39 (0.87)	0.28	25.4	62	123.5 (<480)
M	7	Classic	No	1476.5 (1300-2300)	1.3 (0.87)	0.29	39.4	51	127.1 (<480)
F	9	Classic	Yes	1918 (1300-2300)	1.27 (0.87)	0.27	28.7	63	300.9 (<480)
F	17	Classic	No	1855 (1400-2500)	1.07 (0.83)	0.15	16.4	72	372.3 (<600)
F	17	Classic	No	1940.5 (1400-2500)	0.89 (0.83)	0.14	34	56	464.6 (<600)
M	15	Classic	No	2051.4 (1400-2500)	0.91 (0.88)	1.30	29.3	60.32	676.5 (<600)
M	14	Classic	No	2491.1 (1650-3330)	1.22 (0.89)	1.55	30	59	458.6 (<600)
F	15	Mild	Yes	1829 (1400-2500)	1.56 (0.85)	0.21	24	60	190.5 (<600)
M	8	Moderate	Yes	1275.13 (1300-2300)	1.28 (0.87)	0.34	30.05	59.94	142.3 (<480)
M	7	Moderate	Yes	1262.86 (1300-2300)	0.79 (0.87)	0.27	22.57	63.7	387.5 (<480)
M	8	Moderate	Yes	1918 (1300-2300)	1.27 (0.87)	0.34	28.7	63	157.4 (<480)
M	6	Moderate	Yes	1607.58 (1300-2300)	1.19 (0.87)	1.60	29.63	53.19	326.6 (<360)
F	18	Moderate	Yes	1856 (1400-2500)	1.7 (0.82)	0.90	30	59.5	263.4 (<600)
M	18	Moderate	Yes	1266.03 (1400-2500)	0.9 (0.85)	0.29	30.1	58.3	226.1 (<600)
M	17	Mild	Yes	1720 (1400-2500)	0.98 (0.86)	0.54	31.2	54.1	351.1 (<600)
M	12	Classic	No	1102.55 (1650-3330)	0.83 (0.92)	0.22	23.76	72.44	322.4 (<480)

5.6 Biochemical and Haematological Markers.

5.6.1 Total sample: IEM patients and controls biochemical and haematological markers.

The laboratory findings of IEM patients and control groups, mean serum vitamin E (1.69 mg/dL) was found to be lower in patients than the control group (4.38 mg/dL) (p = 0.003). Selenium level also was lower in IEM patients than controls (76.37±18.01 vs. 85.46±8.01 mg/dL, p = 0.000). The frequency of selenium levels below the reference range was higher in IEM patients with 36.4% of them. Mean serum folate levels were found to be increased in patients compared to the control group (17.82 vs. 8.99 ng/mL, p = 0.000). A high level was presented in 44.4% of the IEM patients. Table 62.

Table 62: Mean of biochemical blood analysis for IEM patients and controls

	Patients	Controls	
Blood variable	Mean± SD	Mean± SD	*P*
Prealbumin (mg/dL)	22.98±5.59	22.66±4.56	0.667
RBP (mg/dL)	3.51±0.97	3.72±2.35	0.421
Protein total (g/dL)	7.27±0.35	7.21±0.37	0.283
Albumin (g/dL)	4.64±0.214	4.69±0.207	0.798
Calcium (mg/dL)	9.77±0.33	9.76±0.29	0.771
Zinc (µg/dL)	101.69±17.63	103.3±12.44	0.468
Selenium (µg/L)	76.37±18.01	85.46±8.01	**0.000**
Iron (µg/dL)	89.8±33.19	89.55±34.81	0.967
Ferritin (ng/mL)	38.01±27.64	40.48±38.93	0.620
Transferrin (mg/dL)	291.08±43.01	264.34±46.96	0.685
Vitamin A (mg/dL)	0.39±0.14	0.36±0.11	0.325
Vitamin D (ng/mL)	23.86±11.58	22.33±6.8	0.272
Vitamin E (mg/dL)	1.69±2.01	4.38±4.81	**0.003**
Vitamin k (ng/dL)	0.91±1.45	0.32±0.26	**0.003**
Folate (ng/mL)	17.82±11.41	8.99±3.79	**0.000**
VitaminB12 (pg/mL)	1621.4±8030.14	577.95±221.79	**0.046**
Cholesterol (mg/dL)	157.86±26.44	165.6±27.12	**0.047**
TG (mg/dL)	84.2±43.39	59.77±27.57	**0.000**
HDL (mg/dL)	56.92±51.51	57.71±11.55	0.888
LDL (mg/dL)	87.78±19.65	107.88±110.95	0.126

P significant at <0.05, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, RBP: Retinol Binding Protein, TG: Triglyceride

According to the reference values for serum vitamin D, the frequency of blood analysis values shows a high deficiency and insufficiency of the vitamin in both groups of IEM patients and controls (44.4%, 44.6), respectively. No defect of B12 observed in both groups. Two IEM patients versus seven control presents with vitamin A deficiency. Vitamin K was low in five patients and eleven controls. More normal values were present in controls too. Vitamin A with average values was more frequent in controls than IEM patients with significant differences ($p = 0.021$). Table 63.

Table 63: Frequency of blood analysis for vitamins according to reference values

		Patients		Controls		
		N	%	N	%	*p*
Vitamin D (ng/mL)	deficiency	5	5.1	2	2.2	0.456
	insufficient	39	39.4	39	42.4	0.888
	recommended	55	55.6	51	55.4	0.954
Folate (ng/mL)	low	2	2	3	3.3	0.745
	normal	53	53.4	85	92.4	**0.000**
	high	44	44.4	4	4.3	**0.000**
Vitamin B12 (pg/mL)	low	0	0	0	0	0.945
	normal	96	97	92	100	0.756
	high	3	3	0	0	0.256
Vitamin A (mg/dL)	low	2	2	7	7.6	0.315
	normal	31	1	53	57.6	**0.021**
	high	1	1	0	0	0.756
Vitamin E (mg/dL)	low	1	1	0	0	0.843
	normal	13	13.1	14	15.2	0.542
	high	20	20.2	45	48.9	**0.030**
Vitamin K (ng/dL)	low	5	5.1	11	12	**0.038**
	normal	21	21.2	51	55.4	**0.002**
	high	5	5.1	0	0	0.621

p significant at < 0.05.

Selenium deficiency presented in 36 IEM patients (36.4%) while in controls only one child has selenium deficiency ($p = 0.000$) and more controls are within normal values than IEM patients (n=88 vs. n=62). Iron and transferrin normal values are more frequent in controls than

patients with a significant difference; TG serum values are higher while cholesterol is lower in IEM patients than in controls (157.86 mg/dL vs. 165.6 mg/dL, $p = 0.047$). Table 64.

Table 64: Frequency of blood analysis for nutrients and minerals according to reference value

		Patients		Controls		
		N	%	N	%	*p*
Selenium (µg/L)	low	36	36.4	1	4.3	**0.000**
	normal	62	62.6	88	95.7	**0.026**
	high	1	1	0	0	0.845
TG (mg/dL)	low	0	0	1	1	0.832
	normal	90	90	90	97.8	0.945
	high	9	9	1	1.1	**0.027**
Cholesterol (mg/dL)	low	10	10.1	1	1.1	**0.026**
	normal	89	89.9	91	98.9	0.712
	high	0	0	0	0	0.945
Zinc (µg/dL)	low	1	1	0	0	0.888
	normal	93	93.9	91	98.9	0.768
	high	5	5.1	1	1.1	0.712
RBP (mg/dL)	low	30	30.3	24	26.1	0.546
	normal	68	68.7	61	66.3	0.658
	high	1	1	1	1.1	0.921
Prealbumin (mg/dL)	low	0	0	32	34.8	**0.002**
	normal	32	32.3	60	65.2	**0.003**
	high	67	67.7	0	0	**0.000**
Calcium (mg/dL)	low	0	0	0	0	0.941
	normal	99	100	92	100	0.765
	high	0	0	0	0	0.955
Ferritin (ng/mL)	low	1	1	3	3.3	0.845
	normal	93	94	87	94.6	0.801
	high	0	0	0	0	0.955
Iron (µg/dL)	low	3	3	5	5.4	0.765
	normal	47	47.5	85	92.4	**0.004**
	high	1	1	2	2.2	0.895
Transferrin (mg/dL)	low	1	1	2	2.2	0.865
	normal	27	27.3	47	51.1	**0.034**
	high	3	31.3	10	10.9	0.057

RBP: Retinol binding protein, TG: Triglyceride, *p* significant at <0.05.

Binary logistic regression analysis, with the dependent variable groups (patients versus controls) and the independent variables serum triglyceride, serum folate, and serum selenium, was conducted identified with significant model (chi-square = 75.519, *p* =0.000). The logistic regression analysis identified three risk factors for this pathology: serum triglyceride, serum folate, and serum selenium level.

According to the results, the overall variables predicted correctly at 76.7%. They founded that serum selenium level is expected to be almost 1.54 times higher in the control group compared with the IEM patients. In contrast, serum folate is expected to be 0.86 times lower in the control group than in patients, while serum triglyceride is expected to be lower in controls than the patients. Table 65.

Table 65: Results of binary logistic regression analysis of patients with IEM and controls

	Coefficient	S. E	Wald	*P*	OR
Serum Triglycerides	-0.018	0.006	9.901	**0.002**	0.982
Serum Folate	-0.146	0.032	20.815	**0.000**	0.864
Serum Selenium	0.042	0.015	7.702	**0.006**	1.043

R^2=0.220, *p* significant at < 0.05.
S.E.: standard error, OR: odd ratio, Dependent variable :(0: patients, 1: controls).
Independent variables: serum triglyceride, serum folate, serum selenium.

5.6.2 Biochemical and haematological markers in intemediary metabolism disorders.

In AA metabolism disorders a significant difference was founded in serum transferrin which was lower in AA metabolism disorders patients than in controls (298.68±39.62 vs. 577.95±221.79 mg/dL, *p* = 0.039), cholesterol which was also lower in AA metabolism disorders (156.82±25.47 vs. 165.6±27.12 mg/dL, *p* = 0.043). At the same time, TG was higher in patients with AA metabolism disorders than controls (81.14±39.24 vs. 57.71±27.57 mg/dL, *p* = 0.000).

Among FAO metabolism disorders ferritin was significantly lower in FAO metabolism patients compared to controls (65.33±45.41 vs. 92.00±20.72 ng/dL, *p* = 0.024), TG was higher in the FAO metabolism

patients than in controls (74.2±44.2 vs. 58.08±23.42, $p = 0.032$ mg/dL), in the other hand HDL was lower in FAO metabolism patients than in controls (42.8±10.71 vs. 57.08±9.45mg/dL, $p = 0.025$). High serum TG level founded in CHD patients than in controls (111.92±86.96 vs. 70.00±29.01 mg/dL, $p = 0.041$), while LDL was lower in CHD metabolism patients than controls (86.00±20.07 vs. 155.08±185.36, mg/dL, $p = 0.043$). Table 66.

Table 66: Mean of biochemical blood analysis for intermediary metabolism disorders.

		Patients	Controls	
	Disorders	Mean± SD	Mean± SD	*P*
Prealbumin (mg/dL)	AA	23.18±5.1	22.66±4.6	0.485
	FAO	23.1±7.7	23.92±4.52	0.764
	CHD	21.58±6.86	24.15±4.89	0.399
RBP (mg/dL)	AA	3.6±0.94	3.7±2.4	0.701
	FAO	3.00±1.16	3.75±0.75	0.082
	CHD	3.42±0.99	5.31±5.68	0.139
Protein total (g/dL)	AA	7.26±0.44	7.24±0.46	0.914
	FAO	7.3±0.48	7.42±.52	0.892
	CHD	7.58±0.67	7.31±0.48	0.746
Albumin (g/dL)	AA	4.84±0.37	4.9±0.29	0.257
	FAO	4.8±0.42	5.00±0.01	0.914
	CHD	4.83±0.39	4.92±0.28	0.811
Ferritin (ng/mL)	AA	33.62±23.19	40.49±38.93	0.061
	FAO	65.33±45.41	92.00±20.72	**0.024**
	CHD	45.18±25.55	38.08±24.89	0.268
Transferrin (mg/dL)	AA	298.68±39.62	577.95±221.79	**0.039**
	FAO	247.00±52.33	288.75±26.58	0.091
	CHD	264.13±45.84	290.08±24.89	0.107
Cholesterol (mg/dL)	AA	156.82±25.47	165.6±27.12	**0.043**
	FAO	171.4±14.18	170.25±23.43	0.894
	CHD	153.25±37.18	169.00±30.86	0.061
TG (mg/dL)	AA	81.14±39.24	57.71±27.57	**0,000**
	FAO	74.2±44.2	58.08±23.42	**0.032**
	CHD	111.92±86.96	70.00±29.01	**0.041**
HDL (mg/dL)	AA	59.38±56.86	57.71±11.55	0.787
	FAO	42.8±10.71	57.08±9.45	**0.025**
	CHD	52.36±20.69	60.17±10.24	0.258
LDL (mg/dL)	AA	86.16±19.08	107.88±110.9	0.142
	FAO	110.2±13.06	101.58±22.84	0.446
	CHD	86.00±20.07	155.08±185.36	**0.043**

AA: amino acids, CHD: carbohydrate disorders, FAO: fatty acids disorders, HDL: high density lipoprotein, LDL: low density lipoprotein, RBP: retinol binding protein,
TG: Triglyceride. *P* significant at < 0.05.

Vitamin K among AA metabolism disorders patients compared to controls with a lower concentration (0.91±1.45 vs. 0.32 ±0.26 mg/dL, *p* = 0.033). High vitamin B12 serum level founded in AA metabolism disorders patients (1895.69±909.25 vs. 577.95±221.79 pg/mL, *p* = 0.021). A significant high folate level in AA metabolism disorders patients compared to controls (20.33±11.56 vs. 9.00±3.81 ng/dL, *p* = 0.000). Selenium serum level was lower in AA metabolism disorders patients than in controls (74.55±18.68 vs. 85.46±8.01 μg/L, *p* = 0.011).

FAO metabolism disorders observed with significant low iron (56.01 vs. 92.01 μg/dL), vitamin E (1.04 vs. 4.38 mg/dL) and vitamin D (15.9 vs. 20.92 ng/mL). No significant differences recorded in CHD. Table 67.

Table 67: Means of serum blood vitamins and minerals analysis for intermediary metabolism disorders.

	Disorders	Patients Mean± SD	Controls Mean± SD	*P*
Vitamin A (mg/dL)	AA	0.39±0.14	0.36±0.11	0.391
	FAO	0.34±0.12	0.36±0.11	0.451
	CHD	0.33±0.11	0.36±0.11	0.431
Vitamin D (ng/mL)	AA	25.36±12.14	22.33±6.80	0.062
	FAO	15.9±7.32	20.92±4.23	**0.045**
	CHD	20.83±6.97	21.00±4.95	0.745
Vitamin E (mg/dL)	AA	3.69±2.00	4.38±4.81	0.812
	FAO	1.04±0.29	4.38±4.81	**0.003**
	CHD	3.98±3.2	4.38±4.8	0.745
Vitamin k (ng/dL)	AA	0.91±1.45	0.32±0.26	**0.033**
	FAO	0.21±0.42	0.32±0.26	0.455
	CHD	0.31±0.25	0.32±0.26	0.741
Folate (ng/mL)	AA	20.33±11.56	9.00±3.81	**0.000**
	FAO	10.4±5.85	10.75±4.71	0.878
	CHD	8.33±3.94	9.46±3.48	0.712
VitaminB12 (pg/mL)	AA	1895.69±909.25	577.95±221.79	**0.021**
	FAO	648.7±168.5	658.67±374.8	0.939
	CHD	672.00±214.74	728.46±329.34	0.514
Calcium (mg/dL)	AA	9.78±0.33	9.76±0.29	0.853
	FAO	9.75±0.27	9.84±0.27	0.745
	CHD	9.75±0.38	9.92±0.26	0.565
Zinc (µg/dl)	AA	101.6±16.9	103.3±12.44	0.455
	FAO	101.05±0.12	104.67±11.51	0.544
	CHD	99.75±23.85	108.00±13.74	0.268
Selenium (µg/L)	AA	74.55±18.68	85.46±8.01	**0.011**
	FAO	83.03±5.56	84.75±10.74	0.245
	CHD	82.14±13.87	88.15±7.91	0.192
Iron (µg/dL)	AA	94.69±33.12	89.55±34.81	0.423
	FAO	56.01±2.83	92.01±0.27	**0.035**
	CHD	70.14±26.14	85.31±24.41	0.212

AA: amino acids, FAO: fatty acid oxidation, CHD: carbohydrate, *P* significant at <0.05)

6. DISCUSSION

6.1 Anthropometric Characteristics.

This study shows significant findings in body composition measurements of IEM patients, such as reduced height, increased waist circumferences, increased prevalence of overweight/obesity, and increased FM.

An important finding of this study is that IEM patients have significantly lower height z-scores than controls (z-score mean= -0.28±1.23 vs. 0.16±0.93), with the lowest z-score recorded was -2.94. In this study, we found that height is lower in males patients comparing to controls males. This agrees with a study of Wilcox *et al*., in which they reported a reduced height in both sexes of patients with IEIPM compared to controls (Wilcox *et al*., 2005).

CHD presented the lowest height value z-score (z-score mean= -1.173±1.04) with a significant difference from the control group, followed by the AA group. Panis *et al*., showed in children aged 3-17 years old with classic galactosemia on dietary treatment a significantly decreased mean height z-score ($p< 0.001$) (Panis *et al*, 2004). Reduced height and weight-for-height in childhood and early adolescence have been reported in treated classic galactosemia patients aged from 2 weeks to 37 years by Waggoner *et al.,* (Waggoner *et al*., 1990). In the same line, Schweitzer *et al*. showed decreased height and weight in patients with classic galactosemia aged from 9 months to 33 years (Schweitzer *et al*., 1993). Pronicka *et al*., showed in children with HFI, aged 3-20years old, height deficiency with the lowest recorded z-score was -2.6. Those patients were followed a diet restricted in fructose, but 6 of them have a daily fructose intake above the recommended (2 g/day). A height deficit of HFI patients could be due to the presence of trace amounts of fructose in the diet or to quantitative and qualitative dietary deficiencies that are a side effect of diet elimination (Pronicka *et al*., 2007). Several authors have reported reduced height as common problems in GSD patients. Height was significantly reduced in patients with GSD I and III, with a median age of 11 years old compared to

controls (Dos Santos, B. B., *et al*., 2017). Also, height was significantly reduced in the European Study (ESGSD I) for patients with GSD I (ESGSD I), median age 10.4 years (Rake *et al*., 2007).

Regarding AA disorders, multiples early studies showed differences in body composition parameters to children with PKU, MMA, PA, and UCD (Batshaw *et al*., 2014; Hauser *et al*., 2011; Evans *et al.*, 2017). Growth outcomes in MMA were poor in height, and body composition showed a significantly increased percentage of FM (Manoli *et al*., 2016). Evans *et al.* found that patients with MMA/PA had the lowest median height and weight z-scores (Evans *et al.*, 2017). In contrast, patients with UCD presented normal weight but decreased linear growth. The differences in dietary patterns could explain this fact. UCD patients consumed a diet with restriction of total proteins and, consequently, higher CH and lipids Although MMA/PA is consuming a diet with restriction of a natural protein, but not total protein in the total caloric value (Batshaw *et al*., 2014).

Reduced height z-scores were reported in PKU patients (Aldámiz-Echevarría *et al*., 2014; Couce *et al*., 2015). Other studies found similar weight and BMI z-scores in PKU and controls (Allen *et al*., 1995; Adamczyk *et al*., 2011; Belanger-Quintana and Martínez-Pardo, 2011). However, it was also reported that children with PKU weight more than control (White *et al.*, 1982; Scaglioni; Giovannini *et al*., 2007). One study on children with PKU showed lower weight than controls (Dobbelaere *et al*., 2003). These differences could be due to the type of PKU, complaints to treatment, and the number of dietary products with phenylalanine restriction consumption and small cohorts with limited statistical power.

According to the FAO metabolism disorders, our study recorded the highest weight z-score comparing with control. Few reports have studied obesity in FAO, and most of them only documented the problem as a trend rather than a statistically significant issue without exploring its consequences. The diet regimen in FAO disorders based on reduced fat and diet that applied to prevent hypoglycemia by increased food consumption. This diet records a tendency to overweight; this has been

observed in several studies with MCADD (Derks *et al*., 2006). But normal growth and weight have been recorded in other studies in MCADD patients (Wilcken *et al*., 2009). Although IEM patients trend for increased, we found no differences in weight and BMI z-scores mean between patients and controls, probably in relation to that more percentage of patients are within the highest and lowest percentage than controls. In order to know adiposity, we could evaluate BMI; in this study, the WHO BMI percentage and z-scores were used to assess overweight and obesity in children. 25.65% of IEM patients having a BMI percentage above 85th when compared to controls, only 6.3% having BMI above 95th.

AA and FAO disorders presented a significant higher BMI z-score comparing to controls. In CHD, no significant difference in BMI z-score means and controls; however, 4 out of 12 patients with CHD tend to have higher BMI percentile >85th.

The prevalence of overweight and obesity was higher in PKU patients aged 10–16 years than controls of similar age (Rocha, J. C., *et al*., 2012). Couce *et al*., showed that BMI z-score in PKU patients had a high value in 37 patients (26.24 %) [25 (67.6 %) were overweight, and 12 (32.4 %) were obese (z-score ≥2)] (Couce *et al*., 2016). On the other hand, the PKU population on a low phenylalanine diet trend of becoming overweight and obese is very similar to the healthy UK population (Robertson *et al*., 2013).

According to FAO matbolism disorders, increased BMI z-score in our studied patient appears to be due to the high FFM z-score was presented. Multiple studies founded increased proportion of overweight in prepubertal patients (Derks *et al*., 2006; Iafolla *et al*., 1994).

High rates of excess weight (overweight 28%; obesity 40%) were found among patients with GSD, 2 of 5 patients with GSD III and IXa/b were overweight, while 5 out of 6 with GSD Ib and 10 out of 14 with GSD Ia had excess weight (overweight or obesity), (Dos Santos, B. B., *et al*., 2017). Chen *et al*., found a high frequency of obesity in a study of 13 GSD I patients treated with uncooked cornstarch (Chen *et al*., 1993).

The WC has founded to be higher in IEM patients than in controls (z-score mean= -0.08 vs. -0.58, respectively). The highest WC was found to be in the AA metabolism disorders. This result is in line with a previous study in patients with HPA, which found that WC was significantly higher in PKU patients than in the MHPA group (Couce *et al*., 2016). A similar pattern of results was obtained in PKU patients showed increased WC in 46.34% PKU patients vs. 27.9% in controls (Hermida-Ameijeiras *et al*., 2017). On the other hand, Rocha *et al*., study did not demonstrate an increased WC in PKU patients when comparing to controls (Rocha *et al.*, 2013). WC measurement is a useful method for assessing risk for obesity-related diseases. It correlates with abdominal adipose tissue, though better with total abdominal fat than intra-abdominal fat.

6.2 Body Composition Assessment

No significant differences in FM z-score observed between patients and controls in the total sample. But a significant difference was founded when comparing FM (kg) between IEM patients and controls with higher fat mass in IEM patients (12.25±8.11 vs. 10.03±6.45, p = 0.040)

Results of intermediary metabolic groups showed that the FM z-score means higher among AA metabolism disorders than in controls (z-score mean=0.315±1.83vs. -0.071±1.35 p <0.05). In PKU patients, we founded significant differences in FM compared to controls (12.73±8.7 kg vs. 10.03±6.45 kg, p = 0.381). Also, FFM was lower in PKU patients than in controls (28.1± 8.3 vs. 30.62±11.9, p =0.044). A study showed an increased body fat percentage in PKU females above 11 years of age comparing with males (Albersen *et al*., 2010). On the contrary, several studies showed that no significant difference in FM recorded in PKU patients. Huemer *et al.*, found that no significant differences in FM were presented between PKU patients and healthy populations either at birth or during the study period (Huemer *et al*., 2007). Also, no significant differences were found between PKU patients and controls regarding FM and FFM (Rocha *et al.*, 2013).

Some AA disorders patients follow a low protein diet, which is comparable with a vegetarian diet, leading to more contribution of carbohydrates and allowed fat to complete their energetic need, which can explain the increased FM in AA patients.

Regarding CHD and FAO metabolism disorders there were no significant differences between groups patients and controls in respect to FM z-score mean.

In contrary to our finding FM mean z-score was found te be lower in patients with classic galactosemia assessed by BIA (Panis, *et al.*, 2005). An imbalance between FFM and FM z-score assessed by DEXA, in patients with classic galactosemia was observed with high FM than FFM (Doulgeraki, A., 2014). Also, FM assessed by BIA found significantly greater in patients with GSD and GSD III than in controls, those patients showed low physical activity, and were reciving uncooked cornstarch, which may explain the increased FM (dos Santos, *et al.*, 2017).

Patients with LCHAD deficiency assessed by DEXA, aged 7-17 years old, tended to have a higher FM and decrease in lean mass than controls, in such patuents as body ability to oxidize fat is impaired, they will be more trend for to have higher body fat content (Gillingham, M. B., *et al*., (2013).

According to DEXA measured variables IEM patients diagnosed have lower BMD when compared with matched healthy controls: total body BMD (p = 0.000), BMD in femur neck (p = 0.044), BMD in femur trochanter (p = 0.012), BMD in femur ward (p = 0.023).

Total body BMD z-score was lower in AA metabolism disorders patients than in controls (0.833±0.92 vs. 1.66±1.35, p = 0.000). In AA metabolism disorders according to the femur, three sites bone density (femur neck, trochanter, and ward) founded to be significantly lower from reference controls (p <0.05). In PKU patients also we founded lower measured BMD z-scores means in BMD of total body (1.01 vs. 1.78), low z-score mean BMD trochanter, ward and femure witj (p<0.05) in all measured sites. Our findings are agreed with many

previous studies. Low lumbar spine BMD was detected in patients with PKU (Koura *et al.*, 2011). Both lumbar spine BMD and BMD total body were decreased too in PKU patients (Zeman *et al.*, 1999). Adamczyk *et al*., described a group of PKU children (mean age 13.8 ± 5.2 years) and concluded lower z-score mean lumbar spine BMD -0.572 ± 1.270 and total body BMD (-0.117 ± 1.347). They also found that in sexually mature patients, those who were non-adherent to the diet had a significantly lower BMD than those who adhered to diet (Adamczyk *et al*., 2011). Furthermore, Barat *et al*., investigated a group of pediatric patients with PKU, reported a low mean lumbar spine BMD z-score of -1.36 ± 1.586. Touati *et al*., showed that bone density z-score was low in MMA patients (Touati *et al*., 2006). In UCD patients, BMD was lower compared with age-matched control (Wilcox *et al.*, 2005). For UCD, the composition of the therapeutic low protein diet and/or the disorders itself may cause low BMD. Limited data about BMD and influencing factors are available.

It should be taken into account that Phe-free formulas or other formulas for AA metabolism are fortified with minerals and vitamins including Ca and vitamin D. PKU and MMA patients who are more compliant with the restricted diet may reach normal levels of Ca and vitamin D, despite of low BMD. In this sense, an adequate intake of Ca, P and vitamin D is not enough for normal bone development when natural protein intake is decreased, which plays a more important role in BMD development in PKU and other AA metabolism patients.

In CHD, low BMD could be contributed to their particular diet, which may result in nutritional deficiency such as calcium necessary for healthy bone density. BMD has been reported to be below the average values for children and adults with classic galactosemia, included in the 2017 meta-analysis by (van Erven *et al*., 2017), this meta-analysis concluded that 10–25% had a significantly reduced bone density (z-score ≤ -2). In a study for a group of children with classic galactosemia, the researcher recorded a significant low BMD in femur and spine compared with controls (Panis *et al*., 2004). BMD (total body) of children with classic galactosemia under dietary treatment was

decreased when compared with the reference value, and BMD (femoral neck) z-score was significantly reduced (Rubio-Gozalbo *et al*., 2002). Factors contributing to low BMD in classic galactosemia are reduced dietary of calcium and vitamin D intake, reduced physical activity, and in some patients and potentially the metabolic disorder itself (Batey *et al.,* 2013; Waisbren *et al.,* 2012). Low BMD has been described in GSD I type a and b, GSD II, GSD III, GSD V, and GSD IX (van den Berg *et al.,* 2010; Schwahn *et al*., 2002; Rodríguez-Gómez *et al*., 2018; Rake *et al.,* 2003). In GSD type 1, a possible factor that may contribute to the reduction in BMD is poor metabolic control (Melis *et al*., 2014; Wong *et al.,* 2017).

A significant inverse negative correlation was presented between the age of and BMD of the spine, femur, and trochanter, indicating that the bone density of mentioned sites is more prone to osteopenia development and/or osteoporosis (decreased bone density) with age. A significant positive correlation was recorded between weight and BMI, with almost all bone measured sites. Generally, the association between body composition and bone health is conflicting. In children, it is proposed that adipose tissue may stimulate bone growth (Clark *et al*., 2006), and it has been reported that FM was positively associated with bone mass in children (Leonard *et al*., 2004; Timpson *et al*., 2009).

6.2.1 Mineral bone diseases osteopenia/ osteoporosis

The definitions of osteopenia and osteoporosis are highly heterogeneous between the studies of World Health Organization (WHO) and the International Society for Clinical Densitometry (ISCD) based on BMD measurement (Demirdas *et al.,* 2015). According to the ISCD z-score < -2 is considered normal, and fracture history must be assessed along-side BMD z-score before the diagnosis can be made (Crabtree *et al.,* 2014).

In the patient's group 33.3% presented osteopenia risk and seven with osteoporosis risk 7.1%. Among the controls group, 20.4% presented osteopenia risk, and no one has a very low bone density (z <

-2.5) with significant differences between the patients and the controls group ($p = 0.036$).

Numerous studies have examined osteopenia among IEM patients. Although many sub-types of IEM require different kinds of diets, despite adequate intake of Ca, P, and vitamin D, but could be insufficient for healthy bone development, because of dairy products and foods rich in protein restriction.

In this thesis, AA metabolism disorders presented the highest prevalence risks of osteopenia and osteoporosis than the other groups. Twenty-five AA metabolism disorders children had risk of osteopenia, and five with osteoporosis risk. CHD comes next with six children having osteopenia and two with osteoporosis. In FAO disorders, only two patients have a risk of osteopenia.

Several studies reported the occurrence of osteopenia/osteoporosis in AA disorders. Al-Qadreh *et al*., showed severe osteopenia (z-score<-2 SD) in 22/48 in PKU patients (Al-Qadreh *et al*., 1998). Despite adequate calcium and vitamin D content of Phe-free L-amino acid supplements, osteopenia is still identified in PKU patients on a strict diet (Mirás *et al*., 2013). Osteopenia has not been observed in untreated mild HPA (Porta *et al*., 2011). Classic homocystinuria patients have presented with risk of osteoporosis and osteopenia (Weber *et al.*, 2016).

Osteopenia/osteoporosis has been noted in patients with GSD III (Mundy *et al*., 2008). A recent study in four males with GSD I (a and b) had either osteoporosis or osteopenia (Wong *et al*., 2017). BMD has been reported to be below the average values for the general population in several studies in children and adults with classic galactosemia (van Erven *et al*., 2017).

6.3 Physical Activity and Body Composition in IEM Patients.

Lower level of physical activity presented in IEM patients comparing with controls. Most of our IEM patients have practiced less vigorous exercise < 3 days/week and fewer hours < 7 hours/week following the WHO recommendation for healthy sports practice. Physical activity is a significant factor affecting body composition, especially muscle and FM and BMD.

In recent years, we have been experiencing a favorable process of awareness regarding rare diseases. However, the lack of literature that deals with sports practice in IEM patients are noteworthy. Sports practice is of undoubted importance in their personal and social development. (Organization *et al*., 2003).

Serrano *et al*., made some recommendations and observations for some of the most frequent IEM. With patients under control like PKU, the practice of sport implies no additional risks if attention is paid to calorie intake and protein catabolism (Serrano *et al*., 2010).

Patients with classic galactosemia might be less physically active due to motor dysfunction (Rubio-Agusti *et al.*, 2013; Gubbels *et al*., 2011). In classic galactosemia, there are no risks involved with sports practice for patients; in fact, moderate exercise practicing can enhance bone mass development.

In FA β-oxidation disorders, patients have difficulty obtaining energy from fatty acids when the energy supplied from glucose is insufficient. Prolonged exercise is therefore affected, and it is essential to ensure glucose supply (before, during, and after activity), or patients may suffer ketotic hyperglycinemia. Carnitine or middle chain fatty acids are often administered in LC-FAOD. There should be careful control of metabolism, the possible cardiovascular complications generated by the illness, and contraindicating or limiting sports and physical exercise.

In this thesis, moderate and vigorous physical activity showed a positive correlation with spine and femur bone density; it is also related positively to muscle mass. No research articles search about the effect of physical activity level with body composition and BMD in patients with IEM. Children and adolescents with appreciable physical activity have higher BMD values than those who have sedentary activity. A study compared children who engage in a competitive sport with untrained controls showed significant associations between physical activity and higher BMD (Specker *et al*., 2015)

Elite athletes, runners, and gymnasts have higher BMD values than sedentary individuals. Controlled studies in adolescents with varying degrees of physical activity have shown that prolonged exercise stimulates bone mass development. (Bailey *et al*., 1996). Bone growth and maintenance are critically stimulated by physical activity, which is important at all ages (Going and Farr, 2010). Regular physical activity is the key to optimizing peak bone mass, thus preventing risk of osteoporosis later in life (Zulfarina *et al*., 2016).

6.4 Dietary Intake and Correlation with Body Composition

The diet and type of energy consumed differed between patients and controls; we observed a significantly lower daily intake of total protein, protein natural, and energy from the protein in IEM patients compared with controls. There was increased intake of carbohydrate in IEM patients than in controls.

According to intermediary metabolism groups, we found that total protein and total natural protein intake differ significantly between the group with the lowest consumption was among patients with AA metabolism disorders followed by CHD group. The total intake of fat observed with a significant difference and the lowest intake value recorded in patients with FAO metabolism disorders followed by AA metabolism disorders.

Protein intake shows a non-significant positive correlation with weight and BMI. In muscle mass, a significant positive relationship between protein intake and muscle mass have observed. Regarding the diet carried out by IEM patients and their influence on the growth, a previous study tried to relate the total protein intake, natural proteins and protein substitute with growth, and height in children with PKU, and founded that neither protein nor energy intake correlated with height and growth (Hoeksma *et al.*, 2005).

Intake of protein total and natural protein was positive significant related to BMD total and ward. Energy intake has a significant positive correlation with body weight. Several studies have shown that protein intake deficiency can influence bone mass (Hannan *et al*., 2000).

In our study, we found natural protein intake was positively and significantly correlated with total body BMD (r = 0.186, r= 0.264, p <0.01). Also, protein natural was positives and significant related with femur (r = 0.208, p = 0.003), trochanter (r = 0.264, p = 0.000) and ward (r = 0.287, p = 0.001). Bone health also depends on protein structure quality. (Demirdas *et al*., 2015). Mirás *et al*., identified the absence of bone disease in 12/12 PKU patients treated by BH4, which allowed a higher natural protein intake. She described 43 patients with classic PKU on a strict low-Phe diet divided into two groups with and without the mineral bone disease (MBD) following definitions of bone density levels: A T-score of -1.1 to -2.4 indicates osteopenia risk. A T-score ≤-2.5 means a diagnosis of osteoporosis risk. The main difference between the group with and without MBD was the natural protein intake (14.33 ± 8.95 g/day in the group with MBD versus 21.25 ±20.85 in the group without MBD) (Mirás *et al*., 2013).

Our IEM patients reported significantly lower total and femur trochanter BMD than control (p = 0.001 and p = 0.012, respectively), despite the higher daily intake of calcium and magnesium by IEM patients than controls.

Carson *et al.*, using the lumbar spine CT, reported a significant reduction in mineral content of trabecular bone in 4 out of 11 patients with PKU. The authors also found a significant correlation between

BMC and dietary assessment of calories, natural protein, calcium, and copper (Carson *et al.*, 1990).

An adequate intake of minerals as calcium, potassium, phosphorous, iron, and zinc has been recorded. In our study, both calcium and vitamin D showed no correlation with bone mineral density. An adequate intake of Ca, P, and vitamin D is necessary for optimal bone development, but it is not enough for healthy bone development.

In patients with classic galactosemia, galactose restricted diet influences nutritional deficiencies, including a calcium deficiency. Therefore, dietary calcium intake needs to be monitored, as is also recommended for other metabolic diseases in which bone health is often affected.

As we observed in our study both calcium and vitamin D intake have no relation with BMD, this finding is compatible with that of Olgac *et al*., where he found no statistically significant differences between vitamin D and BMD of patients and healthy controls (Olgac *et al*., 2019). Many studies, including meta-analyses, have evaluated the relationship of 25-OHD levels and BMD of children with IEM consuming protein-restricted diets (mainly PKU), and could not detect any significant correlation. (Demirdas *et al*., 2017; Baldan *et al.*, 2018; Mendes *et al*., 2012). On the contrary, researchers found that vitamin D supplements improved BMD in a cohort of patients with inadequate dietary intake (Pérez-Dueñas *et al*., 2002).

Allen *et al.*, showed that no correlation founded between BMD and dietary compliance in PKU patients, this may be explained because they studied only prepubertal subjects with a mean age 7.7 years old (Allen *et al.*, 1995).

It should be noted that taking all reported data into account, including the present results, appears that a problem exists with bone mineralization at all ages. This problem tends to escalate in adolescence, probably owing to poor dietary compliance.

Although vitamin D effect on bone health is well known, BMD is influenced by many factors, including genetic, environmental, and endocrine factors. In many types of IEM, there is a real need to analyze and regulate potential contributors to the bone loss observed in these diseases.

6.4.1 Adherence to dietary treatment in PKU patient

According to our results, Phe blood concentration found to be high among patients in the adolescent period with the age range 14-19 years old despite the low protein dietary intake. Several studies reported that Phe blood level changed by age. In a study in British and Australian centers involved in the management of PKU found that almost 30% of Phe concentrations were higher than the target ranges for children younger than four years old. Control worsened with age, and by 15–19 years of age, almost 80% of concentrations were above the target range (Walter *et al*., 2002). In a German study of children treated for PKU, 42 of 89 patients (47%) followed the recommendations over their first nine years of life (Burgard *et al*., 1996). Multi European centers experienced in PKU management provided data from 1,921 patients with PKU treated with diet over one year. In the early years, Phe blood level was well controlled but worsen with age (Gokmen-Ozel *et al*., 2009).

The dietary treatment of PKU is multi-various, challenging, and lifelong. Key nutritional behaviors associated with optimal control of blood Phe level include avoidance of high protein foods consumption, and distribution of protein substitutes throughout the day and adequate energy intake (MacDonald *et al.*, 2006). Sub-optimal compliance is generally associated with higher blood Phe concentrations (Walter *et al*., 2002), which, in turn, is associated with less positive neurocognitive outcomes in school-age children (Zeman *et al.*, 1996).

Dietary compliance is influenced by cognitive, emotional, physiological, and cultural factors, and studies examining interventions to improve dietary compliance in PKU are limited and mainly

uncontrolled. In some protocols, the adequate protein intake in PKU patients follow the RDA for total protein intake (Acosta *et al*., 2003; McBurnie *et al*., 1991), while in others, an increase in protein intake above the RDA is recommended (Hoeksma *et al*., 2005; Dobbelaere *et al*., 2003).

It is essential to keep in mind if protein intake excessive in early life is related to the appearance in later ages of obesity, renal dysfunction, and even worse neurological prognosis. Therefore, caution should be exercised regarding the recommendation to provide excessive amounts of protein (protein substitute) to PKU children.

6.5 Biochemical and Haematological Markers

In this thesis, we found some vitamins and minerals deficiency in IEM children comparing with healthy matched group and reference values. Supplements are commonly unpalatable or incomplete. The recommended dose may not deliver the Reference Nutrient Intake (RNI), and long-term adherence may be an issue with either caregiver forgetting to administer daily preparations or children refusing to accept them (MacDonald *et al.*, 2008).

Selenium was low in 36.4% of the total patients with a significant difference from controls. Our patients generally had adequate calcium and phosphorous levels. High serum folate concentrations were detected in 44.4% of patients.

We found low levels of 25-OHD in 44% of them. We think this finding may be related to the patient's origin, being that the patients coming from the north of Spain (Galicia) more prompted to suffer this deficit as they exposure to few sunshine hours.

Other vitamins that have been found lower in patients are vitamin A, and folate. Iron (Fe) deficiency recorded in three patients. RBP was low in 32.3% of the patient's group. Lower cholesterol levels also presented more frequent in patients than in controls. Higher serum TG was presented in patients than in controls.

In AA metabolism disorders selenium found to be very low than in controls. Food rich in protein contains more selenium than those low in protein. Seafood and liver are the most selenium-rich foods (400-1500 μg/kg), meat (100-400 μg/kg), grains, vegetables, and fruits usually contain much less. AA metabolism disorders patient's diets are restricted with a high rich protein food; this can explain the lower serum selenium level in their blood.

High serum TG in AA metabolism disorders patients compared with controls (81.14±39.24 vs. 57.71±27.57mg/dl), is possibly associated with the high intake of dietary carbohydrate. Type and amount of carbohydrate consumed have a direct effect on serum triglyceride levels; our body convert excess carbohydrates that are not used for energy into TG.

A significant high serum folate was found in AA metabolism disorders when compared with controls. Similar founding has been reported in previous studies in PKU patients (MacDonald *et al.*, 2011). High serum folate may be explained as patients with AA metabolism disorders tends to consume more vegetables and fruits. Although serum folate is a useful marker of folate status, it may be variable, with recent consumption of a high folate meal or folate supplements, and medications (Organization *et al.*, 2015). There is some suggestion that excess folic acid intake interferes with zinc homeostasis but in this study no correlation between folate and zinc levels was found.

The diet indicated in PKU patients also consists of a series of advantages and limitations derived from the low contribution of food of animal origin, like those produced in vegan diets. On the one hand, the benefits would be the lower levels of saturated fat, cholesterol, and higher levels of carbohydrates, fiber, magnesium, and potassium. The limitations, such as the low contribution of micronutrients, include vitamins A, C, E, selenium, vitamin B2, B6 and B12, folates, iron, and zinc, carnitine, and LCPUFAs. These deficits are even more crucial in poorly nourished PKU patients than in vegans since the latter can include cereals and nuts in their diet.

In AA metabolism disorders, protein restriction enforces an alternative source to cover the minimum requirement of essential nutrients (Acosta, 2010). Dietary restrictions also increase essential fatty acids deficiency risks, such as docosahexaenoic acid (DHA) and arachidonic acid (ARA), which has been described in PKU patients (Koletzko *et al.*, 2009). There is also a risk of deficiencies in Fe, zinc, selenium, among other minerals, so it is essential to evaluate the type of specialized formula that the patient receives and whether the additional supplementation is necessary or not (Colombo, 2003).

Many clinical and biochemical vitamin and mineral deficiencies have been reported in children on limited/restricted protein diets (Ihara *et al.*, 2011; Yannicelli *et al.*, 1992). Natural sources of some vitamins and minerals as B12, zinc, selenium, and Fe are limited or with reduced bioavailability unless small amounts of foods containing protein with high biological value are allowed.

Among FAO metabolism disorders ferritin was significantly lower in FAO patients compared to controls (65.33±45.41 vs. 92.00±20.72 ng/dl, $p = 0.024$), TG was higher in the FAO disorders patients than in controls (74.2±44.2 vs. 58.08±23.42, $p = 0.032$ mg/dl), in the other hand HDL cholesterol was lower in FAO disorders patients than in controls (42.8±10.71vs. 57.08±9.45mg/dl, $p = 0.025$). In relation to the others nutrinets zinc, B12, iron, and folate, no significant differences have been founded between the IEM patient group and controls.

FAO patients, especially children, on very low-fat diets are at high risk for fat-soluble vitamin deficiencies (Gillingham., 2015). We found in our thesis patients wit FAO significant lower serum level of vitamin D than controls (15.9±7.32 vs. 20.92±4.23). As we know vitamin D is a fat-soluble vitamin. Our FAO sample consists of MCADD patients following a diet restricted in fat, in parallel to low sun exposure diet may contribute to such deficiency. Also, vitamin E serum level was lower in FAO patients than controls (1.04±0.29 vs. 4.38±4.8, $p<0.05$).

High serum TG level founded in CHD patients than in controls (111.9±86.96 vs. 70.0±29.01 mg/dl, $p = 0.041$), while LDL was lower in CHD disorders than controls (86.00±20.07 vs. 155.08±185.36,

mg/dl, $p = 0.043$). Among the others remains nutrients zinc, B12, iron, and folate; no significant differences have been founded between the patient's group and controls. Despite normal serum calcium and vitamin D in CHD patients, it recommended that individuals with galactosemia who do not consume adequate calcium and vitamin D from their diet require supplementation to at least meet the DRI.

Normal nonsignificant serum folate found in CHD patients compared to controls, but it was the lowest among the intermediary metabolism groups. Patients with classic galactosemia could be at risk of folate deficiency, as naturally rich sources of folic acid such as legumes, organ meats, and some grain products are not permitted. Highly restrictive diets in infancy may lead to a reduction in fruit and/or vegetable consumption (Coelho, *et al.,* 2017).

The nutritional treatment of IEM disorders is complicated, and a fundamental part that represents the most significant challenge is ensuring healthy growth and development of the patients.

Our study has some limitations. The main one is that it's a cross sectional study. Besides the number of subjects is short, especially in fat and carbohydrate metabolism disorders. In relation to dietary intake and physical activity, we use validated questionnaires, not objective measurements.

Based on our results, there are differences in anthropometric and body composition among patients with IEM compared to controls mainly reduced height, increased FM and WC, and low BMD. AA metabolism disorders are the most affected intermediary metabolism group.

Documentation of body composition and biochemical parameters in larger patient series is important to elucidate whether these results reflect increased risks (hence opportunities for prevention) of bone disease, vitamins and minerals deficiency and metabolic syndrome in this population. It is necessary to carry out a longitudinal study which allows exploring the variability in the nutritional status of these patients and integrating it with biochemical controls that will enable metabolic control to be correlated with nutritional status and treatment adherence.

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7. CONCLUSIONS

1. Patients with IEM of intermediary metabolism present a significantly lower height than the controls. However, many other factors can influence height during development, including hormones, medical condition, pharmacological treatment, and microbiota.

2. BMI percentage distribution showed a high frequency of patients within the most upper and lower percentiles and an increased waist circumference. So, it is very important monitoring nutritional status in these pediatric patients, because of BMI is obesity diagnosis and increased waist circumferences is associated to higher metabolic risk.

3. In children with IEM, particularly in these with amino acids metabolism disorders tend to be shorter, higher in BMI, higher waist circumferences, and lower bone mineral density. The nature of the diet of these patients based on highly restricted natural protein plays probabley a vital role in such deterioration.

4. Low physical activity in these patients and the positive correlation with muscle mass and bone mineral density in them indicate that regular physical activity plays a main role to optimize body composition aside with a balanced diet.

5. Low bone mineral density associated with risk of osteopenia or osteoporosis in patients guides us to look for possible factors related to this defect, espeially if our patients are consuming adequate supplements that positively affect bone density, such as Ca, P, and vitamin D.

6. Serum vitamins and minerals fluctuation in our patients, as reduced selenium, insufficient vitamin D, and high folate level, are mainly associated with diet modifications for each intermediary metabolism group disturbances, except for vitamin D which probably may correlate to low exposure to the sun in the region of the research.

7. Diet showed a significant relation with body composition (muscle mass, fat mass, and bone mineral density). Attention should be made to avoid the undesirable effect of diet modification in development and growth.

8. In our study physical activity founded to be positively and significantly correlated with socioeconomic and education levels of parents. Physical activity and exercise are a significant contributor to a healthy lifestyle. The influence of IEM patient parents' education and socioeconomic level is undoubtedly crucial in helping to optimize healthy life by allow practicing more physical activity.

9. The clinical outcome of children with IEM depends on multiple factors, including the type of the disorder, the severity of the underlying metabolic defect, the ability to make the diagnosis early, availability of specific and adequate dietary treatment options, and the definitive therapeutic intervention. Depending on all these variables, some IEM could have a relatively better prognosis than others.

BIBLIOGRAPHY

Acosta, P. B. Nutrition management of patients with inherited metabolic disorders. 2nd rev ed. Massachusetts: Sudbury, 2010.

Acosta, P. B., Yannicelli, S., Ryan, A. S., Arnold, G., Marriage, B. J., Plewinska, M., et al. Nutritional therapy improves growth and protein status of children with a urea cycle enzyme defect. Mol Genet Metab. 2005; 86:448–455.

Acosta, P. B., Yannicelli, S., Singh, R., Mofidi, S., Steiner, R., De Vincentis, E., et al. Nutrient intakes and physical growth of children with phenylketonuria undergoing nutrition therapy. J Am Diet Assoc. 2003; 103:1167–1173.

Adamczyk, P., Morawiec-Knysak, A., Płudowski, P., Banaszak, B., Karpe, J. Pluskiewicz, W. Bone metabolism and the muscle–bone relationship in children, adolescents, and young adults with phenylketonuria. J Bone Miner Metab. 2011; 29:236–244.

Adeva-Andany, M. M., González-Lucán, M., Donapetry-García, C., Fernández-Fernández, C., Ameneiros-Rodríguez, E. Glycogen metabolism in humans. BBA clinical. 2016; 5:85–100.

Al-Qadreh, A., Schulpis, K., Athanasopoulou, H., Mengreli, C., Skarpalezou, A., Voskaki, I. Bone mineral status in children with phenylketonuria under treatment. Acta Paediatr. 1998; 87:1162–1166.

Albersen, M., Bonthuis, M., de Roos, N. M., den van Hurk, D. A., Carbasius Weber, E., Hendriks, M. M., et al., Whole body composition analysis by the bodpod air-displacement plethysmography method in children with phenylketonuria shows a higher body fat percentage. J Inherit Metab Dis. 2010; 33:283–288.

Aldámiz-Echevarría, L., Bueno, M. A., Couce, M. L., Lage, S., Dalmau, J., Vitoria, I., et al. Anthropometric characteristics and nutrition in a cohort of pah-deficient patients. Clin Nutr. 2014; 33:702–717.

Alfadhel, M., Al-Thihli, K., Moubayed, H., Eyaid, W., Al-Jeraisy, M. Drug treatment of inborn errors of metabolism: a systematic review. Arch Dis Child. 2013; 98:454–461.

Allen, J. R., McCauley, J. C., Waters, D. L., O'connor, J., Roberts, D., Gaskin, K. Resting energy expenditure in children with phenylketonuria. Am J Clin Nutr. 1995; 62:797–801.

American academy of Pediatrics, A. A. et al. Newborn screening: toward a uniform screening panel and system. Genet Med. 2006; 8:1S–252S.

Angle, B. and Burton, B. K. Risk of sudden death and acute life-threatening events in patients with glutaric acidemia type II. Mol Genet Metab. 2008; 93:36–39.

Applegarth DA, Toone JR. Incidence of inborn errors of metabolism in British Columbia, 1969–1996. Pediatrics. 2000 Jan 1;105: e10.

Armstrong, L. E., Kenefick, R. W., Castellani, J. W., Riebe, D., Kavouras, S. A., Kuznicki, J. T., et al., Bioimpedance spectroscopy technique: intra-, extracellular, and total body water. Med Sci Sports Exerc. 1997; 29:1657–1663.

B. Heymsfield, S., Wang, Z., Baumgartner, R. N., Ross, R. (1997). Human body composition: advances in models and methods. Annu Rev Nutr. 1997; 17:527–558.

Bailey, D. A., Faulkner, R. A., McKAY, H. A. Growth, physical activity, and bone mineral acquisition. Exerc Sport Sci Rev.1996; 24:233–266.

Baldan, A., Tagliati, S., Saccomandi, D., Brusaferro, A., Busoli, L., Scala, A., et al., Assessment of lactose-free diet on the phalangeal bone mineral status in italian adolescents affected by adult type hypolactasia. Nutrients. 2018; 10:558.

Barends, M., Pitt, J., Morrissy, S., Tzanakos, N., Boneh, A. Biochemical and molecular characteristics of patients with organic acidaemias and urea cycle disorders identified through newborn screening. Mol Genet Metab. 2014; 113:46–52.

Barness LA. The metabolic and molecular bases of inherited disease. Am J Med Genet.1996; 66:87-89.

Bartrina, J. A., Majem, L. S., Verdú, J. M. Evaluación del estado nutricional. En: Lluís Serra Majem; Javier Aranceta Bartrina; Mataix Verdú J., Nutrición y salud pública: métodos, bases científicas y aplicaciones, Barcelona. 2006; p.114–135.

Batey, L., Welt, C., Rohr, F., Wessel, A., Anastasoaie, V., Feldman, H., Guo, C.Y., Rubio-Gozalbo, E., Berry, G., and Gordon, C. Skeletal health in adult patients with classic galactosemia. Osteoporos Int. 2013; 24:501–509.

Batshaw, M. L., Tuchman, M., Summar, M., Seminara, J., Consortium, U. C. D., et al. A longitudinal study of urea cycle disorders. Mol Genet Metab. 2014; 113:127–130.

Baumgartner, M. R., Hörster, F., Dionisi-Vici, C., Haliloglu, G., Karall, D., Chapman, K. A., et al., Proposed guidelines for the diagnosis and management of methylmalonic and propionic acidemia. Orphanet J. Rare Dis. 2014; 9:130.

Belanger-Quintana, A. Martínez-Pardo, M. Physical development in patients with phenylketonuria on dietary treatment: a retrospective study. Mol Genet Metab. 2011; 104:480–484.

Bickel, H. Influence of phenylalanine intake on pheny-lketonuria. Lancet. 1953; 812.

Bodamer, O. A., Hoffmann, G. F., Lindner, M. Expanded newborn screening in europe 2007. J. Inherit. Metab. Dis. 2007; 30:439–444.

Boelens, J., Rocha, V., Aldenhoven, M., Wynn, R., O'Meara, A., Michel, G., et al. risk factor analysis of outcomes after unrelated cord blood transplantation in patients with hurler syndrome. Biol Blood Marrow Transplant. 2009; 15:618–625.

Bosch, A. M. Classical galactosaemia revisited. J Inherit Metab. Dis. 2006; 29:516–525.

Brambilla, P., Manzoni, P., Sironi, S., Simone, P., Del, A. M., Chiumello, G., et al., Peripheral and abdominal adiposity in childhood obesity. Int J Obes Relat Metab Disord, 1994; 18:795–800.

Brosco, J. P., Mattingly, M., Sanders, L. M. Impact of specific medical interventions on reducing the prevalence of mental retardation. Arch Pediatr Adolesc Med. 2006; 160:302–309.

Brunetti-Pierri, N., Parenti, G., Andria, G. Inborn errors of metabolism. In: Giuseppe Buonocore; Rodolfo Bracci; Michael Weindling. Neonatology. Milano. 2012. p. 949–966.

Brusilow, S., Valle, D., Batshaw, M. New pathways of nitrogen excretion in inborn errors of urea synthesis. The Lancet. 1979; 314:452–454.

Brusilow, S. W. Phenylacetylglutamine may replace urea as a vehicle for waste nitrogen excretion. Pediatr Res.1991; 29:147–150.

Burgard, P., Schmidt, E., Rupp, A., Schneider, W., Bremer, H. J. Intellectual development of the patients of the german collaborative study of children treated for phenylketonuria, Eur J Pediatr.1996;155: S33–S38.

Burton, B. K. Inborn errors of metabolism in infancy: a guide to diagnosis. Pediatrics. 1998; 102: e69–e69.

Burwinkel, B., Scott, J. W., Bührer, C., Van Landeghem, F. K., Cox, G. F., Wilson, C. J; et al., Fatal congenital heart glycogenosis caused by a recurrent activating r531q mutation in the g2-subunit of amp-activated protein kinase (prkag2), not by phosphorylase kinase deficiency. Am J Hum Genet. 2005; 76:1034–1049.

Campeau, P. M., Scriver, C. R., Mitchell, J. J. A 25-year longitudinal analysis of treatment efficacy in inborn errors of metabolism. Mol Genet Metab. 2008; 95:11–16.

Carson, D., Greeves, L., Sweeney, L., Crone, M. Osteopenia, and phenylketonuria. Pediatr Radiol. 1990; 20:598–599.

Chace, D. H., Pons, R., Chiriboga, C. A., McMahon, D. J., Tein, I., Naylor, E. W., et al., Neonatal blood carnitine concentrations: normative data by electrospray tandem mass spectometry. Pediatr Res. 2003; 53:823–829.

Chen, Y. T., Bazzarre, C. H., Lee, M. M., Sidbury, J. B., Coleman, R. A. Type I glycogen storage disease: nine years of management with cornstarch. Eur J Pediatr.1993; 152: 56-59.

Chuang, D. T., Chuang, J. L., Wynn, R. M. Lessons from genetic disorders of branched-chain amino acid metabolism. J Nut. 2006; 136:243S–249S.

Chumlea, W; Guo, S. S. Assessment, and prevalence of obesity: application of new methods to a major problem. Endocr J. 2000; 13:135–142.

Chumlea, W. C; Guo, S. S. Bioelectrical impedance, and body composition: present status and future directions. Nutr Rev. 1994; 52:123–131.

Chumlea, W. C., Guo, S. S., Kuczmarski, R. J., Flegal, K. M., Johnson, C. L., Heymsfield, S. B., et al., Body composition estimates from nhanes iii bioelectrical impedance data. Int J Obes. 2002; 26:1596–1609.

Chumlea, W. C., Guo, S. S., Steinbaugh, M. L. Prediction of stature from knee height for black and white adults and children with application to mobility-impaired or handicapped persons. J Am Diet Assoc. 1994; 94:1385–1391.

Chumlea, W. C., Guo, S. S., Wholihan, K., Cockram, D., Kuczmarski, R. J., Johnson, C. L. Stature prediction equations for elderly non-hispanic white, non-hispanic black, and mexican-american persons developed from NHANES III data. J Am Diet Assoc. 1998; 98:137–142

Clarke, J. T. A clinical guide to inherited metabolic diseases. 3rd rev ed. New York: Cambridge, 2005.

Cleary, M., Green. Developmental delay: when to suspect and how to investigate for an inborn error of metabolism. Arch Dis Child. 2005; 90:1128–1132.

Cocho de Juan, J., Castiñeiras Ramos, D., Fraga Bermúdez, J. Cribado neonatal de los errores congénitos del metabolismo. En: Diagnóstico y tratamiento de las enfermedades metabólicas hereditarias. 4ª Ed. Sanjurjo P, Baldellou A ed. Ergón ed. Madrid 2014. p. 47–61.

Coelho, A. I., Rubio-Gozalbo, M. E., Vicente, J. B., Rivera, I. Sweet and sour: an update on classic galactosemia. J Inherit Metab Dis. 2017; 40: 325-342.

Cole, T. J., Bellizzi, M. C., Flegal, K. M., Dietz, W. H. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000; 320: 1240.

Collins, J; Leonard, J. The dietary management of inborn errors of metabolism. Human nutrition. J Appl Nutr. 1985; 39:255–272.

Colombo, M. Errores innatos en el metabolismo del niño. Editorial universitaria. 2nd rev ed. Santiago de chile, 2003.

Colonetti, K., Roesch, L. F., Schwartz, I. V. D. The microbiome and inborn errors of metabolism: Why we should look carefully at their interplay? Genet Mol Biol. 2018; 1:515–532.

Couce, M. L., Castiñeiras, D. E., Bóveda, M. D., Baña, A., Cocho, J. A., Iglesias, A. J., et al., Evaluation and long-term follow-up of infants with inborn errors of metabolism identified in an expanded screening programme. Mol Genet Metab. 2011;104: 470–475.

Couce, M. L., Sánchez-Pintos, P., Aldámiz-Echevarría, L., Vitoria, I., Navas, V., Martín-Hernández, E., et al. Evolution of tyrosinemia type 1 disease in patients treated with nitisinone in spain. Medicine. 2019; 98: e17303.

Couce ML, Vitoria I, Aldámiz-Echevarría L, Fernández-Marmiesse A, Roca I, Llarena M, et al., Lipid profile status and other related factors in patients with Hyperphenylalaninaemia. Orphanet J Rare Dis. 2016; 11:123.

Couce ML, Fernández JR, Fraga BJ. Enfermedades congénitas del metabolismo en el período neonatal. Protocolos de la Asociación Española de Pediatría. Madrid: 2008.

Cox, T. M., Drelichman, G., Cravo, R., Balwani, M., Burrow, T. A., Martins, A. M., et al., Eliglustat compared with imiglucerase in patients with gaucher's disease type 1 stabilised on enzyme replacement therapy: a phase 3, randomised, open-label, non-inferiority trial. The Lancet. 2015; 385:2355–2362.

Crabtree, N. J., Arabi, A., Bachrach, L. K., Fewtrell, M., Fuleihan, G. E.-H., Kecskemethy, H. H., et al., Dual-energy x-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD pediatric official positions. J Clin Densitom. 2014; 17:225–242.

Demirdas, S., Coakley, K. E., Bisschop, P. H., Hollak, C. E., Bosch, A. M., Singh, R. H. Bone health in phenylketonuria: a systematic review and meta-analysis. Orphanet J Rare Dis. 2015; 10:17.

Demirdas, S., van Spronsen, F. J., Hollak, C. E., van der Lee, J. H., Bisschop, P. H., Vaz, F. M., et al., Micronutrients, essential fatty acids and bone health in phenylketonuria. Ann Nutr Metab. 2017; 70:111–121.

Den Boer, M. E., Dionisi-Vici, C., Chakrapani, A., van Thuijl, A. O., Wanders, R. J., Wijburg, F. A. Mitochondrial trifunctional protein deficiency: a severe fatty acid oxidation disorder with cardiac and neurologic involvement. J Pediatr. 2003;142: 684–689.

Derks, T. G., Reijngoud, D.J., Waterham, H. R., Gerver, W.J. M., Van Den Berg, M. P., Sauer, P. J., et al., The natural history of medium-chain acyl coa dehydrogenase deficiency in the netherlands: clinical presentation and outcome. J Pediatr. 2006; 148:665–670.

Desnick, R. Schuchman, E. Enzyme replacement therapy for lysosomal diseases: lessons from 20 years of experience and remaining challenges. Annu Rev Genomics Hum Genet. 2012; 13:307–335.

Dewey, K., Beaton, G., Fjeld, C., Lönnerdal, B., Reeds, P. Protein requirements of infants and children discussion. Eur J Clin Nutr. 1996; 50: S119–S150.

Dobbelaere, D., Michaud, L., Debrabander, A., Vanderbecken, S., Gottrand, F., Turck, D., et al., Evaluation of nutritional status and pathophysiology of growth retardation in patients with phenylketonuria. J Inherit Metab Dis. 2003; 26:1–11.

Dobrowolski, S., Pey, A., Koch, R., Levy, H., Ellingson, C., Naylor, E., and Martinez, A. Biochemical characterization of mutant phenylalanine hydroxylase enzymes and correlation with clinical presentation in hyperphenylalaninaemic patients. J Inherit Metab Dis. 2009; 32:10.

Dos Santos, B. B., Nalin, T., Grokoski, K. C., Perry, I. D., Refosco, L. F., Vairo, F. P., et al., Nutritional Status and Body Composition in Patients with Hepatic Glycogen Storage Diseases Treated with Uncooked Cornstarch—A Controlled Study. J Inborn Errors Metab Screen. 2017; 5:2326409817733014.

Douglas, T. D., Kennedy, M. J., Quirk, M. E., Sarah, H. Y., Singh, R. H. Accuracy of six anthropometric skinfold formulas versus air displacement plethysmography for estimating percent body fat in female adolescents with phenylketonuria. J Inherit Metab Dis. 2012; 10: 23–31.

Doulgeraki, A., Skarpalezou, A., Theodosiadou, A., Monopolis, I., Schulpis, K. Body composition profile of young patients with phenylketonuria and mild hyperphenylalaninemia. Int J Endocrinol Metab. 2014; 12: e16061.

Dulín-Íñiguez, E., Espada, M., Eguileor-Gurtubai, I. Programas de cribado neonatal. An Pediatr Contin. 2006; 4:61–65.

El-Gharbawy A, Vockley J. Inborn errors of metabolism with myopathy: defects of fatty acid oxidation and the carnitine shuttle system. Pediatric Clinics. 2018; 65:317-35.

El-Hattab, A. W. Inborn errors of metabolism. Clin Perinatol. 2015; 42:413 –439.

Ellis, K. J. Human body composition: in vivo methods. Physiol Rev. 2000; 80:649–680.

Enns, G. M., Berry, S. A., Berry, G. T., Rhead, W. J., Brusilow, S. W., Hamosh, A. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. N Engl J Med. 2007; 356:2282–2292.

Evans, M., Truby, H., Boneh, A. The relationship between dietary intake, growth, and body composition in inborn errors of intermediary protein metabolism. J Pediatr. 2017; 188:163–172.

Ezgu, F. Inborn errors of metabolism. Adv Clin Chem. 2016; 195–250.

Fernandes, J., Leonard, J., Moses, S., Odievre, M., Di Rocco, M., Schaub, J., et al., Glycogen storage disease: recommendations for treatment. Eur J Pediatr. 1988; 147:226–228.

Fernandes, J., Saudubray, J.-M., Van den Berghe, G., Walter, J. H. Inborn metabolic diseases: diagnosis and treatment. 4th rev ed. Heidelberg. 2006.

Ferreira, C. R., van Karnebeek, C. D., Vockley, J., Blau, N. A proposed nosology of inborn errors of metabolism. Genet Med. 2019; 21:102–106.

Frazier, D. Medium Chain Acyl CoA dehydrogenase deficiency (MCADD). GMDI: Nutrition Guidelines. Update 2008.

Frazier, D. M., Allgeier, C., Homer, C., Marriage, B. J., Ogata, B., Rohr, F., et al., Nutrition management guideline for maple syrup urine disease: an evidence-and consensus-based approach. Mol Genet Metab. 2014; 112:210–217.

Frisard, M. I., Greenway, F. L., DeLany, J. P. Comparison of methods to assess body composition changes during a period of weight loss. Obes Res. 2005; 13:845–854.

Froissart, R., Piraud, M., Boudjemline, A. M., Vianey-Saban, C., Petit, F., Hubert-Buron, A., et al., Glucose-6-phosphatase deficiency. Orphanet J Rare Dis. 2011; 6:27.

Fulgoni III, V. L. Current protein intake in america: analysis of the national health and nutrition examination survey, 2003–2004. Am J Clin Nutr. 2008; 87:1554S–1557.

Gába, A., Kapuš, O., Cuberek, R., Botek, M. Comparison of multi-and single-frequency bioelectrical impedance analysis with dual-energy x-ray absorptiometry for assessment of body composition in post-menopausal women: effects of body mass index and accelerometer-determined physical activity. J Hum Nutr Diet. 2015; 28:390–400.

Gajewska, J., Ambroszkiewicz, J., Radomyska, B., Chelchowska, M., Oltarzewski, M., Laskowska-Klita, T., and Milanowski, A. Serum markers of bone turnover in children and adolescents with classic galactosemia. Adv Med Sci. 2008; 53:214.

Gallagher, R. C., Van Hove, J. L., Scharer, G., Hyland, K., Plecko, B., Waters, P. J., Mercimek-Mahmutoglu, S., et al. Folinic acid–responsive seizures are identical to pyridoxine-dependent epilepsy. Ann Neurol. 2009; 65:550–556.

Galton, D. Archibald e. garrod (1857-1936). J Inherit Metab Dis. 2008; 31:561.

Gambello, M. J. Li, H. Current strategies for the treatment of inborn errors of metabolism. J Genet Genomics. 2018; 45:61–70.

Gardeitchik, T., Humphrey, M., Nation, J., Boneh, A. Early clinical manifestations and eating patterns in patients with urea cycle disorders. J Pediatria. 2012; 161:328–332.

Ghosh, A., Schlecht, H., Heptinstall, L. E., Bassett, J. K., Cartwright, E., Bhaskar, S. S., et al., Diagnosing childhood-onset inborn errors of metabolism by next-generation sequencing. Arch Dis Child. 2017; 102: 1019-1029.

Gillingham, M. B. Nutrition studies in long-chain fatty acid oxidation disorders: Diet composition and monitoring. In: Bernstein LE, Rohr F, Helm JR. Nutrition Management of Inherited Metabolic Diseases. Cham: Switzerland Springer. 2015; 255–270.

Gillingham, M. B., Scott, B., Elliott, D., Harding, C. O. Metabolic control during exercise with and without medium-chain triglycerides (MCT) in children with long-chain 3-hydroxy acyl-coa dehydrogenase (LCHAD) or trifunctional protein (TFP) deficiency. Mol Genet Metab. 2006; 89:58–63.

Gillingham, M. B., Weleber, R. G., Neuringer, M., Connor, W. E., Mills, M., Van Calcar, S., et al., Effect of optimal dietary therapy upon visual function in children with long-chain 3-hydroxyacyl coa dehydrogenase and trifunctional protein deficiency. Mol Genet Metab. 2005; 86:124–133.

Giovannini, M., Verduci, E., Salvatici, E., Fiori, L., Riva, E. Phenylketonuria: dietary and therapeutic challenges. J Inherit Metab Dis. 2007; 30:145–152.

Going, S. B. Farr, J. N. Exercise and bone macro-architecture is childhood a window of opportunity for osteoporosis prevention. Int J Body Compos Res. 2010; 8:1.

Gokmen-Ozel, H., Ahring, K., Belanger-Quintana, A., Dokupil, K., Lammardo, A., MacDonald, A., et al., Blood phenylalanine control in pku: a survey of ten european centres. Mol Genet Metab. 2009; 98: 30–31.

Gubbels, C. S., Maurice-Stam, H., Berry, G. T., Bosch, A. M., Waisbren,S., Rubio-Gozalbo, M. E., et al., Psychosocial developmental milestones in men with classic galactosemia. J Inherit Metab Dis. 2011; 34:415–419.

Guo, S., Huang, C., Maynard, L., Demerath, E., Towne, B., Chumlea, W. C., et al., Body mass index during childhood, adolescence and young adulthood in relation to adult overweight and adiposity: the fels longitudinal study. Int J Obes. 2000; 24:1628–1635.

Guo, S. S., Wu, W., Chumlea, W. C., Roche, A. F. Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence. Am J Clin Nutr. 2002; 76:653–658.

Häberle, J., Boddaert, N., Burlina, A., Chakrapani, A., Dixon, M., Huemer, M., et al. Suggested guidelines for the diagnosis and management of urea cycle disorders. Orphanet J Rare Dis. 2012; 7:32.

Haglind, C. B., Stenlid, M. H., Ask, S., Alm, J., Nemeth, A., Döbeln, U., et al., Growth in long-chain 3-hydroxyacyl-coa dehydrogenase deficiency. J Inherit Metab Dis. 2012; 5: 81–90.

Hannan, M. T., Tucker, K. L., Dawson-Hughes, B., Cupples, L. A., Felson, D. T., Kiel, D. P. Effect of dietary protein on bone loss in elderly men and women: the framingham osteoporosis study. J Bone Miner Res. 2000; 15:2504–2512.

Hart, C. E., Race, V., Achouri, Y., Wiame, E., Sharrard, M., Olpin, S. E., et al. Phosphoserine aminotransferase deficiency: a novel disorder of the serine biosynthesis pathway. Am J Hum Genet. 2007; 80:931–937.

Hassan, F. A., El-Mougy, F., Sharaf, S. A., Mandour, I., Morgan, M. F., Selim, L. A., et al. Inborn errors of metabolism detectable by tandem mass spectrometry in egypt: the first newborn screening pilot study. J Med Screen. 2016; 23:124–129.

Hauser, N. S., Manoli, I., Graf, J. C., Sloan, J., Venditti, C. P. Variable dietary management of methylmalonic acidemia: metabolic and energetic correlations. Am J Clin Nutr. 2011; 93:47–56.

Heaney, R. P; Layman, D. K. Amount and type of protein influences bone health. Am J Clin Nutr. 2008; 87:1567S–1570S.

Hermida-Ameijeiras, A., Crujeiras, V., Roca, I., Calvo, C., Leis, R., Couce, M.-L. Arterial stiffness assessment in patients with phenylketonuria. Medicine. 2017; 96: e9322.

Hidvégi, E., Arató, A., Cserháti, E., Horváth, C., Szabó, A., Szabó, A. Slight decrease in bone mineralization in cow milk–sensitive children. J Pediatr Gastroenterol Nutr. 2003; 36:44–49.

Hillman, L., Schlotzhauer, C., Lee, D., Grasela, J., Witter, S., Allen, S., et al., Decreased bone mineralization in children with phenylketonuria under treatment. Eur J Pediatr.1996; 155: S148–S152.

Hoeksma, M., Van Rijn, M., Verkerk, P. H., Bosch, A. M., Mulder, M. F., de Klerk, J. B., et al. The intake of total protein, natural protein and protein substitute and growth of height and head circumference in dutch infants with phenylketonuria. J Inherit Metab Dis. 2005; 28:845–854.

Hoffman, J. R. Falvo, M. J. Protein–which is best? J Sports Sci Med. 2004; 3:118.

Hsia, D. Y.-Y., Walker, F. A. Variability in the clinical manifestations of galactosemia. J Pediatr. 1961; 59:872–883.

Huemer, M., Huemer, C., Möslinger, D., Huter, D., Stöckler-Ipsiroglu, S.Growth and body composition in children with classical phenylketonuria: results in 34 patients and review of the literature. J Inherit Metab Dis. 2007; 30:694.

Humphrey, M., Truby, H., and Boneh, A. New ways of defining protein and energy relationships in inborn errors of metabolism. Mol Genet Metab. 2014; 112:247–258.

Hynynen, J., Komulainen, T., Tukiainen, E., Nordin, A., Arola, J., Kälviäinen, R., et al., Acute liver failure after valproate exposure in patients with polg1 mutations and the prognosis after liver transplantation. Liver Transplant. 2014; 20:1402–1412.

Iafolla, A. K., Thompson Jr, R. J., Roe, C. R. Medium–chain acyl-coenzyme a dehydrogenase deficiency: Clinical course in 120 affected children. J Pediatr. 1994; 124:409–415.

Ibdah, J. A., Perlegas, P., Zhao, Y., Angdisen, J., Borgerink, H., Shadoan, M. K., et al., Mice heterozygous for a defect in mitochondrial trifunctional protein develop hepatic steatosis and insulin resistance. Gastroenterology. 2005; 128:1381–1390.

Ihara, K., Abe, K., Hayakawa, K., Makimura, M., KOJIMA-ISHII, K., and Hara, T. Biotin deficiency in a glycogen storage disease type 1b girl fed only with glycogen storage disease-related formula. Pediatr Dermatol. 2011; 28:339–341.

Merritt J. L. M., Norris, M., and Kanungo, S. Fatty acid oxidation disorders. AnnTransl Med. 2018; 6:473

Illsinger, S. Das, A. M. Impact of selected inborn errors of metabolism on prenatal and neonatal development. IUBMB life. 2010; 62: 403–413.

Kanis, J. A., McCloskey, E. V., Johansson, H., Cooper, C., Rizzoli, R., Reginster, J.Y., et al. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int. 2013; 24(1):23–57.

Kaufman, F. R., Loro, M. L., Azen, C., Wenz, E., Gilsanz, V. Effect of hypogonadism and deficient calcium intake on bone density in patients with galactosemia. J Pediatr.1993;123:365–370.

Kaur, M., Kabra, M., Das, G., Suri, M., Verma, I. Clinical and biochemical studies in homocystinuria. Indian Pediatr. 1995; 32:1067–1076.

Kelly, D. A., Portmann, B., Mowat, A. P., Sherlock, S., Lake, B. D. Niemann-pick disease type c: diagnosis and outcome in children, with particular reference to liver disease. J Pediatr.1993; 123:242–247.

Kishnani, P. S., Austin, S. L., Arn, P., Bali, D. S., Boney, A., Case, L. E., et al., Glycogen storage disease type III diagnosis and management guidelines. Genet Med. 2010; 12:446–463.

Kishnani, P. S. Beckemeyer, A. A. New therapeutic approaches for pompe disease: enzyme replacement therapy and beyond. Pediatr Endocrinol Rev. 2014; 12:114–124.

Kisler, J. E., Whittaker, R. G., McFarland, R. Mitochondrial diseases in childhood: a clinical approach to investigation and management. Dev Med Child Neurol. 2010; 52:422–433.

Koletzko, B., Beblo, S., Demmelmair, H., Hanebutt, F. L. Omega-3 lc-pufa supply and neurological outcomes in children with phenylketonuria (PKU). J Pediatr Gastroenterol Nutr. 2009; 48: S2–S7.

Kölker, S., Valayannopoulos, V., Burlina, A. B., Sykut-Cegielska, J., Wijburg, F. A., Teles, E. L., et al. The phenotypic spectrum of organic acidurias and urea cycle disorders. part 2: the evolving clinical phenotype. J Inherit Metab Dis. 2015; 38:1059–1074

Koura, H. M., Ismail, N. A., Kamel, A. F., Ahmed, A. M., Saad-Hussein, A., Effat, L. K. A long-term study of bone mineral density in patients with phenylketonuria under diet therapy. Arch Med Sci. 2011; 7:493-500.

Kuczmarski, R. J., Ogden CL., Grummer-Strawn LM., Flegal KM., Guo SS., Wei R., et al.,; 2000 CDC Growth Charts: United States. Adv Data. 2000. p. 1-27.

Kuriyan, R. Body composition techniques. Iidian J Med Res. 2018; 148:648.

Leahy, S., O'Neill, C., Sohun, R., Jakeman, P. A comparison of dual energy x-ray absorptiometry and bioelectrical impedance analysis to measure total and segmental body composition in healthy young adults. Eur J Appl Physiol. 2012; 112:589–595.

Leonard, J. Morris, A. Inborn errors of metabolism around time of birth. The Lancet. 2000; 356(9229):583–587.

Lim, J., Tan, E., John, C., Poh, S., Yeo, S., Ang, J., et al. Inborn error of metabolism (IEM) screening in singapore by electrospray ionization-tandem mass spectrometry (ESI/MS/MS): An 8-year journey from pilot to current program. Mol Genet Metab. 2014; 113:53–61.

Lindner, M., Gramer, G., Haege, G., Fang-Hoffmann, J., Schwab, K. O., Tacke, U., et al., Efficacy and outcome of expanded newborn screening for metabolic diseases-report of 10 years from south-west germany. Orphanet J Rare Dis. 2011; 6:44.

Lohman, T., Wang, Z., Going, S. B. Human body composition. 2nd rev ed. USA, Philadelphia. 2005.

MacDonald, A., Chakrapani, A., Hendriksz, C., Daly, A., Davies, P., Asplin, D., et al., Protein substitute dosage in PKU: how much do young patients need? Arch Dis Child. 2006; 91:588–593.

MacDonald, A., Daly, A., Chakrapani, A. Dietary management of amino acid inborn errors of metabolism. Middle East Paediatr. 2004; 9:49–54.

MacDonald, A., Lee, P., Davies, P., Daly, A., Lilburn, M., Ozel, H. G., et al., Long-term compliance with a novel vitamin and mineral supplement in older people with PKU. J Inherit Metab Dis. 2008; 31:718–723.

MacDonald, A., Rocha, J., Van Rijn, M., Feillet, F. Nutrition in phenylketonuria. Mol Genet Metab. 2011; 104: S10–S18.

Malinová, V., Wegrzyn, G., Narajczyk, M. The use of elevated doses of genistein-rich soy extract in the gene expression-targeted isoflavone therapy for sanfilippo disease patients. JIMD Rep. 2012; 5: 21–25.

Manoli, I., Myles, J. G., Sloan, J. L., Shchelochkov, O. A., Venditti, C. P. A critical reappraisal of dietary practices in methylmalonic acidemia raises concerns about the safety of medical foods. part 1: isolated methylmalonic acidemias. Genet Med. 2016; 18:386–395.

Marín Soria, J., Aldamiz-Echevarría, L., Castiñeiras Ramos, D. E., Dalmau Serra, J., Fernández Sánchez, A., González Lamuño, D., et al., Programas de cribado neonatal en españa: Actualización y propuestas de futuro. Documento del consenso. Madrid. 2011.

Marra, M., Sammarco, R., De Filippo, E., Caldara, A., Speranza, E., Scalfi, L., et al., Prediction of body composition in anorexia nervosa: Results from a retrospective study. Clin Nutr. 2018; 37:1670–1674.

Martins, A. M. Inborn errors of metabolism: a clinical overview. Sao Paulo Med J. 1999; 117:251–265.

Masurel-Paulet, A., Poggi-Bach, J., Rolland, M.-O., Bernard, O., Guffon, N., Dobbelaere, D., Sarles, J., et al., NTBC treatment in tyrosinaemia type I: long-term outcome in french patients. J Inherit Metab Dis. 2008; 31:81–87.

Matalonga, L., Gort, L., Ribes, A. Small molecules as therapeutic agents for inborn errors of metabolism. J Inherit Metab Dis. 2017; 40:177–193.

Mattar, L., Godart, N., Melchior, J. C., Falissard, B., Kolta, S., Ringuenet, D., et al., Underweight patients with anorexia nervosa: comparison of bioelectrical impedance analysis using five equations to dual x-ray absorptiometry. Clin Nutr. 2011; 30:746–752.

Mayatepek, E., Hoffmann, B., and Meissner, T. Inborn errors of carbohydrate metabolism. Best Pract Res Clin Gastroenterol. 2010; 24:607–618.

Mazariegos, G., Shneider, B., Burton, B., Fox, I. J., Hadzic, N., Kishnani, P., et al., Liver transplantation for pediatric metabolic disease. Mol Genet Metab. 2014; 111:418–427.

Mazzola, P. N., Nalin, T., Castro, K., van Rijn, M., Derks, T. G., Perry, I. D., et al, Analysis of body composition and nutritional status in brazilian phenylketonuria patients. Mol Genet Metab Rep. 2016; 6:16–20.

McBurnie, M., Kronmal, R., Schuett, V., Koch, R., Azeng, C. Physical growth of children treated for phenylketonuria. Ann Hum Biol. 1991; 18:357–368.

Melis, D., Pivonello, R., Cozzolino, M., Della Casa, R., Balivo, F., Del Puente, A., et al. Impaired bone metabolism in glycogen storage disease type 1 is associated with poor metabolic control in type 1a and with granulocyte colony-stimulating factor therapy in type Ib. Horm Res Paediatr. 2014; 81:55–62.

Mendes, A., Martins, F., Cruz, W., Da Silva, L., Abadesso, C., Boaventura, G. Bone development in children and adolescents with PKU. J Inherit Metab Dis. 2012; 35:425–430.

Millington, D., Kodo, N., Norwood, D., Roe, C. Tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism. J Inherit Metab Dis. 1990; 13:321–324.

Millward, D. J. Macronutrient intakes as determinants of dietary protein and amino acid adequacy. J Nutr. 2004; 134:1588S–1596S.

Millward, D. J., Layman, D. K., Tomé, D., Schaafsma, G. Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health. Am J Clin Nutr. 2008; 87:1576S–1581S.

Mirás, A., Bóveda, M. D., Leis, M. R., Mera, A., Aldámiz-Echevarría, L., Fernández-Lorenzo, J. R., et al. Risk factors for developing mineral bone disease in phenylketonuric patients. Mol Genet Metab. 2013; 108:149–154.

Moammar, H., Cheriyan, G., Mathew, R., Al-Sannaa, N. Incidence and patterns of inborn errors of metabolism in the eastern province of saudi arabia, 1983-2008. Ann Saudi Med. 2010; 30:271–27.

Mouchegh, K., Zikánová, M., Hoffmann, G. F., Kretzschmar, B., Kühn, T., Mildenberger, E., et al. Lethal fetal and early neonatal presentation of adenylosuccinate lyase deficiency: observation of 6 patients in 4 families. J Pediatr. 2007; 150:57–61.

Mundy, H., Williams, J., Lee, P., Fewtrell, M. Reduction in bone mineral density in glycogenosis type iii may be due to a mixed muscle and bone deficit. J Inherit Metab Dis. 2008; 31:418–423.

Nassogne, M. C., Heron, B., Touati, G., Rabier, D., Saudubray, J. Urea cycle defects: management and outcome. Inherit Metab Dis. 2005; 28:407–414.

Neovius, M., Hemmingsson, E., Freyschuss, B., Uddén, J. Bioelectrical impedance underestimates total and truncal fatness in abdominally obese women. J Obes. 2006; 14:1731–1738.

Olgac, A., Inci, A., Okur, I., Ezgu, F., Biberoglu, G., Tumer, L. Vitamin d levels and bone mineral density in inborn errors of metabolism requiring specialised diets. J Coll Physicians Surg Pak. 2019; 29:1207–1211.

Olsen, R. K., Olpin, S. E., Andresen, B. S., Miedzybrodzka, Z. H., Pourfarzam, M., Merinero, B., et al., ETFDH mutations as a major cause of riboflavin-responsive multiple acyl-CoA dehydrogenation deficiency. Brain. 2007; 130:2045–2054.

Organization, W. H. Obesity: preventing and managing the global epidemic. Number 894.2000.
https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/

Organization, W. H. et al. Health and development through physical activity and sport. Technical report, 2003.
https://apps.who.int/iris/handle/10665/67796

Cordero AM, Crider KS, Rogers LM, Cannon MJ, Berry RJ. Optimal serum and red blood cell folate concentrations in women of reproductive age for prevention of neural tube defects. World Health Organization guidelines. Morb Mortal Wkly Rep. 2015; 64:421-3.

WHO J, Consultation UE. Protein and amino acid requirements in human nutrition. World Health Organ Tech Rep Ser. 2007; 935:1-265.

Palau, F. Estrategia en enfermedades raras del sistema nacional de salud. ministerio de sanidad y política social 2009 (revised in 2014).

Panis, B., Forget, P. P., Van Kroonenburgh, M., Vermeer, C., Menheere, P., Nieman, F., et al., Bone metabolism in galactosemia. Bone. 2004; 35:982–987.

Panotopoulos, G., Ruiz, J. C., Guy-Grand, B., Basdevant, A. Dual x-ray absorptiometry, bioelectrical impedance, and near infrared interactance in obese women. Med Sci Sports Exerc. 2001; 33:665–670.

Parenti, G., Andria, G., Ballabio, A. Lysosomal storage diseases: from pathophysiology to therapy. Annu Rev Med. 2015; 66:471–486.

Parisi, E., Nicotera, A., Alagna, A., Di Rosa, G. Neonatal seizures, and inborn errors of metabolism: an update. Int J Pharm Compd. 2015.

Patel, D. R., Greydanus, D. E., Omar, H. A., Merrick, J. Neurodevelopmental Disabilities: Clinical Care for Children and Young Adults. 2nd ver ed. New york Springer. 2011; 53-67.

Pateyjohns, I. R., Brinkworth, G. D., Buckley, J. D., Noakes, M., Clifton, P. M. Comparison of three bioelectrical impedance methods with dxa in overweight and obese men. Obesity. 2006; 14:2064–2070.

Patterson, M. C., Vecchio, D., Prady, H., Abel, L., Wraith, J. E. Miglustat for treatment of niemann-pick c disease: a randomised controlled study. Lancet Neurol. 2007; 6:765–772.

Pei KJ, Rajikan RB, Hock NL, Jamil K. Growth and Nutritional Status of Children with Urea Cycle Defects (UCD): A 6-months Follow up Study in Institute of Pediatric, Hospital Kuala Lumpur. Int J Res. 2014; 2:41-52.

Peña-Quintana, L., Llarena, M., Reyes-Suárez, D., and Aldámiz-Echevarria, L. Profile of sodium phenylbutyrate granules for the treatment of urea-cycle disorders: patient perspectives. Patient Prefer Adherence. 2017; 11:1489.

Pérez-Dueñas, B., Cambra, F., Vilaseca, M., Lambruschini, N., Campistol, J., Camacho, J. New approach to osteopenia in phenylketonuric patients. Acta Paediatr. 2002; 91:899–904.

Poggi-Travert, F., Martin, D., de Villemeur, T. B., Bonnefont, J., Vassault, A., Rabier, D., et al., Metabolic intermediates in lactic acidosis: compounds, samples, and interpretation. J Inherit Metab Dis. 1996; 19:478–488.

Porta, F., Mussa, A., Zanin, A., Greggio, N. A., Burlina, A., Spada, M. Impact of metabolic control on bone quality in phenylketonuria and mild hyperphenylalaninemia. J Pediatr Gastroenterol Nutr. 2011; 52:345–350.

Prietsch, V., Lindner, M., Zschocke, J., Nyhan, W., Hoffmann, G. Emergency management of inherited metabolic diseases. J Inherit Metab Dis. 2002; 25:531–546.

Pronicka, E., Adamowicz, M., Kowalik, A., Płoski, R., Radomyska, B., Rogaszewska, M., et al., Elevated carbohydrate-deficient transferrin (CDT) and its normalization on dietary treatment as a useful biochemical test for hereditary fructose intolerance and galactosemia. Pediatr Res. 2007; 62:101-105.

Raghuveer, T. S., Garg, U., Graf, W. D. Inborn errors of metabolism in infancy and early childhood: an update. Am Fam Physician. 2006; 73:1981–1990.

Rake, J., Visser, G., Labrune, P., Leonard, J. V., Ullrich, K., Smit, P. G. Glycogen storage disease type I: diagnosis, management, clinical course and outcome. Results of the European Study on Glycogen Storage Disease Type I (ESGSD I). Eur J Pediatr. 2002; 161: S20-S34.

Rake, J., Visser, G., Huismans, D., Huitema, S., Van Der Veer, E., Piers, D., Smit, G. Bone mineral density in children, adolescents and adults with glycogen storage disease type Ia: a cross-sectional and longitudinal study. J Inherit Metab Dis. 2003; 26:371–384.

Rake JP, Visser G, Smit GP. Disorders of carbohydrate and glycogen metabolism. In: Blau N, Clarke JT, Hoffmann GF, Leonard J, editors. Physician's guide to the treatment and follow-up of metabolic diseases. Berlin: Heidelberg, 2006. p.161-180.

Ramos, R. L., Armán, J. A., Galeano, N. A., Hernández, A. M., Gómez, J. G., Molinero, J. G. Dual energy x-ray absorptimetry: fundamentals, methodology, and clinical applications. Radiología. (2012); 54: 410–423.

Robertson, L. V., McStravick, N., Ripley, S., Weetch, E., Donald, S., Adam, S., et al., Body mass index in adult patients with diet-treated phenylketonuria. J Hum Nutr Diet. 2013; 26:1-6.

Rocha, J. C., van Spronsen, F. J., Almeida, M. F., Soares, G., Quelhas, D., Ramos, E., et al., Dietary treatment in phenylketonuria does not lead to increased risk of obesity or metabolic syndrome. Mol Genet Metab. 2012; 107: 659-663.

Rocha, J. C., van Spronsen, F. J., Almeida, M. F., Ramos, E., Guimarães, J. T., Borges, N. Early dietary treated patients with phenylketonuria can achieve normal growth and body composition. Mol Genet Metab. 2013;110: S40–S43.

Rodríguez-Gómez, I., Santalla, A., Diez-Bermejo, J., Munguía-Izquierdo, D., Alegre, L. M., Nogales-Gadea, G., et al., A new condition in mcardle disease: poor bone health-benefits of an active lifestyle. Med Sci Sports Exerc. 2018; 50:3–10.

Roe, C. R., Sweetman, L., Roe, D. S., David, F., Brunengraber, H. Treatment of cardiomyopathy and rhabdomyolysis in long-chain fat oxidation disorders using an anaplerotic odd-chain triglyceride. J Clin Invest. 2002; 110:259–269.

Rohr, F. Calcar, S. Very long chain acyl coa dehydrogenase deficiency (VLCADD). Genetic Metabolic Dietitians International: Nutrition Guidelines. 2008. http://www.gmdi.org/SearchResults?search=Nutrition%20Guidelines.

Rome LH, Weissmann B, Neufeld EF. Direct demonstration of binding of a lysosomal enzyme, alpha-L-iduronidase, to receptors on cultured fibroblasts. Proc. Natl Acad. Sci. 1979; 76:2331-2334.

Rubio-Agusti, I., Carecchio, M., Bhatia, K. P., Kojovic, M., Parees, I., Chandrashekar, H. S., et al. Movement disorders in adult patients with classical galactosemia. Mov Disord. 2013; 28:804–810.

Rubio-Gozalbo, M., Hamming, S., Van Kroonenburgh, M., Bakker, J., Vermeer, C., Forget, P. P. Bone mineral density in patients with classic galactosaemia. Arch Dis Child. 2002; 87:57–60.

Rush, E., Chandu, V., Plank, L. Prediction of fat-free mass by bioimpedance analysis in migrant asian indian men and women: a cross validation study. Int J Obes. 2006; 30:1125–1131.

Sanderson, S., Green, A., Preece, M., Burton, H. The incidence of inherited metabolic disorders in the west midlands, uk. Arch Dis Child. 2006; 91:896–899.

Saudubray, J., Martin, D., De Lonlay, P., Touati, G., Poggi-Travert, F., Bonnet, D., et al. Recognition and management of fatty acid oxidation defects: a series of 107 patients. J Inherit Metab Dis. 1999; 22:487–502.

Saudubray JM. Clinical approach to inborn errors of metabolism in paediatrics. In: Jean-Marie Saudubray; Georges van den Berghe; John H. Walter. Inborn metabolic diseases. Berlin, Heidelberg, 2012. p. 3-54.

Saudubray, J.-M. Garcia-Cazorla, À. Inborn errors of metabolism overview: pathophysiology, manifestations, evaluation, and management. Pediatr Clin. 2018; 65:179–208.

Saudubray, J.-M., Mochel, F., Lamari, F., Garcia-Cazorla, A. Proposal for a simplified classification of imd based on a pathophysiological approach: A practical guide for clinicians. J Inherit Metab Dis. 2019; 42:706–727.

Saudubray, J.-M., Sedel, F., Walter, J. Clinical approach to treatable inborn metabolic diseases: an introduction. J Inherit Metab Dis. 2006; 29:261–274.

Scaglioni, S., Verduci, E., Fiori, L., Lammardo, A., Rossi, S., Radaelli, G., et al., Giovannini, M. Body mass index rebound and overweight at 8 years of age in hyperphenylalaninaemic children. Acta Paediatr. 2004; 93:1596–1600.

Schiffmann, R. The consequences of genetic and pharmacologic reduction in sphingolipid synthesis. J Inherit Metab Dis. 2015; 38:77–84.

Schwahn, B., Rauch, F., Wendel, U., Schönau, E. Low bone mass in glycogen storage disease type 1 is associated with reduced muscle force and poor metabolic control. J Pediatr. 2002; 141:350–356.

Schwarz, M., Thiel, C., Lübbehusen, J., Dorland, B., de Koning, T., von Figura, K., et al., Deficiency of GDP-Man: GlcNAc2-PP-dolichol mannosyltransferase causes congenital disorder of glycosylation type IK. Am J Hum Genet. 2004; 74:472–481.

Schweitzer, S., Shin, Y., Jakobs, C., Brodehl, J. Long-term outcome in 134 patients with galactosaemia. Eur J Pediatr. 1993; 152:36–43.

Sedel F. Inborn errors of metabolism in adult neurology. Rev Neurol (Paris). 2013;169: S63-9.

Serra-Majem, L., Ribas, L., Pérez-Rodrigo, C., García-Closas, R., Peña-Quintana, L., Aranceta, J. Determinants of nutrient intake among children and adolescents: results from the enkid study. Ann Nutr Metab. 2002; 46: 31–38.

Serrano M, Vilaseca MA, Campistol J. Errores congénitos del metabolismo y práctica deportiva. Apunts Medicina de l'Esport. 2010; 45:185-9.

Singh RH. Nutritional management of patients with urea cycle disorders. J Inherit Metab Dis. 2007; 30:880.

Singh RH, Rohr F, Frazier D, Cunningham A, Mofidi S, Ogata B, et al., Recommendations for the nutrition management of phenylalanine hydroxylase deficiency. Genet Med. 2014; 16:121-31.

Sirrs S, Hollak C, Merkel M, Sechi A, Glamuzina E, Janssen MC, Lachmann R, et al., The frequencies of different inborn errors of metabolism in adult metabolic centres: report from the SSIEM Adult Metabolic Physicians Group. J Inherit Metab Dis. 2015: 85-91.

Sirrs SM, Lehman A, Stockler S, van Karnebeek CD. Treatable inborn errors of metabolism causing neurological symptoms in adults. Mol Genet Metab. 2013; 110:431-8.

Smith SR, Lovejoy JC, Greenway F, Ryan D, deJonge L, de la Bretonne J, et al., Contributions of total body fat, abdominal subcutaneous adipose tissue. Metab Clin Exp. 2013; 110:431-8.

Smith SR, Lovejoy JC, Greenway F, Ryan D, deJonge L, de la Bretonne J, et al., Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. Metab Clin Exp. 2001; 50:425-35.

Specker B, Thiex NW, Sudhagoni RG. Does exercise influence pediatric bone? A systematic review. Clin Orthop Relat Res. 2015; 473:3658-72.

Spiekerkoetter U. Effects of a fat load and exercise on asymptomatic VLCAD deficiency. J Inherit Metab Dis. 2007; 30:405.

Spiekerkoetter U, Lindner M, Santer R, Grotzke M, Baumgartner MR, Boehles H, et al., Management and outcome in 75 individuals with long-chain fatty acid oxidation defects: results from a workshop. J Inherit Metab Dis. 2009; 32:488-97.

Spiekerkoetter, U., Lindner, M., Santer, R., Grotzke, M., Baumgartner, M., Boehles, H., et al. Treatment recommendations in long-chain fatty acid oxidation defects: consensus from a workshop. J Inherit Metab Dis. 2009b; 32:498–505.

Spiekerkoetter, U., Sun, B., Zytkovicz, T., Wanders, R., Strauss, A. W., Wendel, U. Ms/ms-based newborn and family screening detects asymptomatic patients with very-long-chain Acyl-Coa dehydrogenase deficiency. J Pediatria. 2003; 143:335–342.

Stewart, S., Bramley, P., Heighton, R., Green, J., Horsman, A., Losowsky, M., et al., Estimation of body composition from bioelectrical impedance of body segments: comparison with dual-energy x-ray absorptiometry. Br J Nutr.1993; 69:645–655.

Tezel B, Dilli D, Bolat H, Şahman H, Özbaş S, Acıcan D, et al., Scientific Committee of Turkish National Newborn Screening Programme. The development and organization of newborn screening programs in Turkey. J Clin Lab Anal. 2014; 28:63-9.

Therrell Jr BL, Lloyd-Puryear MA, Camp KM, Mann MY. Inborn errors of metabolism identified via newborn screening: ten-year incidence data and costs of nutritional interventions for research agenda planning. Mol Genet Metab. 2014; 113:14-26.

Thomson R, Brinkworth GD, Buckley JD, Noakes M, Clifton PM. Good agreement between bioelectrical impedance and dual-energy X-ray absorptiometry for estimating changes in body composition during weight loss in overweight young women. Clin Nutr. 2007;26:771-7.

Touati G, Valayannopoulos V, Mention K, de Lonlay P, Jouvet P, Depondt E, et al., Methylmalonic and propionic acidurias: management without or with a few supplements of specific amino acid mixture. J Inherit Metab Dis. 2006; 29:288-298.

Underhaug J, Aubi O, Martinez A. Phenylalanine hydroxylase misfolding and pharmacological chaperones. Curr Top Med Chem. 2012; 12:2534-45.

V Rodrigues J, J Henriques B, G Lucas T, M Gomes C. Cofactors and metabolites as protein folding helpers in metabolic diseases. Curr Top Med Chem. 2012; 12:2546-59.

Van den Berg LE, Zandbergen AA, van Capelle CI, de Vries JM, Hop WC, van den Hout JM, et al., Low bone mass in Pompe disease: muscular strength as a predictor of bone mineral density. Bone. 2010; 47:643-649.

Van Erven B, Welling L, Van Calcar SC, Doulgeraki A, Eyskens F, Gribben J, et al., Bone health in classic Galactosemia: systematic review and meta-analysis. JIMD Rep. 2017; 35:87-96.

Van Hove JL, Lohr NJ. Metabolic and monogenic causes of seizures in neonates and young infants. Mol Genet Metab. 2011; 104:214-230.

Van Karnebeek CD, Shevell M, Zschocke J, Moeschler JB, Stockler S. The metabolic evaluation of the child with an intellectual developmental disorder: diagnostic algorithm for identification of treatable causes and new digital resource. Mol Genet Metab. 2014; 111:428-38.

Van Karnebeek CD, Stockler S. Treatable inborn errors of metabolism causing intellectual disability: a systematic literature review. Mol Genet Metab. 2012; 105:368-81.

Van Spronsen, F., Smit, G., Erwich, J. Inherited metabolic diseases and pregnancy. BJOG. 2005; 112:2–11.

Vassili V, Tien PT. Diagnostic work-up in acute conditions of inborn errors of metabolism and storage diseases. In: Oscar H. Del Brutto, Hector H. Garcia. Handbook of clinical neurology. New York. 2013. p.1553-1562.

Vernon HJ. Inborn errors of metabolism: advances in diagnosis and therapy. JAMA pediatrics. 2015; 169:778-82.

Visapää I, Fellman V, Vesa J, Dasvarma A, Hutton JL, Kumar V, et al., GRACILE syndrome, a lethal metabolic disorder with iron overload, is caused by a point mutation in BCS1L. Am J Hum Genet. 2002;71: 863-76.

Weber DR, Coughlin C, Brodsky JL, Lindstrom K, Ficicioglu C, Kaplan P, et al., Low bone mineral density is a common finding in patients with homocystinuria. Mol Genet Metab. 2016; 117:351-4.

Waggoner DD, Buist NR, Donnell GN. Long-term prognosis in galactosaemia: results of a survey of 350 cases. J Inherit Metab Dis. 1990; 13:802-18.

Waisbren SE. Newborn screening for metabolic disorders. JAMA. 2006; 296:993-5.

Waisbren, S. E., Potter, N. L., Gordon, C. M., Green, R. C., Greenstein, P., Gubbels, C. S., et al., The adult galactosemic phenotype. J Inherit Metab Dis. 2012; 35:279–286.

Wald NJ. Guidance on terminology. J Med Screen. 1994; 1:76.

Walter JH, Patterson A, Till J, Besley GT, Fleming G, Henderson MJ. Bloodspot acylcarnitine and amino acid analysis in cord blood samples: efficacy and reference data from a large cohort study. J Inherit Metab Dis. 2009; 32:95.

Walter JH, White FJ, Hall SK, MacDonald A, Rylance G, Boneh A, et al., How practical are recommendations for dietary control in phenylketonuria? The Lancet. 2002; 360:55-7.

Walter JH, MacDonald A. The use of amino acid supplements in inherited metabolic disease. J Inherit Metab Dis. 2006; 29:279-80.

Waters D, Adeloye D, Woolham D, Wastnedge E, Patel S, Rudan I. Global birth prevalence and mortality from inborn errors of metabolism: a systematic analysis of the evidence. J Glob Health. 2018;8: 021102.

Wertheim-Tysarowska K, Gos M, Sykut-Cegielska J, Bal J. Genetic analysis in inherited metabolic disorders--from diagnosis to treatment. Own experience, current state of knowledge and perspectives. Dev Period Med. 2015; 19:413-31.

White JE, Kronmal RA, Acosta PB. Excess weight among children with phenylketonuria. J Am Coll Nutr. 1982; 1:293-303.

Wilcken B. An Introduction to Nutritional Treatment in Inborn Errors of Metabolism-Different Disorders, Different Approaches. Asian Pac J Trop Med. 2004; 34:198-201.

Wilcken B. The consequences of extended newborn screening programmes: Do we know who needs treatment? J Inherit Metab Dis. 2008; 31:173-7.

Wilcken B, Haas M, Joy P, Wiley V, Bowling F, Carpenter K, et al., Expanded newborn screening: outcome in screened and unscreened patients at age 6 years. Pediatrics. 2009;124: e241-8.

Wilcken B, Wiley V, Hammond J, Carpenter K. Screening newborns for inborn errors of metabolism by tandem mass spectrometry. N Engl J Med. 2003; 348:2304-12.

Wilcox G, Strauss BJ, Francis DE, Upton H, Boneh A. Body composition in young adults with inborn errors of protein metabolism-A pilot study. J Inherit Metab Dis. 2005; 28:613-26.

Williams JE, Wells JC, Wilson CM, Haroun D, Lucas A, Fewtrell MS. Evaluation of Lunar Prodigy dual-energy X-ray absorptiometry for assessing body composition in healthy persons and patients by comparison with the criterion 4-component model. Am J Clin Nutr. 2006; 83:1047-54.

Winter SC. Treatment of carnitine deficiency. J Inherit Metab Dis. 2003; 26:171-80.

Wong, E. M., Lehman, A., Acott, P., Gillis, J., Metzger, D. L., Sirrs, S. Hypogonadotropic hypogonadism in males with glycogen storage disease type 1. JIMD Rep. 2017; 36: 79–84.

Yannicelli S, Hambidge KM, Picciano MF. Decreased selenium intake and low plasma selenium concentrations leading to clinical symptoms in a child with propionic acidaemia. J Inherit Metab Dis. 1992; 15:261-8.

Yasawy, M. I., Folsch, U. R., Schmidt, W. E., Schwend, M. Adult hereditary fructose intolerance. World J Gastroenterol. 2009; 15:2412-3.

Yoon HR, Lee KR, Kang S, Lee DH, Yoo HW, Min WK, et al., Screening of newborns and high-risk group of children for inborn metabolic disorders using tandem mass spectrometry in South Korea: a three-year report. Clinica Chimica Acta. 2005; 354:167-80.

Yuan Y, Su W, Zhu M. Threshold-free measures for assessing the performance of medical screening tests. Public Health Front. 2015; 3:57.

Yunus ZM, Rahman SA, Choy YS, Keng WT, Ngu LH. Pilot study of newborn screening of inborn error of metabolism using tandem mass spectrometry in Malaysia: outcome and challenges. J Pediatr Endocr Met. 2016; 29:1031-9.

Zanchetta JR, Plotkin H, Filgueira MA. Bone mass in children: normative values for the 2–20-year-old population. Bone. 1995;16: S393-9.

Zeman J, Bayer M, Štěpán J. Bone mineral density in patients with phenylketonuria. Acta Paediatr. 1999; 88:1348-51.

Zeman J, Pijackova A, Behulova J, Urge O, Saligova D, Hyanek J. Intellectual and school performance in adolescents with phenylketonuria according to their dietary compliance. Eur J Pediatr. 1996;155: S56.

Zulfarina MS, Sharkawi AM, AQILAH-SN ZS, Mokhtar SA, Nazrun SA, Naina-Mohamed I. Influence of adolescents' physical activity on bone mineral acquisition: a systematic review article. Iran J Public Health. 2016; 45:1545.

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APPENDIX

Appendix **A**

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XUNTA DE GALICIA
CONSELLERÍA DE SANIDADE
Secretaría Xeral Técnica

Secretaría Técnica
Comité Autonómico de Ética da Investigación de Galicia
Secretaría Xeral. Consellería de Sanidade
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DICTAMEN DEL COMITÉ DE ÉTICA DE LA INVESTIGACIÓN DE SANTIAGO-LUGO

Guillermo José Prada Ramallal, Secretario del Comité de Ética de la Investigación de Santiago-Lugo,

CERTIFICA:

Que este Comité evaluó en su reunión del día 21 de septiembre de 2017 el estudio:

Título: Evaluación de la composición corporal y estado nutricional en pacientes con trastornos metabólicos hereditarios
Promotor: María Rosaura Leis Trabazo, M.ª Luz Couce Pico
Tipo de estudio: Outros
Versión:
Código del Promotor:
Código de Registro: 2017/310

Y, tomando en consideración las siguientes cuestiones:

- La pertinencia del estudio, teniendo en cuenta el conocimiento disponible, así como los requisitos legales aplicables, y en particular la Ley 14/2007, de investigación biomédica, el Real Decreto 1716/2011, de 18 de noviembre, por el que se establecen los requisitos básicos de autorización y funcionamiento de los biobancos con fines de investigación biomédica y del tratamiento de las muestras biológicas de origen humana, y se regula el funcionamiento y organización del Registro Nacional de Biobancos para investigación biomédica, la ORDEN SAS/3470/2009, de 16 de diciembre, por la que se publican las Directrices sobre estudios Postautorización de Tipo Observacional para medicamentos de uso humano, y la Circular nº 07/2004, de investigaciones clínicas con productos sanitarios.
- La idoneidad del protocolo en relación con los objetivos del estudio, justificación de los riesgos y molestias previsibles para el sujeto, así como los beneficios esperados.
- Los principios éticos da Declaración de Helsinki vigente.
- Los Procedimientos Normalizados de Trabajo del Comité.

Emite un dictamen **FAVORABLE** para la realización del estudio **por el/la investigador/a del centro**:

Centros	**Investigadores Principales**
C.H. Universitario de Santiago	María Rosaura Leis Trabazo, M.ª Luz Couce Pico

En Santiago de Compostela, a 29 de septiembre de 2017.

El Secretario del Comité Territorial de Ética de la Investigación de Santiago Lugo,

XUNTA DE GALICIA CONSELLERÍA DE SANIDADE COMITÉS DE ÉTICA DA INVESTIGACIÓN

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Guillermo José Prada Ramallal

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Guillermo José Prada Ramallal, Secretario del Comité de Ética de la Investigación de Santiago-Lugo,

HACE CONSTAR QUE:

1.- El Comité Territorial de Ética de la Investigación de Santiago-Lugo cumple tanto en su composición como en sus PNTs los requisitos legales vigentes (RD 1090/2015 de ensayos clínicos, y la Ley 14/2007 de Investigación Biomédica).

2.- La composición actual del Comité Territorial de Ética de la Investigación de Santiago-Lugo es:

- **Juan Manuel Vázquez Lago (Presidente).** Médico especialista en Medicina Preventiva y Salud Pública. Área de Gestión Integrada de Santiago.
- **Pilar Rodríguez Ledo (Vicepresidenta).** Médico especialista en Medicina Familiar y Comunitaria. Área de Gestión Integrada de Lugo.
- **Guillermo José Prada Ramallal (Secretario).** Médico especialista en Farmacología Clínica. Área de Gestión Integrada de Santiago. Fundación Ramón Domínguez.
- **Lorenzo Armenteros del Olmo (Vicesecretario).** Médico especialista en Medicina Familiar y Comunitaria. Área de Gestión Integrada de Lugo.
- **Francisco Campos Pérez.** Biólogo. Instituto de Investigación Sanitaria de Santiago de Compostela.
- **Rosana Castelo Domínguez.** Farmacéutica de Atención Primaria. Área de Gestión Integrada de Santiago.
- **Ricardo García Martínez.** Licenciado en Derecho. Área de Gestión Integrada de Lugo.
- **Jaime Gulín Dávila**. Farmacéutico especialista en Farmacia Hospitalaria. Área de Gestión Integrada de Lugo.
- **Victor Herrán Carreira.** Paciente. ADIL-Asociación de Diabéticos Lucense.
- **María Jesús Lamas Díaz.** Farmacéutica especialista en Farmacia Hospitalaria. Área de Gestión Integrada de Santiago.
- **Carlos Rodríguez Moreno.** Médico especialista en Farmacología Clínica. Área de Gestión Integrada de Santiago.
- **Rafael Carlos Vidal Pérez.** Médico especialista en Cardiología. Área de Gestión Integrada de Lugo.
- **María Jesús Wandosell Picatoste.** Enfermera. Área de Gestión Integrada de Santiago.

Para que conste donde proceda, y a petición del promotor/investigador, en Santiago de Compostela, a 29 de septiembre de 2017.

El Secretario del Comité Territorial de Ética de la Investigación de Santiago Lugo,

XUNTA DE GALICIA SECRETARÍA XERAL TÉCNICA COMITÉS DE ÉTICA DA INVESTIGACIÓN CONSELLERÍA DE SANIDADE

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Appendix B

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1) FOLLA DE INFORMACIÓN ÓS PAIS/TUTORES LEGAIS (Gallego)

TÍTULO DO ESTUDO: Avaliación da composición corporal e o estado nutricional en pacientes con enfermidades metabólicas hereditarias.

INVESTIGADOR PRINCIPAL: MARIA ROSAURA LEIS TRABAZO, MARIA LUZ COUCE PICO.

CENTRO: Departamento de Pediatría. Hospital Clínico Universitario de Santiago de Compostela (CHUS).

Este documento ten como obxectivo ofrecerlle información sobre un estudo de investigación en que se convida a participar ó seu fillo. Este estudo foi aprobado polo Comité de Ética de Investigación de Santiago-Lugo.

Si decide participar no mesmo, debe recibir información personalizada do investigador, **ler antes este documento** e facer todas preguntas que precise para comprender os detalles sobre o estudo. Se así o desexa, pode levar o documento, consultalo con outras persoas, e tomarse o tempo necesario para decidir se participa ou non.

A participación neste estudo é completamente **voluntaria**. Vostede pode decidir non participar ou, se acepta facelo, cambiar de parecer retirando o consentimento en calquera momento sen ter que dar explicacións. Asegurámoslle que esta decisión non afectará á relación co seu médico nin á asistencia sanitaria á que o seu fillo ten dereito.

¿Cal é o propósito deste estudo?

O obxectivo deste estudo é avaliar a composición corporal e o estado nutricional de pacientes con estes trastornos. Esto axudará a mellorar a práctica e o asesoramento do estilo de vida destes pacientes, con fin de garantirlles unha mellor calidade de vida.

¿Por que lle ofrecen participar ó meu fillo?

O seu fillo foi convidado a participar neste estudo porque foi diagnosticado con algún tipo de enfermidade metabólica hereditaria E o que se segue no CHUS.

¿En qué consiste a participación do meu fillo?

A participación do seu fillo consiste na realización dun seguimento clínico e analítico rutinario, que se fai coincidir coas visitas médicas que motivan a súa asistencia á nosa consulta. Tamén se recollerá información dun cuestionario sobre o exercicio físico realizado. Adicionalmente se realizará unha análise da súa composición corporal, que durará uns dez minutos e que non suporá ningún tipo de molestia nin efecto secundario.

¿Qué molestias ou inconvenientes ten?

As únicas molestias serán as asociadas á propia venopunción das extraccións de sangue, que coincidirán coas analíticas sanguíneas programadas.

¿Obterei algún beneficio por participar?

Os nenos participantes non obterán ningún beneficio directo de participar no estudo, pero os datos obtidos poderán axudar a comprender millor o efecto directo dos trastornos metabólicos sobre a composición corporal e o estado nutricional, e sobre os factores de risco que poden relacionarse con eles.

¿Recibirei a información que se obteña do estudo?

Se vostede o desexa, se lle facilitarán os resultados das probas realizadas.

¿Publicaranse os resultados deste estudo?

Os resultados deste estudo serán remitidos a publicacións científicas para a su difusión, pero non se transmitirá ningún dato que poida levar á identificación dos participantes.

¿Cómo se protexerá a confidencialidade dos datos e mostras do meu fillo?

En todo momento, poderá acceder ós datos do seu fillo, opoñerse, correxilos ou cancelalos, solicitándoo ante o investigador.

Só o equipo investigador, e as autoridades sanitarias, que teñen o deber de gardar a confidencialidade, terán acceso a todos os datos recollidos no estudo. Poderase transmitir a terceiros información que non poida ser identificada. No caso de que algunha información sexa transmitida a outros países, realizarase cun nivel de protección dos datos equivalentes, como mínimo, ó esixido pola normativa do noso país.

Os datos do seu fillo e as mostras biolóxicas serán recollidas dun modo codificado. Os datos manteranse codificados mentras dure o estudo, o que quere dicir que poseen un código a través do cal, o equipo investigador poderá coñecer a quen pertencen. Unha vez remate o estudo, eses datos se anonimizarán se Vd. o autoriza. As mostras destruiranse unha vez sexan analizadas conforme á LIB.

Os responsables da custodia dos datos e mostras serán Maria ***Luz Couce Pico e M. Rosaura Leis Trabazo*** e o lugar de realización das análises previstas neste estudo é nas *consultas externas de pediatría do CHUS.*

¿Existen intereses económicos neste estudo?

O investigador non recibirá compensación económica pola súa dedicación ó estudo. Vostede non vai ser retribuido pola participación do seu fillo neste estudo.

¿Cómo contactar co-equipo investigador deste estudo?

Vd. pode contactar con M. Luz Couce Pico no teléfono 981951134, e con M. Rosaura Leis Trabazo no teléfono 981951112

Moitas gracias pola súa colaboración.

DOCUMENTO DE CONSENTIMENTO PARA A PARTICIPACIÓN NUN ESTUDO DE INVESTIGACIÓN

TÍTULO: Avaliación da composición corporal e o estado nutricional en pacientes con enfermedades metabólicas hereditarias.

Eu, _____

- *Lin a folla de información ó participante do estudo arriba mencionado que me foi entregado, puiden conversar con _____ e facer todo as preguntas sobre o estudo necesarias.*
- *Comprendo que a participación do meu fillo é voluntaria, e que poido retirarme do estudo cando queira, sen ter que dar explicacións e sen que isto repercuta nos coidados médicos do meu fillo.*
- *Accedo a que se utilicen os datos e mostras do meu fillo nas condicións descritas na folla de información o participante.*
- *Presto libremente a miña conformidade para participar neste estudo.*

Respecto a conservación dos DATOS recollidos neste estudo

☐ Non accedo a que sexan conservados unha vez remate o estudo

☐ Accedo a que sexan conservados anonimizados.

Asinado: Nai/pai/titor do participante

Nome e apelidos: _____

((Só cubra se un pai non está presente): Confirmo que o outro proxenitor non se opón á participación do noso fillo no estudo.

Asinado: Na/pai/titor do participante:

Nome e apelidos: _____

Relación familiar: _____

Asinado: O/A investigador/a que solicita o consentimento

Nome e apelidos: _____

Asentimento para nenos máis de 12 anos de idade (Gallego)

TÍTULO DO ESTUDO: Avaliación da composición corporal e o estado nutricional en pacientes con enfermidades metabólicas hereditarias.

INVESTIGADOR PRINCIPAL: MARIA ROSAURA LEIS TRABAZO, MARIA LUZ COUCE PICO.

CENTRO: Departamento de Pediatría. Hospital Clínico Universitario de Santiago de Compostela (CHUS).

Este documento ten como obxectivo ofrecerlle información sobre un estudo de investigación en que se convida a participar ó seu fillo. Este estudo foi aprobado polo Comité de Ética de Investigación de Santiago-Lugo.

Si decide participar no mesmo, debe recibir información personalizada do investigador, ler antes este documento e facer toda as preguntas que precise para comprender os detalles sobre o estudo. Se así o desexa, pode levar o documento, consultalo con outras persoas, e tomarse o tempo necesario para decidir se participa ou non. A participación neste estudo é completamente voluntaria. Vostede pode decidir non participar ou, se acepta facelo, cambiar de parecer retirando o consentimento en calquera momento sen ter que dar explicacións. Asegurámoslle que esta decisión non afectará á relación co seu médico nin á asistencia sanitaria á que o seu fillo ten dereito.

¿Por que me ofrecer implicados?

Vostede foi convidado a participar neste estudo porque foi diagnosticado con algún tipo de enfermidade metabólica hereditaria e está a ser seguido en CHUS.

¿Cal é a miña parte?

A súa participación é constituída por clínico e de laboratorio siga rutina visitas médicas xogos que motivan a participación na nosa clínica. Tamén se dará información sobre un cuestionario sobre o exercicio. Ademais, a composición corporal, que levará uns dez minutos, e non implicará serán analizados os efectos secundarios ou incomodidade.

¿Que problema ou inconveniente que ten?

O único inconveniente será asociado a punção venosa propio sangue empates, que pode coincidir cos exames de sangue programadas.

Vou recibir calquera beneficio de participar?

Non vai obter calquera beneficio directo de participar no estudo, pero os datos poden axudar a comprender mellor o efecto directo de trastornos metabólicos na composición corporal e de estado e factores de risco nutricional que poden dicir respecto a estes casos.

Vou recibir información a partir do estudo?

Se se desexa, especificar os resultados das probas.

¿Son os resultados deste estudo serán publicados?

Os resultados deste estudo serán sometidos a revistas científicas para a transmisión, pero non hai datos que poderían levar á identificación dos participantes serán transmitidos.

¿Como podo protexer os meus datos confidenciais e mostras?

En todo momento, pode acceder os seus datos, oporse, corrixir ou cancelalalos, pedindo información ao investigador.

Só o equipo de investigación e de saúde autoridades, que teñen o deber de manter a confidencialidade, ten acceso a todos os datos recollidos no estudo. No caso de que calquera información é transmitida a outros países, realizarase cun nivel de protección polo menos equivalente ao esixido polos regulamentos de nosos datos de campo. Os datos e mostras biolóxicas serán recollidos en forma cifrada. Os datos permanecen criptografada durante o estudo, o que significa que ten un código a través do cal o equipo de investigación pode saber quen eles pertencen. Tras concluír o estudo, os datos serán anónimas se acepta. As mostras serán destruídas cando analizados segundo lib.

Responsable da garda de datos e mostras será María Luz Couce Pico e M. Rosaura Leis Trabazo eo lugar da análise proporcionada neste estudo é en pediátrica CHUS.

¿Hai intereses económicos neste estudo?

O investigador non recibirá unha compensación económica pola súa dedicación ao estudo. Non pagará a túa participación neste estudo.

¿Como contactar con equipo de investigación deste estudo?

Pódese contactar con M. Luz Couce Pico no teléfono 981951134 e con M. Rosaura Leis Trabazo no teléfono 981951112

Moitas grazas pola vosa axuda

Lin este documento e se me explicou o seu contido. Comprendín o propósito deste estudo e os procedimentos que se me realizarán durante o mesmo. Outorgo libremente o meu consentimento para participar neste estudo, tal como se me describiu neste documento. Comprendo que recibiréi unha copia deste documento firmado. Este asentimento é válido, polo menos ata que o revoque.

Paciente **Sinatura**

(nome e apelidos do paciente)

Representante legal **Sinatura**

(se o paciente é un menor ou
legalmente autorizado para
actuar como representante personal
para firmar por nome do paciente)

Teste muña **Sinatura**

(se o paciente outorga
consentimento oral, non firmado)

Investigador **Sinatura**

Persoa que presenta/
explica o documento

2) HOJA DE INFORMACIÓN A LOS PADRES / TUTOR LEGAL (Castellano)

TÍTULO DEL ESTUDIO: Evaluación de la composición corporal y estado nutricional en pacientes con enfermedades metabólicas hereditarias.

INVESTIGADOR PRINCIPAL: MARIA ROSAURA LEIS TRABAZO, MARIA LUZ COUCE PICO.

CENTRO: Departamento de Pediatría. Hospital Clínico Universitario de Santiago de Compostela (CHUS)

Este documento tiene como objetivo ofrecerle información sobre un **estudio de investigación** en el que se invita a participar a su hijo. Este estudio ha sido aprobado por el Comité de Ética de Investigación de Santiago-Lugo.

Si decide participar en el mismo, debe recibir información personalizada del investigador, **leer antes este documento** y hacer todas las preguntas que precise para comprender los detalles sobre el estudio. Si así lo desea, puede llevar el documento, consultarlo con otras personas, y tomarse el tiempo necesario para decidir si participa o no.

La participación en este estudio es completamente **voluntaria**. Usted puede decidir no participar o, si acepta hacerlo, cambiar de parecer retirando el consentimiento en cualquier momento sin tener que dar explicaciones. Le aseguramos que esta decisión no afectará a la relación con su médico ni a la asistencia sanitaria a la que su hijo tiene derecho.

¿Cal es el propósito de este estudio?

El objetivo de este estudio es valorar la composición corporal y el estado nutricional en pacientes con este tipo de trastornos. Esto ayudará a mejorar la práctica clínica y el asesoramiento adecuado de su estilo de vida, con el fin de garantizarles una mejor calidad de vida.

¿Por qué le ofrecen participar a mi hijo?

Su hijo ha sido invitado a participar en este estudio porque ha sido diagnosticado de algún tipo de enfermedad metabólica hereditaria y está siendo seguido en el CHUS.

¿En qué consiste la participación de mi hijo?

La participación de su hijo consiste en la realización del seguimiento clínico y analítico rutinario que coincide con las visitas médicas que motivan su asistencia a nuestra consulta. Se le recogerá también información de un cuestionario sobre el ejercicio físico realizado. Adicionalmente se realizará el análisis de su composición

corporal, lo que le llevará unos diez minutos, y que no supondrá ningún tipo de molestia ni efecto secundario.

¿Qué molestias o inconvenientes tiene?

Las únicas molestias serán las asociadas a la propia venopunción de las extracciones de sangre, que se harán coincidir con las analíticas sanguíneas programadas.

¿Obtendré algún beneficio por participar?

Los niños participantes no obtendrán ningún beneficio directo de participar en el estudio, pero los datos obtenidos podrán ayudar a comprender mejor el efecto directo de los trastornos metabólicos sobre la composición corporal y el estado nutricional y sobre los factores de riesgo que pueden relacionarse con estos casos.

¿Recibiré la información que se obtenga del estudio?

Si usted lo desea, se le facilitarán los resultados de las pruebas realizadas.

¿Se publicarán los resultados de este estudio?

Los resultados de este estudio serán remitidos a publicaciones científicas para su difusión, pero no se transmitirá ningún dato que pueda llevar a la identificación de los participantes.

¿Cómo se protegerá la confidencialidad de los datos y muestras de mi hijo?

El tratamiento, comunicación y cesión de los datos de su hijo se hará conforme a lo dispuesto por la Ley Orgánica 15/1999, de 13 de diciembre, de protección de datos de carácter personal. En todo momento, Vd. podrá acceder a los datos de su hijo, oponerse, corregirlos o cancelarlos, solicitándolo ante el investigador.

Sólo el equipo investigador, y las autoridades sanitarias, que tienen deber de guardar la confidencialidad, tendrán acceso a todos los datos recogidos en el estudio. En el caso de que alguna información sea transmitida a otros países, se realizará con un nivel de protección de los datos equivalente, como mínimo, al exigido por la normativa de nuestro país.

Los datos de su hijo y las muestras biológicas serán recogidos de un modo codificado. Los datos se mantendrán codificados mientras dure el estudio, lo que quiere decir que poseen un código a través del cual, el equipo investigador podrá conocer a quién pertenecen. Una vez termine el estudio, esos datos se anonimizarán si Vd. lo autoriza. Las muestras se destruirán una vez sean analizadas conforme a la LIB.

Los responsables de la custodia de los datos y muestras serán **Maria Luz Couce Pico** y **M. Rosaura Leis Trabazo y** el lugar de realización de los análisis previstos en este estudio es en las consultas externas de pediatría del CHUS.

¿Existen intereses económicos en este estudio?

El investigador no recibirá retribución económica por su dedicación al estudio.

Vd. no será retribuido por la participación de su hijo en este estudio.

¿Cómo contactar con el equipo investigador de este estudio?

Vd. puede contactar con María Luz Couce Pico en el teléfono 981951134, y con María Rosaura Leis Trabazo en el teléfono 981951112

Muchas gracias por su colaboración.

DOCUMENTO DE CONSENTIMENTO PARA LA PARTICIPACIÓN EN UN ESTUDIO DE INVESTIGACIÓN (Castellano)

TÍTULO: Evaluación de la composición corporal y estado nutricional en pacientes con enfermedades metabólicas hereditarias.

Yo, _____

- *He leído la hoja de información al participante del estudio arriba mencionado que se me ha entregado, he podido conversar con _____y hacer todas las preguntas sobre el estudio necesarias.*
- *Comprendo que la participación de mi hijo es voluntaria, y que puedo retirarme del estudio cuando quiera, sin tener que dar explicaciones y sin que esto repercuta en los cuidados médicos de mi hijo.*
- *Accedo a que se utilicen los datos y muestras de mi hijo en las condiciones detalladas en la hoja de información al participante.*
- *Presto libremente mi conformidad para participar en este estudio.*

Respecto a la conservación de los DATOS recogidos en este estudio

☐ No accedo a que sean conservados una vez terminado el estudio

☐ Accedo a que sean conservados anonimizados.

Fdo: Madre/padre/tutor del participante

Nombre y apellidos: _____ Fecha: _____

(Sólo cubrir en el caso de uno de los dos progenitores no esté presente): **Mediante está firma confirmo que el otro progenitor no se opone a la participación de nuestro hijo en el estudio.**

Fdo: Padre/Madre del participante:

Nombre y apellidos: _____ Fecha: _____

Relación de parentesco: _____ Fecha: _____

Fdo: El/la investigador/a que solicita el consentimiento

Nombre y apellidos: _____Fecha: _____

Asentimiento para niños mayores de 12 años de edad

TITULO: Evaluación de la composición corporal y estado nutricional en pacientes con enfermedades metabólicas hereditarias.

INVESTIGADOR PRINCIPAL: MARIA ROSAURA LEIS TRABAZO**,** MARIA LUZ COUCE PICO.

CENTRO: Departamento de Pediatría. Hospital Clínico Universitario de Santiago de Compostela (CHUS)

Este documento tiene como objetivo ofrecerle información sobre un estudio de investigación en el que se invita a participar a su hijo. Este estudio ha sido aprobado por el Comité de Ética de Investigación de Santiago-Lugo.

Si decide participar en el mismo, debe recibir información personalizada del investigador, leer antes este documento y hacer todas las preguntas que precise para comprender los detalles sobre el estudio. Si así lo desea, puede llevar el documento, consultarlo con otras personas, y tomarse el tiempo necesario para decidir si participa o no.

La participación en este estudio es completamente voluntaria. Usted puede decidir no participar o, si acepta hacerlo, cambiar de parecer retirando el consentimiento en cualquier momento sin tener que dar explicaciones. Le aseguramos que esta decisión no afectará a la relación con su médico ni a la asistencia sanitaria a la que su hijo tiene derecho.

¿Por qué me ofreces participar?

Usted ha sido invitado a participar en este estudio porque ha sido diagnosticado con alguna forma de enfermedad metabólica hereditaria y está siendo seguido en CHUS.

¿Cuál es mi participación?

Su participación consiste en el seguimiento clínico y analítico de rutina que coincide con las visitas médicas que motivan su asistencia a nuestra consulta. También se le dará información sobre un cuestionario sobre el ejercicio físico. Además, se analizará la composición corporal, que tomará unos diez minutos, y no implicará ningún tipo de molestia o efecto secundario.

¿Qué molestias o inconvenientes tiene?

Las únicas molestias serán las asociadas a la propia venopunción de las extracciones de sangre, que se harán coincidir con las analíticas sanguíneas programadas.

¿Recibiré algún beneficio por participar?

No obtendrá ningún beneficio directo de participar en el estudio, pero los datos obtenidos pueden ayudar a comprender mejor el efecto directo de los trastornos metabólicos sobre la composición corporal y el estado nutricional y sobre los factores de riesgo que pueden relacionarse con estos casos.

¿Recibiré la información obtenida del estudio?

Si lo desea, se le proporcionarán los resultados de las pruebas realizadas.

¿Se publicarán los resultados de este estudio?

Los resultados de este estudio serán enviados a publicaciones científicas para difusión, pero no se transmitirán datos que puedan conducir a la identificación de los participantes.

¿Cómo protegeré la confidencialidad de mis datos y muestras?

El tratamiento, comunicación y transferencia de sus datos se realizará de acuerdo con lo establecido en la Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal. En todo momento, usted puede acceder a sus datos, oponerse, corregirlos o cancelarlos, solicitando su información ante el investigador.

Sólo el equipo de investigación y las autoridades sanitarias, que tienen el deber de mantener la confidencialidad, tendrán acceso a todos los datos recogidos en el estudio. En el caso de que cualquier información se transmita a otros países, se llevará a cabo con un nivel de protección de datos equivalente al menos al requerido por las regulaciones de nuestro país.

Sus datos y muestras biológicas se recogerán de manera codificada. Los datos permanecerán cifrados durante el estudio, lo que significa que usted tiene un código a través del cual el equipo de investigación puede saber a quién pertenecen. Una vez completado el estudio, los datos serán anónimos si usted lo autoriza. Las muestras serán destruidas una vez que se analizan según LIB.

Los responsables de la custodia de los datos y muestras serán María Luz Couce Pico y M. Rosaura Leis Trabazo y el lugar de realización de los análisis previstos en este estudio es en las consultas externas de pediatría del CHUS.

¿Hay intereses económicos en este estudio?

El investigador no recibirá compensación financiera por su dedicación al estudio. Usted no pagará por su participación en este estudio.

¿Cómo contactar al equipo de investigación de este estudio?

Puede ponerse en contacto con M. Luz Couce Pico al teléfono 981951134, y con M. Rosaura Leis Trabazo al teléfono 981951112

Muchas gracias por su ayuda.

He leído este documento y se me ha explicado su contenido. He comprendido el propósito de este estudio y los procedimientos que se me realizarán durante el mismo. Otorgo libremente mi consentimiento para participar en este estudio, tal como se me ha descrito en este documento. Comprendo que recibiré una copia de este documento firmado. Este asentimiento es válido, a menos que y hasta que lo revoque.

Paciente	**Firma**	**Fecha**
(nombre y apellidos del paciente)		
Representante legal	**Firma**	**Fecha**
(si el paciente es un menor o legalmente autorizado para actuar como representante personal para firmar por nombre del paciente)		
Testigo	**Firma**	**Fecha**
(si el paciente otorga consentimiento oral, no firmado)		
Investigador	**Firma**	**Fecha**
Persona que presenta/ explica el documento		

Appendix C

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Fecha de la entrevista □□/□□/□□□□

día mes año

ESTUDIO:

EVALUACIÓN DE LA COMPOSICIÓN CORPORAL Y ESTADO NUTRICIONAL EN PACIENTE CON TRASTORNOS METABÓLICOS GENÉTICOS

CUADERNO DE RECOGIDA DE DATOS

1) DATOS DEMOGRAFICOS

- Fecha de nacimiento ☐☐/☐☐/☐☐☐☐
  Día Mes Año
- Edad: ☐☐ años ☐☐ meses
- Sexo:
  - ☐1 Hombre
  - ☐2 Mujer

CLASE SOCIAL

Índique el nivel de estudios del padre/madre del niño/a:

¿Qué estudios ha realizado el **padre** del niño/a?	¿Qué estudios ha realizado la **madre** del niño/a?
☐1 Sin estudios	☐1 Sin estudios
☐2 No sabe leer o escribir	☐2 No sabe leer o escribir
☐3 Estudios de 1º Grado (Estudios primarios, EGB hasta 5º)	☐3 Estudios de 1º Grado (Estudios primarios, EGB hasta 5º)
☐4 Estudios de 2º Grado, primer ciclo (Graduado escolar, EGB hasta 8º, Bachiller elemental)	☐4 Estudios de 2º Grado, primer ciclo (Graduado escolar, EGB hasta 8º, Bachiller elemental)
☐5 Estudios de 2º grado, segundo ciclo (Bachiller Superior, FP, BUP, Aprendizaje y Maestría industrial, COU)	☐5 Estudios de 2º grado, segundo ciclo (Bachiller Superior, FP, BUP, Aprendizaje y Maestría industrial, COU)
☐6 Estudios de 3º grado, primer ciclo (Perito, Ingeniero técnico, Escuelas Universitarias, Magisterio)	☐6 Estudios de 3º grado, primer ciclo (Perito, Ingeniero técnico, Escuelas Universitarias, Magisterio)
☐7 Estudios de 3º grado, segundo y tercer ciclo (Ingeniero superior, Licenciado, Doctorado, Master)	☐7 Estudios de 3º grado, segundo y tercer ciclo (Ingeniero superior, Licenciado, Doctorado, Master)
☐98 NS/NC	☐98 NS/NC

Está el **padre** del niño trabajando en la actualidad:

☐0 No
☐1 Sí
☐98 NS/NC

Si la respuesta fué no, ¿Por qué motivo?

☐1 Jubilado
☐2 En paro, con subsidio
☐3 En paro, sin subsidio
☐4 Estudiando
☐5 Invalidez
☐98 NS/NC
☐99
Otros……Especificar……..

Está la **madre** del niño trabajando en la actualidad:

☐0 No
☐1 Sí
☐98 NS/NC

Si la respuesta fué no, ¿Por qué motivo?

☐1 Jubilado
☐2 En paro, con subsidio
☐3 En paro, sin subsidio
☐4 Estudiando
☐5 Invalidez
☐98 NS/NC
☐99
Otros…… Especificar…..…

Asignación del padre a un subgrupo de ocupación (3 dígitos) según el CNO-1994 (ver Anexo 1) ☐☐☐

Asignación de la madre a un subgrupo de ocupación (3 dígitos) según el CNO-1994 (ver Anexo 1) ☐☐☐

- Estado civil de los padres

☐0 Casados

☐1 Separados

☐2 Divorciados

☐3 Viudo/a

☐4 Pareja de hecho

-¿Quien pasa la mayor parte del tiempo con el niño?

☐0 Madre

☐1 Padre

☐2 Ambos

☐3 Otros (abuelos, tios…)

2) Historia de la madre durante el embarazo

- Ganancia ponderal durante el embarazo: ☐☐ kg

- Diabetes gestacional:
  - ☐0 No
  - ☐1 Sí (En caso de que sea afirmativa responder a la siguiente pregunta)

  ***Tratamiento diabetes gestacional**:*
  - ☐0 Dieta
  - ☐1 Insulina
  - ☐2 Ambos
  - ☐3 Otros

- Hipertensión durante el embarazo
  - ☐0 No
  - ☐1 SI

- Hábitos tóxicos durante el embarazo:
  - ☐0 No
  - ☐1 Tabacos
  - ☐2 Alcohol
  - ☐3 Tabaco más alcohol
  - ☐4 Drogas

- Toxi-infecciones:
  - ☐0 No
  - ☐1 Si. *Especificar:*

- Tipo de parto:
  - ☐0 Espontáneo
  - ☐1 Cesárea
  - ☐2 Forcéps
  - ☐3 Ventosa

3) Historia del niño:

- Edad gestacional: ☐☐ semanas
- Peso al nacimiento: ☐☐☐☐ g
- Longitud al nacimiento: ☐☐, ☐ cm
- Perímetro de cráneo al nacimiento: ☐☐, ☐ cm (***Opcional)***
- Tiempo de diagnóstico: _____ día/ mes/año

- Antecedentes familiares de la enfermedad.

Quien	Edad	Enfermedad
Padre		
Madre		
Hermana		
Hermano		
De parte Madre (tío, tía, abuela, abuelo, sobrino)		
De parte Padre (tío, tía, abuela, abuelo, sobrino)		

4) EXPLORACION FISICA

Medidas antropométricas del niño/a participante (por la investigator)

Antropometría	Medida 1	Medida 2	Medida 3
Peso (kg)	□□□,□□	□□□,□□	□□□,□□
Talla (cm)	□□□,□□	□□□,□□	□□□,□□
Perímetro cintura (cm)	□□□,□□	□□□,□□	□□□,□□
Perímetro cadera (cm)	□□□,□□	□□□,□□	□□□,□□
Pliegue cutáneo: biceps (mm)	□□,□□	□□,□□	□□,□□
Pliegue cutáneo: tríceps (mm)	□□,□□	□□,□□	□□,□□
Pligue cutáneos: subescapular (mm)	□□,□□	□□,□□	□□,□□
Pliegue cutáneso: suprailiaco (mm)	□□,□□	□□,□□	□□,□□

Estadio Tanner:

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

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6) CUESTIONARIO DE ACTIVIDAD FÍSICA

¿En que vas al colegio?

☐1 Caminando.

☐2 Transporte público.

☐3 Transporte particular.

- Si tu respuesta fue caminando, ¿Cuánto tiempo te lleva llegar al colegio?
☐ mins.

¿Cuantas horas a la semana realizas de educación física durante el horario escolar?

1 ninguna

2 1 hora a la semana

3 2 horas a la semana

4 3 horas a la semana

Si realizas algún deporte más de una hora, especificar cual:

¿Cuánto tiempo al día/semana dedicas a las siguientes actividades, ya sea en el colegio o en casa?

	Días a la semana	**Horas cada día**	**NS/NC**
Actividades que no requieren actividad física (lectura, TV, sentado/caminar poco)	☐ días	☐☐ horas	☐98
Caminar bastante, sin esfuerzos vigoros, (pasear, ir en bici…)	☐ días	☐☐ horas	☐98
Caminar bastante, con esfuerzos vigorosos (correr, esquiar, tenis, bailar, juegos de pelota)	☐ días	☐☐ horas	☐98

Esfuerzos vigorosos y de mucha actividad (entrenamiento…)	☐ días	☐☐ horas	☐98
Actividades en el hogar (ayudar en las tareas domésticas)	-	-	☐98
Actividad física en familia (pasear, actividades de pelota, tenis…)	-	-	☐98

¿Es miembro su hijo/a de algún club deportivo?

❍1 Sí
❍2 No

¿Cuánto tiempo pasa al día haciendo ejercicio en el club deportivo?

|___|___| horas/día |___|___| horas/semana |___|___| días/semana

¿Qué tipo de deporte practica su hijo/a en el club deportivo?
Por favor, marque la opción que corresponda.

❍ fútbol
❍ natación
❍ tenis
❍ gimnasia rítmica
❍ Otra. Por favor, especificar:

7) CUESTIONARIO DE SEDENTARISMO Y PATRONES DE CONSUMO

Cuestionarios sobre comportamientos sedentario

¿Cuánto tiempo suele ver su hijo/a la televisión/vídeo/DVD por día?

	Nada en absoluto	Menos de 30 min. por día	Menos de 1 hora por día	Aprox. 1-2 horas por día	Aprox. 2-3 horas por día	Más de tres horas por día	NS/NC
Entre semana	❍1	❍2	❍3	❍4	❍5	❍6	❍98
Sábado/domingo	❍1	❍2	❍9	❍4	❍5	❍6	❍98

¿Cuánto tiempo suele usar su hijo/a el ordenador (Internet, videojuegos.)?

	Nada en absoluto	Menos de 30 min. por día	Menos de 1 hora por día	Aprox. 1-2 horas por día	Aprox. 2-3 horas por día	Más de tres horas por día	NS/NC
Entre semana	❍$_1$	❍$_2$	❍$_3$	❍4	❍5	❍6	❍98
Sábado/domingo	❍$_1$	❍$_2$	❍$_9$	❍4	❍5	❍6	❍98

¿Cuánto tiempo suele usar su hijo la consola al día?

	Nada en absoluto	**Menos de 30 min. por día**	**Menos de 1 hora por día**	**Aprox. 1-2 horas por día**	**Aprox. 2-3 horas por día**	**Más de tres horas por día**	**NS/NC**
Entre semana	❍1	❍2	❍3	❍4	❍5	❍6	❍98
Sábado/domingo	❍1	❍2	❍9	❍4	❍5	❍6	❍98

¿Cuánto tiempo suele usar el móvil al día?

	Nada en absoluto	**Menos de 30 min. por día**	**Menos de 1 hora por día**	**Aprox. 1-2 horas por día**	**Aprox. 2-3 horas por día**	**Más de tres horas por día**	**NS/NC**
Entre semana	❍1	❍2	❍3	❍4	❍5	❍6	❍98
Sábado/domingo	❍1	❍2	❍9	❍4	❍5	❍6	❍98

¿Cuáles de los siguientes aparatos tiene su hijo/a en su habitación? ¿y en el hogar?

Por favor, marque todas las opciones que correspondan

❍$_1$**habitación**	❍$_2$ **hogar**

- ❍ Televisor ❍$_1$ ❍$_2$
- ❍ Ordenador ❍$_1$ ❍$_2$
- ❍ Conexión a Internet ❍$_1$ ❍$_2$
- ❍ Vídeo/DVD ❍$_1$ ❍$_2$
- ❍ Equipo musical ❍$_1$ ❍$_2$
- ❍ Consola de videojuegos ❍$_1$ ❍$_2$

❍ Móvil ❍1 ❍2

❍ Ninguno de ellos

¿Cuándo suele ver su hijo/a la televisión?

Por favor, marque todas las opciones que correspondan.

❍1 No la ve
❍2 Pronto por la mañana (6-9 a.m.) **SI** ❍1 **NO**❍2
❍3 Por la mañana (9-12 a.m.) **SI** ❍1 **NO**❍2
❍4 Al mediodía (12-3 p.m.) **SI** ❍1 **NO**❍2
❍5 Después de comer (3-6 p.m.) **SI** ❍1 **NO**❍2
❍6 Por la tarde (6-9 p.m.) **SI** ❍1 **NO**❍2
❍7 Por la noche (9-12 p.m.) **SI** ❍1 **NO**❍2
❍98 NS/NC

¿Con quién suele ver su hijo/a la televisión?

Por favor, marque la situación más habitual.

❍1 Solo
❍2 Con sus padres/tutores
❍3 Con sus hermanos/as
❍4 Con sus amigos/as
❍5 Apenas ve la televisión.

¿Comes enfrente del TV?:

☐1 Nunca
☐2 Casi nunca
☐3 Casi siempre
☐4 siempre

¿Cuántas horas sueles dormir a diario durante la semana?

☐☐ horas

¿Cuántas horas sueles dormir los días de fin de semana? ☐☐ horas

¿Cuántas horas al día dedicas a hacer los deberes o tareas escolares fuera del horario del colegio?

☐1 Ninguna
☐2 Media hora al día
☐3 1 Hora al día
☐4 2 Horas al día
☐5 Más de 3 horas
☐98 NS/NC

8) CUESTIONARIO DE FRECUENCIA DE CONSUMO

En el último mes, ¿con qué frecuencia ha consumido su hijo/a los siguientes alimentos y bebidas?

Indicar en cada uno de los alimentos con qué frecuencia lo consume, eligiendo una de las 9 casillas que aparecen a la derecha. Si consumé 2 veces al día ese alimento poner una cruz dentro de la casilla 2-3 AL DÍA.

Por favor, limítese a las cuatro últimas semanas y excluya las comidas del colegio.

LACTEOS		Nunca o casi nunca ☐1	Veces al día: 1 ☐2	2-3 ☐3	4-6 ☐4	+6 ☐5	A la semana: 1 ☐6	2-4 ☐7	5-6 ☐8	Al mes: 1-3 ☐9	NS/NC ☐98	TIPO
Leche	Sin azúcar ☐1											☐1 Desnatada ☐2 Semidesnatada ☐3 Entera
	Con azúcar ☐2											☐1 Desnatada ☐2 Semidesnatada ☐3 Entera
Yogurt	Natural o kéfir sin azúcar ☐1											☐1 Desnatada ☐2 Entera
	Yogur azucarado ☐2											☐1 Desnatada ☐2 Entera
	Bebidas lácteas fermentadas (actimel®, LCR®, etc)											☐1 Desnatada ☐2 Entera
Queso												☐1 Fresco
												☐2 Curado/semicurado
												☐3Untar (ej.Philadelphia)
												☐4 Queso rallado
Nata												
Batidos lácteos												

HUEVO, CARNES Y PESCADOS	Nunca o casi nunca ☐1	Veces al día				A la semana			Al mes	NS/ NC ☐ 98	TIPO
		1 ☐ 2	2-3 ☐3	4-6 ☐4	+6 ☐5	1 ☐ 6	2-4 ☐7	5-6 ☐8	1-3 ☐9		
Huevo											☐1 Frito/ revuelto/ tortilla ☐2 Duro/ escalfado
Pollo/pavo											☐1 Fresco cocinado ☐2 Frita
Carne de ternera o vaca											☐1 Fresca cocinada ☐2 Frita
Carne de cerdo											☐1 Fresca cocinada ☐2 Frita
Carne de cordero											
Productos loncheados y conservados listos para cocinar (embutidos, jamón, lomo,etc)											
Pescado blanco, varitas de pescado											☐1 Cocinado ☐2 Frito
Pescado azul											☐1 Cocinado ☐2 Frito
Mariscos											

VERDURAS Y HORTALIZAS	Nunca o casi nunca ☐1	Veces al día				A la semana			Al mes	NS/ NC ☐ 98	TIPO
		1 ☐ 2	2-3 ☐3	4-6 ☐4	+6 ☐5	1 ☐ 6	2-4 ☐7	5-6 ☐8	1-3 ☐9		
Vegetales crudos (mezclados en la ensalada, zanahoria, pepino, lechuga, escarola, endibias, tomate, etc)											
Vegetales cocinados (Col, coliflor, brócoles, judías verdes, etc)											
Patatas											☐1 Cocinadas ☐2 Fritas

FRUTAS	Nunca o casi nunca ☐1	Veces al día				A la semana			Al mes	NS/ NC ☐ 98	TIPO
		1 ☐ 2	2-3 ☐3	4-6 ☐4	+6 ☐5	1 ☐ 6	2-4 ☐7	5-6 ☐8	1-3 ☐9		
Frutas frescas (también licuadas) **sin azúcar** añadido											
Frutas frescas (también licuadas) **con azúcar** añadido o en almíbar											
Zumos de frutas naturales											
LEGUMBRES	**Nunca o casi nunca** ☐1	**Veces al día**				**A la semana**			**Al mes**	**NS/ NC**	**TIPO**
		1 ☐ 2	**2-3** ☐3	**4-6** ☐4	**+6** ☐5	**1** ☐ 6	**2-4** ☐7	**5-6** ☐8	**1-3** ☐9	☐ 98	
Lentejas											
Garbanzos											
Alubias (pintas, blancas, negras)											
AZÚCARES	**Nunca o casi nunca** ☐1	**Veces al día**				**A la semana**			**Al mes**	**NS/ NC**	**TIPO**
		1 ☐ 2	**2-3** ☐3	**4-6** ☐4	**+6** ☐5	**1** ☐ 6	**2-4** ☐7	**5-6** ☐8	**1-3** ☐9	☐ 98	
Azúcar añadido											
Miel											
Membrillo											
Mermeladas, confituras											
Cacao soluble en polvo											
Nocilla o crema de avellanas											

CEREALES, PASTA, ARROZ	Nunca o casi nunca ☐1	Veces al día				A la semana			Al mes	NS/ NC ☐ 98	TIPO
		1 ☐ 2	2-3 ☐3	4-6 ☐4	+6 ☐5	1 ☐ 6	2-4 ☐7	5-6☐ 8	1-3 ☐9		
Pan											☐1 Pan blanco
											☐2 Biscottes
Pan integral											☐1 Pan integral
											☐2 Biscottes
Pan de molde											☐1 Blanco
											☐2 Integral
Galletas sin azúcar, integrales, de cereales…											
Cereales de desayuno											☐ 1Azucarados, muesli azucarado, chocolateados (corn flakes, crispies, etc
											☐2No azucarados, muesli natural, copos de avena
											☐3 Barritas de cereales
Pasta, fideos											☐1 Normal
											☐2 Integral
Arroz											☐1 Normal
											☐2 Integral
Pizza como plato principal											

SNACKS, APERITIVOS DULCES	Nunca o casi nunca ☐1	Veces al día				A la semana			Al mes	NS/ NC ☐ 98	TIPO
		1 ☐ 2	2-3 ☐3	4-6 ☐4	+6 ☐5	1 ☐ 6	2-4 ☐7	5-6☐ 8	1-3 ☐9		
Tortas o bollos, pasteles (ej.tarta de manzana, crepes, palmeras de hojaldre, etc)											
Chocolate, barritas de chocolate (Mars, Lion, Kit Kat, etc)											
Galletas, Pasteles envasados, tartas (ej. donuts, bollycao, cañas de chocolate, etc)											
Caramelos, chucherías, gominolas, etc.											
Helados, polos, sorbetes de fruta (ej. magnum, calippo, etc)											
SNACKS, APERITIVOS SALADOS	**Nunca o casi nunca ☐1**	**Veces al día**				**A la semana**			**Al mes**	**NS/ NC ☐ 98**	**TIPO**
		1 ☐ 2	**2-3 ☐3**	**4-6 ☐4**	**+6 ☐5**	**1 ☐ 6**	**2-4 ☐7**	**5-6☐ 8**	**1-3 ☐9**		
Frutos secos y semillas (pipas, cacahuetes,…)											
Patatas fritas, aperitivos de maíz, palomitas de maíz,(cheetos, gusanitos..)											

ACEITES Y GRASAS	Nunca o casi nunca ☐1	Veces al día				A la semana			Al mes	NS/ NC ☐98	TIPO
		1 ☐2	2-3 ☐3	4-6 ☐4	+6 ☐5	1 ☐6	2-4 ☐7	5-6 ☐8	1-3 ☐9		
Mantequilla											
Margarina											
Aceite de oliva											
Aceite de girasol											
Mahonesa y derivados de la mahonesa (ej. salsa rosa, tártara,etc)											
Ketchup											

BEBIDAS	Nunca o casi nunca ☐1	Veces al día				A la semana			Al mes	NS/ NC ☐98	TIPO
		1 ☐2	2-3 ☐3	4-6 ☐4	+6 ☐5	1 ☐6	2-4 ☐7	5-6 ☐8	1-3 ☐9		
Agua											☐1 Natural
											☐2 Sabor añadido (naranja,melocotón,etc
Bebidas azucaradas: refrescos, té embotellado, etc											
Bebidas light o bebidas refrescantes sin azúcar (ej.coca cola light, coca cola zero, etc)											
Bebidas deportivas, energéticas (aquarius®, isostar®, etc)											
Zumos envasados de frutas (naranja, manzana, piña, etc)											
Café											
Té											
Infusiones											

ALIMENTOS PRECOCINADOS	Nunca o casi nunca ☐1	Veces al día				A la semana			Al mes	NS/ NC ☐98	TIPO
		1 ☐2	2-3 ☐3	4-6 ☐4	+6 ☐5	1 ☐6	2-4 ☐7	5-6 ☐8	1-3 ☐9		
Alimentos precocinados (croquetas, empanadillas, lasañas, barritas de pescado, San Jacobo, etc)											
Pizza											
Hamburguesa											
Productos sustitutivos de la carne y productos de soja	**Nunca o casi nunca ☐1**	**Veces al día**				**A la semana**			**Al mes**	**NS/ NC ☐98**	**TIPO**
		1 ☐2	2-3 ☐3	4-6 ☐4	+6 ☐5	1 ☐6	2-4 ☐7	5-6 ☐8	1-3 ☐9		
Tofu, tempé, leche de soja, yogures de soja, etc											

9) Registro del consumo de alimentos de 3 días

Por favor, mantenga un registro de todo lo que COMER y BEBER durante 3 días - 2 días laborales y 1 día de fin de semana. Incluya todas las comidas, aperitivos y bebidas. Elija por favor los días que son TÍPICOS para sus patrones de alimentación actuales.

Nombre: _____ Fecha: _____

Primer Día	**Alimento**	**Preparación**	**Medida casera**	**Peso aproximado**
Desayuno				
Media mañana				
Comida				
Merienda				
Cena				
Segundo Día				
Desayuno				
Media mañana				
Comida				
Merienda				
Cena				
Tercer Día				
Desayuno				
Media mañana				
Comida				
Merienda				
Cena				

ANEXO 1: CLASIFICACIÓN NACIONAL DE OCUPACIONES (CON-94)

0	**FUERZAS ARMADAS**
00	FUERZAS ARMADAS
001	Escala superior
002	Escala media
003	Escala básica
1	**DIRECCIÓN DE LA EMPRESAS Y DE LAS ADMINISTRACIONES PÚBLICAS**
1A	**DIRECCIÓN DE LAS ADMINISTRACIONES PÚBLICAS Y DE EMPRESAS DE 10 O MÁS ASALARIADOS**
10	PODER EJECUTIVO Y LEGISLATIVO Y DIRECCIÓN DE LAS ADMINISTRACIONES PÚBLICAS; DIRECCIÓN DE ORGANIZACIONES DE INTERÉS
101	Poder ejecutivo y legislativo, y consejo general del poder judcial
102	Personal directivo de las administraciones públicas
103	Gobierno local
104	Dirección de organizaciones de interés
11	DIRECCIÓN DE EMPRESAS DE 10 O MÁS ASALARIADOS
111	Dirección general y presidencia ejecutiva
112	Dirección de departamento de producción
113	Dirección de áreas y departamentos especializados
1B	**GERENCIA DE EMPRESAS CON MENOS DE 10 ASALARIADOS**
12	GERENCIA DE EMPRESAS DE COMERCIO CON MENOS DE 10 ASALARIADOS
121	Gerencia de empresas de comercio al por mayor con menos de 10 asalariados
122	Gerencia de empresas de comercio al por menor con menos de 10 asalariados
13	GERENCIA DE EMPRESAS DE HOSTELERÍA Y RESTAURACIÓN CON MENOS DE 10 ASALARIADOS
131	Gerencia de empresas de hospedaje con menos de 10 asalariados
132	Gerencia de empresas de restauración con menos de 10 asalariados
14	GERENCIA DE OTRAS EMPRESAS CON MENOS DE 10 ASALARIADOS
140	Gerencia de otras empresas con menos de 10 asalariados
1C	**GERENCIA DE EMPRESAS SIN ASALARIADOS**
15	GERENCIA DE EMPRESAS DE COMERCIO SIN ASALARIADOS
151	Gerencia de empresas de comercio al por mayor sin asalariados
152	Gerencia de empresas de comercio al por menor sin asalariados
16	GERENCIA DE EMPRESAS DE HOSTELERÍA SIN ASALARIADOS

161	Gerencia de empresas de hospedaje sin asalariados
162	Gerencia de empresas de restauración sin asalariados
17	GERENCIA DE OTRAS EMPRESAS SIN ASALARIADOS
170	Gerencia de otras empresas sin asalariados
2	**TÉCNICOS Y PROFESIONALES CIENTÍFICOS E INTELECTUALES**
2D	**PROFESIONES ASOCIADAS A TITULACIONES DE 2º Y 3ER CICLO UNIVERSITARIO Y AFINES**
20	PROFESIONES ASOCIADAS A TITULACIONES DE 2º Y 3ER CICLO UNIVERSITARIO EN CIENCIAS FÍSICAS, QUÍMICAS, MATEMÁTICAS E INGENIERÍA
201	Físicos, químicos y asimilados
202	Matemáticos, actuarios, estadísticos y asimilados
203	Profesionales de la informática de nivel superior
204	Arquitectos, urbanistas e ingenieros planificadores de tráfico
205	Ingenieros superiores
21	PROFESIONALES ASOCIADOS A TITULACIONES DE 2º Y 3ER CICLO UNIVERSITARIO EN CIENCIAS NATURALES Y SANIDAD
211	Profesionales en ciencias naturales
212	Médicos y odontólogos
213	Veterinarios
214	Farmacéuticos
219	Otros profesionales de nivel superior de la sanidad
22	PROFESIONES ASOCIADAS A TITULACIONES DE 2º Y 3ER CICLO UNIVERSITARIO EN LA ENSEÑANZA
221	Profesores de universidades y otros centros de enseñanza superior
222	Profesores de enseñanza secundaria
223	Otros profesionales de la enseñanza
23	PROFESIONALES DEL DERECHO
231	Abogados y fiscales
232	Jueces y magistrados
239	Otros profesionales del derecho
24	PROFESIONALES EN ORGANIZACIONES DE EMPRESAS, PROFESIONALES EN LAS CIENCIAS SOCIALES Y HUMANAS ASOCIADAS A TITULACIONES DE 2º Y 3ER CICLO UNIVERSITARIO
241	Profesionales en organización y administración de empresas
242	Economistas
243	Sociólogos, historiadores, filósofos, filólogos, psicólogos y asimilados
25	ESCRITORES, ARTISTAS Y OTRAS PROFESIONES ASOCIADAS
251	Escritores y artistas de la creación o de la interpretación

252 Archiveros, bibliotecarios y profesionales asimilados
253 Diversos profesionales de las administraciones públicas que no pueden ser clasificados en apartados anteriores

2E PROFESIONES ASOCIADAS A UNA TITULACIÓN DE 1ER CICLO UNIVERSITARIO Y AFINES

26 PROFESIONES ASOCIADAS A UNA TITULACIÓN DE 1ER CICLO UNIVERSITARIO EN CIENCIAS FÍSICAS, QUÍMICAS, MATEMÁTICAS, INGENIERÍA Y ASIMILADOS
261 Profesionales asociados a una titulación de 1er ciclo universitario en ciencias físicas, químicas y asimilados
262 Profesionales asociados a una titulación de 1er ciclo universitario en matemáticas, estadística y asimilados
263 Profesionales de nivel medio de informática
264 Arquitectos técnicos
265 Ingenieros técnicos
27 PROFESIONES ASOCIADAS A UNA TITULACION DE 1ER CICLO UNIVERSITARIO EN CIENCIAS NATURALES Y SANIDAD, EXCEPTO ÓPTICOS, FISIOTERAPEUTAS Y ASIMILADOS
271 Profesionales asociados a una titulación de 1er ciclo universitario en ciencias naturales
272 Enfermeros
28 PROFESIONES ASOCIADAS A UNA TITULACIÓN DE 1ER CICLO UNIVERSITARIO EN LA ENSEÑANZA
281 Profesores de enseñanza primaria e infantil
282 Profesores de educación especial
283 Profesorado técnico de formación profesional
29 OTRAS PROFESIONES ASOCIADAS A UNA TITULACIÓN DE 1ER CICLO UNIVERSITARIA
291 Diplomados en contabilidad y graduados sociales y técnicos de empresas y actividades turísticas
292 Ayudantes de archivo, biblioteca y asimilados
293 Diplomados en trabajo social
294 Sacerdotes de las distintas religiones
295 Otros profesionales de las administraciones públicas que no pueden ser clasificados en apartados anteriores

3 TÉCNICOS Y PROFESIONALES DE APOYO
3F TÉCNICOS Y PROFESIONALES DE APOYO

30 TÉCNICOS DE LAS CIENCIAS FÍSICAS, QUÍMICAS E INGENIERÍAS
301 Delineantes y diseñadores técnicos
302 Técnicos de las ciencias físicas, químicas y de las ingenierías
303 Profesionales técnicos de la informática

304	Operadores de equipos ópticos y electrónicos
305	Profesionales en navegación marítima
306	Profesionales en navegación aeronáutica
307	Técnicos en edificación, seguridad en el trabajo y control de calidad
31	TÉCNICOS DE LAS CIENCIAS NATURALES Y DE LA SANIDAD
311	Técnicos de las ciencias naturales y profesionales auxiliares asimilados
312	Técnicos de sanidad
313	Diversos técnicos de sanidad no clasificados en rúbricas anteriores
32	TÉCNICOS EN EDUCACIÓN INFANTIL, INSTRUCTORES DE VUELO, NAVEGACIÓN Y CONDUCCIÓN DE VEHÍCULOS
321	Técnicos en educación infantil y educación especial
322	Instructores de vuelo, navegación y conducción de vehículos
33	PROFESIONALES DE APOYO EN OPERACIONES FINANCIERAS Y COMERCIALES
331	Profesionales de apoyo en operaciones financieras y algunas operaciones comerciales
332	representantes de comercio y técnicos de venta
34	PROFESIONALES DE APOYO A LA GESTIÓN ADMINISTRATIVA
341	Profesionales de apoyo de la gestión administrativa, con tareas administrativas generales
342	Profesionales de carácter administrativo de aduanas, de tributos y asimilados que trabajan en tareas propias de las administraciones públicas
35	OTROS TÉCNICOS Y PROFESIONALES DE APOYO
351	Consignatarios y agentes en la contratación de mano de obra
352	Técnicos especialistas de las Fuerzas de Seguridad y detectives privados
353	Profesionales de apoyo de promoción social
354	Profesionales del mundo artístico, del espectáculo y de los deportes
355	Auxiliares laicos de las religiones
4	**EMPLEADOS DE TIPO ADMINISTRATIVO**
4G	**EMPLEADOS DE TIPO ADMINISTRATIVO**
40	EMPLEADOS EN SERVICIOS CONTABLES, FINANCIEROS, Y DE SERVICIOS DE APOYO A LA PRODUCCIÓN Y AL TRANSPORTE
401	Auxiliares contables y financieros
402	Empleados de registro de materiales, de servicios de apoyo a la producción y al transporte
41	EMPLEADOS DE BIBLIOTECAS, SERVICIOS DE CORREOS Y ASIMILADOS
410	Empleados de bibliotecas, servicios de correos y asimilados
42	OPERADORES DE MÁQUINAS DE OFICINA
421	Taquígrafos y mecanógrafos
422	Grabadores de datos

43	AUXILIARES ADMINISTRATIVOS SIN TAREAS DE ATENCIÓN AL PUBLICO NO CLASIFICADOS ANTERIORMENTE
430	Auxiliares administrativos sin tareas de atención al público no clasificados anteriormente
44	AUXILIARES ADMINISTRATIVOS CON TAREAS DE ATENCIÓN AL PÚBLICO NO CLASIFICADOS ANTERIORMENTE
440	Auxiliares administrativos con tareas de atención al público no clasificados anteriormente
45	EMPLEADOS DE TRATO DIRECTO CON EL PÚBLICO EN AGENCIAS DE VIAJE, RECEPCIONISTAS Y TELEFONISTAS
451	Empleados de información y recepcionistas en oficinas
452	Empleados de agencias de viajes, recepcionistas en establecimientos distintos de oficinas y telefonistas
46	CAJEROS, TAQUILLEROS Y OTROS EMPLEADOS ASIMILADOS EN TRATO DIRECTO CON EL PÚBLICO
460	Cajeros, taquilleros y otros empleados asimilados en trato directo con el público
5	**TRABAJADORES DE LOS SERVICIOS DE RESTAURACIÓN, PERSONALES, PROTECCIÓN Y VENDEDORES DE LOS COMERCIOS**
5H	**TRABAJADORES DE LOS SERVICIOS DE RESTAURACIÓN Y DE SERVICIOS PERSONALES**
50	TRABAJADORES DE LOS SERVICIOS DE RESTAURACIÓN
501	Cocineros y otros preparadores de comidas
502	Camareros, bármanes y asimilados
503	Jefes de cocineros, de camareros y asimilados
51	TRABAJADORES DE LOS SERVICIOS PERSONALES
511	Auxiliares de enfermería y asimilados
512	Trabajadores que se dedican al cuidado de personas y asimilados (excepto auxiliares de enfermería)
513	Peluqueros, especialistas en tratamiento de belleza y trabajadores asimilados
514	Trabajadores que atienden a viajeros y asimilados
515	Mayordomos, ecónomos y asimilados
519	Otros trabajadores de servicios personales
5J	**TRABAJADORES DE LOS SERVICIOS DE PROTECCIÓN Y SEGURIDAD**
52	TRABAJDORES DE SERVICIOS DE PROTECCIÓN Y SEGURIDAD
521	Guardias civiles
522	Policías
523	Bomberos
524	Funcionario de prisiones
525	Guardias jurados y personal de seguridad privado

529	Otros trabajadores de los servicios de protección y seguridad
5K	**DEPENDIENTES DE COMERCIO Y ASIMILADOS**
53	DEPENDIENTES DE COMERCIO Y ASIMILADOS
531	Modelos de moda, arte y publicidad
532	Encargado de sección dentro de un comercio y asimilados
533	Dependientes y exhibidores en tiendas, almacenes, quioscos y mercados
6	**TRABAJADORES CUALIFICADOS EN LA AGRICULTURA Y EN LA PESCA**
6L	**TRABAJADORES CUALIFICADOS EN LA AGRICULTURA Y EN LA PESCA**
60	TRABAJADORES CUALIFICADOS EN ACTIVIDADES AGRÍCOLAS
601	Trabajadores cualificados por cuenta propia
602	Trabajadores cualificados por cuenta ajena en actividades agrícolas
61	TRABAJADORES CUALIFICADOS EN ACTIVIDADES GANADERAS
611	Trabajadores cualificados por cuenta propia en actividades ganaderas
612	Trabajadores cualificados por cuenta ajena en actividades ganaderas
62	TRABAJADORES CUALIFICADOS EN OTRAS ACTIVIDADES AGRARIAS
621	Trabajadores cualificados por cuenta propia en actividades agropecuarias
622	Trabajadores cualificados por cuenta propia en actividades forestales y asimilados
623	Trabajadores cualificados por cuenta ajena en actividades agropecuarias
624	Trabajadores cualificados por cuenta ajena en actividades forestales y asimilados
63	PESCADORES Y TRABAJADORES CUALKIFICADOS EN ACTIVIDADES PISCÍCOLAS
631	Pescadores y trabajadores cualificados por cuenta propia en actividades piscícolas
632	Pescadores y trabajadores cualificados por cuenta ajena en actividades piscícolas
7	**ARTESANOS Y TRABAJADORES CUALIFICADOS DE LAS INDUSTRIAS MANUFACTURERAS, LA CONSTRUCCIÓN, Y LA MINERÍA, EXCEPTO LOS OPERADORES DE INSTALACIONES Y MAQUINARIA**
7M	**TRABAJADORES CUALIFICADOS DE LA CONSTRUCCIÓN, EXCEPTO LOS OPERADORES DE MAQUINARIA**
70	ENCARGADOS DE OBRA Y OTROS ENCARGADOS EN LA CONSTRUCCIÓN
701	Encargados y jefes de equipo en obras estructurales de la construcción
702	Jefes de taller y encargados de trabajadores de acabado de edificios
703	Encargados de pintores, empapeladores y asimilados
71	TRABAJADORES EN OBRAS ESTRUCTURALES DE CONSTRUCCIÓN Y ASIMILADOS
711	Albañiles y mamposteros

712 Trabajadores en hormigón armado, enfoscadores, ferrallistas y asimilados
713 Carpinteros (excepto carpinteros de estructuras metálicas)
714 Otros trabajadores de las obras estructurales de construcción
72 TRABAJADORES DE ACABADO DE CONSTRUCCIONES Y ASIMILADOS; PINTORES Y OTROS ASIMILADOS
721 Revocadores, escayolistas y estuquistas
722 Fontaneros e instaladores de tuberías
723 Electricistas de construcción y asimilados
724 Pintores, barnizadores, empapeladores y asimilados
725 Personal de limpieza de fachadas de edificios y deshollinadores
729 Otros trabajadores de acabado de construcción y asimilados
7N TRABAJADORES CUALIFICADOS DE LAS INDUSTRIAS EXTRACTIVAS, DE LA METALURGIA, LA CONSTRUCCIÓN DE MAQUINARIA Y ASIMILADOS
73 ENCARGADOS EN LA METALURGIA Y JEFES DE TALLERES MECÁNICOS
731 Jefes de taller y encargados de moldeadores, soldadores montadores de estructuras metálicas y afines
732 Jefes de taller de vehículos de motor
733 Jefes de taller de máquinas agrícolas e industriales y motores de avión
734 Jefes de equipo de mecánicos y ajustadores de equipos eléctricos y electrónicos
74 TRABAJADORES DE LAS INDUSTRIAS EXTRACTIVAS
741 Encargados y capataces de la minería
742 Mineros, canteros, pegadores y librantes de la piedra
75 SOLDADORES, CHAPISTAS, MONTADORES DE ESTRUCTURAS METALICAS, HERREROS, ELABORADORES DE HERRAMIENTAS Y ASIMILADOS
751 Moldeadores, soldadores, chapistas, montadores de estructuras metálicas y trabajadores asimilados
752 Herreros, elaboradores de herramientas y asimilados
76 MECÁNICOS Y AJUSTADORES DE MAQUINARIA Y EQUIPOS ELÉCTRICOS Y ELECTRÓNICOS
761 Mecánicos y ajustadores de maquinaria
762 Mecánicos y ajustadores de equipos eléctricos y electrónicos
7P TRABAJADORES CUALIFICADOS DE INDUSTRIAS DE ARTES GRÁFICAS, TEXTIL Y DE LA CONFECCIÓN, DE LA ELABORACIÓN DE ALIMENTOS, EBANISTAS, ARTESANOS Y OTROS ASIMILADOS

77	MECÁNICOS DE PRECISIÓN EN METALES, TRABAJADORES DE ARTES GRÁFICAS, CERAMISTAS, VIDRIEROS Y ARTESANOS DE LA MADERA, TEXTIL Y DEL CUERO
771	Mecánicos de precisión en metales y materiales similares
772	Trabajadores de artes gráficas y asimilados
773	Ceramistas, vidrieros y asimilados
774	Artesanos de la madera, de textiles, del cuero y materiales similares
78	TRABAJADORES DE LA INDUSTRIA DE LA ALIMENTACIÓN, BEBIDAS Y TABACO
780	Trabajadores de la industria de la alimentación, bebidas y tabaco
79	TRABAJADORES QUE TRATAN LA MADERA, EBANISTAS, TRABAJADORES DE LA INDUSTRIA TEXTIL, CONFECCIÓN PIEL, CUERO, CALZADO Y ASIMILADOS
791	Trabajadores que tratan la madera y asimilados
792	Ebanistas y trabajadores asimilados
793	Trabajadores de la industria textil, la confección y asimilados
794	Trabajadores de la industria de la piel, del cuero y del calzado
8	**OPERADORES DE INSTALACIONES Y MAQUINARIA, Y MONTADORES**
8Q	**OPERADORES DE INSTALACIONES INDUSTRIALES, DE MAQUINARIA FIJA; MONTADORES Y ENSAMBLADORES**
80	JEFES DE EQUIPO Y ENCARGADOS EN INSTALACIONES INDUSTRIALES FIJAS
801	Encargados en instalaciones mineras
802	Encargados en instalaciones de procesamiento de metales
803	Encargados de taller de vidriería, cerámica y asimilados
804	Encargados de taller de madera y jefes de equipo en la fabricación de papel
805	Jefes de equipo en instalaciones de tratamiento químico
806	Jefes de equipo en instalaciones de producción de energía y asimilados
807	Jefes de equipo de operadores de robots industriales
81	OPERADORES DE INSTALACIONES INDUSTRIALES FIJAS Y ASIMILADOS
811	Operadores en instalaciones de la extracción y explotación de minerales
812	Operadores en instalaciones para la obtención y transformación de metales
813	Operadores en instalaciones para la obtención, transformación y manipulado del vidrio y la cerámica y asimilados
814	Operadores en instalaciones para el trabajo de la madera y la fabricación de papel
815	Operadores en plantas industriales químicas
816	Operadores en plantas para producción de energía y similares
817	Operadores de robots industriales
82	ENCARGADO DE OPERADORES DE MÁQUINAS FIJAS

821 Encargado de operadores de máquinas para trabajar metales
822 Encargado de operadores de máquinas para fabricar productos químicos
823 Encargado de operadores de máquinas para fabricar productos de caucho y de material plástico
824 Encargado de operadores de máquinas para fabricar productos de madera
825 Jefes de taller de imprenta, encuadernación y fabricación de productos de papel
826 Encargado de operadores de máquinas para fabricar productos textiles y artículos de piel y cuero
827 Encargado de operadores de máquinas para elaborar productos alimenticios, bebidas y tabaco
828 Encargado de montadores
83 OPERADORES DE MÁQUINAS FIJAS
831 Operadores de máquinas para trabajar metales y otros productos minerales
832 Operadores de máquinas para fabricar productos químicos
833 Operadores de máquinas para fabricar productos de caucho y plástico
834 Operadores de máquinas para fabricar productos de madera
835 Operadores de máquinas para imprimir, encuadernar y para fabricar productos de papel y cartón
836 Operadores de máquinas para fabricar productos textiles, artículos de piel y de cuero
837 Operadores de máquinas para elaborar productos alimenticios, bebidas y tabaco
84 MONTADORES Y ENSAMBLADORES
841 Montadores y ensambladores
849 Otros montadores y ensambladores
8R CONDUCTORES Y OPERADORES DE MAQUINARIA MÓVIL
85 MAQUINISTAS DE LOCOMOTORA, OPERADOR DE MAQUINARIA AGRÍCOLA Y DE EQUIPOS PESADOS MÓVILES, Y MARINEROS
851 Maquinistas de locomotoras y asimilados
852 Encargado de operadores de maquinaria de movimiento de tierras y de materiales
853 Operadores de maquinaria agrícola móvil
854 Operadores de otras máquinas móviles
855 Marineros de cubierta de barco y asimilados
86 Conductores de vehículos para el transporte urbano o por carretera
861 Taxistas y conductores de automóviles y furgonetas
862 Conductores de autobuses
863 Conductores de camiones
864 Conductores de motocicletas y ciclomotores
9 TRABAJADORES NO CUALIFICADOS
9S TRABAJADORES NO CUALIFICADOS EN SERVICIOS (EXCEPTO TRANSPORTES)
90 TRABAJADORES NO CUALIFICADOS EN EL COMERCIO
900 Vendedores ambulantes y asimilados

91	EMPLEADOS DOMÉSTICOS Y OTRO PERSONAL DE LIMPIEZA DE INTERIOR DE EDIFICIOS
911	Empleados del hogar
912	Personal de limpieza de oficinas, hoteles y otros trabajadores asimilados
92	CONSERJE DE EDIFICIOS, LIMPIACRISTALES Y VIGILANTES
921	Conserjes de edificios, limpiacristales y asimilados
922	Vigilantes, guardianes y asimilados
93	OTROS TRABAJADORES NO CUALIFICADOS EN OTROS SERVICIOS
931	Limpiabotas y otros trabajadores de oficios callejeros
932	Ordenanzas
933	Mozos de equipaje y asimilados
934	Lectores de contadores (agua…) y recolectores de dinero de máquinas expendedoras
935	Recogedores de basura y obreros asimilados
9T	**PEONES DE LA AGRICULTURA, PESCA, CONSTRUCCIÓN, INDUSTRIAS MANUFACTURERAS Y TRANSPORTES**
94	PEONES AGROPECUARIOS Y DE LA PESCA
941	Peones agrícolas
942	Peones ganaderos
943	Peones agropecuarios
944	Peones forestales
945	Peones de la pesca
95	PEONES DE LA MINERÍA
950	Peones de la minería
96	PEONES DE LA CONSTRUCCIÓN
960	Peones de la construcción
97	PEONES DE LAS INDUSTRIAS MANUFACTURERAS
970	Peones de industrias manufactureras
98	PEONES DEL TRANSPORTE Y DESCARGADORES
980	Peones del transporte y descargadores