Review Article

Hepatic response to aluminum toxicity: Dyslipidemia and liver diseases

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ABSTRACT

Aluminum (Al) is a metal toxin that has been implicated in the etiology of a number of diseases including Alzheimer's, Parkinson's, dialysis encephalopathy, and osteomalacia. Al has been shown to exert its effects by disrupting lipid membrane fluidity, perturbing iron (Fe), magnesium, and calcium homeostasis, and causing oxidative stress. However, the exact molecular targets of aluminum's toxicity have remained elusive. In the present review, we describe how the use of a systems biology approach in cultured hepatoblastoma cells (HepG2) allowed the identification of the molecular targets of Al toxicity. Mitochondrial metabolism is the main site of the toxicological action of Al. Fe-dependent and redox sensitive enzymes in the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS) are dramatically decreased by Al exposure. In an effort to compensate for diminished mitochondrial function, Al-treated cells stabilize hypoxia inducible factor-1α (HIF-1α) to increase ATP production by glycolysis. Additionally, Al toxicity leads to an increase in intracellular lipid accumulation due to enhanced lipogenesis and a decrease in the β-oxidation of fatty acids. Central to these effects is the alteration of α-ketoglutarate (KG) homeostasis. In Al-exposed cells, KG is preferentially used to quench ROS leading to succinate accumulation and HIF-1α stabilization. Moreover, the channeling of KG to combat oxidative stress leads to a reduction of L-carnitine biosynthesis and a concomitant decrease in fatty acid oxidation. The fluidity and interaction of these metabolic modules and the implications of these findings in liver-related disorders are discussed herein.

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Contents

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Introduction

Environmental pollutants, such as heavy metals and organic molecules, are known to participate in the pathogenesis of various diseases in humans. While, heavy metals like mercury (Hg), lead (Pb), and cadmium (Cd) have been shown to induce neurodegeneration, carcinogenesis, and anemia [1–3], organic pollutants, such as phthalates, have also been implicated in numerous disorders including type 2 diabetes and obesity [4,5]. Aluminum (Al) toxicity has also garnered considerable interest due to its bioavailability and adverse effects on human health and persistence in the environment. Indeed, Al toxicity is associated with various pathological conditions including Alzheimer's, Parkinson's, osteomalacia, anemia, and obesity [6–12]. The toxicity of Al has been attributed to its ubiquity in the environment and the frequency of exposure. Dietary sources of Al, such as foods, cookware, and drinking water, are important contributors to Al exposure and uptake [8] (Fig. 1). Owing to aluminum's hard acid properties, valency, and pro-oxidant capabilities, this trivalent metal readily perturbs Fe homeostasis, disrupts biological membranes, enhances ROS formation, and damages DNA [6,8,9,13,14]. Exposure of neurons and astrocytes to Al is known to activate apoptotic cascades, provoke cell cycle arrest, and interfere with cell signaling pathways [14–18] (Fig. 1). Al uptake in the human body can occur via a number of routes. However, dietary absorption is a common route for Al accumulation in the body. Following the dietary exposure to Al, it is subsequently distributed throughout the human body. Various tissues have been shown to accumulate Al however, the highest levels of Al are found in the bone and liver and to a certain extent the brain [19–21] (Fig. 1).

Hepatocytes are the functional units of the liver and rely on a series of complex metabolic networks in order to maintain energy homeostasis in the human body. Various environmental toxins have been shown to perturb liver physiology. However, the influences of pollutants, such as Al, on the molecular networks, which govern the physiological functions of hepatocytes, remain poorly resolved. The response of cellular systems to stimuli is complex with numerous functionally diverse networks interacting to manifest an appropriate response. Investigating individual pathways has successfully uncovered the various components involved in the adjustment of cell behavior to different conditions. Systems biology has been employed to decipher the transport and distribution of systemic Al [22,23]. However, to our knowledge this experimental paradigm has never been employed to investigate the effects of Al on cell physiology and function. In this review, we discuss how we used systems biology to identify the impact of Al toxicity on the metabolic networks in HepG2 cells. Al toxicity induced mitochondrial dysfunction in HepG2 cells leading to enhanced anaerobiosis and lipid accumulation. The role of α-ketoglutarate (KG) in mitigating Al-induced abnormalities is also explained.

Mitochondrial dysfunction: Al toxicity perturbs hepatic energy metabolism

Al disrupts the tricarboxylic acid (TCA) cycle and aerobic respiration

Mitochondrial metabolism and aerobic respiration are central to the physiological function of hepatocytes. The mitochondria in
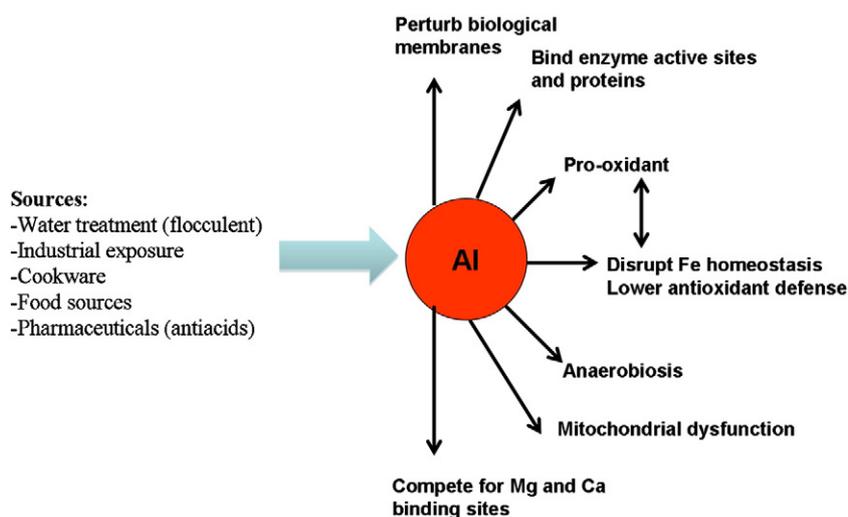

Fig. 1 – Exposure to Al and its toxicological impacts. Dietary uptake represents the major route of Al uptake. Due to the chemical properties of Al, this trivalent metal disrupts multiple cellular processes. The ability of Al to exert these negative effects on cells has been linked to a number of pathologies.

hepatocytes help adjust the levels of circulating fuels and metabolites by participating in the maintenance of carbohydrate and lipid homeostasis, biosynthetic reactions, and amino acid metabolism. Exposure of HepG2 cells to Al leads to the depletion of bioavailable Fe in the mitochondria and oxidative stress, hallmarks of Al toxicity [24,25]. HepG2 cells exposed to Al also accumulate succinate, a harbinger of anaerobic respiration [26]. Under the influence of Al, the accumulation of TCA cycle intermediates is a common occurrence. Al disrupts metabolic processes in hepatocytes by either disrupting Fe homeostasis and/or inducing oxidative stress. The relationship between perturbed Fe homeostasis and metabolic dysfunction in mitochondria is relatively well characterized [27]. Oxidative stress and metal toxicity are known to alter cellular nucleotide and metabolite levels. The exposure of cardiomyocytes to H_2O_2 causes a drastic fall in cellular ATP levels [28]. Furthermore, oxidative stress is also known to alter NAD/NADH and NADP/NADPH ratios [29–31].

Aconitase (ACN), a Fe–S cluster-containing enzyme, which isomerizes citrate to isocitrate, is sharply reduced in activity and expression in Al-treated hepatocytes [26]. Perturbation of the Fe–S cluster in this enzyme is a common phenomenon observed during metal toxicity and oxidative stress. Indeed, toxic metals including Al, zinc (Zn), and nickel (Ni) are known to disrupt ACN by perturbing the Fe–S cluster [32–35]. Oxidative stress and reduced ACN activity have been linked to several neurological disorders including Alzheimer's disease and obesity [35,36]. Fumarase (FUM) and succinate dehydrogenase (SDH), two other Fe–S cluster dependent enzymes, also displayed decreases in activity and expression in Al-exposed hepatocytes [26]. The loss of a functional SDH and the subsequent accumulation of succinate have been demonstrated in several cancer phenotypes, a situation which has been shown to enhance anaerobic respiration [37,38]. Cytochrome C Oxidase (Cyt C Ox), a respiratory complex which shuttles electrons to the terminal acceptor O_2, displayed a sharp decrease in activity and expression in Al-exposed HepG2 cells [26]. In addition, Cyt C Ox activity and expression have been shown to be diminished in several liver-related pathologies such as obesity and fatty liver disease [39,40]. Hence, Al interferes with several Fe-dependent enzymes within the TCA cycle and electron transport chain (ETC) resulting in the diminished production of ATP by the mitochondria, an event that has major implications in hepatic diseases (Fig. 2).

TCA cycle flux is also perturbed by the inhibition of NAD-dependent isocitrate dehydrogenase (NAD–ICDH) and α-ketoglutarate dehydrogenase (KGDH) by this toxic metal [25,41]. Previous studies by several other groups have shown that KGDH activity is severely compromised in the presence of ROS and metal toxins [42,43]. KGDH plays a key role in TCA cycle metabolism since it is involved in a number of catabolic and anabolic reactions. Additionally, KGDH has also been suggested to serve as an ROS gauge in the mitochondria due to the sensitivity of its lipoic acid moiety to pro-oxidant molecules [43]. Studies have shown that the activity of this enzyme can be perturbed by concentrations of H_2O_2 as low as 40 μM [43]. Indeed, the thiol groups on the lipoic acid residue can be readily oxidized by ROS rendering the enzyme inactive [47,49]. However, the limitation of KGDH activity has the added benefit of ensuring the accumulation of α-ketoglutarate (KG), a powerful anti-oxidant [41,44,45]. Additionally, KGDH has also been shown to generate ROS when NADH levels are high [46]. Thus, the modulation of KGDH by ROS allows the TCA cycle to

participate in the synthesis and degradation of ROS. When ROS is high in concentration, KGDH activity is diminished to reduce NADH formation, a pro-oxidant, and prompt the accumulation of KG, an anti-oxidant at the expense of energy production and mitochondrial dysfunction (Fig. 2).

Anaerobic metabolism: Al promotes anaerobic respiration

Mammalian cells have developed an oxygen sensing system which enhances glucose uptake and anaerobic metabolism in order to maintain cellular ATP levels during hypoxia. Under normoxic conditions, prolyl hydroxylase (PHD) hydroxylates the hypoxia inducible factor-1α (HIF-1α) on key proline residues targeting this gene regulatory protein for proteosomal degradation [47,48]. However, when oxygen becomes limiting, PHD activity is diminished leading to the stabilization of HIF-1α, a key subunit for the transcription factor HIF-1. Stabilization of HIF-1α promotes the expression of genes involved in glucose uptake and anaerobic respiration [49]. Numerous factors have been shown to stabilize HIF-1α; however, mitochondrial-derived ROS and succinate are key modulators of this hypoxic signal. ROS and succinate interfere with PHD activity stabilizing HIF-1α, a scenario which suggests that the efficiency of mitochondrial metabolism can be relayed to the nucleus through the PHD-HIF signaling module [37,50]. The treatment of HepG2 cells with Al abolishes the expression of PHD2, the prolyl hydroxylase isoform primarily responsible for degrading HIF-1α [25]. The complete loss of PHD2 expression was mirrored by the stabilization and subsequent nuclear accumulation of HIF-1α (Fig. 3) [25]. Numerous toxic metals, such as cobalt, nickel, copper, and silicon (possibly through an interaction with Al) have been shown to stabilize HIF-1α by interfering with PHD [51–54].

In the case of Al, this toxic trivalent metal may perturb PHD2 levels by enhancing succinate and ROS production and limiting the availability of KG, a key prosthetic group required for PHD activity [25]. Al-treated HepG2 cells readily accumulated succinate in the intracellular environment, a by-product of the KG-mediated sequestration of ROS. Furthermore, the treatment of Al-treated HepG2 cells with KG restored PHD2 protein levels, diminished HIF-1α, and quenched cellular ROS levels [25]. The proper functioning of PHD2 relies on KG, a cofactor which may become limiting during Al toxicity. KG supplementation has been shown to trigger HIF-1α hydroxylation and degradation [38]. Thus, mitochondrial dysfunction evoked by Al results in the promotion of anaerobiosis. The increase in nuclear HIF-1α in the Al-treated hepatocytes is accompanied by enhanced uptake of glucose and the generation of ATP by glycolysis (Fig. 3) [24]. The enhanced uptake of glucose was attributed to the increased activity and expression of glucose transporter-1 (GLUT-1) and hexokinase (HK), two targets of HIF-1 [24]. Intriguingly, previous studies have established that Al actually impedes HK activity. Exley et al. observed that Al inhibits HK activity in vitro while Xu et al. demonstrated that acute exposure to $AlCl_3$ directly inhibited HK and phosphofructokinase in liver cells [21,55]. In our studies, HepG2 cells were exposed to an Al-citrate complex for 24 h. Lactate dehydrogenase (LDH), pyruvate kinase (PK), and glyceraldehyde-3-phosphate dehydrogenase (GAPD), key glycolytic enzymes required to produce ATP and maintain glycolytic flux, also displayed increases in activity and expression in Al-treated HepG2 cells [24]. Hence, a metabolic shift towards increased glycolysis following Al exposure is required to meet the ATP demands of the cell since Al disables aerobic
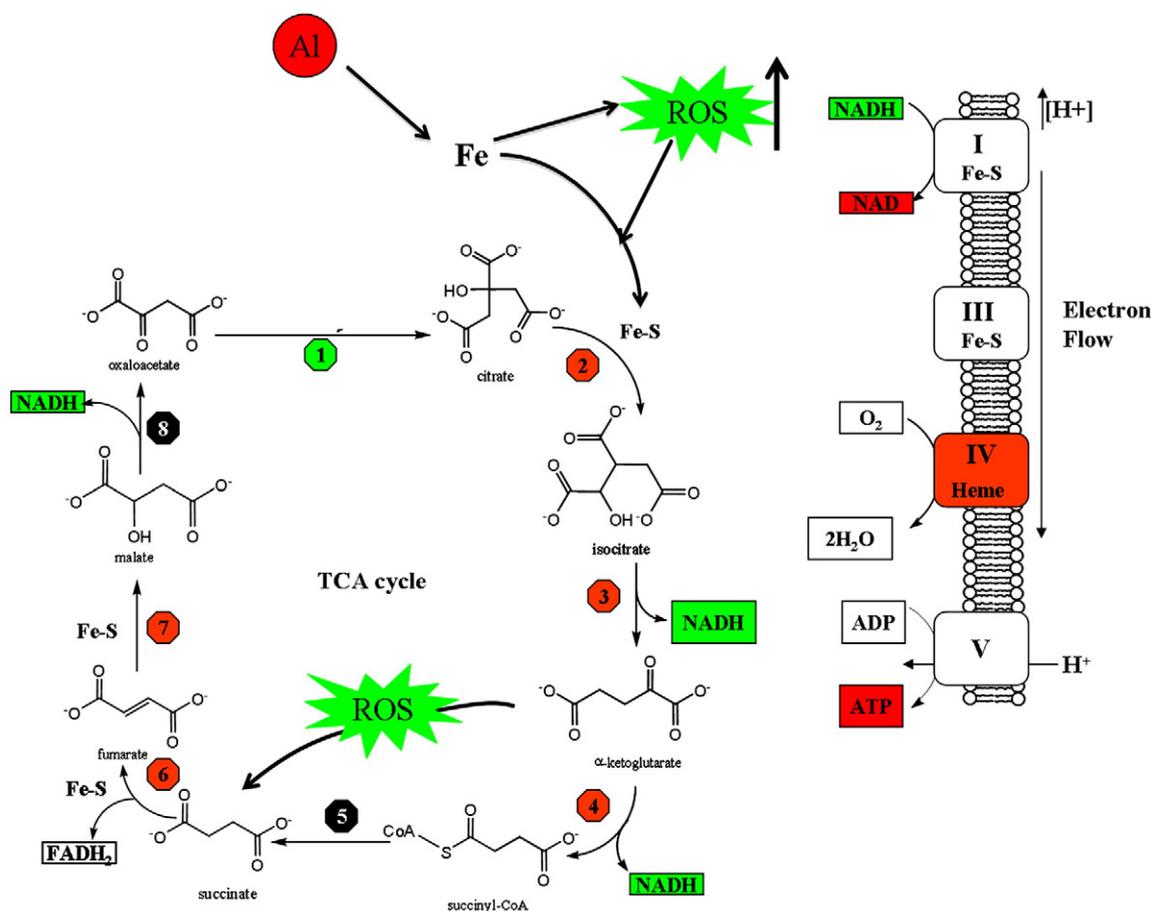
Fig. 2 – Al promotes oxidative stress. Al toxicity disrupts aerobic metabolism in HepG2 mitochondria by displacing bioavailable Fe and inducing oxidative stress. The resulting disruption of TCA cycle flux and oxidative phosphorylation impedes coupled respiration and ATP production. NADH accumulation is due to the disruption of the respiratory chain. Fe-dependent enzymes such as aconitase (2), succinate dehydrogenase (6), fumarase (7), and complex IV were decreased by Al exposure. Enzymes dependent on Fe cofactors (either Fe–S or heme) are indicated in the figure. Likewise, enzymes sensitive to inhibition by oxidative stress including NAD-dependent isocitrate dehydrogenase and α-ketoglutarate dehydrogenase were also diminished by Al toxicity. Inhibition α-ketoglutarate dehydrogenase leads to the accumulation of α-ketoglutarate, which quenches ROS generating succinate as a non-enzymatic by-product. Decreases in enzyme activity or metabolite levels are indicated in red. Increases in enzyme activity or metabolite levels are indicated in green. 1: citrate synthase, 3:NAD-dependent isocitrate dehydrogenase, 4:α-ketoglutarate dehydrogenase, 5: succinyl-CoA synthetase, 8:malate dehydrogenase.

respiration. To this end, Al exposure has a stimulatory effect on glycolysis which is consistent with the previously reported biphasic effect of Al on cells [56]. However, these conditions are conducive to fat accumulation, obesity, and other liver disorders. Indeed, Al-treated hepatocytes show abnormal depots of fats (Fig. 3).

Antioxidant defense and Al toxicity

NADPH is central to any anti-oxidative defense strategy and it fuels most, if not all the enzymes involved in combating oxidative stress [31,57]. In fact, these cellular systems would not be able to function without NADPH. Furthermore, several α-ketoacids, such as KG, have shown ROS-scavenging properties with the spontaneous decarboxylation to succinate. The metabolic shift within the TCA cycle evoked by Al enhances the production of NADPH and the accumulation of KG in hepatocytes [41]. Indeed, the mitochondria from the Al-exposed

hepatocytes display a dramatic increase in the activity and expression of mitochondrial NADP-dependent isocitrate dehydrogenase (mNADP–ICDH) [41]. The increase in mNADP–ICDH and the decrease in KGDH would allow the production of NADPH and KG in an effort to limit the pro-oxidant effects of Al. The activity and expression of cytosolic NADP-dependent isocitrate dehydrogenase (cNADP–ICDH) and glucose-6-phosphate dehydrogenase (G6PDH) are also increased in Al-exposed hepatocytes [7]. Although the upregulation of these enzymes is crucial to maintain the proper functioning of antioxidant defense systems, it is important that the supply of NADP is adequate if these enzymes are to function properly. NAD kinase (NADK), an enzyme which produces NADP by phosphorylating NAD is also enhanced in these Al-challenged hepatocytes [58]. Hence, an intricate NADPH-generating machinery is activated under the influence of Al toxicity, a situation that favors the biosynthesis of fatty acids and the subsequent accumulation of lipids.
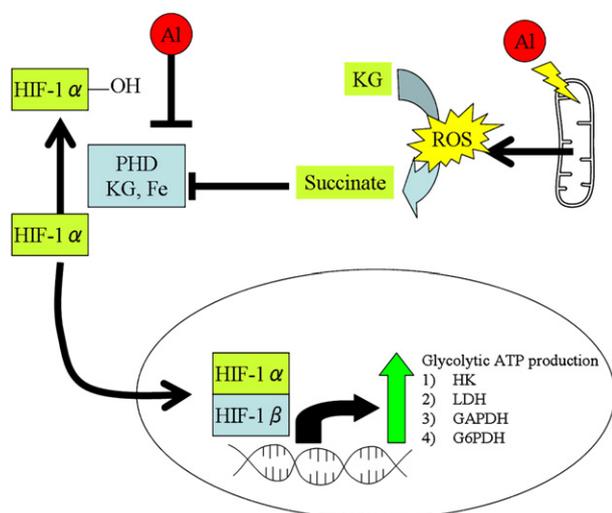
Fig. 3 – Al toxicity and anaerobiosis. The Al-mediated disruption of mitochondrial metabolism and the subsequent dedication of α-ketoglutarate to ROS sequestration stabilizes hypoxia inducible factor-1α (HIF-1α) and enhances the glycolytic production of ATP. HIF-1α, a transcriptional protein required for the induction of glycolytic genes, is stabilized by the inhibition and decreased expression of prolyl hydroxylase (PHD). The decrease in PHD is due to the decrease in α-ketoglutarate availability, a key cofactor required for PHD activity. PHD protein levels are also most likely disrupted by Al, which can displace the Fe in the active site.
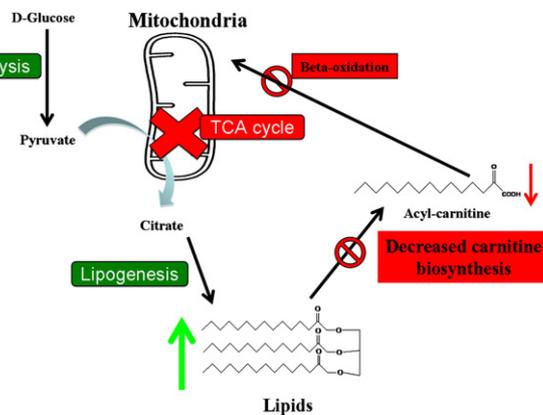

Fig. 4 – Al toxicity and lipogenesis. The Al-induced perturbation of mitochondrial function leads to the subsequent diversion of metabolized carbohydrates towards lipid biosynthesis and triglyceride accumulation. The accretion of lipids is compounded by the Al-mediated depletion of cellular L-carnitine. Perturbation of the TCA cycle prompts the accumulation of citrate which is then exported into the cytosol and acted upon by lipogenic enzymes to generate fatty acyl moieties. Following the esterification of fatty acyl groups with glycerol, the triglyceride is either stored in the cytosol or exported into the surrounding extracellular environment as VLDL. Fatty acid oxidation is also diminished which is attributed to the Al-mediated decrease in L-carnitine. The decrease in L-carnitine in Al-treated cells is due to the diversion of KG towards ROS scavenging. Red or down arrows represent a decrease while green and up arrows represent an increase.

Lipid accumulation: Al toxicity evokes fat accretion in hepatocytes

The conversion of glucose to lipids

Conditions that hinder mitochondrial metabolism have been known to enhance lipid synthesis, accumulation, and secretion. Mitochondrial DNA damage, loss of ACN function, drug toxicity, and the disruption of the respiratory complexes have been shown to contribute to hepatic dysfunction and the pathogenesis of obesity [36,59–61]. Indeed, the deregulation of mitochondrial function is quite common in persons suffering from obesity and hepatic steatosis, conditions characterized by the excessive accumulation of hepatic fat [62]. In the Al-treated HepG2 cells, the inability of the mitochondria to function properly would compel the cell to divert incoming carbohydrates to lipid biosynthesis. The presence of high amounts of very-low density lipoprotein (VLDL) and lipids in the media provides compelling evidence that Al enhances lipid biosynthesis and secretion (Fig. 4). High levels of ApoB-100 and triglycerides are observed in the Al-challenged hepatocytes [7]. Zinc (Zn) and H_2O_2, two known inhibitors of mitochondrial respiration, evoke fat accumulation [7]. HepG2 cells exposed to Al concentrations of 100 μM readily accumulate lipid droplets [63]. Moreover, exposure of cells to H_2O_2 also enhances lipid accretion illustrating the commonality between Al and ROS toxicity [63].

Lipogenesis involves the carboxylation and polymerization of acetyl-CoA with the participation of NADPH. Acetyl-CoA is produced in the cytosol following the cleavage of citrate by ATP-citrate lyase (ATP-CL). In the Al-treated hepatocytes exposed to 30 mM D-glucose, while the activity and expression of ATP-CL and citrate synthase (CS) are higher, ACN undergoes a marked reduction of activity [7]. This biochemical manipulation creates an ideal situation for lipogenesis. The Al-mediated disruption of aerobic respiration would induce the immediate shuttling of incoming catabolic substrates towards lipid production. The enhanced production of lipids in the Al-treated cells exposed to 30 mM D-glucose is also characterized by the increased activity and expression of acetyl-CoA carboxylase (ACC) and glycerol-3-phosphate dehydrogenase (G3PDH) [7]. While ACC catalyzes the rate determining step of lipid synthesis, G3PDH provides the necessary glycerol backbone for triglyceride production [7]. The supply of NADPH by cNADP–ICDH, G6PDH, and NADK would also favor lipogenesis [7,58]. The Al-mediated upregulation of these NADPH-producing enzymes provides the necessary reducing equivalents for both antioxidant defense and lipogenesis. Thus, Al toxicity favors a metabolic shift aimed at preserving cellular ATP levels and the maintenance of a reductive environment with the concomitant preservation of energy substrates in the form of lipids (Fig. 4). Naturally, a cell would not want to haphazardly squander energy substrates. Indeed, the cell may be attempting to retain energy substrates until mitochondrial metabolism can be restored. However, if the mitochondrial dysfunction is not rectified, the accumulation of lipids over an extended period of time will have pathological consequences. Indeed the accumulation of lipids is more pronounced when the cells are fed D-fructose, a hexose known to be a major contributor to obesity [7].

L-carnitine metabolism: disruption by Al toxicity

Intracellular lipid content is maintained by both *de novo* lipogenesis and fatty acid β-oxidation. While the induction of lipogenesis commits acetyl-CoA to the biosynthesis of acyl moieties which are then esterified with glycerol to generate triglyceride, fatty acid oxidation in the mitochondria systematically breaks down acyl chains into acetyl-CoA for combustion and aerobic ATP production. Both pathways are thought not to operate simultaneously since this would lead to the futile cycling of acetyl-CoA groups and the loss of energy as heat. The oxidation of fatty acids requires L-carnitine, an essential metabolite generated by the amino acids lysine and methionine that shuttles fatty acyl moieties into the mitochondria matrix for degradation. Carnitine biosynthesis is reliant on four different enzymes called trimethyllysine dioxygenase (TMLD), hydrotrimethyllysine aldolase (HTMLA), trimethylaminobutyralde-hyde dehydrogenase (TMABD), and butyrobetaine dioxygenase (BBD) [64]. The activities of the two dioxygenase enzymes involved in the hydroxylation of trimethyllysine and butyrobetaine require KG, ascorbate, and Fe [64]. The liver is the main site for L-carnitine biosynthesis in the body. L-carnitine biosynthesis is dependent on KG and Fe as cofactors, two moieties known to be disrupted by Al toxicity and oxidative stress. The substantial accumulation of intracellular lipids and increased levels of VLDL in the Al-treated HepG2 cells are characterized by a marked reduction in β-oxidation. Al-treated HepG2 cells do not only enhance the generation of lipid droplets but the cells are also unable to oxidize palmitic acid [63]. Curiously, the oxidation of palmitic acid in Al-exposed cells is restored by a 24 h incubation in 5 mM KG [63]. This is attributed to a decrease in the cellular levels of L-carnitine, a phenomenon reversed by KG. This decrease in cellular L-carnitine is due to the Al and ROS-mediated decreased expression of BBD [63] (Fig. 4). Fluctuations in L-carnitine levels is often associated with oxidative stress [65]. Both ROS and the metal toxin, namely Al, severely diminish L-carnitine levels that contribute to lipid accumulation in hepatocytes. Moreover, the recovery of L-carnitine with KG illustrates that Al or ROS toxicity diminishes the availability of this α-ketoacid for biosynthetic processes since it is being used for ROS detoxification. The inability to maintain L-carnitine levels is associated with aberrant lipid accumulation in skeletal muscle, heart, liver and kidney [66]. The metabolic networks favoring the production of NADPH and citrate coupled with the decrease in the synthesis of L-carnitine observed in Al-exposed hepatocytes are phenomena associated with the metabolic syndrome and obesity.

Perspectives: Al and hepatic diseases

Numerous liver disorders have been attributed to mitochondrial dysfunction and the inability of hepatocytes to perform their physiological functions [67,68]. Perturbed aerobic metabolism, disruptions of mitochondrial structure, and reduced mitochondrial number have been shown to potentiate the onset of obesity, type II diabetes, and hepatic steatosis [62,69,70]. This is due to the central nature of the mitochondria to the proper functioning of the liver. Indeed, functional mitochondria are essential in energy produc-tion, lipid homeostasis, amino acid metabolism, transcriptional regulation, and calcium storage. The current systems biology approach in evaluating the global metabolic perturbation triggered

by Al toxicity has helped identify the key metabolic participants in dyslipidemia in hepatocytes (Fig. 5). Due to its hard acid and pro-oxidant properties and ability to disrupt Fe binding sites, the primary target for Al toxicity is the mitochondria. Indeed, mitochondrial function is inherently dependent on Fe and a reductive environment, which can easily be disrupted by Al. The Al-mediated increase in oxidative stress and disruption of Fe-dependent enzymes perturbs TCA cycle function and disables the aerobic production of ATP, conditions synonymous with metabolic disorders such as obesity. The Al-mediated disruption of mitochondrial function in the HepG2 cells induced a sequela of events including perturbed lipid metabolism and accumulation and increased anaerobic respiration, conditions often observed during obesity and hepatic steatosis. Indeed, obesity, type II diabetes, and hepatic steatosis are charac-terized by a dramatic decrease in aerobic respiration, the perturba-tion of mitochondrial lipid oxidation, fat accretion, and enhanced VLDL secretion. However, the mechanistic details underlying how these perturbations can lead to these liver-related disorders remain poorly understood. The present review illustrates how the coordi-nated analysis of the metabolic pathways inherent to hepatocytic function can be severely affected by the presence of Al. Furthermore, the simultaneous investigation of these molecular networks has provided novel insights into the role of environmental toxins in liver-related pathologies such as obesity. Metabolic pathways are often presented and studied as discrete entities operating indepen-dently from the rest of the cell. This reductionist approach to research has provided valued information on numerous cellular processes however does not take into account the true complexity of cellular metabolism. Metabolic pathways actually form a complex network functioning in a cooperative fashion to meet the needs of the cell. The Al-mediated alterations in the levels of KG, succinate, NADPH, and L-carnitine render β-oxidation ineffective and promote lipid accumulation, conditions that are the hallmarks of numerous liver diseases.
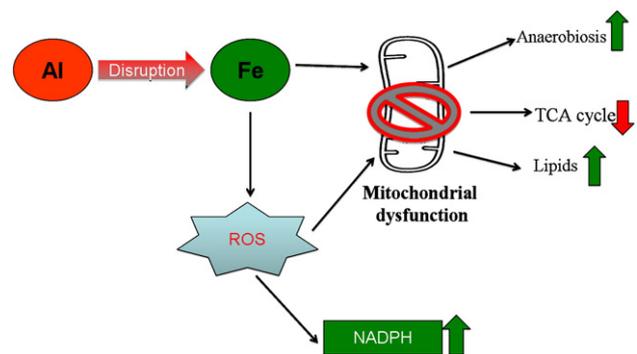
Acknowledgments

Fig. 5 – The metabolic link between Al toxicity and hepatic fat accumulation. Al-mediated decrease in bioavailable Fe leads to increased ROS levels, mitochondrial dysfunction and increased lipogenesis.

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