Abnormal results in common clinical laboratory assays: clues to diagnose rare inborn errors of metabolism?

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ABNORMAL RESULTS IN COMMON CLINICAL LABORATORY ASSAYS: CLUES TO DIAGNOSE RARE INBORN ERRORS OF METABOLISM?

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CONTENTS

1 SUMMARY

The highly specialized field of inborn errors of metabolism (IEM) goes hand in hand with laboratory medicine. Diagnosis and therapeutic monitoring of many IEMs require indeed highly specialized laboratory analyses that are only available in tertiary care and academic hospital facilities. Medical expertise regarding these orphan diseases is also often scarce outside specialized IEM reference centers.

A few basic laboratory tests, that are widely available in most hospitals, may however give important hints to suspect an underlying IEM in “unusual” clinical situations. Thus, biochemical pathways governing the interplay of key intermediates of energy metabolism that are glucose, ketones and lactate are reviewed, as well as pathways influencing more organ specific biomarkers, such as ammonium, creatine kinase, homocysteine and uric acid. Clinical indications to prescribe each of these laboratory analyses and adequate handling of the biological samples are reviewed, since these are prerequisites to providing meaningful test results.

The importance of following laboratory medicine good practice rules is discussed, since the increasing burden of overall medical costs puts pressure on physicians to optimize the way they request and interpret laboratory results. Examples of published algorithms that assist these processes will be shown, highlighting clinical and para-clinical constellations in which an underlying IEM should be suspected.

Biochemistry is taught during the preclinical years of medical training but often neglected during further professional education. IEMs illustrate the links between basic sciences, clinical reasoning and treatment options in an outstanding, logical way. In order to illustrate the educational value of these rare “orphan” diseases, a few treatable IEMs that should come to mind when patients display abnormal results in the aforementioned common clinical biomarkers will be described.

The field of IEMs is complex and requires very sophisticated laboratory tools; however, a sound knowledge of a few essential metabolic pathways and simple laboratory tools should allow any primary care physician to suspect a possible underlying IEM and take appropriate steps for further management and referral.

2 INTRODUCTION

Laboratory analyses have become an essential tool of medical practice and physicians may request laboratory investigations in a great variety of clinical settings. Overall, it seems reasonable to consider that laboratory results influence medical decisions in about 35% of patient encounters [1], as the often cited old claim that laboratory medicine data influence 70% of clinical decisions is likely based on unpublished studies and anecdotal observations and cannot be objectively verified [2]. An obvious indication to request laboratory analyses arises when medical history combined with physical examination fail to provide a definite diagnosis. Laboratory workup, together with imaging techniques will help to narrow down the spectrum of diagnostic hypotheses and to choose the most appropriate therapeutic strategy. In emergency departments 41-56% of patients will undergo laboratory tests [1]. Another common reason to order laboratory analyses is to monitor treatment efficacy in hospitalized patients or chronic conditions, or check for potential therapeutic side effects. Laboratory investigations are also recommended to search for long term complications of chronic illnesses, detect risk factors for various diseases in either population oriented selective or non-selective screening programs, or in the context of health checkups. Whenever using laboratory services, medical practitioners should follow good practice rules such as those summarized by Lundberg in a JAMA editorial published in 1975: « The Modern Clinical Laboratory: Justification, Scope, and Directions: […] Laboratory tests should not be ordered without a plan for using the information gained. What will be done if the test result is normal? high? low? » [3].

In this thesis, I will review the added value of common and widely available laboratory tests playing an essential role in helping the astute clinician to apprehend the diagnosis of an inborn error of metabolism (IEM).

For each relevant biomarker this literature review will provide:

- a description of the biomarker's physiological function in metabolism
- recommendations for adequate prescription in clinical situations
- important preanalytical issues, if warranted
- a short description of selected individual IEM or categories of IEM ascribed to a given abnormal biomarker result.

2.1 LABORATORY ANALYSES IN THE ERA OF EVIDENCE BASED MEDICINE

The roots of evidence based medicine (EBM) can be traced back many years before the term was coined in 1992 [4] and since, its principles are increasingly taught in medical schools and graduate training programs. In the field of laboratory medicine, it wasn't until 2006 that a task force was set up in the US by the Center for Disease Control in order to promote Laboratory Medicine Best Practices [5]. Nevertheless, choosing the right test at the right moment for the right patient remains a difficult task [6]. The potential for underutilization, overutilization or misuse is huge and around one third of ordered laboratory tests may be inappropriate as stated by Zhi *et al.*[7]. To address the problem of overutilization in a wider setting, the choosing wisely campaign was launched in 2012 by the American Board of Internal Medicine, to stimulate measures to avoid wasteful or unnecessary medical tests, treatments, and procedures [8]. A promising strategy to increase the efficiency of the laboratory testing process and reduce diagnostic errors could be brought up by setting up diagnostic management teams of laboratory medicine experts for advising physicians on the selection of necessary tests and the interpretation of complex test results [9]. The importance of providing guidance on good practices in the field of laboratory diagnostics on a broader level has been recognized by the World Health Organization. The WHO has indeed published the first edition of its Model List of Essential In Vitro Diagnostics in 2018 and plans to update it annually through its Strategic Advisory Group of Experts on In Vitro Diagnostics [10].

2.2 INBORN ERRORS OF METABOLISM

Inborn errors of metabolism (IEM) form a large class of rare or ultra-rare genetic diseases, which is rapidly expanding. As of March 2020, over 1400 IEM had been discovered, according to the most comprehensive IEM database maintained by the University of British Columbia (http://www.iembase.org/) with a cumulative incidence exceeding 1 in 800 [11]. The vast majority of IEM are caused by either a failure to synthesize an enzyme, or by the synthesis of a less functional or less stable enzyme, co-enzyme, transporter or receptor that compromises one or more metabolic pathways. The resulting clinical pictures are extremely diverse, ranging from a healthy looking newborn who dies within 48 hours of uncontrollable hyperammonemia due to a urea cycle defect (UCD), to an ageing adult complaining of slowly progressive muscle weakness caused by a lysosomal storage disorders (LSD) such as Pompe disease, or the incidental finding of splenomegaly due to very mild subclinical Gaucher disease: any organ can be involved, any age group can be affected; symptoms may be permanent or intermittent.

The field of IEM has for a long time been considered a pediatric specialty; the number of adult patients affected by IEM is however steadily increasing. Progress in the treatment of classical IEM

and therefore increased survival of many pediatric patients has created the need for adult IEM care facilities. Adolescents and young adults affected by severe neonatal onset IEM such as organic acidemias and UCDs, but also the increasing number of patients diagnosed by classical and expanded newborn screening (with phenylketonuria, galactosemia, fatty acid oxidation disorders (FAO), MSUD …) are now transitioned to adult IEM specialists in many centers; thus, the total number of adult IEM patients will soon outnumber those in the pediatric age group [12,13].

A subset of neurological or neurogenerative diseases that start in adulthood are caused by an IEM with a clinical presentation that is completely different from their pediatric equivalent. Mutations in a same gene (for instance *GBE1)* lead to type IV glycogen storage disease (GSD IV), which can present as a perinatal fatal neuromuscular disease, an infantile progressive hepatic disease, a non-progressive childhood liver disease, or the adult-onset neurodegenerative polyglucosan disease [14]. Thanks to the now widespread availability of genetic next generation sequencing (NGS) techniques, the metabolic community is learning to recognize the full clinical spectrum of many IEM.

How do we diagnose IEM? The importance of diagnosing many IEM early, i.e. before the occurrence of irreversible organ damage, has led to the development of newborn screening (NBS) programs; these started in the 1960s for phenylketonuria (PKU) and were expanded since to screen for a selection of >50 mostly genetic diseases in a non-uniform way among different countries [15,16]. The majority of patients suffering from an IEM are however diagnosed after becoming symptomatic, as NBS is not available for most IEM. The complex field of IEM requires therefore extensive training of clinicians and laboratory staff to efficiently select, run and interpret a range of highly specialized, non-standardized and sometimes costly laboratory assays, in order to identify and confirm these orphan diseases in a timely and efficient way. Diagnostic algorithms have been developed to guide this difficult process in selected clinical situations [17]. A more comprehensive approach is taken by the expert-curated IEMbase (http://www.iembase.org/), which provides extensive up-to-date clinical, epidemiological, biochemical and genetic information in a standardized concise format and which features a prototype diagnosis support system [18]. If one enters for instance the clinical signs « vomiting », « lethargy » and the biochemical feature « hypoglycemia », the mini-expert system suggests the disease « 3-Hydroxy-3-methyl glutaric aciduria», a disorder of branched chain amino acid and ketone metabolism. Clinical signs that are observed in different age groups are listed, as well as abnormalities affecting biochemical markers. If the query is changed to « vomiting », « lethargy » and « hyperammonemia », the system will suggest a list of eight diagnoses; the „DDX“ function highlights differences between selected diseases. Such a support system is obviously no substitute for medical expertise, but may provide diagnostic assistance in the complex field of IEM to both newcomers and experienced physicians.

Last but not least, the website of the Society for the Study of Inborn Errors of Metabolism (SSIEM): https://www.ssiem.org/ provides a very comprehensive list of links to resources, such as textbooks, databases, diagnostic services, journals, learned societies … that can be helpful to the novice as well as the expert in IEMs.

2.3 DO COMMONLY AVAILABLE LABORATORY TESTS HELP IDENTIFYING AN UNDERLYING IEM?

Working up the "accidental finding" of a highly abnormal biochemical parameter amid a "routine follow up laboratory panel" can sometimes lead to the diagnosis of an unsuspected treatable IEM. Published estimates on the frequency of incidental diagnoses are scarce: a retrospective study looking at healthy controls enrolled for clinical studies in a single center revealed that 2.3% out of 990 subjects had to be excluded due to newly diagnosed medical abnormalities, such as familial hypercholesterolemia or nephrotic syndrome [19]. The likelihood that such a "chance finding" reveals a rare IEM should therefore be much lower. Nevertheless, we experienced this in a young adult who had insidiously developed end stage renal failure requiring renal replacement therapy, and in whom the finding of a surprisingly elevated homocysteine level > 250 μmol/l ultimately led to the diagnosis of cobalamin C (CblC) deficiency, an inborn error of intracellular cobalamin metabolism. Treatment with high dosage hydroxycobalamin led to a marked improvement of the patient's rather unspecific neuropsychiatric symptoms caused by this IEM [20]. Thanks to this highly specific treatment, the young man's personality problems improved and he managed for the first time to keep stable relationship both in employment and at the private level. It is likely that earlier recognition and treatment of his CblC deficiency would have prevented renal failure.

A more effective and recommended strategy to reach such a diagnosis is to start by considering the possibility of an underlying IEM in clinically suggestive cases, secondly proceed to selecting appropriate first line biomarkers that will refine the probability of an underlying IEM, and finally confirm or exclude this diagnosis through further, often very specialized laboratory analyses.

If one looks back at the pioneering years of IEM, qualitative urinary colorimetric assays, such as the Benedict's copper reduction, the ferric chloride or the nitroprusside-cyanide tests were used to screen for IEM in newborns and young infants [21]. These historical tests are no longer available in most modern laboratories, as they have been superseded by more sensitive, more specific, quantitative, and often automated analytical methods.

On the other hand, enzymatic determinations of ammonium, glucose, ketones and lactate that have been routinely performed in clinical laboratories since the 1960's [22] are still central to many diagnostic and therapeutic decisions. Interestingly, homocysteine determination was introduced at the same time in a few specialized laboratories following the discovery of the first IEM patients suffering from homocystinuria [23]; technical improvements in measuring total homocysteine in the 1980's boosted homocysteine research unraveling the links between homocysteine, vitamin B12 and folate metabolism, and establishing homocysteine as an independent cardiovascular risk factor [23].

The purpose of this study is to survey commonly available biochemical laboratory markers that give crucial hints in the often complex and sometimes lengthy process of suspecting, working up, diagnosing and finally treating an underlying IEM.

3 METABOLIC BIOMARKERS: FROM AMMONIUM TO URIC ACID

In the following section, a selection of common biomarkers that may point to an underlying IEM will be listed in alphabetical order. The physiological role of the biomarkers will be summarized, emphasizing their metabolic function and the pathways for their synthesis and degradation.

In order to promote efficient clinical utilization of these biomarkers, a framework based on the evidence derived from published literature and recommendations from laboratory service providers will be proposed, aiming at increasing clinician's awareness of rare IEM as potential causes of abnormal or unexpected laboratory results. A few treatable IEM will be briefly described in each section of this chapter to illustrate this far-reaching diagnostic process.

3.1 AMMONIUM

Ammonia (NH3), which at physiological pH predominates as ammonium ion (NH_4^+), is a neurotoxic metabolite produced during the breakdown of proteins and other nitrogen containing compounds. This process occurs mainly in the gastro-intestinal tract, liver, kidney, brain and muscle during numerous metabolic processes. Nitrogen derived from food proteins and bacterial metabolism is exchanged not only through NH3 but also through intermediates such as alanine, glutamine and urea [24].

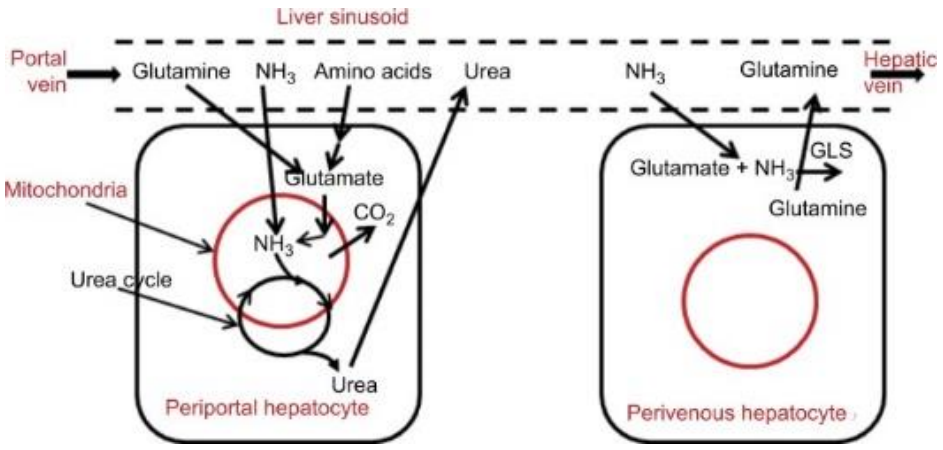

Fig. 1 Pathways of ammonia (NH3) and other nitrogen carriers in the liver

Reproduced from Levitt, D. & Levitt, M. in Clin. Exp. Gastroenterol. Volume 11, 193–215 (2018)

Ammonium as biomarker

Automated enzymatic assays for plasma ammonium have been developed since the late 1970's and are central to diagnosing certain IEM that present most often in newborns and children; this laboratory parameter is therefore better known to pediatricians than to physicians in adult medicine [25,26].

Accurate ammonium quantification is highly sensitive to pre-analytical factors. This key intermediate of nitrogen metabolism is continuously released into the circulation and increases during exercise and after meals. Ammonium release from erythrocytes and deamination of proteins and amino acids by gamma glutamyl transferases continues during the procedure of blood sampling, transportation and specimen processing preceding the analytical phase in the laboratory. It is therefore critical to observe the pre-analytical conditions specified by the Association for Clinical Biochemistry and laboratory medicine [ACB] (http://www.acb.org.uk/whatwedo/science/AMALC.aspx), that states: « A free-flowing venous (or arterial) blood sample should be collected into a specimen tube (preferably pre-chilled) containing either lithium heparin or EDTA as an anticoagulant and which has been determined to be free of ammonia contamination […] The sample should be transported on ice to the laboratory, separated within 15 minutes of collection and analyzed immediately ». These conditions are rarely met in daily routine and up to half of all elevated NH3 results may in fact represent false positives due to a poor compliance to these pre-analytical requirements [27]. A time limit of 120 minutes between blood sampling and analysis was shown to minimize the false positive rate, as in a single center the percentage of normal

results (< 40 µmol/l) dropped abruptly from 41-45 % for samples processed within 120 minutes to 17-26 % for samples requiring longer processing time [28].

When should plasma ammonium be checked?

According to the laboratory tests database of the American Association for Clinical Chemistry (AACC) (https://labtestsonline.org/), plasma ammonium is indicated […] " to help investigate the cause of changes in behavior and consciousness; to help diagnose hepatic encephalopathy or Reye syndrome."

While there is little debate regarding the recommendation that plasma NH3 should be ordered in any adult or child with unexplained encephalopathy, its significance in the pathogenesis of hepatic encephalopathy has been the focus of many studies with contradictory conclusions [29].

In their 2014 Practice Guideline, the American Association for the Study of Liver Diseases (AASLD) recommends: […] "Increased blood ammonia alone does not add any diagnostic, staging, or prognostic value for HE in patients with chronic liver disease. A normal value calls for diagnostic reevaluation. (GRADE II-3, A, 1)[30]. Furthermore, the Canadian Association for the Study of the Liver (CASL) has recently published the following statement: "Don't order serum ammonia to diagnose or manage hepatic encephalopathy (HE)" as top priority among 5 recommendations of their society to the Canadian Choosing Wisely task force [31].

Recommendations regarding ammonium monitoring in the context of acute (fulminant) liver failure in Europe are strikingly different : the European Association for the Study of the Liver (EASL) states that ammonium should be measured when admitting the patient to assess severity of liver failure; ammonium monitoring is recommended when instituting enteral nutrition (evidence level III, grade of recommendation 1) and « ammonia level over 150–200 µmol/L that does not drop with initial treatment interventions (RRT and fluids) » is featured as a criterion to consider invasive intracranial pressure monitoring [32] as persistently elevated serum ammonia >200 µmol/L correlates with increased intracranial pressure in patients with fulminant hepatic failure [33]. Benefit of intracranial pressure monitoring in patients with acute liver failure is however controversial [34].

However, in the absence of acute or chronic severe liver disease patients, the finding of hyperammonemia in the context of unexplained alterations of consciousness, behavioral changes and/or neurological symptoms should prompt the search for an inborn error of metabolism (IEM) that compromises NH3 detoxification through the urea cycle, or a vascular malformation that diverts NH3-rich portal blood from its obligatory detoxifying passage through the liver. In pediatric acute liver failures, 10-34% of patients are ultimately diagnosed with an underlying IEM, as for instance

galactosemia, tyrosinemia, fatty acid oxidation disorders, mitochondrial cytopathies , Wilson disease or urea cycle disorders, and sometimes with portosystemic shunts [35].

3.1.1. PORTOSYSTEMIC SHUNTS

The contribution of hyperammonemia to the neurological and psychiatric manifestations of HE in patients with chronic liver disease is incompletely understood; glutamine and inflammation are two other major players participating in this complex process [36]. Hyperammonemia is thought to result from a combination of liver insufficiency and portosystemic venous shunts (PSS); however, as already stated, hepatic encephalopathy (HE) symptoms correlate poorly with ammonia levels in patients with chronic liver disease, as opposed to those with acute liver failure.

HE symptoms occur also in patients with congenital portosystemic venous shunts (CPSS), in the absence of liver insufficiency. Extra and intrahepatic portosystemic shunts are developmental anomalies with a prevalence around 1/30'000 births that occur generally as isolated malformations, but also in conjunction with cardiovascular anomalies. Patients may become symptomatic already *in utero*, during childhood or adulthood, or remain asymptomatic lifelong; around 25% patients with CPSS will present neurological symptoms of portosystemic encephalopathy [37, 38].

3.1.2 UREA CYCLE DISORDERS

A primary urea cycle disorder (UCD) results from DNA mutations that abolish or greatly reduce the activity of one of the 6 enzymes or 2 transporters required to convert ammonia (NH3) into urea. Efficient NH3 elimination through the urea cycle may also be compromised in situations where the substrate or cofactor of one of these enzymes is lacking, due to another underlying genetic condition and/or drug toxicity [39].

Early recognition is of paramount importance for decreasing morbidity and mortality of patients with urea cycle disorders (UCD). A panel of IEM experts "strongly recommend considering a UCD at any age in any acute or intermittent neurological deterioration or psychiatric illness, acute liver failure, suspected intoxication, or in the differential diagnosis of neonatal sepsis. Catabolism or protein load may represent triggering factors (quality of evidence moderate)" [40].

In neonatal onset forms, the clinical course of proximal UCDs is often dramatic; within 48-72h, a well appearing newborn in the delivery room will gradually display lethargy, refusal to feed, vomiting, and relentlessly progress to coma with various neurological features of encephalopathy. Neonatal onset forms represent around one quarter of all UCD patients or less than 40% of all

symptomatic patients (OTC females may remain asymptomatic) [41]. Less severe mutations in the genes coding for UCD enzymes or transporters result in partial deficiencies with attenuated late-onset phenotypes that present in infancy, childhood or even adulthood. Undiagnosed patients may develop strategies to avoid high nitrogen loads by avoiding protein-rich meals and display only non-specific intermittent symptoms, such as irritability, aggressive behavior or drowsiness. More evident symptoms of hyperammonemic encephalopathy may be precipitated by triggers as diverse as valproic acid treatment, postpartum stress, short-bowel syndrome, kidney disease, gastro-intestinal or internal bleeding, parenteral nutrition with high nitrogen intake or other high protein diet strategies, intravenous or high dose glucocorticoids or chemotherapy that affect protein turnover … [42]. Neurological deterioration in UCD patients often progresses rapidly towards permanent sequelae or fatal outcome; thus, ammonium measurement should be included early when the differential diagnosis comprises UCDs.

3.2 CREATINE KINASE

Creatine kinases (CK) play a pivotal role in cellular energy homeostasis through interconversion of creatine and phosphocreatine, which buffer ATP and ADP levels in tissues with rapidly changing energy demands, such as muscle and brain [43,44]. The two octameric mitochondrial isoforms located in the cristae and intermembrane space cooperate with the cytosolic dimeric MM-CK, BB-CK or MB-CK isoforms in various tissues [43,44]. They are well known to clinicians as first-line biomarkers of muscle damage; plasma CK activity increase in a wide variety of diseases affecting myocyte integrity, of both acquired and genetic origin, as well as following strenuous exercise [45].

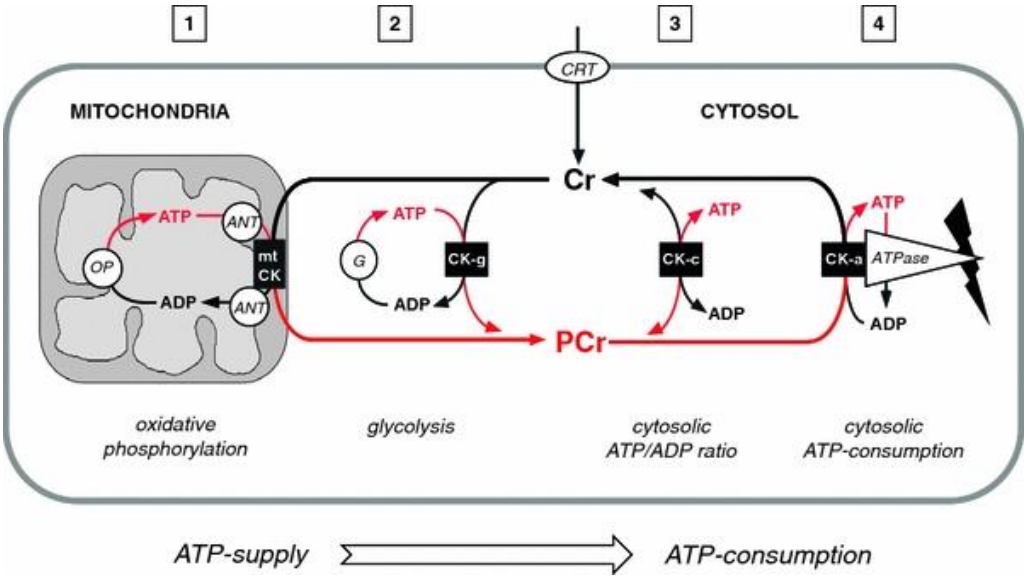

Fig. 2 Creatine-kinase /phosphocreatine (CK/PCr) system for energy buffering in the cell and coupling. 1: oxidative phosphorylation, 2: glycolysis, 3: cytosolic ATP/ADP ratio and 4: ATP consumption.

Reproduced from Wallimann, T., Tokarska-Schlattner, M. & Schlattner, U. in Amino Acids 40, 1271–1296 (2011).

Creatine kinase (CK) as biomarker

Before the advent of high sensitivity cardiac troponin (hsTn) assays, serial total CK and measurement of the CK-MB isoenzyme were used to help diagnosing myocardial infarction; this strategy is hampered by poor sensitivity and specificity of this marker. Some authors consider that determination of CK-MB should no longer be part of investigations performed in the clinical context of acute coronary syndromes [46]. Peak CK-MB activities correlate however with infarct size, left ventricular ejection fraction and 1-year major adverse cardiac events; this marker may therefore still be used as a prognostic rather than diagnostic biomarker [47].

Total CK determination is nowadays indicated for the detection and monitoring of suspected muscle damage in various conditions and clinical presentations, as specified in the AACC database (https://labtestsonline.org/).

On the other hand, incidental finding of sometimes markedly elevated total CK activities "hyperCKemia" without or with only minimal muscle-related symptoms such as exercise intolerance or cramps can be a diagnostic challenge. Besides performing additional investigations as described hereafter, it is worth mentioning that some patients display falsely elevated serum CK activities in the absence of muscle disease, due to the presence of abnormal macroenzymes in their sera.

Macroenzymes are specific antigen-antibody complexes made of IgG or IgA and enzymes such as alanine-amino transferase (AST), amylase, lactate dehydrogenase (LDH) or CK [48] that can be revealed by techniques such as chromatography or immunoelectrophoresis [22]. The incidence of macro-CK has been reported to be in the range of 0.2% in blood donors, but >4% in patients with cardiac disease; macroenzymes are also more frequent in cancer patients than in other disease groups [48].

The European Federation of the Neurological Societies guidelines on the diagnostic approach to paucisymptomatic or asymptomatic hyperCKemia that where published in 2010[49] recommend considering a nerve conduction study and electromyogram in case of persistent CK elevation > 1.5 times the upper limit of normal (ULM). CK should be measured after instructing patients to avoid strenuous exercise for 7 days before testing and there should be at least 2 determinations 1 month apart; muscle biopsies should be restricted to younger patients with CK activities persistently > 3x ULM under the aforementioned conditions.

With the advent of next generation sequencing (NGS), the diagnostic approach may rapidly change, as genetic causes may be found in up to 50% of asymptomatic or minimally symptomatic patients with elevated CK activities with currently available techniques [50]. Among the diagnosed conditions, many have no specific treatment. However, the clinical course of several inborn errors of metabolism that present with permanent or intermittent elevation of plasmatic CK activities can be greatly improved, once a specific diagnosis is reached, as exemplified hereafter.

3.2.1 Carnitine palmitoyl transferase II (CPT II) deficiency

Carnitine palmitoyl transferase II (CPT II) deficiency is a disorder of long chain fatty oxidation; the more common “adult form” is a typical cause of intermittent rhabdomyolysis that may be complicated by acute renal or respiratory failure [51]. Attacks of myalgia, weakness and dark urine are most often triggered by exercise, fasting, infections and exposure to cold, but emotional stress, decreased fluid intake and medications such as Ibuprofen may increase the risk; between attacks, the majority of patients are asymptomatic, displaying normal CK levels [52]. Attacks can be prevented or attenuated by dietary adjustments such as providing regular carbohydrate intake and fluids before and during exercise, avoiding fasting, limiting cold exposure and avoiding exercise during febrile illnesses. These measures are very important for limiting the risk of life threatening acute renal failure [52,53]. CK levels are typically markedly elevated during attacks and often completely normal between these episodes [53].

3.2.2 LATE ONSET POMPE DISEASE (LOPD)

Pompe disease is caused by a deficiency in alpha-glucosidase (GAA) - also called acid maltase -, a lysosomal enzyme that hydrolyzes glycogen. Complete absence of this enzyme results in severe cardiomyopathy and hypotonia in the first months of life ("floppy baby syndrome"); less severe mutations in the *GAA* gene with some residual enzymatic activity result in late onset Pompe disease (LOPD), a progressive myopathy that affects predominantly the proximal muscles and diaphragm but causes also many systemic manifestations [54,55,56]. Enzyme replacement therapy is available to treat Pompe disease since 2006. 95% of LOPD patients permanently have mildly elevated CK activities (usually <1000 U/l) [55,57]. This rare potentially treatable disorder should not be overlooked in patients with « unclassified limb-girdle-muscular dystrophy » [57].

3.3 GLUCOSE

Glucose is the universal energy vehicle of the organism. Blockage or regulatory dysfunction in any of the energy supplying metabolic pathways may lead to disruption of glucose homeostasis.

When should glucose levels be checked?

Glucose differs from other laboratory parameters, as it most often is measured by the patient himself. In 2009, the worldwide sales of the self-monitoring of blood glucose industry reached $8.8 billion, representing 22% of the overall $39 billion *in vitro* diagnostic market [58]. Close to 13 billion test stripes were sold worldwide in 2016 [59]. This is not surprising if one considers that the 2014 global age adjusted prevalence of diabetes has been estimated around 9.0% (95% confidence interval 7.2-11.1%) in men and 7.9% (6.4- 9.7%) in females, amounting to a total of 420 million adults, of which many are undiagnosed [60].

Indications for glucose measurements are obviously not limited to screening, diagnosis and therapeutic monitoring of diabetes, a topic *per se* that will not be covered in this thesis.

Monitoring glycaemia after birth

Transition from intrauterine life, during which glucose is continuously supplied to the fetus, to postnatal life, where various nutriments are provided intermittently, requires complex metabolic and endocrine adaptations. Newborns with the following conditions and symptoms are at increased risk of neonatal hypoglycemia and should undergo glucose measurements soon after birth, as clinical symptoms in newborns are unspecific and not sensitive enough to suspect hypoglycemia in a reliable way:

	Indications for glycemia measurements in newborns
1.	Unspecific symptoms of hypoglycemia (irritability, tremor, jitteriness, high pitched cry, lethargy, floppiness …)
2.	Large for gestational age (birth weight ≥ p90 or ≤ 4000 or 4500 g)
3.	Small for gestational age (Intrauterine growth restriction: birth weight ≤ p10 or ≤ 2500 g)
4.	Perinatal stress (Birth asphyxia/ischemia; cesarean delivery for fetal distress, Maternal preeclampsia/eclampsia or hypertension, meconium aspiration syndrome)
5.	Premature delivery
6.	Postmature delivery
7.	Infant of diabetic mother (DM type I, type II or gestational diabetes)
8.	Family history of a genetic form of hypoglycemia
9.	Congenital syndromes (eg, Beckwith-Wiedemann), abnormal physical features (eg, midline facial malformations, microphallus)

Adapted from Preisler, N. et al. in Mol. Genet. Metab. 110, 287–289 (2013) and Hughes, M. D. in J. Diabetes Sci. Technol. 3, 1219–1223 (2009)

There is however no consensus regarding normal glucose values in newborns as evidence for defining a safe minimal threshold is lacking. Glucose levels are physiologically lower during the first 72h after birth than later in life. The process of metabolic adaptation from intra-uterine to extra-uterine life is characterized by a failure of insulin suppression at low glucose levels, due to a lower threshold for insulin secretion in fetal islet cells [61]. A survey of various published studies and recommendations reveals no consensus for the timing of the first glucose measurement in newborns, nor whether to measure it in the fed or unfed state. Thresholds for recommending intervention (feeding or intravenous glucose infusion) in newborns < 72h are commonly situated between 2.2 and 2.6 mmol/l [62–66].

Pitfalls in glucose measurements

Whole blood glucose values are about 10-15% lower than in serum or plasma as the water content differs between erythrocytes (~71%) and plasma (~93%) and glucose exchanges freely between these two compartments [67]. Venous blood concentrations are ~10% lower than in arterial and capillary blood, as glucose diffuses from the capillary to the interstitial fluid [68]. Delays between sampling and processing in the laboratory will result in a drop of 0.3 mmol/L/hour if the sample is not collected in a tube containing a glycolysis inhibitor. On the other hand, the inaccuracy of point-of-care devices in the hypoglycemia range varies between 0.6-0.8 mmol/l [63,66]. Laboratory measurements are more reliable, but require larger sample volumes, and the time needed to obtain the results and correct hypoglycemia is often not compatible with patient's safety.

Investigating persistent hypoglycemia

Investigations for persistent hypoglycemia should be considered in newborns not earlier than on their third day of life, ideally only in patients that fulfill Whipple's triad: 1 - symptoms and/or signs consistent with hypoglycemia, 2 - a documented low plasma glucose (PG) concentration, and 3 - relief of signs/symptoms when PG concentration is restored to normal. However, since neurogenic signs and symptoms are difficult to recognize in this population, newborns and infants with recurrent plasma glucose < 3.3 mmol/l should be investigated [63].

The differential diagnosis of persistent hypoglycemia is large. To narrow down the list of possible causes, it is helpful to observe when hypoglycemia occurs with respect to the feeding schedule, and to measure other key metabolic intermediates, such as lactate, ketones and free fatty acids (FFA) during hypoglycemia.

Hypoglycemia in the fed state is suggestive of hyperinsulinism; occurrence a few hours after meals is typical of enzymatic deficiencies in the glycogenolysis pathway while fasting hypoglycemia is more commonly observed in fatty oxidation or neoglucogenesis defects, as these pathways take over the processes of releasing energy and maintaining glucose homeostasis when feeding is withheld for short or long term fasting [69–71]. The following figure (fig. 3), reproduced from the review "Adaptive reciprocity of lipid and glucose metabolism in human short-term starvation" by Soeters *et al.* [72] illustrates the relative contributions of carbohydrate and lipid oxidation to meeting the body's energy demand during the transition from a fed to fasted state.

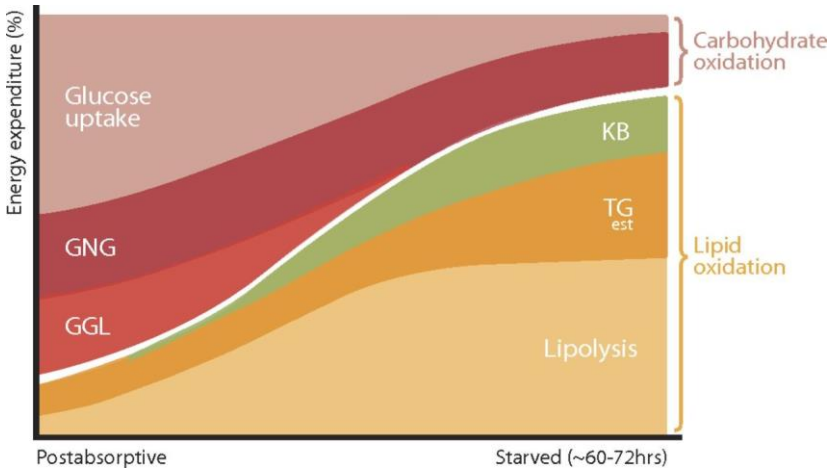

Fig. 3 Schematic diagram depicting approximate changes in substrate metabolism during the transition from the postabsorptive phase to short time starvation

GNG = gluconeogenesis, GGL = glycogenolysis, KB = ketone bodies, TG est = triglyceride reesterification.

Reproduced from Soeters M. R. et al. in Am. J. Physiol. Endocrinol. Metab. 303, E1397-1407 (2012).

Insufficient release of any of the counterregulatory hormones (glucagon , epinephrine, growth hormone, cortisol) will disrupt glucose homeostasis through ineffective activation of the aforementioned pathways when blood glucose levels are falling; the effects of glucagon and epinephrine release occur within minutes, while those of cortisol and growth hormone have longer latencies and duration of action [71].

The following algorithm (fig. 4) reproduced from the 2015 Recommendations from the Pediatric Endocrine Society for Evaluation and Management of Persistent Hypoglycemia in Neonates, Infants, and Children [63] may help selecting the most appropriate further investigations to reach a definite diagnosis.

Whenever possible, a “critical sample” obtained before correction of hypoglycemia should be obtained, in order to measure simultaneously plasma glucose (PG), beta-hydroxybutyrate (BOHB) and lactate, with extra plasma and serum spared for specific tests. Since available plasma and serum is often limited in infants and young children, the most useful specialized tests may be selected once the most likely cause of hypoglycemia is identified through the clinical information and PG/BOB/lactate profile.

Many institutions will however perform a comprehensive panel including insulin, C-peptide, cortisol, GH, FFA and acylcarnitine profile, regardless of the PG/BOB/lactate profile since endocrine profiles are nowadays frequently measured on high throughput corelab facilities and diagnostic categories are not always as clearcut as depicted in fig. 4; limited BOHB production may for instance be observed in some patients with Fatty Acid oxidation disorders [73].

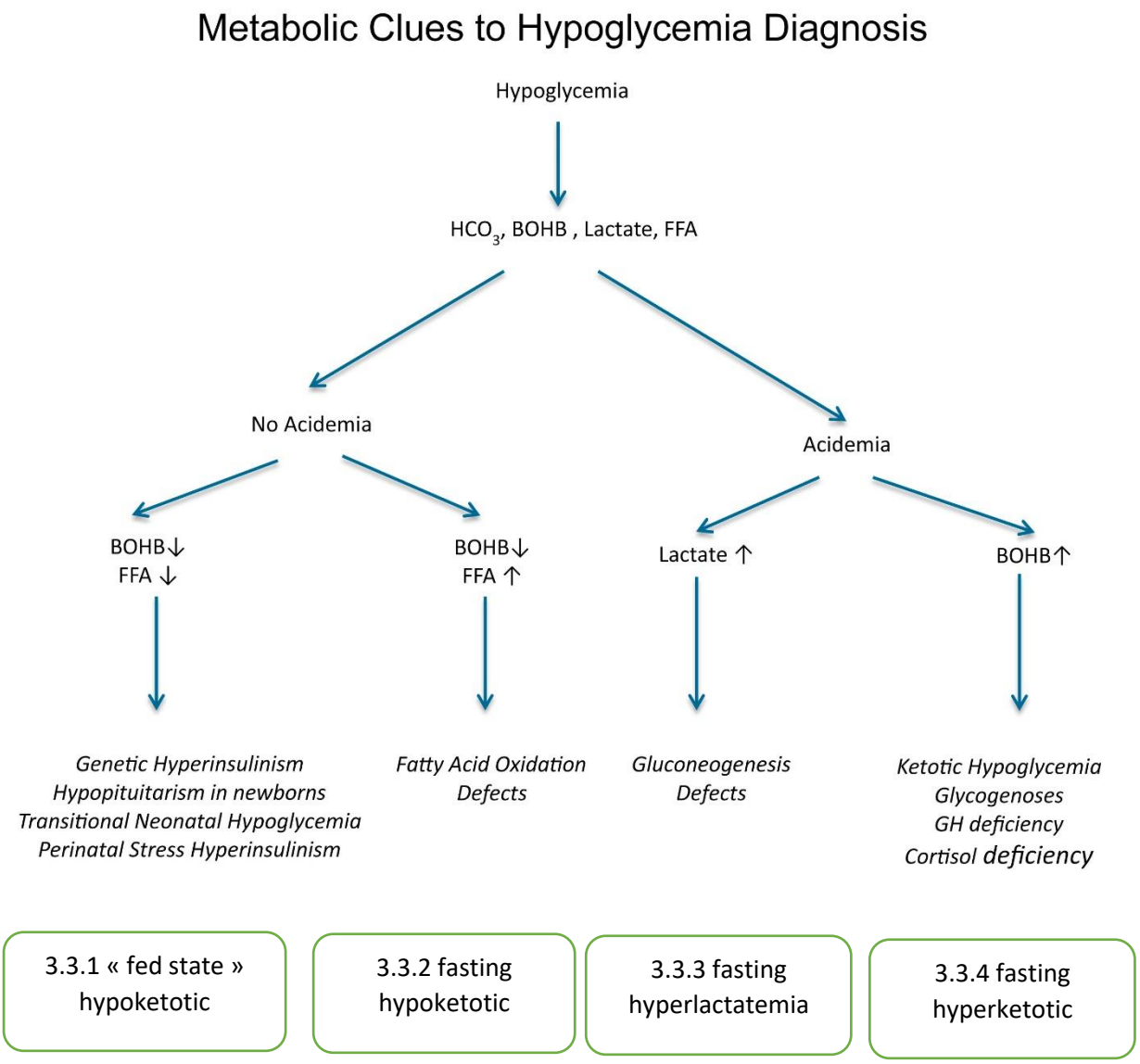

Figure 4. **Algorithm showing how patients presenting with hypoglycemia can be subdivided according to the biomarker profile of the "critical sample" obtained during hypoglycemia**

BOHB = beta-hydroxybutyrate, FFA = free fatty acids, GH = growth hormone, HCO3 = bicarbonate

Reproduced from Thornton, P. S. et al. in J. Pediatr. 167, 238–245 (2015).

3.3.1 HYPOKETOTIC HYPOGLYCEMIA IN THE FED STATE

Hypoglycemia occurring within two hours after the last meal results most often from transitory or permanent hyperinsulinism [74] and occasionally from deficiency of one or several of the counter regulatory hormones in the context of congenital hypopituaritism [75].

Less severe isolated growth hormone or isolated cortisol deficiency can also present as fasting hypoglycemia. Activation of lipolysis and ketone synthesis are repressed by inappropriately high levels of insulin, explaining low FFA and low BOHB concentrations during hypoglycemia.

Further details on the pathophysiology and clinical presentation of hyperinsulinism, growth hormone and cortisol deficiencies are beyond the scope of this thesis.

3.3.2 FASTING HYPOKETOTIC HYPOGLYCEMIA

In the fed state, ATP production in the liver or muscle occurs predominantly through the pathways of glycolysis that converts glucose to pyruvate, followed by pyruvate oxidation in the tricarboxylic acid cycle (TCA) and oxidative phosphorylation [70]. Interestingly, recent work by Hui et al. revealed that even in the fed state, there is no direct coupling between glycolysis and the TCA except in brain and muscles, allowing independent tissue-specific regulation of these two processes. Overall, circulating lactate rather than glucose serves as the predominant shuttle to provide carbohydrates to the TCA [76].

In the postabsorptive state, lipolysis and ketone synthesis are turned on to spare glucose produced by glycogenolysis and gluconeogenesis for the brain and erythrocytes. Enzymatic deficiencies involving fatty acid oxidation (FAO), the carnitine cycle or ketogenesis compromise the activation of these glucose-sparing sources of energy and affected subjects may therefore present hypoketotic hypoglycemia, when glucose supply is low.

<u>Fatty acid oxidation disorders</u>

Disorders of mitochondrial fatty acid oxidation (FAOD) and the associated carnitine shuttle constitute a group of frequent inborn errors of metabolism with a combined incidence approximating 1/9'000 in Caucasian populations [77]. Knowledge about this group of diseases has greatly benefitted from the advent of tandem mass spectroscopy in the 1990s, which has revolutionized the detection of biomolecules. FAODs are nowadays increasingly diagnosed by newborn screening (NBS); there is however no international consensus regarding the selection of inborn errors of metabolism (IEM), endocrinopathies or other diseases included in national, or even regional NBS programs [78].

Mutations in two thirds of the genes coding for the ~20 proteins involved in FAO are known to cause diseases in humans; these disorders vary greatly in their clinical presentation, according to the

affected enzyme and the type of mutations which translate in varying degrees of blockage of the energy producing pathway and buildup of toxic metabolites [79].

FAO remains active - even in the fed state - in the heart, skeletal muscle and kidney. While the fetal heart relies mostly on glucose and lactate to satisfy its energy demand, it switches to fatty sources immediately after birth and FAO ultimately provides over half of the required ATP; this process requires the induction of mitochondrial biogenesis [79].

The most common clinical symptom of a metabolic block in FAO is hypoketotic hypoglycemia, often accompanied by hepatomegaly caused by fatty liver infiltrates; this occurs typically in catabolic states such as poor feeding, fasting, fever, intercurrent illnesses and exercise. Long chain FAO disorders will also present with hypertrophic or dilated cardiomyopathy, arrhythmias and conduction defects. Rhabdomyolysis, muscle weakness and/or myalgias are symptoms of skeletal muscle involvement that are also more prominent during catabolic states. Patients with isolated deficiencies, such as long-chain (S)-3-hydroxyacyl-CoA dehydrogenase (LCHAD) may present less common symptoms, such as peripheral neuropathy and retinopathy [79,73]. Treatment of medium chain acyl-CoA dehydrogenase deficiency (MCADD) – by far the most prevalent FAOD - relies simply on avoidance of fasting, with the occasional requirement of intravenous glucose infusions during intercurrent illnesses or surgery as a life-saving measure. Avoidance of catabolic states is not sufficient for treating severe long chain FAOD; affected patients require also dietary restriction of long chain triglycerides and compensatory addition of medium chain triglycerides to fuel their TCA and allow ketone synthesis downstream of the metabolic bloc. Therapeutic supplementation of carnitine is nowadays mainly restricted to patients with primary carnitine deficiency; caution is advised regarding pharmacological administration in secondary deficiencies, as this may promote arrhythmias in long chain FAOD [73,79].

Ketone synthesis defects

Inappropriately low ketones associated with fasting hypoglycemia may also result from an inborn error of ketone synthesis, an important pathway downstream of fatty acid oxidation, which provides energy to the brain and other organs when glucose supply is scarce. When a FAO is strongly suspected in the differential diagnosis, but cannot be identified, one should consider ketone synthesis defects; these diseases are covered in section 3.5.1.

3.3.3 Fasting hypoglycemia with elevated lactate

Elevated lactate in the context of fasting hypoglycemia reflects ineffective activation of gluconeogenesis. This may occur in inborn errors of metabolism that compromise either gluconeogenesis, gluconeogenesis and glycogenolysis, or gluconeogenesis and the TCA, as reviewed

by Weinstein *et al.* [80]. The incidence of isolated gluconeogenesis disorders is very rare. Deficiency of glucose-6-phosphatase (Glycogen storage disease type Ia) which compromises the common final step of glycogenolysis and gluconeogenesis is characterized by severe hypoglycemia, occurring within 2.5-4 hours after meals and often massive hepatomegaly manifesting in infancy [80]. Lactate levels normalize in the fed state.

Further details on the characteristics of gluconeogenesis disorders are provided in ref. [80].

3.3.4 HYPERKETOTIC HYPOGLYCEMIA

Occasionally, healthy children aged 6 months – 6 years may present to emergency services with ketotic hypoglycemia, often triggered by poor food intake in the context of gastroenteritis or other intercurrent illnesses. Their most prevalent clinical presenting signs are lethargy and altered mental status. These children should be evaluated to exclude an underlying endocrinopathy (cortisol or growth hormone (GH) deficiency), or an inborn error of metabolism, e.g. a glycogen storage disorder (GSD), may be found in a small fraction of these children, which require specialized follow-up and interventions. The majority of these will eventually end up with a diagnosis of idiopathic ketotic hypoglycemia (KH) [81], or may be considered to represent the "lower tail of the Gaussian distribution of fasting tolerance in children" [82].

No consensus exists regarding the optimal workup in children with one or several episodes of KH. Hepatomegaly may point towards a GSD and poor longitudinal growth towards GH deficiency, but these are inconsistent findings. In children with severe, "atypical" or recurrent episodes of KH, measurements of GH, cortisol and FFA in a critical sample are required to appreciate whether the levels of these counterregulatory hormones are appropriate to activate the pathways of lipogenesis, glycogenolysis and gluconeogenesis in order to avoid hypoglycemia. Further details on these endocrine disorders are beyond the scope of this thesis.

<u>Glycogen storage diseases (GSD)</u>

As depicted in Fig. 5, hepatic glycogenolysis is the first line of defense to maintain glucose homeostasis, when exogenous carbohydrate supply is faltering. Glycogen storage diseases (GSD) are due to mutations resulting in enzymatic deficiencies hindering glycogen synthesis (GSD type 0) or more commonly glycogen degradation (GSD types I, III, VI, IX) in the liver.

Patients display fasting intolerance, and except for GSD type 0, abnormally stored glycogen will result in hepatomegaly. Some patients with enzymatic defects compromising degradation of both liver and

muscle glycogen, such as GSD IIIa, may have additional signs, such as exercise intolerance or muscle cramps. Ketones are elevated in GSD type III, VI and IX, while lactate remains normal during hypoglycemia, as opposed to GSD type I, which is associated with elevated lactate during hypoglycemia due to the associated gluconeogenesis block, while absent ketosis is explained by inhibition of carnitine palmitoyl transferase I (CPT1) through elevated acetyl CoA and ensuing elevated malonyl CoA [83]. Postprandial hyperglycemia and hyperlactatemia are observed in the rarely diagnosed GSD type 0 [80].

Guidelines for the diagnosis and treatment of (non ketotic) GSD type I [84] and (ketotic) GSDbtype III [85], VI and IX [86] have been published recently.

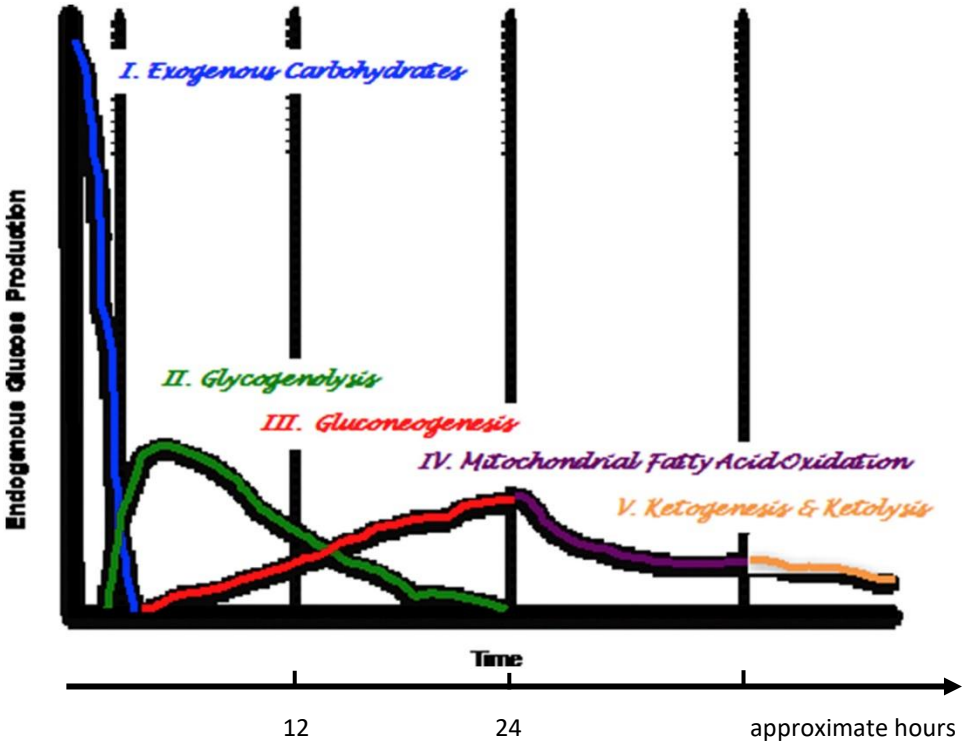

Fig. 5 Sources of endogenous glucose production with fasting. Contribution of major metabolic pathways responsible for glucose homeostasis

Reproduced from Weinstein, D. A. et al. in Pediatr. Clin. North Am. 65, 247–265 (2018).

Disorders of ketone transport and utilization

As depicted in fig. 3 and 5, activation of ketone synthesis during fasting emerges as a significant energy provider, once glycogen stores are depleted. Therefore, if ketones are synthesized but cannot

be metabolized to provide energy, while glucose is only supplied by gluconeogenesis, energy shortage will result in hypoglycemia.

Defects in one of the rarely encountered disorders of ketone transport and degradation are covered in section 3.5.2.

3.4 HOMOCYSTEINE

Homocysteine is an important metabolite at the intersection of the methionine, folate and transsulfuration pathways. As shown in fig. 6, homocysteine can be either remethylated to methionine or catabolized to cysteine by the transsulfuration pathway [87].

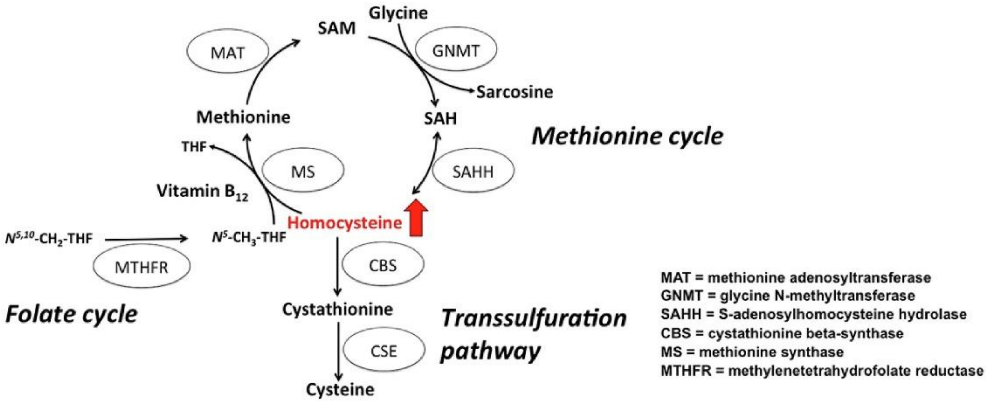

Fig. 6 Pathways in homocysteine homeostasis. Homocysteine stands at the crossroads of the folate and methionine cycles and the transsulfuration pathway.

Reproduced from Hannibal, L. & Blom, H. J. in Mol. Aspects Med. 53, (2017).

Lack of vitamin B12, the cofactor of methionine synthase (MS), as well as low folate which serves as precursor for the methyl donor 5-methyl tetrahydrofolate, slow down the remethylation pathway that converts homocysteine back to methionine. Deficiency of vit. B6, the cofactor of cystathionine beta-synthase (CBS), blocks the transsulfuration pathway that degrades homocysteine to cysteine.

When should homocysteine levels be checked?

Homocysteine is described by the American Association for Clinical Chemistry [88] as a laboratory parameter indicated to search for vitamin B6, folate or B12 deficiency, or to investigate patients that have suffered from cardiovascular events in the absence of traditional risk factors.

Serum homocysteine is indeed a very sensitive marker for folate or B12 deficiencies, as homocysteine starts to rise before serum levels of these vitamins fall below their respective reference ranges [89]. Elevated homocysteine levels have moreover been associated with both increased cardiovascular risks and cognitive decline in many studies [87].

Nevertheless, homocysteine should not (no longer) be ordered to stratify patients regarding their risk of ischemic heart disease (IHD) or stroke, as already stated by the American Heart Association in 1999. This recommendation has been strengthened by the demonstration that lowering of mildly elevated homocysteine through B12 and folate supplements failed to improve the outcome regarding cardiovascular events in several well conducted large randomized controlled trials [90]. New trials are still being published on homocysteine lowering strategies, but no change in the overall conclusion has occurred according to the third update of the Cochrane on this topic which was published in 2017 [91].

Less data have been published to support recommendations for or against homocysteine testing in patients with cognitive impairment. The higher sensitivity of homocysteine as opposed to serum B12 level is put forward by some experts, who consider that patients may present neuropsychological signs of B12 deficiency despite B12 levels in the low normal range [92,93]. Other experts disagree with this statement, as a meta-analysis encompassing 22'000 patients enrolled in trials looking at the effect of B12 supplements on cognitive decline failed to demonstrate any benefit for individual cognitive domains or global cognitive function [94].

Although the rationale for widespread measurements of homocysteine in the context of IHD, stroke or cognitive decline is now controversial, this biomarker remains essential to identify and follow patients with rare inborn errors of vitamin B12 metabolism [95] and homocystinuria due to cystathionine beta-synthase deficiency (CBS) [96]. Clinicians should keep in mind the possibility of such an underlying genetic predisposition, when relatively common conditions, such as for instance thrombotic events, occur at an unusually young age, with an atypical presentation, in association with unexplained neuro-psychiatric symptoms or with a positive family history.

A detailed clinical description of the whole spectrum of inborn errors of metabolism featuring elevated homocysteine is beyond the scope of this thesis. Severe cases will often present in infancy or childhood and be either picked up by newborn screening or diagnosed with the help of very specialized laboratory assays such as a chromatography of plasma amino acids and organic acids in urine. Milder forms can however present in adulthood and require a good clinical sense to be recognized. Three of the most frequently encountered enzymatic defects leading to elevated

homocysteine are presented hereafter. Homocysteine levels below the reference range have not been linked to inborn errors of metabolism.

3.4.1 Methylenetetrahydrofolate reductase (MTHFR) deficiency

MTHFR deficiency can present in adulthood with unexplained progressive neurological symptoms, such as epilepsy, spastic paraparesis, behavioural changes, frank psychotic episodes or unexplained encephalopathy and mild learning disabilities, which may be associated with recurrent thrombo-embolic events [97,98]. In these patients, homocysteine levels are often markedly elevated (>100 µmol/l) and should prompt referral for specialized investigations and treatment.

3.4.2 Cobalamin C (CblC) disease

Cobalamin C (CblC) disease, that results from an impaired conversion of vit. B12 into its metabolically active forms, can present from infancy to adulthood with a similar combination of unexplained neuro-psychiatric syndromes and thrombo-embolic events [20,98,99]. Therefore, homocysteine should be quantified in patients with renal thrombotic microangiopathy [54] or atypical hemolytic uremic syndrome, even more so if these are associated with neuropsychiatric symptoms, since this may be a revealing sign of CblC disease. Both homocysteine and methylmalonic acid (MMA) are elevated in CblC patients.

3.4.3 Cystathionine beta-synthase (CBS) deficiency

Cystathionine beta-synthase (CBS) deficiency can present in adolescence or young adulthood with thrombotic events, often affecting atypical locations such as the cerebral sinuses [100,101]. Coexistence of myopia, lens ectopia, scoliosis or Marfanoid features should increase the clinical suspicion of CBS deficiency.

MTHFR, CblC and CBS deficiencies are examples of rare inborn errors of metabolism (IEM) that can be detected through elevated homocysteine; plasma levels are typically > 100. However, in patients affected by milder genetic defects, the homocysteine level can sometimes normalize when patients take over the counter supplements of vitamin B6 (CBS defects) or B12 (CblC defects). An underlying IEM should therefore be considered in patients with Homocysteine above 20 µmol/l and normal renal function (30 µmol/l, if renal failure), in the absence of nutritional deficiencies of folate or vitamin B12. As diagnostic procedures may be complex, these patients should be referred to a specialized IEM consultation.

3.5 KETONES

Acetoacetate (AcAc) and beta-hydroxybutyrate (3OHB or 3HB) produced via reduction of AcAc, are the principal ketone bodies (KB) produced by the liver. The major precursor for ketone synthesis is acetyl-CoA, derived from fatty acid oxidation. In the fed state, precursors for ketones synthesis originate from the degradation of leucine and other ketogenic amino acids.

As shown in Fig. 7, KB are released into the blood to be taken up by extrahepatic tissues, most importantly the brain, when glucose supply is low [102]. Ketones represent an alternative metabolic fuel also for other high energy-consuming tissues, such as the heart and skeletal muscles; overall their contribution to the body's energy expenditure ranges from 5 % in the fed state to 20% after exercise and fasting.

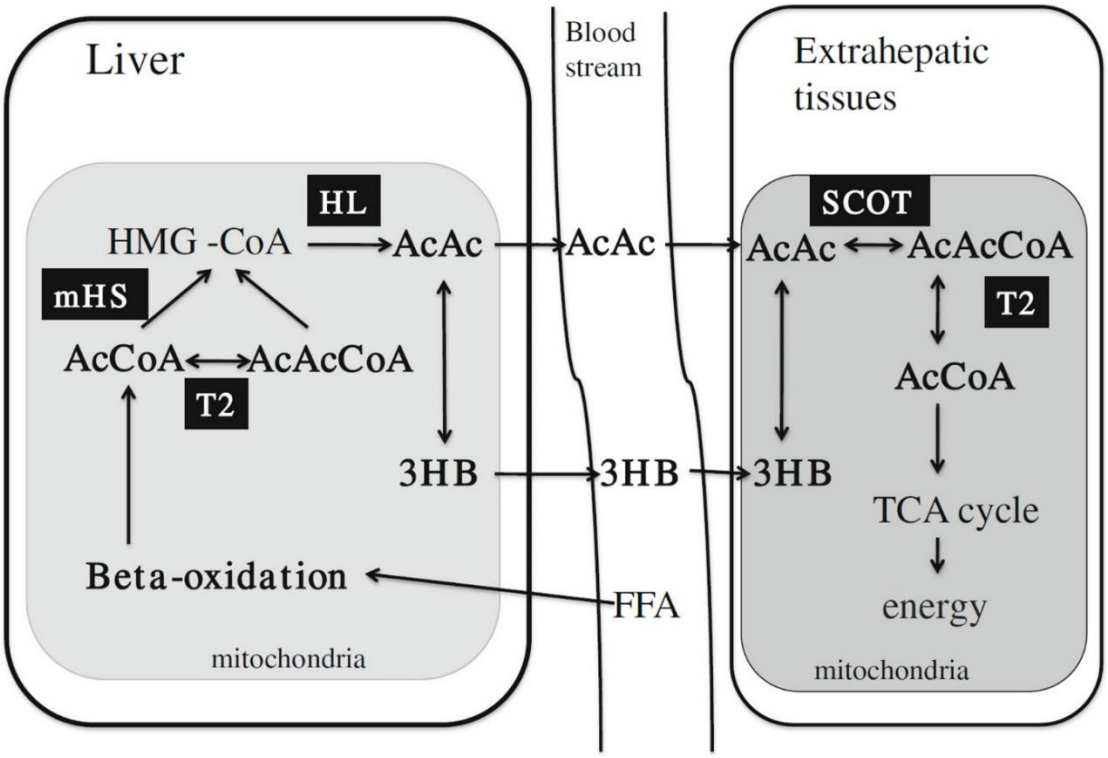

Fig. 7 Overview of ketone synthesis, transport and utilization. AcAc = acetoacetate; 3HB = 3-hydroxybutyrate; FFA = free fatty acids; mHS = HMG-CoA synthase ; HL = HMG-CoA lyase; SCOT = succinyl-CoA:3-oxoacid CoA transferase; T2 = mitochondrial acetoacetyl-CoA thiolase; TCA = tricarboxylic acid cycle

Reproduced from Fukao, T. et al. in J. Inherit. Metab. Dis. 37, 541–551 (2014).

Spontaneous decarboxylation of AcAc to the volatile acetone occurs when total ketone bodies (AcAc + 3OHB) are elevated; a fruity smell can be detected at levels >7 mM [102]. Ketone synthesis is repressed by insulin and stimulated by glucagon and catecholamines. Recent studies have uncovered that, next to their better known function as vital metabolic fuel, ketones also play pivotal roles in

post-translational modification of proteins, and as signaling mediators or modulators of inflammation and oxidative stress [102].

When and how should ketone levels be checked?

The most frequent indication to monitor ketones is by far the prevention of diabetic ketoacidosis. Advice given to parents of diabetic children to check ketones in urine every 4 hours during significant illness was already standard of care 30 years ago [103]. Nowadays, measuring ketones in capillary blood rather than urine is favored, as blood levels reflect current rather than average levels since last void. Moreover, hand-held ketone meters measure plasma 3OHB, whereas urine ketone stick give a semi-quantitative estimation of AcAc. The relative fraction of 3OHB/AcAc varies depending on the mitochondrial redox state, and strongly favors 3OHB during diabetic ketoacidosis (DKA) [104,105]. Plasma readings are more sensitive, accurate and precise, but more expensive than urine dipsticks. Nowadays, dual hand-held meters which measure both glucose and 3OHB on distinct test strips are increasingly used.

Ketosis is not always a feared complication of diabetes, but sometimes the result of a carefully planned ketogenic diet, an increasingly popular treatment, not only for drug resistant epilepsy, but also for a range of other neurological conditions, such as autism, Alzheimer's disease [106] or cancer [107], or as lifestyle and to boost athletic performances [108]. In the field of inborn errors of metabolism, ketogenic diet is a first line treatment for patients with GLUT1 or Pyruvate dehydrogenase (PDH) deficiency and may have benefits in selected other patients [109]. Measurement of ketone bodies is part of the management of ketogenic diets.

Measurement of ketone levels is indicated in newborns or children presenting with hypoglycemia, as detailed above in chapter 3.3.

Interpretation of 3OHB or total ketone bodies' (TKB) levels is complex, as their synthesis and degradation reflects dynamic processes which integrate energy fluxes from several energy providing pathways. Identifying abnormally high or abnormally low levels requires thorough clinical information on patient's age, height and weight, presence of stresses, such as fever, vomiting and diarrhea, fasting duration and details regarding most recent food intake and levels of other metabolic markers, such as glucose and free fatty acids.

As exemplified in fig. 8, fasting tolerance is lowest in infants and increases progressively during childhood. A twelve hours fast will cause a physiological elevation of TKB in infants, but not in the majority of older children and adults on a regular diet.

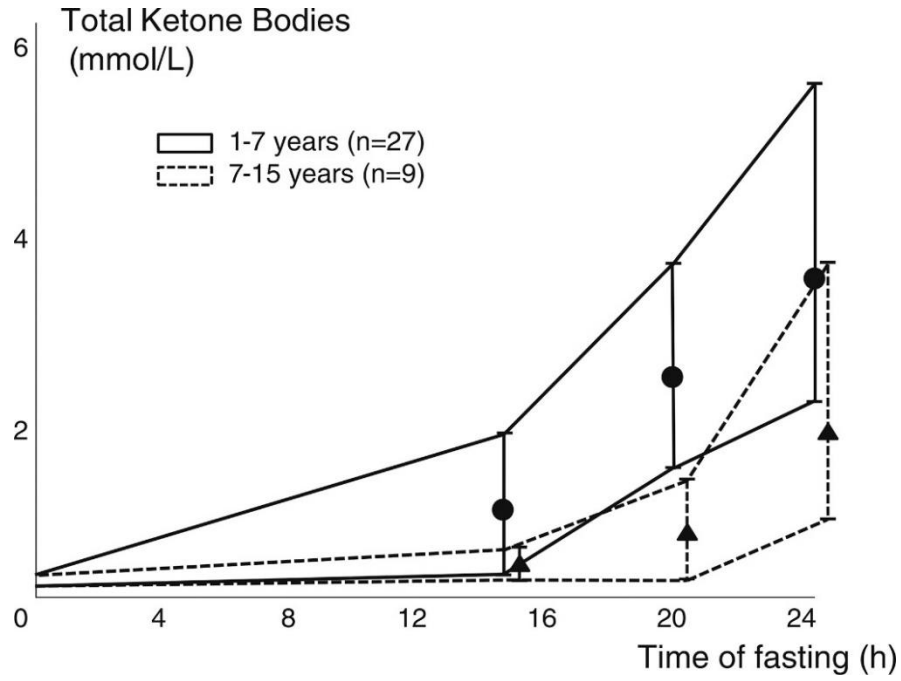

Fig. 8 Level of total ketone bodies (TKB) in relationship to fasting duration in younger vs older children; results are expressed as 10–90 percentiles with mean values

Reproduced from Fukao, T. et al. in J. Inherit. Metab. Dis. 37, 541–551 (2014).

3.5.1 Inborn errors in ketone synthesis (ketogenesis)

Patients with inborn errors of ketone synthesis due to either deficiency of mitochondrial HMG-CoA synthase (mHS) or deficiency of HMG-CoA lyase (HL) will most often present during an episode of gastroenteritis with hypoglycemia and severely altered consciousness; workup will reveal abnormally low TKB in relationship to the level of free fatty acids (FFA) which rise due to activation of lipolysis and fatty acid oxidation [104]. An overview of the pathways of ketone synthesis and degradation is depicted in figure 7. Details on clinical presentations and specialized workup can be found in the review by Fukao *et al*. [104]. Some patients have died during initial presentation of their disease. Treatment consists in avoiding catabolism.

3.5.2 Inborn errors in ketone transport & degradation

Patients with inborn errors of ketone degradation will often present in infancy with severe sometimes life-threatening bouts of ketoacidosis induced by catabolic states, such as during infections. Some patients display elevated ketones in the fed state [104].

Inborn errors in ketone metabolism are extremely rare but should be considered in the differential diagnosis of patients in whom a fatty acid oxidation defect is suspected but not confirmed, in

patients with ketosis without hyperglycemia in the fed state, or in those with severe ketoacidosis with or without fasting hypoglycemia.

3.6 LACTATE

As shown in fig. 9, lactate is produced through reduction of pyruvate following glycolysis; this crucial metabolite stands at the crossroads of glycolysis, oxidative phosphorylation and gluconeogenesis [110].

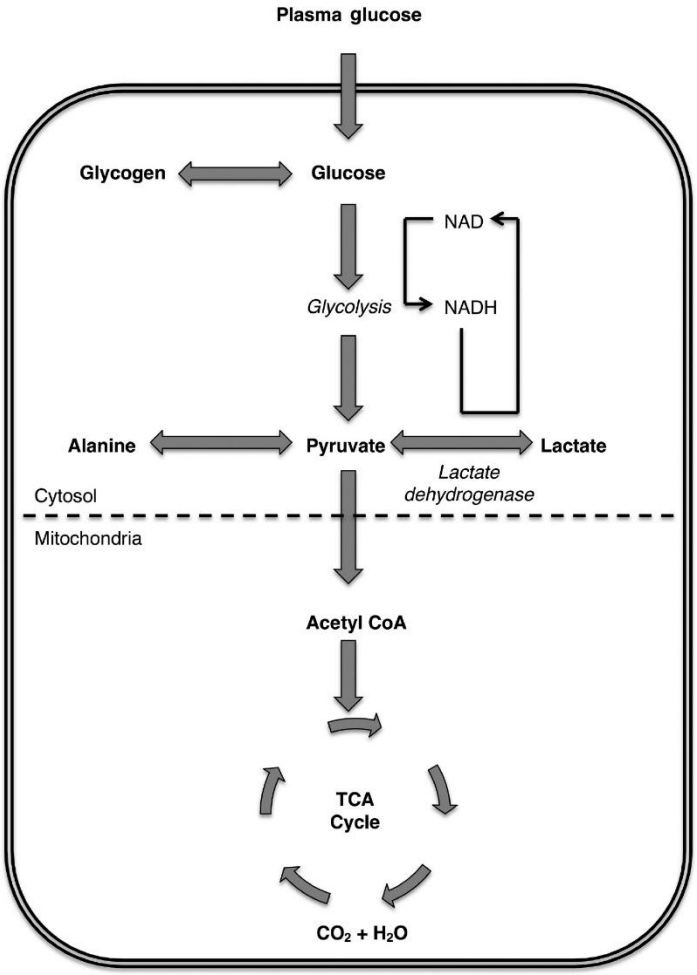

Fig. 9 Glycolytic pathway showing the production of lactate from pyruvate, catalyzed by NAD-dependent lactate dehydrogenase

Reproduced from Seheult, J. et al. in Clin. Chem. Lab. Med. CCLM 55, 322–333 (2017).

In the postabsorptive state, two thirds of plasma lactate is derived from glucose, while 16-20% are generated from the transamination of alanine and pyruvate catalyzed by alanine aminotransferase (ALT) [111].

At rest, plasma lactate levels reflect production by muscle (25%), skin (25%), central nervous system (20%), erythrocytes (20%) and gastro-intestinal tract (10%). During stress or strenuous exercise,

activation of glycogenolysis and glycolysis increases pyruvate production; when the capacity of oxidative phosphorylation in the TCA becomes limiting, pyruvate accumulates and is converted to lactate by lactate dehydrogenase. This allows recycling of NAD^+ to NADH which is necessary to keep glycolysis ongoing [110]. During stress or exercise, lactate is actively taken up by the heart and brain as an alternative fuel, whereas during recovery, lactate is either converted back to glucose by gluconeogenesis or degraded by oxidative phosphorylation in the liver and to a minor extent in the kidney [98]. These dynamic fluxes are partially depicted in fig. 10, in which gluconeogenesis has been omitted.

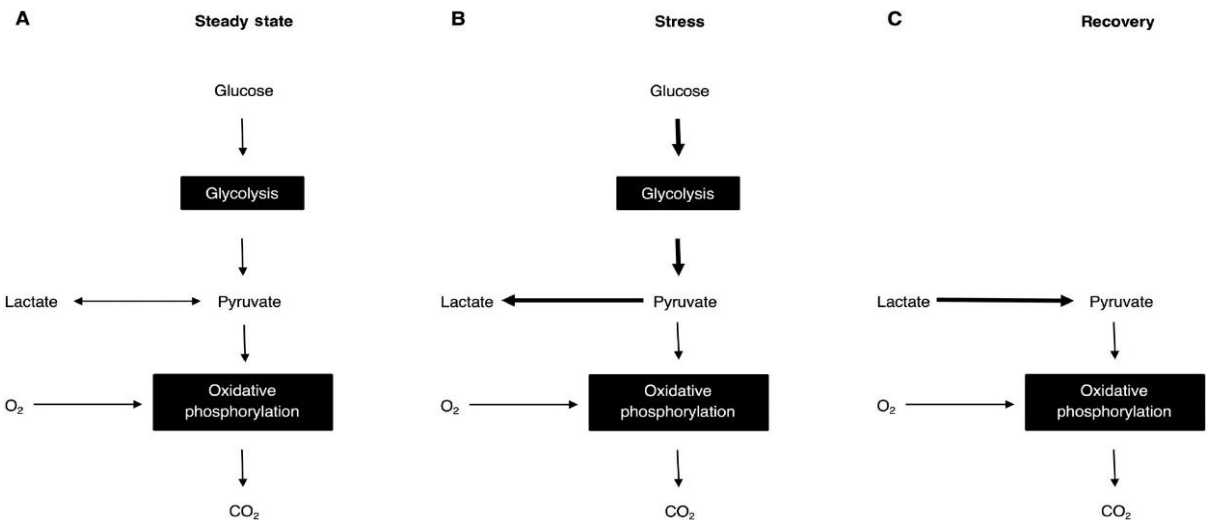

Fig. 10 Dominant fluxes in lactate metabolism during A: steady state, B: stress and C: recovery Nb During recovery, lactate will also fuel gluconeogenesis [112] (not shown)

Reproduced from Seheult, J. et al. in Clin. Chem. Lab. Med. CCLM 55, 322–333 (2017).

Pitfalls in lactate measurements

Blood specimens for lactate measurement should be analyzed within 15 minutes from drawing blood to minimize spurious elevations due to ongoing glycolysis; if immediate processing cannot be guaranteed, glycolysis may be reduced by quickly cooling the sample on ice or by using a collection tube containing sodium fluoride, which inhibits glycolysis [112,113]. Tourniquet use during phlebotomy has no significant impact on venous lactate levels [112,113]. Studies on the correlation between capillary, arterial and venous lactate measurements on handheld devices vs hospital laboratory or blood gas

analyzer reach contradictory conclusions; finger prick capillary sampling in critically ill patients may result in higher values [114,115].

How to use lactate in clinical practice?

Measurement of lactate is mainly indicated in severely ill patients in emergency and intensive care departments to help identifying patients with shock or hypoperfusion, to guide resuscitation and provide prognostic information. Elevated lactate levels and inability to clear lactate during initial resuscitation is associated with higher mortality rates as verified in patients with a wide variety of underlying causes, such as infections, trauma, hemorrhage, cardiac arrest or shock [112]. An elevated lactate level should be considered a red flag calling for immediate intervention; as stated by Bakker *et al.*: "the higher the lactate level, the higher the urgency" [116].

Understanding why lactate is or stays elevated in a septic patient in intensive care is challenging. Lactate production may increase through activation of glycolysis by the inflammatory response, as well as by the use of catecholamines or steroids, whereas hepatic clearance of lactate may decrease due to ischemic hepatitis caused by shock [117]. Various common drugs and toxins (eg acetaminophen, nucleoside reverse-transcriptase inhibitors, propofol, metformin) have suspected or proven mitochondrial toxicity which reduces overall capacity for oxidative phosphorylation; carbon monoxide intoxication will increase lactate by 2 mechanisms: 1) competitive inhibition of oxygen binding to hemoglobin which diminishes arterial oxygen content and 2) inhibition of oxidative phosphorylation through binding to cytochrome A [112]. Renal failure reduces lactate consumption through gluconeogenesis by the kidney; hepatic cirrhosis limits hepatic clearance through oxidative phosphorylation; lactate accumulates also in thiamine deficiency, since thiamine is an essential cofactor of the enzyme pyruvate dehydrogenase (PDH). Moreover, some hematological malignancies with very high cell turnover and a glycolytic phenotype may greatly increase lactate production [110,112].

Any acquired or inborn defect in gluconeogenesis or oxidative phosphorylation, including the PDH complex, TCA and mitochondrial respiratory chain may result in lactate accumulation [111]. Primary mitochondrial disorders will be briefly described in section 3.6.1.

In all above mentioned instances, hyperlactatemia is caused by accumulation of L-lactate. In patients with short-bowel syndrome, undigested carbohydrates may be transformed into D-lactate by micro-organisms, such as *lactobacillus acidophilus* or *streptococcus bovis* which contain D-lactate dehydrogenase [111].

3.6.1 MITOCHONDRIAL DISEASES

Primary mitochondrial diseases are caused by mutations in over 200 nuclear genes and in all mitochondrial encoded genes [118]. Clinical presentations are very diverse and symptoms may present at any age: severe compromise of mitochondrial energy production may lead to antenatal structural brain abnormalities as for example in pyruvate dehydrogenase complex (PDH) deficiency. Organs with high energy demand, such as the central nervous system (brain, retina), heart and skeletal muscle are frequently affected and progressive multi-organ involvement as featured in figure 11 reproduced from ref [119] should raise suspicion of a mitochondrial disease.

Lactate concentrations may be elevated in blood and CSF of patients with primary mitochondrial diseases; however, determination of normal lactate levels does not exclude these disorders [118].

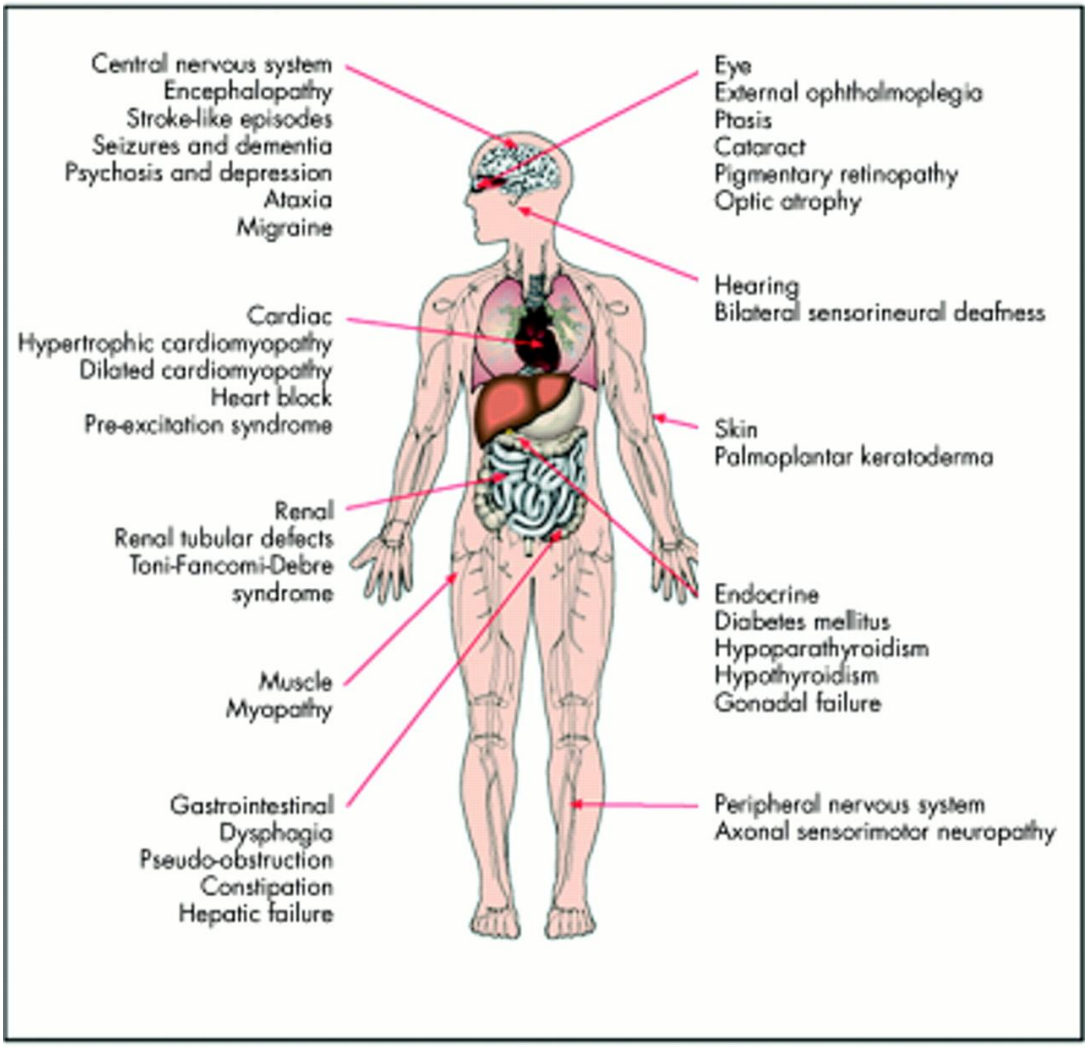

Fig. 11 Clinical features of mitochondrial disease. Patients may present with single organ involvement (sensorineural deafness, diabetes, visual failure, myopathy, or cardiomyopathy), or progressive multisystem involvement.

Reproduced from Kaufmann, P. et al. in Neurology 77, 1965–1971 (2011).

MELAS

The acronym “MELAS” stands for “Mitochondrial Encephalopathy Lactic Acidosis Stroke”, one of the better known clinically distinct clusters of symptoms and features that is most often associated with the maternally inherited m.3243A>G mutation in mitochondrial DNA [118]. A high proportion of affected mitochondria correlates with earlier involvement, more rapid progression to severe multi-organ disease and hence shortened survival; carrier relatives with a low proportion of mutated mitochondria may stay asymptomatic or slowly progress to present symptoms such as exercise intolerance, diabetes or night blindness [120].

3.7 URIC ACID

Uric acid is synthesized through purine degradation in the liver. Two thirds are eliminated by the kidney by filtration, tubular reabsorption and secretion, while one third is secreted into the gut. The metabolic pathways controlling urate homeostasis are complex and highly regulated, as shown in figure 12.

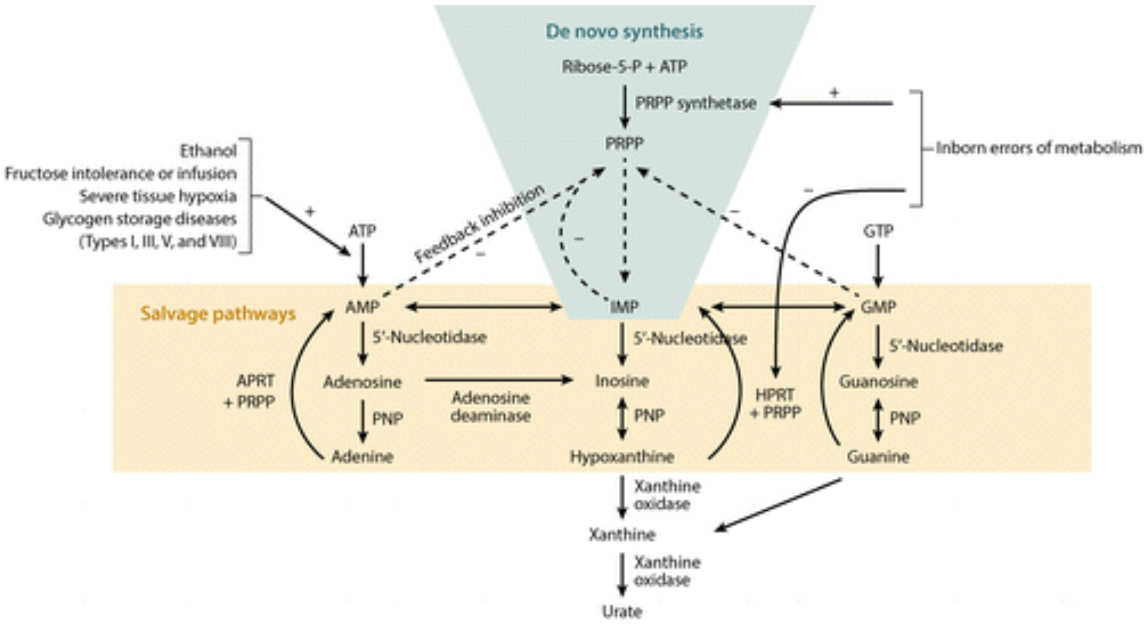

Fig. 12 Pathways of purine metabolism leading to urate synthesis.

APRT = adenine phosphoribosyltransferase; HPRT = hypoxanthine phosphorylbosyltransferase; PNP = purine nucleoside phosphorylase, PRPP = phosphoribosylpyrophosphate

Reproduced from Mandal, A. K. & Mount, D. B. in Annu. Rev. Physiol. 77, 323–345 (2015).

Interestingly, humans and chimpanzees differ from other mammalians as they share truncating loss of function mutations in the uricase gene, which codes for an otherwise well conserved enzyme which degrades urate. Urate levels in humans and hominoids are therefore about tenfold higher than in other mammals and this led to speculations regarding the beneficial effects of increased urate levels selected during evolution. Reported advantages are reduced oxidant stress, decreased cancer incidence, ability to survive under conditions of low dietary salt and even increased intelligence! These possible benefits are however balanced by the risk of gout and nephrolithiasis [121].

Overproduction of urate may be observed in patients with increased precursor loads (consumption of alcohol and a purine-rich diet, rapid tissue lysis with nucleotide release) and in rare patients with inborn errors of purine metabolism that carry mutations activating the PRPP synthase or inhibiting HPRT function, thereby preventing the recycling of hypoxanthine and increasing urate production.

Urate production increases also following consumption of a high fructose diet. Worldwide increased consumption of sucrose and high-fructose corn syrup correlates with the epidemic of diabetes, obesity, metabolic syndrome, fatty liver, and hyperuricemia. Increased urate production following fructose consumption is caused by fructose-induced ATP depletion in the liver, as shown in Fig. 13, reproduced from ref. [122].

This ATP depletion is also the cause for the increased purine degradation and urate production observed in several inborn errors of metabolism, e.g. in a subset of glycogen storage disorders and in aldolase b deficiency.

Decreased urate clearance secondary to increased reabsorption is observed in diabetic ketoacidosis and other situations characterized by increased serum lactate or ketone levels [121]. On the other hand, increased urate clearance is observed when renal reabsorption is decreased [123].

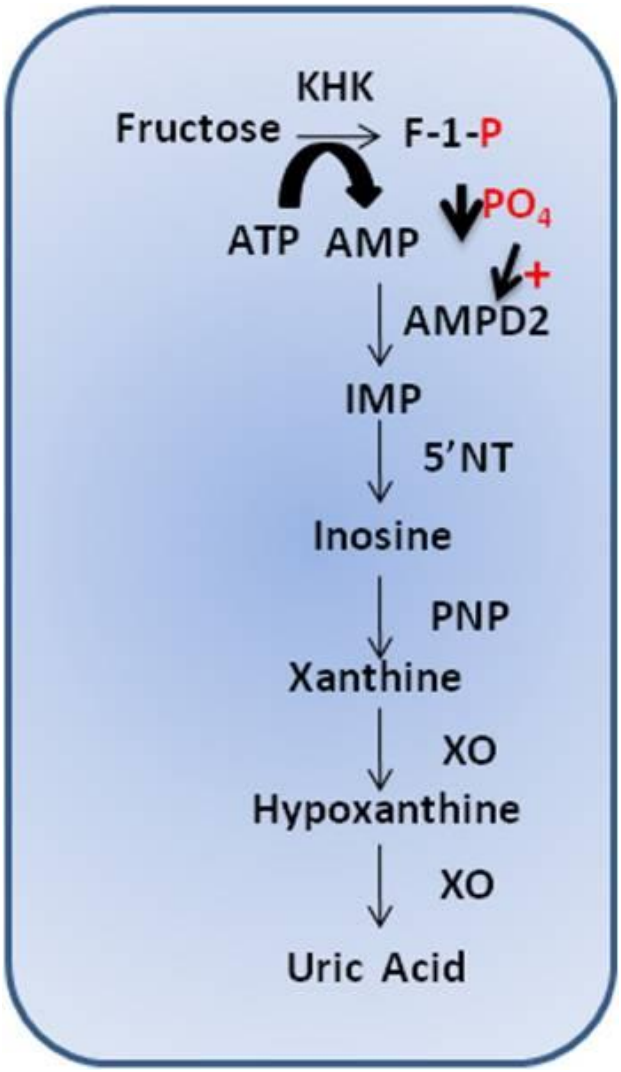

Fig. 13 Increased uric acid synthesis following fructose intake.

KHK = ketohexokinase (or fructose kinase). Phosphorylation of fructose uses ATP, giving raise to AMP, stimulating in turn the activity of AMP deaminase 2 (AMPD2), which converts AMP to inosine monophosphate (IMP).

Reproduced from Mandal, A. K. & Mount, D. B. in Annu. Rev. Physiol. 77, 323–345 (2015).

When to check urate levels?

Determination of serum uric acid levels is indicated as a diagnostic parameter in patients presenting symptoms suggestive of gout or to monitor treatment in gout patients. Patients undergoing chemotherapy or radiation therapy are also at risk of developing hyperuricemia [121,124]. In patients with nephrolithiasis, uric acid determination should occur in both a 24-hour urine collection [124], and in serum [125]; increased and decreased serum and urinary urate levels can be found, pointing to different pathologies, as described below.

Although hyperuricemia is frequently encountered in patients with hypertension and with the metabolic syndrome, treatment with xanthine oxidase inhibitors is generally not recommended in patients with asymptomatic hyperuricemia according to currently available evidence, except for patients with very elevated levels, to prevent nephrotoxicity [126]. Indications for uric acid determination in these patients should be adapted accordingly.

3.7.1 Inborn Errors of Purine metabolism

Inborn errors of purine synthesis, salvage or degradation pathways may be characterized by decreased, normal or increased serum urate levels, depending on the affected enzyme. Clinical manifestations comprise not only gout and kidney stones, but also neurological features, such as self-mutilations, autistic traits, epilepsy, spasticity or psychomotor retardation; immunodeficiency, anemia, myopathy, liver dysfunction, visual abnormalities, hearing loss. Clinical severity, age of onset and existence of therapeutic options are highly variable and reviewed in reference [127]. Only a minority of gout patients have an underlying defect in purine metabolism [121].

Phosphoribosyl-pyrophosphate (PRPP) Synthetase activation

As illustrated in fig. 12, mutations in the X-linked PRPP gene causing overexpression or superactivity lead to hyperuricemia and gout at an unusually young age compared to classical gout patients. Severe presentations also feature mental retardation, hypotonia and sensorineural deafness in young boys, while female carriers may present gout in adolescence. Treatment modalities include the xanthine oxidase inhibitor Allopurinol (or febuxostat), urine alkalinisation to reduce urinary reabsorption, high fluid intake and a low purine/ low fructose diet [127].

Hypoxanthine phosphorylbosyltransferase (HPRT) deficiency

Inactivating mutations in the HPRT gene inhibit the purine salvage pathway resulting also in hyperuricemia and gout. Complete absence of the X chromosome localized HPRT enzyme results in Lesh-Nyhan disease, characterized by psychomotor delay, dystonia, choreoathetosis, self-mutilation, impulsive aggression and megaloblastic anemia. Minor impairments cause less severe neurological features, associated with gout and nephrolithiasis. HPRT deficiency is treated in the same way as hyperuricemia due to PRPP synthase activation [127].

Adenine phosphorylbosyltransferase (APRT) deficiency

Inactivating mutations in the APRT gene limit also the purine salvage pathway resulting in hyperuricemia and gout. Treatment combines Allopurinol, high fluid intake, low-purine diet and if required, urological treatment of stones [127].

Hereditary Xanthinuria

Deficient conversion of xanthine to uric acid by Xanthine dehydrogenase/oxidase (XO), the last step of purine degradation, may lead to xanthine stones and renal failure with very low plasma and urinary urate levels [127–129]. Treatment combines high fluid intake, low-purine diet and if required, urological treatment of stones.

3.7.2 Secondary Hyperuricemia in other Inborn Errors of metabolism (IEM)

Clinicians specialized in caring for patients with IEM may follow uric acid levels in patients with selected glycogen storage disorders to monitor dietary therapy. Elevated uric acid levels correlate indeed with dietary errors leading to insufficient energy intake and/or hypoglycemia. When gout symptoms occur in a patient with sometimes unrecognized muscle weakness or myalgias, an underlying muscular glycogen storage disorder may therefore be uncovered [130].

3.7.3 Renal Hypouricemia

Renal hypouricemia (RHUC) is defined as serum urate levels < 120 µmol/l with increased fractional urinary excretion or urate clearance. Increased urinary excretion is caused by mutations disrupting URAT1/SLC22A12 or GLUT9/SLC2A9, the dominant urate exchangers of the human proximal tubule that are responsible for 90-95% reabsorption of the filtered urate load [123,131,132].

RHUC has been most extensively studied in Japan, where it has a prevalence of 0.3 % [131]. Affected patients may remain asymptomatic or suffer from urolithiasis and/or exercise induced acute kidney injury. Treatment consists in preventing these complications through lifestyle recommendations; a xanthine oxidase inhibitor such as Allopurinol may be considered in high-risk patients [131].

4 SELECTED CLINICAL INDICATIONS TO ORDER METABOLIC LABORATORY INVESTIGATIONS

Whereas section 3 covered an alphabetically ordered selection of common biomarkers of which most bear key functions in energy metabolism, section 4 will start from a few clinical situations, in which these biomarkers may suggest the presence of an underlying IEM, and describe how these may help to select further more specialized diagnostic investigations.

4.1 DIAGNOSIS OF "INTOXICATION-TYPE" IEM IN NEWBORNS

Birth is a high risk process, during which 2-5% term delivered newborns may suffer from a significant adverse event that may or may not lead to lifelong impairment or death [133,134]. These infants will most likely be admitted in intensive care units. Clinical symptoms have very limited specificity in newborns; the ill-appearing neonate may present with increased or decreased temperature, respiratory distress, apnea, bradycardia, poor sucking, hypotonia, lethargy or seizures secondary to more common causes such as birth asphyxia and sepsis, or in rare instances due to an underlying IEM [135]. In a French study conducted in a tertiary pediatric intensive care unit, 2% of all admitted newborns underwent specialized investigations to diagnose or exclude an underlying IEM [136]. A lack of awareness regarding these individually rare diseases will often cause diagnostic delay which may translate in poorer outcome.

Acute neurological deterioration in a term infant with clinical signs such as lethargy, coma, hiccups, poor sucking, hypothermia, hypotonia, hypertonia, abnormal movements, large amplitude tremor, myoclonic jerks, or abnormal odor developing after a symptom-free interval may suffer from an "intoxication-type" IEM characterized by accumulation of a toxic molecule upstream of a metabolic blockade. Since intoxication-type IEM are often treatable, basic investigations including at least blood cell count, electrolytes, transaminases, prothrombin time, blood gases, ammonium, glucose, ketones and lactate should be obtained at once [135].

The following table classifies classical IEM manifesting in newborns or infants according to the results of basic investigations and suggests further specialized laboratory investigations.

Classification of IEM presenting with acute neurological deterioration in newborns and infants

(modified from ref. [135,137]):

Disease	Acidosis ketones	Ammonia (NH3) lactate	other : CBC, glucose, …	Specialized laboratory analyses
Organic acidurias: MMA, PA, IVA, MCD…	acidosis ++ ketones ++	NH3 ↑ +/++ lac N / ↑ +	WBC N/↓ , platelets N/↓ gluc : N or ↑ +	Urinary organic acids Plasma amino acids Acylcarnitines
Urea Cycle Disorders	acidosis 0 ketones 0 respiratory alkalosis +/-	NH3 ↑ +/+++ lac N / ↑ +	CBC N glucose N +/- coagulation def.	Plasma + urine amino acids orotic acid
MSUD	acidosis +/+++ ketones +/+++	NH3 ↑ + lac N	CBC N glucose N	Plasma + urine amino acids
NKH	acidosis 0 ketones 0	NH3 N lac N / ↑ +	CBC N gluc : N (EEG burst-suppression)	Plasma + urine +/- CSF amino acids
Sulfite oxidase def. Molybdenum cofactor def. (MOCO)	acidosis 0 ketones 0	NH3 N lac N / ↑ +	CBC N, gluc : N Uric acid ↓ (MOCO)	Urinary sulfites ↑
Congenital lactic acidosis : PC,PDH,TCA,RCD	acid +/+++ ketones ++	NH3 ↑ + lac +/+++	CBC N / anemia gluc : N / ↓ +	Lac/pyruvate, acetoacetate/ β-OH-butyrate Urinary organic acids
Fatty acid oxidation defects (FAO)	acidosis ++ ketones 0/+	NH3 ↑ +/++ lac +/++	CBC N gluc : ↓ +/++	Urinary organic acids Acylcarnitines

CBC = Cell blood count, WBC = white blood cells
MMA = methylmalonic aciduria, PA = propionic aciduria, IVA = isovaleric aciduria,
MCD = multiple carboxylase deficiency, MSUD = maple sirup urine disease,
NKH = non-ketotic hyperglycinemia, PC = pyruvate carboxylase,
PDH = pyruvate dehydrogenase, TCA = tricarboxylic acid cycle,
RCD = respiratory chain disease, FAO = fatty acid oxidation

4.2 Atypical psychiatric clinical presentations

Several IEM may present for the first time in adolescence or adulthood with psychiatric manifestations. Some of these IEM respond well to metabolic treatment, which will not only cure the psychiatric symptoms, but also prevent occurrence of further irreversible somatic symptoms.

Psychiatrists are often unfamiliar with IEMs and patients may be wrongly diagnosed as suffering from atypical psychosis, schizophrenia, personality disorder or bipolar disorder. This under-recognized issue has been addressed by a few authors over the last 20 years [138–140]. A simple practical classification has been proposed by Sedel *et al.* [140] as shown hereafter:

Classification of IEM with primary psychiatric symptoms

	Symptoms	Disorder (+/- link to section)
Emergencies	acute (recurrent) attacks of confusion or behavioral changes visual hallucinations, delusions catatonia impulsivity mania, restlessness, insomnia anxiety	Urea cycle disorders (3.1.2) Remethylation disorders (3.4.1;3.4.2) Porphyrias
Chronic treatable diseases	Behavioral problems delusions, hallucinations depression obsessive-compulsive disorder personality disorder impulsivity, disinhibition	Wilson Disease Homocystinuria (3.4.3) Cerebrotendinous xanthomatosis Creatine synthesis defects
Chronic non-treatable diseases	Behavioral problems psychosis, depression, mania mental retardation aggressiveness	Lysosomal storage disorders Adrenoleukodystrophy

Adapted from Sedel, F. et al. in J. Inherit. Metab. Dis. 30, 631–641 (2007), Bonnot, O. et al. in Orphanet J. Rare Dis. 9, 65 (2014) and Demily, C. & Sedel, F. in Ann. Gen. Psychiatry 13, (2014).

Should all psychiatric patients be screened for an underlying IEM? This seems not feasible, given the high prevalence of psychiatric problems, low prevalence of IEM, suboptimal sensitivity and specificity of the biomarkers as well as the practical obstacles to meeting the stringent pre-analytical prerequisites for quantifying biomarkers such as ammonium in a psychiatric ward (see section 3.1).

Screening for an underlying IEM should therefore be prioritized in patients with “atypical psychosis” i.e. displaying visual rather than auditory hallucinations, mental confusion, catatonia, fluctuation of symptoms, resistance to treatment, progressive cognitive decline.

Likelihood of an underlying IEM is heightened by the following criteria: early onset, acute onset, intellectual impairment and unusual or severe side effects [138,139], as shown in figure 14.

Psychiatric Red flags	Strengthening features
Confusion	Acute onset
Visual > auditory hallucinations	Early onset
Catatonia	Intellectual disability
Progressive dementia	Unusual or severe side-effect
Treatment resistance	
Fluctuating symptoms	

Other clinical features	Disease
Headache, nausea, vomiting, selective diet	UCD
Recurrent thrombosis	Remethylation disorders (MTHFR, CblC)
Marfanoid habitus,, severe myopia ectopia lentis,	Homocystinuria (CBS)
Corneal Kayser-Fleischer ring	Wilson Disease
Abdominal pain, dysautonomia, dark urine, photosensitivity	porphyrias
Juvenile cataract, xanthomas, diarrhea	Cerebrotendinous xanthomatosis

Imaging	Disease
Brain MRI	UCD
Brain +/- spinal MRI	Remethylation disorders (MTHFR, CblC)
Brain MRI: basal ganglia changes?	Wilson Disease
Brain MRI	Cerebrotendinous xanthomatosis

Screening IEM biomarker	Disease
ammonia	UCD
homocysteine	Remethylation disorders (MTHFR, CblC) Homocystinuria (CBS)
Ceruloplasmin, copper	Wilson Disease
Urinary porphobilinogen	porphyrias

Specialized IEM analysis	Disease
Amino acids	UCD
Amino acids	Remethylation disorders (MTHFR, CblC) Homocystinuria (CBS)
Sterol (cholestanol)	Cerebrotendinous xanthomatosis
porphyrins	porphyrias

Fig. 14 Diagnostic algorithm for patients with atypical psychosis

Redrawn using data from Sedel, F. et al. in J. Inherit. Metab. Dis. 30, 631–641 (2007) and Bonnot, O. et al. in Orphanet J. Rare Dis. 9, 65 (2014).

If suspicion of an underlying IEM arises based on clinical signs, basic IEM biomarkers and imaging, the patient should be referred for further metabolic investigations to a metabolic specialist. These highly specialized analyses are not available in most hospital and community serving laboratories and must often be performed in tertiary facilities, in order to comply with the stringent pre-analytical handling of the samples.

5 CONCLUSION

This thesis focuses on laboratory analyses, an essential and powerful tool of medical practice.

Utilization of laboratory tests may be improved by educating physicians on the appropriate prescription of diagnostic procedures, as promoted by guidelines or consensus statements from expert medical societies. Laboratory professionals should be responsible for requesting and reinforcing appropriate pre-analytical sample handling; they may also assist physicians in the sometimes challenging task of interpreting significant results in their clinical, metabolic and pathophysiological context.

Basic laboratory analytes such as glucose, ketones and lactate are key players of energy metabolism. In an era were an important part of the world's population suffers from obesity, diabetes and the "metabolic syndrome", whereas at the same time, thousands of children are still starving or suffering from malnutrition, a thorough understanding of the pathophysiology influencing these widely available markers is essential. With such a "prepared mind", a pediatrician or general practitioner should be able to recognize when these biomarkers don't fit together in the usual way and suspect an underlying inborn error of metabolism. Severe liver insufficiency leads to hyperammonemia in patients with cirrhosis, but also in rare instances in a newborn or child with a grossly intact liver but a lacking urea cycle enzyme, a critical but rarely encountered situation every pediatrician should bear in mind.

The field of rare inborn errors of metabolism provides also unique opportunities to reveal the fundamental importance of teaching basic sciences such as biochemistry to medical students, as IEM highlight the clinical consequences of a single metabolic dysfunction.

Experience gained in the understanding and treatment of these very rare metabolic outliers may also help to gain insight in the complex pathophysiology of more common multifactorial diseases und reveal or refine better treatment strategies.

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