Biochimica et Biophysica Acta 1842 (2014) 2021–2029

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

From genome to phenome—Simple inborn errors of metabolism as complex traits ☆

C.M.L. Touw [a,b,c,*], T.G.J. Derks [a,c], B.M. Bakker [b,c], A.K. Groen [b,c], G.P.A. Smit [a,c,1], D.J. Reijngoud [b,c,d]

[a] Section of Metabolic Diseases, University Medical Centre of Groningen, Groningen, The Netherlands
[b] Research Laboratory of Paediatrics, Beatrix Children's Hospital, University Medical Centre of Groningen, Groningen, The Netherlands
[c] Center for Liver, Digestive and Metabolic Diseases, University Medical Centre of Groningen, Groningen, The Netherlands
[d] Laboratory of Metabolic Diseases, Department of Laboratory Medicine, University of Groningen, University Medical Centre of Groningen, Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 31 January 2014
Received in revised form 30 April 2014
Accepted 28 May 2014
Available online 3 June 2014

Keywords:
Mitochondrial fatty acid oxidation
Oxidative phosphorylation
Electron transport chain
NADH
$FADH_2$
Signaling pathway

ABSTRACT

Sporadically, patients with a proven defect in either mFAO or OXPHOS are described presenting with a metabolic profile and clinical phenotype expressing concurrent defects in both pathways. Biochemical linkages between both processes are tight. Therefore, it is striking that concurrent dysfunction of both systems occurs so infrequent. In this review, the linkages between OXPHOS and mFAO and the hypothesized processes responsible for concurrent problems in both systems are reviewed, both from the point of view of primary biochemical connections and secondary cellular responses, *i.e.* signaling pathways constituting nutrient-sensing networks. We propose that affected signaling pathways may play an important role in the phenomenon of concurrent defects. Recent data indicate that interference in the affected signaling pathways may resolve the pathological phenotype even though the primary enzyme deficiency persists. This offers new (unexpected) prospects for treatment of these inborn errors of metabolism. This article is part of a Special Issue entitled: From Genome to Function.

1. Introduction

Inborn errors of metabolism arise from single enzyme deficiencies but behave as complex traits [1]. The broad spectrum of clinical presentations that can be observed in patients with the same enzyme deficiency is a clear example of this. Apparently, cellular metabolism and physiology have many ways to adapt to perturbations in biochemical pathways, and each of these (mal)-adaptations comes with their own set of clinical symptoms. Unbiased '-omics' approaches *i.e.* transcriptomics and metabolomics, can be of great help to go beyond textbook biochemistry and generate hypotheses concerning possible mechanisms that account for these adaptations. Only recently application of these approaches to the field of inborn errors of metabolism has started in the field of mitochondrial diseases.

In the mitochondrion, mFAO, OXPHOS, and the tricarboxylic acid (TCA) cycle function together in the formation of adenosine triphosphate (ATP). Mitochondrial FAO takes care of the repetitive shortening of acyl-CoA esters for the generation of acetyl-CoA, NADH, and $FADH_2$. OXPHOS is the process of ATP synthesis *via* the transfer of electrons from NADH and $FADH_2$, which have been generated in mFAO or the TCA cycle, to oxygen over the electron transport chain (ETC). According to textbook biochemistry, this continuous regeneration of NAD^+ and FAD by the ETC and oxidation of the acetyl-residue of acetyl-CoA in the Krebs cycle to release free CoA (CoASH) are essential for the mFAO to proceed. It is inherent to this that dysfunctional OXPHOS should accompany impairment of mFAO. However, the opposite is observed. In humans, deficiencies in many of the enzymes important in mFAO and OXPHOS have been described [2,3]. When defects and resulting clinical symptoms are considered, mFAO and OXPHOS usually behave as independently functioning systems (for reviews on individual mFAO and OXPHOS defects and their respective clinical presentation we refer to Table 1 and [3–8]). It is sporadically observed that patients with an established OXPHOS or mFAO defect present with clinical symptoms or metabolite patterns that imply concurrent defects in both systems. The question then arises what molecular mechanisms determine this sporadic occurrence of signs of a combined defect.

Abbreviations: ANT, Adenine nucleotide transporter; CoASH, Free CoA; CPT, Carnitine palmitoyltransferase; DHA, Docosahexaenoic acid; EMA, Ethylmalonic acid; ETC, Electron transport chain; ETF, Electron transfer flavoprotein; ETF:QO, ETF:ubiquinone oxidoreductase; FAD, Flavin adenine dinucleotide; HADH, 3-hydroxyacyl-CoA dehydrogenase; KB, Ketone bodies; LCAD, Long-chain acyl-CoA dehydrogenase; LCHAD, Long-chain 3-hydroxyacyl-CoA dehydrogenase; MADD, Multiple acyl-CoA dehydrogenase deficiency; MCAD, Medium-chain acyl-CoA dehydrogenase; mFAO, Mitochondrial fatty acid oxidation; mTORC, Mammalian target of rapamycin; MTP, Mitochondrial trifunctional protein; OXPHOS, Oxidative phosphorylation; PDHc, Pyruvate dehydrogenase complex; SCAD, Short-chain acyl-CoA dehydrogenase; SCHAD, Short-chain 3-hydroxyacyl-CoA dehydrogenase; TCA, Citric acid cycle; UQ, Ubiquinone; VLCAD, Very long-chain acyl-CoA dehydrogenase

☆ This article is part of a Special Issue entitled: From Genome to Function.

* Corresponding author at: Centre for Liver, Digestive, and Metabolic Diseases, University Medical Centre Groningen, PO Box 30 001, CA84, 9700 RB Groningen, The Netherlands. Tel.: +31 50 3611262; fax: +31 50 3611746.
E-mail address: n.touw@umcg.nl (C.M.L. Touw).

[1] Supported by a grant of Metakids, The Netherlands.

http://dx.doi.org/10.1016/j.bbadis.2014.05.032
0925-4439/

In this review we like to distinguish between two major responses of the body to defects in mFAO and OXPHOS; the 'primary biochemical linkages' and 'secondary cellular response'. In Fig. 1 we depict several levels of integration. At the metabolic level the conversion of substrates into biomass, energy, and products takes place. At this level all biochemical reactions take place, as well as the metabolite-driven regulation of enzyme activity. Until now, the concurrence of mFAO and OXPHOS defects has mainly been discussed at the level of 'primary biochemical linkages', *i.e.* at this metabolic level. Indirectly, metabolism is regulated *via* the other 3 levels, which are collectively considered the 'secondary cellular responses'. The second level represents the nutrient-sensing signaling networks, which influence the biochemical reaction by changing enzyme activities by post-translational modification like phosphorylation. The third level represents the level in which nutrient-sensing transcription factors are active. Since these factors are proteins themselves, they can also undergo post-translational modifications. The reactions in the second and third level are driven by changes in concentration of metabolites, *i.e.* AMP, NAD+, fatty acids or phosphorylated sugars. The fourth level is the genome, in which transcription of proteins feeds back on biochemically active enzymes, signaling proteins and transcription factors.

In the next paragraphs, we will first discuss the known primary biochemical and structural linkages between OXPHOS and mFAO, the reported clinical cases and related *in vitro* studies on the presentation of concurrent OXPHOS and mFAO dysfunction. Next, we will discuss the emerging literature on 'secondary cellular responses', and their role in mitochondrial metabolism. Moreover, we will suggest possible interventions in the pathological signaling as new treatment options.

2. Primary biochemical linkages between OXPHOS and mFAO

Biochemically, mFAO and OXPHOS are coupled by the TCA cycle, complex I of the ETC and electron transfer flavoprotein (ETF) (Fig. 2). During each cycle of mFAO, one molecule of acetyl-CoA, one NADH, and one $FADH_2$ are formed. Acetyl-CoA enters the TCA cycle, generating more NADH and $FADH_2$, or is alternatively used for the production of ketone bodies (KB) or lipogenesis. NADH donates its electrons directly to complex I of the ETC. $FADH_2$ is oxidized by transfer of electrons to ETF. Reduced ETF subsequently transfers its electrons to ETF-ubiquinone oxidoreductase (ETF-QO), which donates the electrons to the ETC *via* ubiquinone (UQ) [9] (Fig. 2).

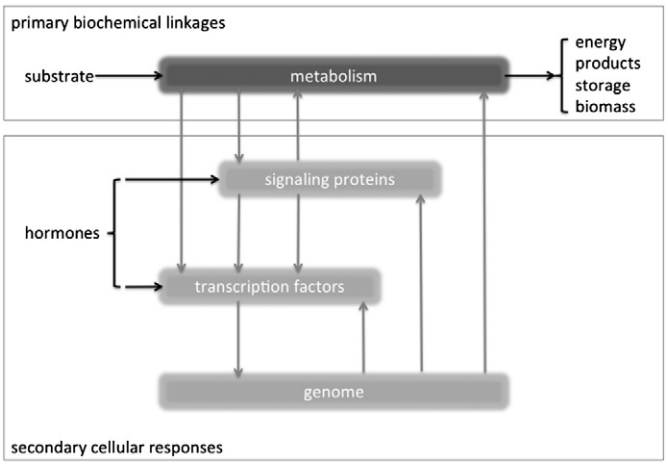

Fig. 1. General scheme of metabolic regulation. Legends: PTM: post-translational modification.

During electron transfer through the ETC, an electrochemical proton gradient over the inner mitochondrial membrane is generated, and subsequently dissipated while ATP is formed by complex V (Fig. 2) [2]. The formed ATP can be exported out of the mitochondria in exchange for ADP by the adenine nucleotide transporter (ANT).

The mitochondrial NADH/NAD^+ ratio is important for proper functioning of mitochondrial metabolism, as the NAD^+-linked 3-hydroxyacyl-CoA dehydrogenases (HADHs: respectively short- and long-chain 3-hydroxyacyl-CoA dehydrogenase, SCHAD and LCHAD) are extremely sensitive to changes in the NADH/NAD^+ ratio [10]. To a lesser extent, NAD^+ is required for functioning of the TCA cycle (isocitrate dehydrogenase-3, α-ketoglutarate dehydrogenase complex, and malate dehydrogenase), and pyruvate dehydrogenase complex (PDHc) [3,11–13]. When an altered mitochondrial NADH/NAD^+ ratio results in an altered cytosolic NADH/NAD^+ ratio, the lactate/pyruvate ratio will change [2,11,14,15]. Furthermore, the $FADH_2$/FAD ratio is important for proper functioning of the group of ACADs in mFAO and complex II in the TCA cycle and ETC [16].

Proteins involved in OXPHOS and mFAO are not only linked kinetically *via* metabolite concentrations, but they also associate into physical complexes. In humans, attachment of VLCAD to the inner mitochondrial membrane enables a direct interaction between mFAO and OXPHOS [17,

Table 1
Clinical characteristics of OXPHOS and mFAO defects.
Adapted from fig. 99-2 in [2], and [3].

OXPHOS defects	mFAO defects
Metabolic profile	
Lactic acidemia, worsening upon glucose administration	(Hypo)ketotic hypoglycemia Abnormal acylcarnitine profile Secondary free carnitine deficiency Organic aciduria
Muscle	
Muscle weakness, atrophy, hypotonia, myoglobinuria, hypertrophic cardiomyopathy	Musculoskeletal or cardiac muscle weakness (VLCAD, LCHAD, MTP, SCAD)
Gastro-intestinal	
Pancreatic dysfunction, diabetes mellitus; renal failure and tubulopathy; diarrhea, villous atrophy	Hepatomegaly, fatty liver
Hematology	
Anemia, neutropenia, trombopenia, myelodysplasia	HELLP syndrome in mothers carrying a fetus with LCHAD deficiency
Nervous system	
Hypotonia, cerebellar ataxia, leukodystrophy, hereditary spastic paraplegia, peripheral neuropathy, sensorineural deafness, optic/retinal atrophy, ptosis	
Hormonal	
Hypothyroidism, hypoparathyroidism, hypothalamic hypocorticism, GH deficiency	