25

The Molecular Connection Between Aluminum Toxicity, Anemia, Inflammation and Obesity: Therapeutic Cues

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1. Introduction

Anemia is reported to be the most common blood disorder. A variety of anemic conditions affecting various groups of people exist and each of the types of anemia has different underlying causes. Iron (Fe) deficiency, a potent instigator of anemia, is the most common mineral deficiency and its effects have been linked to slow physical development and impaired cognitive function along with behavioral and learning disturbances (De Giudice *et al*, 2009). Exacerbating the iron deficiency epidemic is obesity, a disease defined by an excess accumulation of body fat leading to adverse health effects. It has been demonstrated that there is an association between poor iron status and obesity. The relationship between the two conditions has been shown in children, adolescents and adults including post-menopausal women.

Obesity is now considered an independent factor contributing to iron deficiency (McClung *et al,* 2009). Gaining incredible importance as a global health issue, obesity rates are increasing worldwide. The World Health Organization estimated that in 2005, 1.6 billion adults were overweight (body mass index (BMI) =25), and over 400 million adults were obese (BMI =30). Notably, there is an increase in the incidence of childhood and adolescent obesity in industrialized countries where the number of affected population has more than doubled over the past few decades. A similar trend has been observed in developing countries such as Egypt, Brazil and Mexico. What used to be considered a "rich" country issue has now become a worldwide epidemic and the situation is continuously worsening (Hintze *et al*, 2010).

2. Hepcidin: Function and regulation

A significant proportion of the world's population is iron-deficient, obese, or both. This makes an understanding of the mechanisms underlying obesity-induced iron deficiency and anemia crucial. Recently, the discovery of the peptide hormone hepcidin, a regulator of organismal iron metabolism has shed light on the relationship between anemia and obesity. Hepcidin is a 25 amino acid long peptide secreted by the liver and adipose tissue that was initially studied for its modulation of iron-homeostasis during infection. Increases in

hepcidin levels cause the depletion of serum iron levels and prevent the efflux of iron through the cellular iron exporter ferroportin from hepatocytes, enterocytes and macrophages (**Figure 1**). Therefore, hepcidin is the iron/inflammation/oxygen sensor that can act as a signal for numerous physiological responses including i) the decreased dietary iron uptake during an overload situation, ii) the anemia seen during infection to prevent free iron from promoting pathogen proliferation and iii) an increase of iron uptake during hypoxia (Atkinson *et* al, 2011; Choi *et al,* 2007; Vecchi *et al*, 2009).

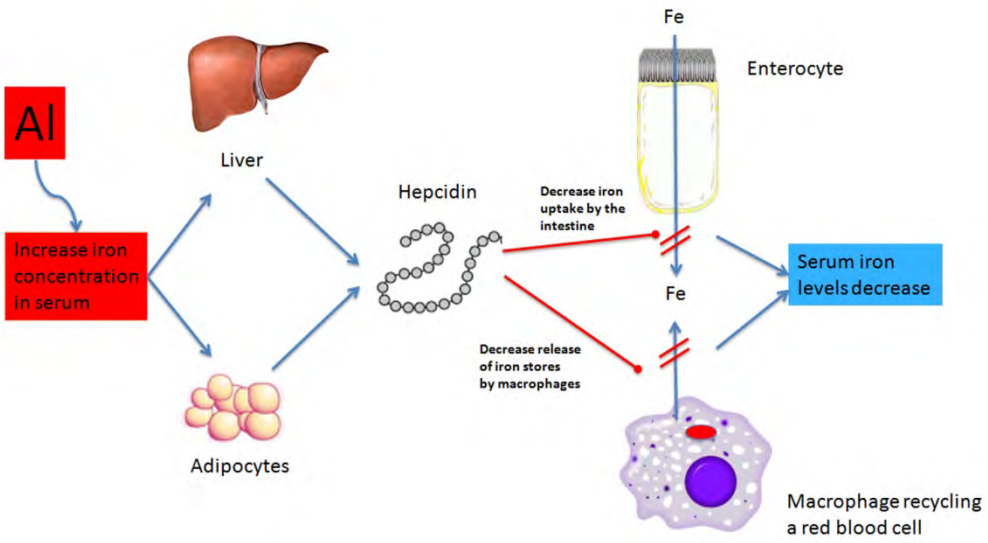

Fig. 1. Al toxicity leads to an increased Fe concentration in the serum of the body. High blood Fe levels cause the liver and adipose tissue to secrete hepcidin, a Fe homeostasis regulator, which signals the decrease in Fe absorption by enterocytes as well as a decrease in Fe release from cellular stores such as red blood cells.

Interestingly, chronic inflammation is one of the hallmarks of obesity (Ausk *et al*, 2008, Yanoff *et al,* 2007). Adipose tissue in obese mice has been shown to be considerably more hypoxic than adipose tissue from lean mice. As a result of the difference in O_2 tension there is an important shift in gene expression, not only for expected genes induced by hypoxia but also the increased levels of inflammatory cytokines including interleukin-6 (IL-6) (Lee *et al*, 2005). The latter appears to be the mediator of the induction of hepatic hepcidin secretion during inflammation. IL-6 and other hepcidin-inducing factors such as the adipokine leptin may explain the increased hepcidin levels in obese individuals compared to healthy patients (Barisani *et al,* 2008; Choi *et al,* 2007; Hintze *et al,* 2010).

3. The link between Fe-deficiency, anemia, inflammation and obesity

When studied separately, the development of anemia, inflammation and obesity have all been associated with aluminum (Al) toxicity. It is well established that Al disrupts iron homeostasis leading to the dysfunction of essential biochemical processes dependent on this redox-active

ion. Al negatively influences the absorption of iron via the intestine, hinders its transport in the serum, and displaces iron by binding to transferrin (**Figure 2**) (Turgut *et al,* 2007).

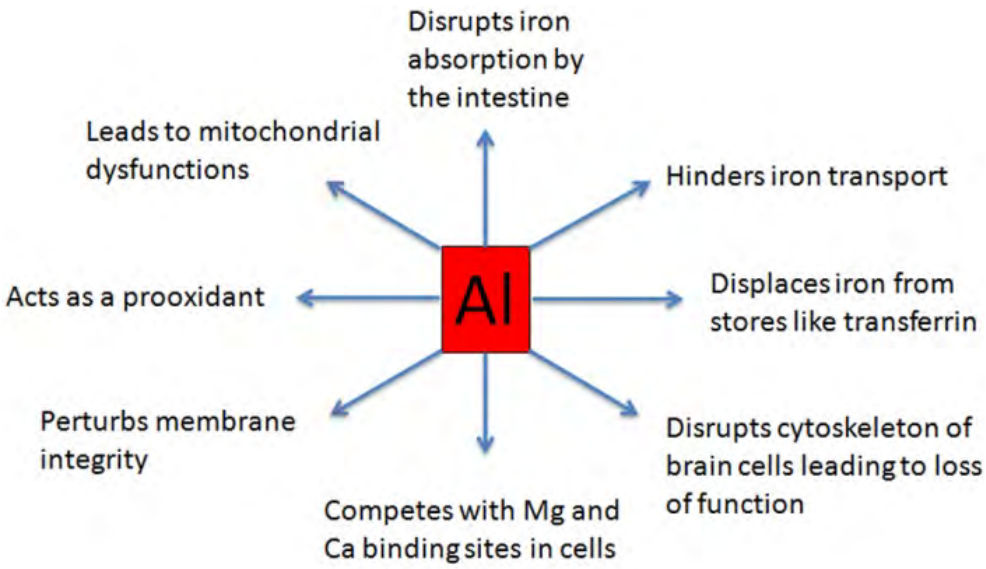

Fig. 2. Al has various toxic effects on cellular processes, notably on Fe homeostasis.

Al plays an important role in the immune response by being a trigger of the inflammatory cascade. For this reason, Al salts are administered with vaccines and act as adjuvants. Al stimulates an inflammatory response which promotes the effectiveness of the immune system to respond to the vaccine and acquire immunity. The effect of Al toxicity on fatty acid metabolism is just starting to emerge. The modulation of energy metabolism at the mitochondrial level caused by this metal leads to an accumulation of triglycerides and an increase in fat deposits. In addition, there is a marked increase in very low density lipoprotein (VLDL) secretion caused by Al toxicity. This phenomenon is directly related to the accumulation of fatty tissue observed during obesity. The information presented in this chapter delineates the molecular mechanism of Al toxicity and thus affords insights into the implication of this metal in dysfunctional Fe homeostasis, inflammation, obesity and anemia.

4. Aluminum and the environment

Aluminum is an environmentally abundant element that has a wide variety of uses and industrial applications. Industrialization and anthropogenic activities have led to a further increase in bioavailable Al. Exposure to this non-redox active metal occurs in daily life where the major sources of exposure for the average individual are diet and drinking water. Additional aluminum exposure can be brought upon by certain medications such as

antacids, while individuals can also be subjected to high Al levels due to occupational exposure from breathing in contaminated air. Although drinking water was first thought to be the main delivery method of Al to the body, it was shown that 95% of the exposure to the element occurs through diet whereas the soluble water form of Al only accounts for 1-2%. The average human Al intake ranges between a total of 4 to 9 mg/day. Although small, these values are easily influenced by factors such as the type of food consumed in one's diet, the country/place of residence, one's age and sex. Alternate sources contribute to the amount of Al an individual is subjected to (**Table 1**), with antacids representing a significant dose, as they are generally composed of aluminum hydroxide salts (Lopez *et al,* 2002; *et al,* 2006; Yokel *et al*, 2008b).

Source	Al exposure contribution
Antacids	5000000 µg/day
Environmental air inhalation	4-20 µg/day
Industrial air inhalation	25000 µg/day
Antiperspirants	70000 µg/day
Cigarettes	500-2000 µg/cigarette
Vaccines	1-8 µg/day
Allergy immunotherapy	7-40 µg/day

Table 1. Various sources of Al and their average Al exposure contribution (Yokel *et al*, 2008b).

The use of aluminum salts is not restricted to stomach acidity neutralizing agents, but are rather quite frequently used in the food industry. Sodium aluminum phosphates (SALPs) are generally recognized as safe (GRAS) FDA-approved food additives that contribute the most important source of Al to the diet. Basic SALP ($Na_8Al_2(OH_2)((PO)_4)_4$) is one of the many emulsifying salts added to processed cheese, cheese food and cheese spread. This salt is added to cheese since it reacts with proteins resulting in modifications that produce a smooth, uniform film around each fat droplet, preventing separation and bleeding of fat from cheese. Ultimately this allows for a soft texture, easy melting characteristics and desirable slicing properties. The FDA approves up to 3% concentrations of basic SALP. Unprocessed cheese has been shown to have an Al concentration of < 10 mg Al/kg. In contrast the aluminum levels in processed cheese can range from 320-1440 mg Al/kg. Cheese is not the only processed food containing higher levels of aluminum. Both fruit juices and soft drinks contain aluminum levels ranging from 49.3 to 1144.6 µg/l in fruit juices and from 44.6 to 1053.3 µg/l in soft drinks respectively (Lopez *et al,* 2002). The benefits of processed foods include a longer shelf life, change in taste and texture, minimal meal preparation and lower cost. The increased consumption of processed food has been shown to be related to the rise in obesity rates in industrialized nations for many reasons including the high simple sugar levels. Perhaps the high levels of Al found in processed food are amplifying the risk of obesity that is associated with food processing (**Figure 3**) (Yokel *et al,* 2008a; 2008b; 2006).

The food processing industry is responsible for an increase in bioavailable Al, however it is not working alone. Industrialization has led to a higher frequency of acid rain which in

turn lowers the pH in soil. Consequently, Al is leaching into groundwater thus causing the bioaccumulation of this toxic metal in various non-processed food such as vegetation and livestock. Once consumed, these sources increase the accumulation of aluminum in the body. Various fresh food sources contain relatively high levels of Al, as shown in **Table 2.**

Food Group	Mean Al concentration (mg/kg of fresh weight)	Food Group	Mean Al concentration (mg/kg of fresh weight)
Bread	6.6	Potatoes	0.9
Poultry	0.3	Canned vegetables	0.97
Fish	6.1	Fresh fruit	0.29
Oils & fats	1.1	Fruit products	0.82
Eggs	0.14	Milk	0.07
Green vegetables	3.1	Nuts	4.0

Table 2. Occurrence of Al in food according to the Food Standards Agency, United Kingdom.

Aluminum toxicity has been extensively studied for its implication in neurodegenerative diseases such as Alzheimer's disease (AD) and multiple sclerosis (MS). The brain is an important Al accumulating organ, yet Al can also concentrate more severely in other tissues of the human body (**Figure 4**) (Gomez *et al,* 2008; Rondeau *et al, 2008*). Nonetheless, aluminum salts such as aluminum hydroxides and aluminum phosphates are routinely used in medical practices as the only licensed adjuvants in vaccines. Aluminum adjuvants, referred to as "alum", are proven effective in stimulating an immune response towards the vaccine being administered. However, the mode of action remains unclear. It is generally accepted that the alum particles adhere to the surface of the antigen (Ag), forming an Ag deposit at the injection site. This process maximizes the interaction time between the Ag and the immune system's antigen presenting cells (APCs) which initiate a response cascade (**Figure 4**). Alum have also been shown to act on the immune system's compliment system of the immune machinery, along with causing the formation of granulomas containing antibody (Ab)-producing cells and other immune response mediators. Recent studies have illustrated the ability of alum to stimulate the release of pro-inflammatory cytokines such as IL-1β and IL-18 that have pleiotropic functions, including adjuvant capacity. Although several mechanisms are proposed (**Figure 5**), the exact way by which aluminum promotes inflammation has yet to be solved (Aimanianda *et al,* 2009; HogenEsch, 2002; Li *et al,* 2008). Nonetheless, Al is an active instigator of inflammation (Campbell *et al,* 2002). Regardless of the source of Al the reality remains that the element is bioavailable and at significant concentrations. For this reason, the toxic effects of Al are of interest to the human population and the study of these effects could help explain Al-associated dysfunctions such as neurodegenerative diseases, obesity and chronic inflammation. More importantly, the link between these disease states, aluminum toxicity and anemia is beginning to be understood.

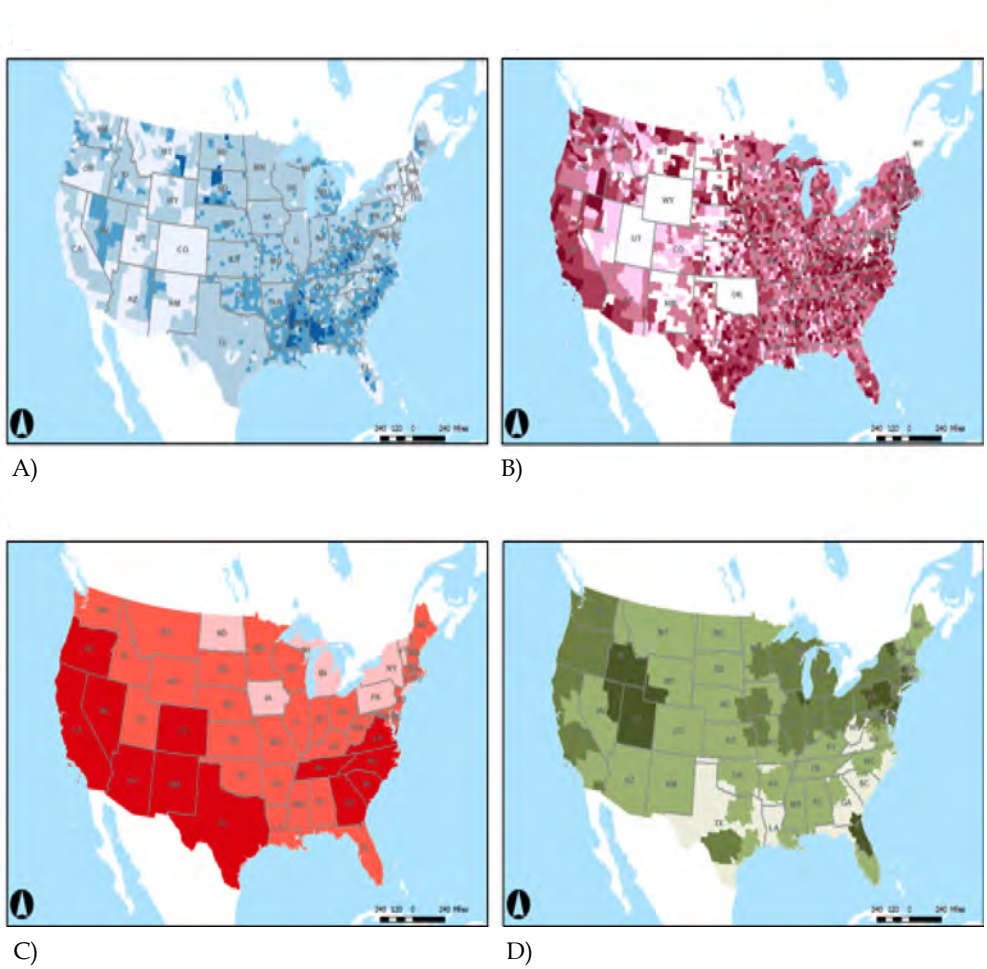

Fig. 3. These maps illustrate the link between processed food (commonly served in the fast food industry or found in prepared meals) and the obesity epidemic in the United-States. **A) Adult obesity rate in 2007**. Grey (12.5% - 25%). Light blue (25.1% - 30%). Blue (30.1% - 35%). Dark blue (35.1% - 43.5%); **B) Low-income preschool obesity rate in 2008.** Light pink (2.1% - 10%). Pink (10.1% - 14%). Red (14.1% - 18%). Dark red (18.1% - 39.7%); **C) Fast food expenditure per capita in 2007**. Light pink ($402.10 - $500.00). Pink ($500.01 - $700.00). Red ($700.01 - $1,043.86). **D) Prepared food (lbs) per capita in 2006**. Grey (229 - 280lbs). Light green (281 - 300lbs). Green (301 - 320lbs). Dark green (321-374lbs). (Centers for Disease Control and Prevention).

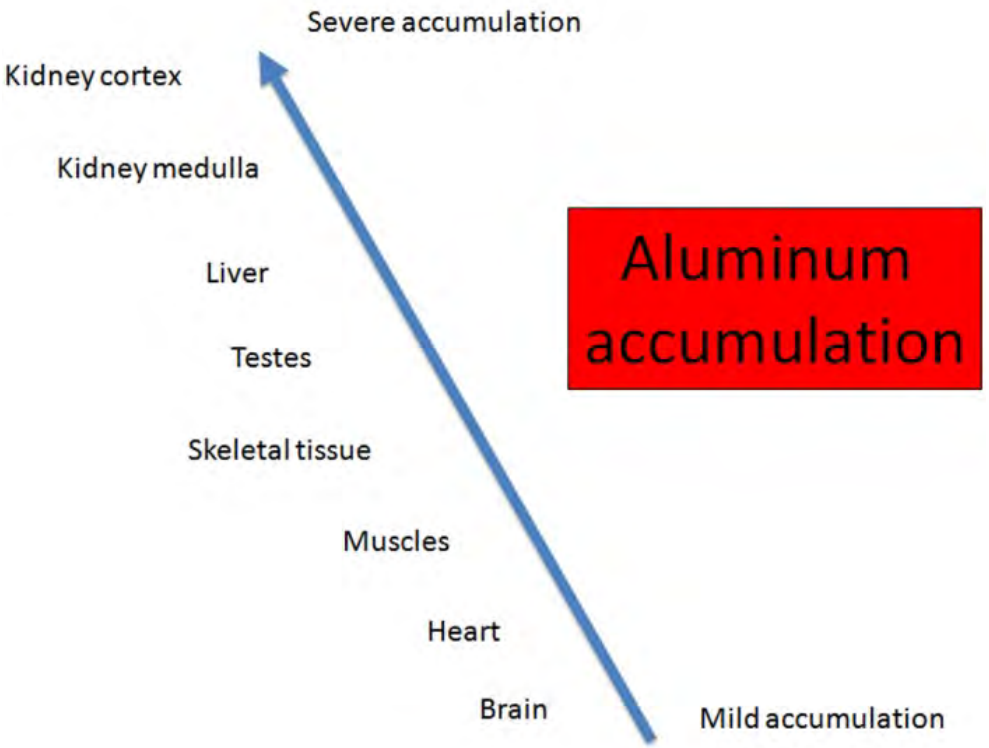

Fig. 4. The hierarchy of Al accumulation in the various tissues of the human body. Although the brain is an important location for Al accumulation, there are many other organs that accumulate much greater concentrations of Al (Ward *et al,* 2001).

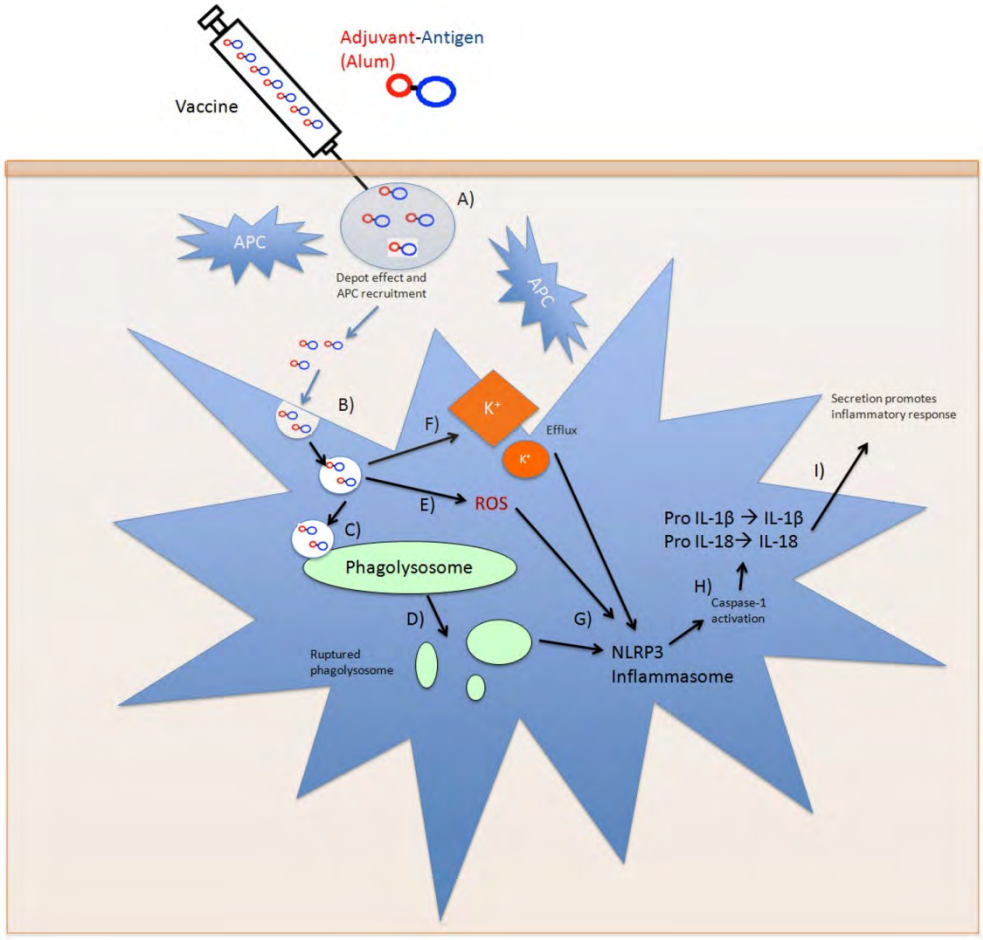

Fig. 5. Proposed mechanism of action for Al being used as an adjuvant A) As a vaccine is injected into the skin, the alum adjuvant bound to the Ag form a deposit at the injection site. This promotes APC recruitment and maximizes the interaction between the Ag and the immune system's cells. B) The innate immune system cells intake the Alum salts. C) The engulfed adjuvants interact with the phagolysosome of the APCs. D) Intereaction with the Alum salts leads to rupture of the phagolysosomes and the release of activators of NLRP3 inflammasome (not shown in image). E) Al within the cell promotes the formation of ROS which also activates the NLRP3 inflammasome. F) The Alum salts also lead to potassium (K^+) efflux, thus further activating the NLRP3 inflammasome. G) The NLRP3 inflammasome, an intracellular innate immune response system, senses the K+ efflux, the ROS formation and the lysosomal damage and activates caspase-1. H) Caspase-1 processes pro-IL-1β and pro-IL-18 into IL-1β and IL-18, respectively, resulting in the release of these mature cytokines (Modified from Aimanianda *et al,* 2009; HogenEsch, 2002; Li, 2008).

5. Aluminum and dyslipidemia: A metabolic perspective

Metal toxicity has been linked to cancers, neurological disorders, nephrological complications and pulmonary diseases. Environmental pollution is a growing concern in today's society causing the increased bioavailability of metals that pose a serious threat to living organisms. Toxic elements like mercury (Hg) and lead (Pb) have been extensively studied. While Hg has been shown to react with critical thiol moieties and impede normal immunological responses and mental cognition, Pb dislocates the essential zinc (Zn) found in enzymes responsible for heme production. Ultimately these toxic metals lead to various diseases (Mailloux *et al*, 2007b). The molecular aspects of Al toxicity have begun to emerge. It has been reported that a variety of ion channels are inactivated by micromolar quantities of Al, subsequently disrupting biological membranes. In addition, Al has an ionic radius that resembles that of magnesium (Mg), which leads to its interaction with naturally-occurring Mg-dependent enzymes. Al has also been shown to perturb cytoskeletal structure of astrocytoma cells causing a disruption of their shape and hence their biological function. Exposure to Al, however, is primarily characterized by the disruption of Fe homeostasis which in turn interferes with essential biochemical processes that are dependent on this metal (Lemire *et al,* 2009; Mailloux *et al,* 2011).

Fe is an important cofactor of many enzymes and a structural component of proteins. It is redox-active and therefore can act as an electron acceptor and donor, a critical attribute for its involvement in metabolic processes. Notably, Fe is required for protein components such as iron-sulfur clusters (Fe-S clusters) and hemes. When Al interacts with these constituents, it mimics the Fe atoms forcing the liberation of the transition metal and its subsequent intracellular accumulation. Free Fe poses a threat to cells as it leads to the formation of reactive oxygen species (ROS) through Fenton chemistry (figure 6). The Al-triggered oxidative environment enhances the toxic effect of the trivalent metal. Together, Al and its concomitant ROS greatly affect cellular metabolism. Metabolism is the foundation of any biological system and metabolic processes allow organisms to react and adapt to intracellular and extracellular fluctuations. It enables the maintenance of an environment suitable for the production and storage of energy and for cellular growth (Kim *et al,* 2007; Mailloux *et al,* 2011; Vergara *et al*, 2008).

The importance of Fe as a cofactor in metabolic processes is made evident in energy metabolism. The redox active metal is essential for the ATP-producing machinery in the mitochondria of eukaryotes. The central metabolic pathway known as the tricarboxylic acid cycle (TCA cycle), along with the electron transport chain (ETC) which is responsible for oxidative phosphorylation, are composed of enzymes that depend on Fe for proper functioning. For example, the Fe-containing enzyme aconitase (ACN) is considered the "gatekeeper" to the TCA cycle. ACN are Fe-S cluster proteins that catalyze the reversible isomerization of citrate to isocitrate. The enzymatically active form of the ACN Fe-S clusters are predominantly [4Fe-4S]. In mammalian systems, ACN with [3Fe-4S] clusters play an alternate role, acting as an oxygen sensor therefore aiding in energy homeostasis. This form of the enzyme serves as a regulatory protein that controls the stability and translation of messenger RNAs (mRNAs) encoding proteins involved in iron and energy homeostasis. The regulatory ACN is referred to as iron-responsive protein, which binds to iron-responsible elements localized in the RNA-stem loop (figure 7). This action leads to the modulation of gene expression (Middaugh *et al,* 2005).

It has been demonstrated that cells exposed to Al have severely impeded mitochondrial functions. Most importantly, these cells appear to have limited ATP production due to

diminished TCA cycle and oxidative phosphorylation activity. As the trivalent metal Al displaces Fe in key enzymes of the TCA cycle such as ACN, fumarase (FUM) along with the Fe found in enzymes of the ETC (notably succinate dehydrogenase (SDH) and cytochrome C oxidase (Cyt c ox)), an evident shift in metabolism occurs as these enzymes become inactive. Impairment of ACN by Al triggers a decrease in NADH production by the TCA cycle. NADH is a reducing equivalent essential for oxidative phosphorylation, thus as the levels of NADH diminish and Al displaces Fe from ETC enzymes, mitochondrial production of ATP is hindered (figure 8). This perturbation in oxidative phosphorylation is advantageous during Al exposure since the ETC is a known endogenous ROS generator. Inefficient electron transport through the chain leads to the univalent reduction of oxygen, a process that creates free radical species that exacerbates the toxic effect of Al. It is in a cell's favor to maintain redox homeostasis by limiting its own production of oxidative species during Al-stressed conditions. In order to meet its energy demands, Al-stressed cells evoke the anaerobic respiratory machinery to produce ATP, notably substrate level phosphorylation (SLP) (Mailloux *et al,* 2011; 2007a; 2007b; Middaugh *et al*, 2005; Lemire *et al,* 2011a).

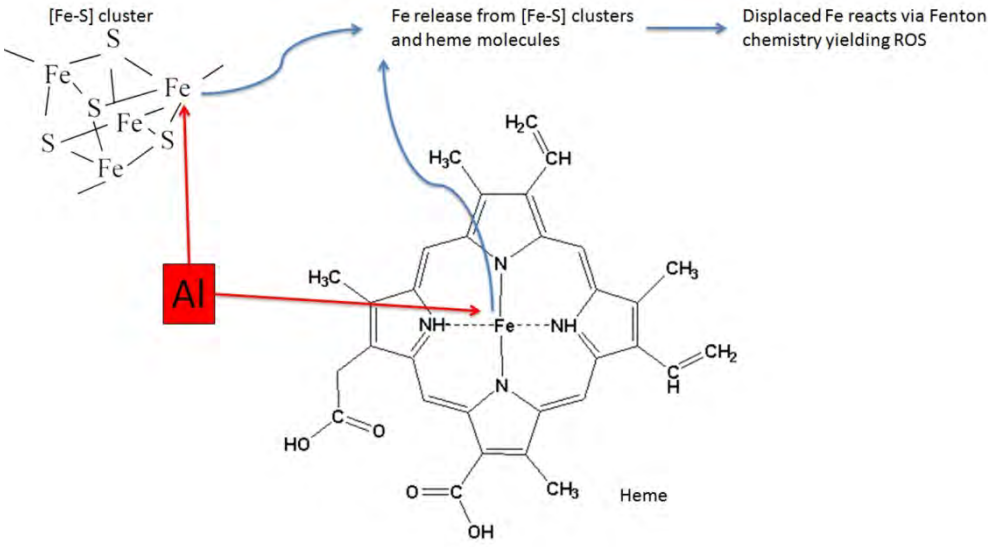

Fig. 6. Al displaces the Fe in biological molecules such as heme and [Fe-S] clusters. The presence of free Fe leads to the formation of ROS.

Al can severely impede mitochondrial function, however the consequences of its effects are not limited to a decrease in energy production. The disruption of oxidative phosphorylation by the trivalent metal evokes not only a limited supply of ATP but also an increased lipid production. This phenomenon is common in obese individuals who tend to experience diminished levels of ATP and an accumulation of fatty tissue. In human liver cells, it has been demonstrated that dyslipidemia is due to the ability of Al to perturb iron metabolism and promote oxidative stress. By displacing Fe in metabolically active enzymes, Al favors a hypoxic environment and stimulates lipogenesis. Lipogenesis is the series of chemical reactions leading to the carboxylation and subsequent polymerization of acetyl CoA

through the use of the anabolic nucleotide NADPH. Under Al toxicity, pivotal enzymes in the lipid production pathway of hepatocytes show an increase in activity. First, acetyl-CoA carboxylase (ACC) is up-regulated under ROS and Al-stress conditions. This enzyme is responsible for the production of malonyl-CoA, an inhibitor of the transport of lipids into the mitochondria and an activator of lipogenesis. Second, glycerol-3-phosphate dehydrogenase (G3PDH) diverts trioses from glycolysis into the lipid generating machinery by producing glycerol, the backbone of triglycerides. Finally, the much-needed supply of NADPH for lipogenesis during Al toxicity is ensured by an ensemble of enzymes including glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH), malic enzyme (ME), $NADP^+$-dependent glutamate dehydrogenase (GDH), NAD kinase (NADK) and $NADP^+$-dependent isocitrate dehydrogenase (IDH) (**Figure 9**). Since Al disrupts Fe homeostasis, rendering the TCA cycle and oxidative phosphorylation inactive, carbon sources coming from upstream metabolism are funneled towards the production of fatty acids. Al toxicity instigates the metabolic shift from NADH production to the synthesis of the anabolic reducing agent and antioxidant, NADPH (**Figure 10**) (Mailloux *et al,* 2011; 2007b; Lemire *et al,* 2008).

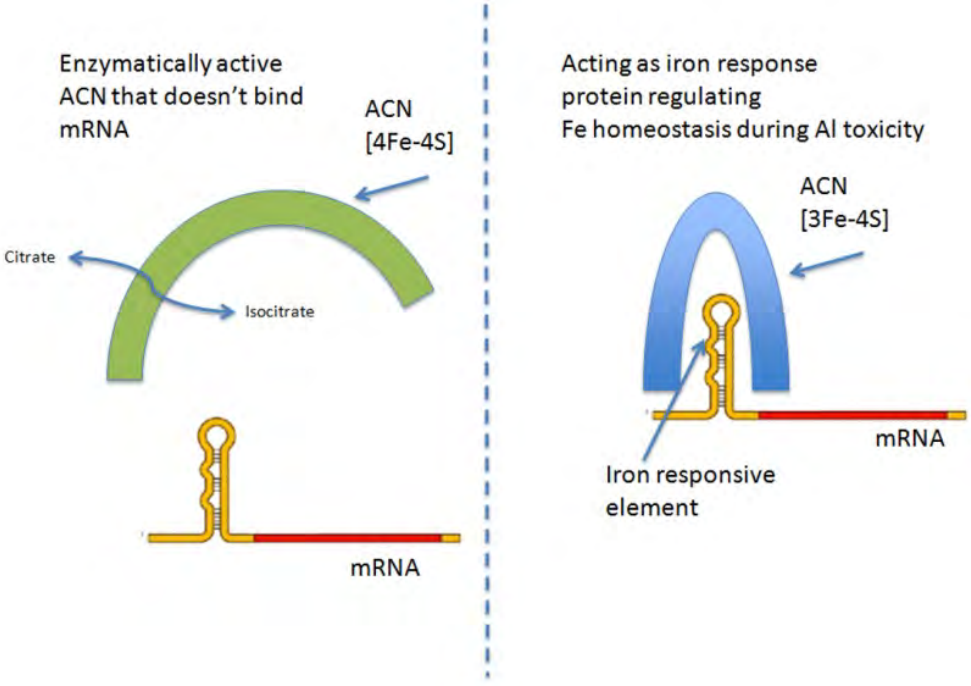

Fig. 7. ACN is recognized as the "gatekeeper" to the TCA cycle. When the [4Fe-4S] cluster is intact, the protein is enzymatically active. However under conditions that favor the [3Fe-4S] form of the enzyme, such as Fe starvation, Al toxicity and oxidative stress, the protein is no longer metabolically active. ACN [3Fe-4S] acts as the Fe response protein which regulates Fe homeostasis at the gene expression level by binding to the Fe responsive element in mRNA coding for various genes involved in Fe metabolism.

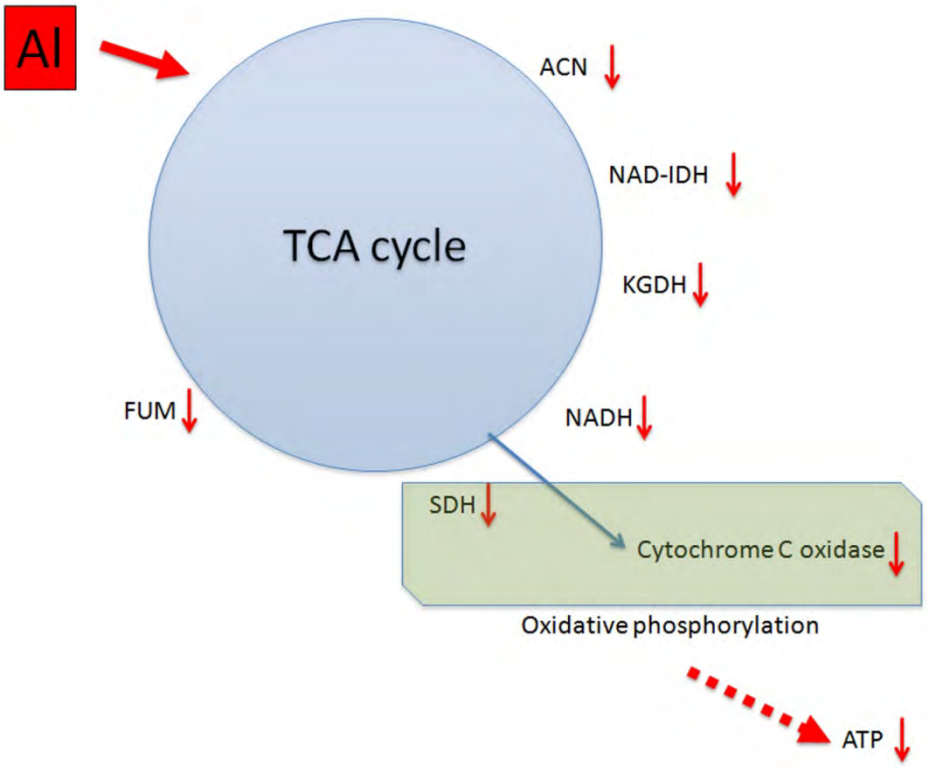

Fig. 8. Al toxicity leads to a disruption of the central metabolic pathways known as the TCA cycle. ACN, IDH (NAD-dependent), KGDH and FUM are all hindered under Al stress. This limits the production of NADH and therefore oxidative phosphorylation lacks the substrate for ATP production. Furthermore, the activity of the ETC enzymes SDH and Cyt C oxidase is perturbed under Al toxicity.

The Al-triggered increase in lipogenesis in liver cells has been recently demonstrated. When cultured hepatocytes are stressed with Al, there is a marked increase in lipoproteins and cholesterol levels in comparison to non-stressed cultures. ApoB-100 is a glycoprotein that plays a critical role in the formation of very low density lipoproteins (VLDL) and low density lipoproteins (LDL). As apoB-100 accumulates, insoluble intracellular aggregates form leading to their co-translational or post-translational degradation. However, nascent apoB-100 is stabilized by the presence of lipids, a process mediated by the microsomal triglyceride transfer protein (MTP), ultimately leading to the formation of VLDL and LDL. Al leads to the increased lipid production needed to stabilize apoB-100, allowing the maturation of VLDL molecules which are subsequently excreted out of the cell. Once out of the cells VLDL molecules can be transported to different organs via a receptor-mediated process. The concentration of the apoB-100 glycoprotein in the spent media of cultured liver cells is directly proportional to the concentration of Al utilized, thus showing direct evidence of the link between Al toxicity, lipogenesis and fatty acid accumulation. What is of

further interest is the fact that the carbon source used to grow the cultured cells has an effect on the concentration of lipids accumulated and the monosaccharide D-fructose, a common product in processed food and a compound chemically linked to cancer development and obesity, lead to enhanced VLDL secretion in the Al-stressed hepatocytes (figure 9) (Mailloux *et al, 2007b)*.

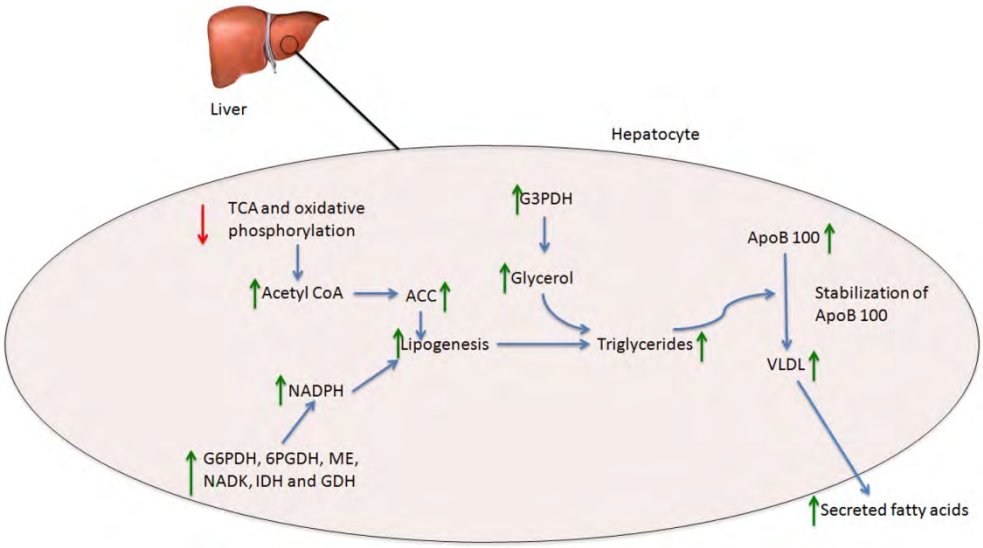

Fig. 9. Al toxicity leads to an increase in lipogenesis which can be observed by the increased levels of VLDL excreted by Al-stressed hepatocytes. As Al disables the TCA cycle and oxidative phosphorylation, there is an accumulation of acetyl-CoA, an important cofactor for lipogensis. Al also induces an increase in NADPH production through IDH and G6PDH along with an increase in glycerol synthesis, two essential substrates for triglyceride synthesis. The elevated levels of lipids in the cell stabilized the glycoprotein ApoB-100, leading to the formation and secretion of VLDL (Mailloux *et al,* 2007b).

The accumulation of lipids during Al exposure is partially due to the increased lipogenesis brought upon by a hindered mitochondrial TCA cycle and ETC. However, the Al stressed cells are also unable to degrade lipids, a phenomenon which also contributes to the accumulation of fatty acids. L-Carnitine is a non-essential amino acid involved in the transport of fatty acid-derived acyl groups into the mitochondria, a key step in the lipid degradation process known as β-oxidation. During Al-stressed conditions, L-carnitine levels have been shown to decrease in both liver and brain cells. The synthesis of L-carnitine is a multistep enzymatic process that requires the participation of lysine, methionine and α-ketoglutarate (KG). The decrease in L-carnitine levels appear to be triggered by the diminished activity and expression of two key enzymes involved in its synthesis, namely γ-butyrobetainealdehyde dehydrogenase (BADH) and butyrobetaine dioxygenase (BBDOX) (Lemire *et al*, 2011b). Along with the downregulated enzymes, the impeded TCA cycle blocks the steady supply of KG needed for L-carnitine synthesis. Al-toxicity is associated with the formation of ROS and under oxidative stress cells undergo metabolic

reconfigurations. As previously mentioned the TCA cycle is modulated during Al exposure and therefore also during oxidative stress. An important aspect of this metabolic adaptation is the downregulation of KG dehydrogenase (KGDH). This enzyme is particularly sensitive to ROS due to the reactivity of its covalently bound lipoic acid cofactor with oxidizing species. It has been shown that oxidation products in the lipid membranes can go on to react with membrane bound proteins. For example the aldehydic product of lipid peroxidation, 4-hydroxy-2-nonenal (HNE), reacts with the lipoic acid of KGDH, leading to the disruption of enzyme activity. The advantage of KGDH downregulation is the accumulation of the potent antioxidant KG. Like other ketoacids such as pyruvate and oxaloacetate, KG reacts with and nullifies ROS releasing succinate (KG's respective product) and CO_2, by a process referred to as non-enzymatic decarboxylation. As KG is being syphoned towards ROS sequestration, L-carnitine synthesis decreases due to a lack of this pivotal cofactor (**Figure 11**) (Lemire *et al*, 2011b).

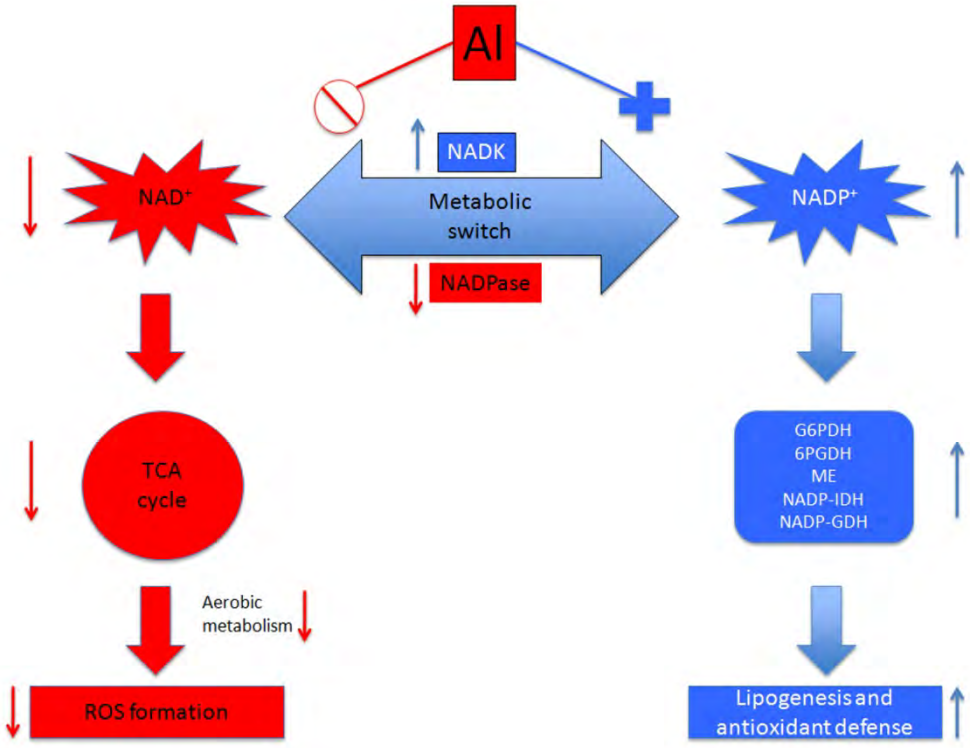

Fig. 10. Al leads to decreased levels of NAD^+ through the inhibition of the TCA cycle. Under Al toxicity, aerobic metabolism comes to a halt in order to prevent further ROS formation by endogenous sources such as the electron transport chain in the mitochondria. Al triggers the metabolic shift gated by NADK towards $NADP^+$ production. This substrate is subsequently reduced by various enzymes to produce NADPH. This last compound is essential in antioxidant defense and just as importantly, for lipogenesis (Mailloux *et al, 2007b).*

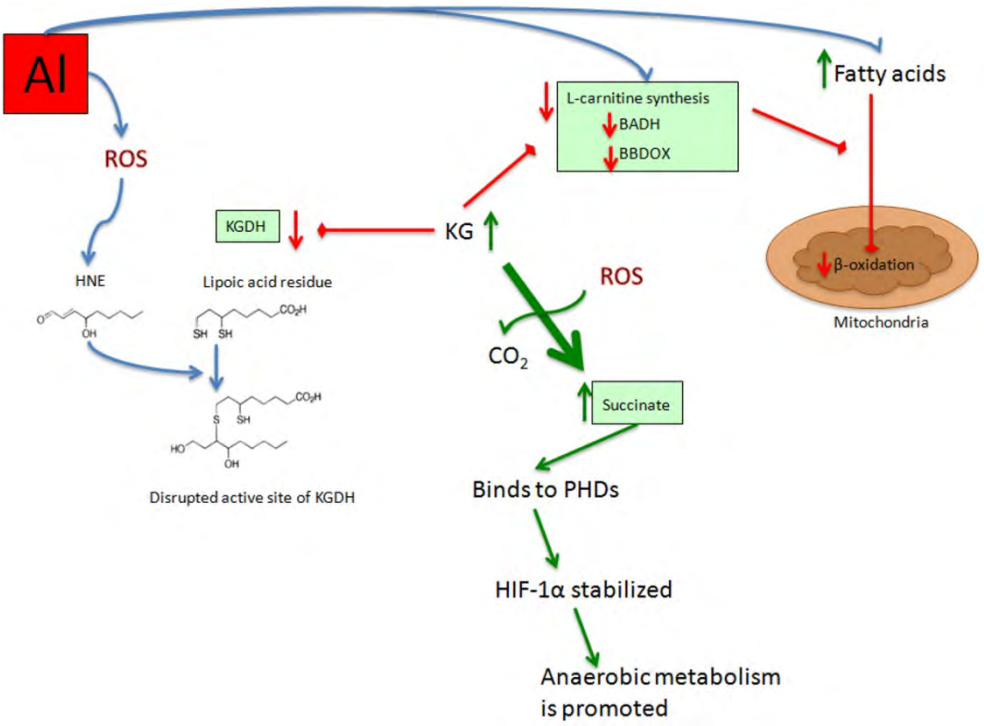

Fig. 11. Al leads to the formation of ROS, which ultimately disrupts KGDH activity (through interactions with the lipoic acid residue in the enzyme's active site). The resulting KG is accumulated in the cell and funneled towards ROS scavenging yielding an accumulation of succinate. Elevated levels of succinate leads to the stabilization of the transcription factor HIF-1α leading to the promotion of anaerobic metabolism. The supply of KG is utilized for sequestration of ROS and L-carnitine synthesis is hindered. Hence, there is decreased expression of key enzymes in the synthesis pathway. This amino acid is responsible for fatty acid transport into the mitochondria for degradation (β-oxidation) and so accumulation of lipids caused by Al is further enabled by a decrease in fatty acid catabolism (Lemire *et al,* 2011b).

The concomitant production of succinate during ROS scavenging by KG is a key contributor to the switch to anaerobic respiration. This adaptive response is initiated by the heterodimeric transcription factor HIF-1. HIF-1 consists of HIF-1α, HIF-2α and HIF-1β subunits. HIF-1α is extremely sensitive to oxygen tension and, under normoxic conditions (when the ETC can function properly), HIF-1α is quickly degraded by prolyl hydroxylases (PHDs) and the ubiquitin-proteosomal degradation pathway. When the proline residues undergo hydroxylation (via PHD), HIF-1α is targeted for degradation by the proteosome. Succinate is known to perturb substrate binding sites in PHD thus interfering with the

degradation of HIF-1α. As Al and ROS lead to an accumulation of succinate due to an altered TCA cycle, HIF-1α is stabilized and anaerobic metabolism is promoted (Mailloux *et al,* 2009; 2007a; 2006; Peyssonnaux *et al*, 2007).

6. Aluminum: The missing link between obesity, chronic inflammation and anemia

It is well established that Al disrupts Fe homeostasis. As exposure to this toxin leads to decreased absorption of Fe in the intestine, hinders its transport in the serum, and displaces Fe by binding to transferrin, the involvement of Al in anemia is evident. In a similar fashion to insulin resistance in diabetic individuals, which leads to high levels of glucose in the serum and low intracellular glucose concentrations, Al toxicity causes a disproportionate ratio of serum and cellular Fe levels. When the body experiences Al toxicity, Fe is displaced and released into the bloodstream. This increase in serum Fe tricks the body into thinking there is an excess of Fe, which would lead to limited Fe uptake and release (regulated by hepcidin), when in fact the Al exposed cells are in an anemic state (Del Giudice *et al,* 2009). However, the effects of Al-altered Fe metabolism extend beyond these aforementioned phenomena. As described in the previous section, Al displaces Fe from important enzymatically active proteins leading to a disruption of metabolic processes. This event results in a dysfunctional mitochondria geared towards lipogenesis rather than energy production. An excess fat accumulation and an increase in adipose tissue evoked by Al toxicity can lead to obesity (Mailloux *et al*, 2011). Obese individuals are faced with many obstacles including being at greater risk for cardiovascular diseases, diabetes, obstructive sleep apnea, certain cancers and osteoarthritis (Ausk *et al,* 2008). One of the hallmarks of obesity is chronic inflammation. Inflammation associated with obesity may be linked to Al exposure, a well known and readily used adjuvant. There exists a correlation between unhealthy eating habits and obesity and so an increased intake of processed and "fast" foods by an individual would suggest an increased exposure to Al. This Al could be responsible for the chronic inflammatory response observed in obese patients.

Fe release from various proteins including the [Fe-S] clusters and hemes of enzymes is facilitated by Al. This would cause an increase in free Fe in the body, an event which is hazardous to an organism due to potential ROS formation and promotion of pathogen infection. The body's response to increase Fe levels is the secretion of the hormonal peptide hepcidin. Secreted by the liver and adipose tissues, this peptide limits absorption of Fe by the intestine and release from stores. When gene deletions for hepcidin were performed in mice, unregulated hyperabsorption of Fe in the intestine and unregulated discharge of Fe from spleen macrophages was observed. Overexpression of the hepcidin gene in the mice led to Fe deficiencies and death. It is therefore obvious that this peptide is the regulator of Fe homeostasis.

As an organism is exposed to Al, multiple effects can be observed. The dysfunctional mitochondria leads to increased intracellular lipid accumulation. Obesity sets in and chronic inflammation occurs. Fe homeostasis is disrupted. Together, these events may favor an increase in hepcidin levels in the serum which would limit further absorption of Fe, ultimately causing Al-induced anemia (figure 12) (Ganz, 2003). As Al is associated with ROS toxicity and metabolic shifts leading to the accumulation of the ketoacid antioxidants, such as KG and pyruvate, perhaps dietary supplementation with these potent scavengers might offset the effects of the metal toxin.

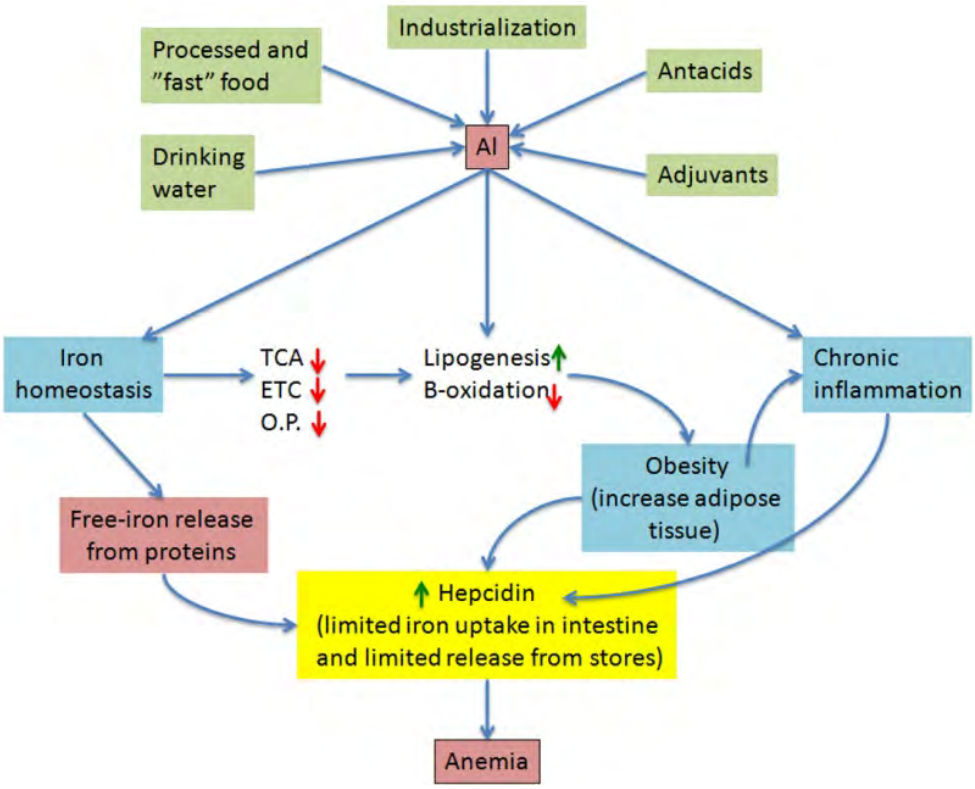

Fig. 12. A global outlook on the implications of Al toxicity leading to disruption of Fe homeostasis, increased lipogenesis and induction of chronic inflammation. Ultimately Al causes the release of Fe into the bloodstream which can signal the secretion of hepcidin by the liver and adipose tissue leading to the limited Fe uptake in the intestine and limited release from cellular stores. Al, by mimicking Fe, tricks the body into thinking it has an overload of the redox active metal and the body response leads to an Al induced anemic state.

7. The therapeutic potential of α-ketoacids

An important factor contributing to Al toxicity is its ability to generate an oxidative environment within the cell. As Al displaces Fe, this redox-active element can participate in ROS-generating reactions leading to the formation of toxic moieties such as hydrogen peroxide (H_2O_2), superoxide (O_2 ·-) and the hydroxyl radical (·OH). Due to the nature of our oxygen-rich environment, cells are constantly challenged with the burden of oxidative stress. Situations such as Al toxicity worsen the oxidative load that aerobic organisms are exposed to. Hence, organisms possess a battery of antioxidant systems that maintain the redox balance of their cells. With the classical antioxidants molecules such as glutathione

(GSH), ascorbic acid (AA) and vitamin E being extensively studied, the importance of α-ketoacids as ROS scavengers has begun to emerge.

α-Ketoacids are organic compounds that contain a carboxylic acid group adjacent to a ketone group. They play an essential role in cellular metabolism as intermediates in many pathways including the TCA cycle and glycolysis. Examples of biologically relevant α-ketoacids include pyruvate, α-ketoglutarate, oxaloacetate and glyoxylate. The antioxidant potential of these substrates has been demonstrated in a variety of ways. For example, in the soil microbe *Pseudomonas fluorescens* the α-ketoacids KG and pyruvate are readily accumulated for ROS scavenging in conditions of oxidative stress induced by exogenous H_2O_2, menadione (a $O_2^{\cdot-}$ generator), and Al (Mailloux *et al,* 2008). Similarly, metabolic adaptations occur in cultured human hepatocytes and astrocytes leading to the accumulation of these α-ketoacids when the cells are exposed to Al and other oxidizing agents (Lemire *et al,* 2011, Mailloux *et al,* 2011).

In a clinical setting, numerous studies have shown the benefits of α-ketoacid supplementation in an effort to prevent or rectify oxidative damage. An area of this research includes the prevention of cataract formation by pyruvate. Cataracts cause the clouding that develops in the crystalline lens of the eye, which can vary from a slight or complete degree of opacity and obstruction of the passage of light. Cataract formation has been linked to diabetes, hypertension and the over-exposure to UV-radiation. Although treatment with surgery and replacement with synthetic implants are options, there is a push for the development of pharmacological means of cataract prevention, which would reduce invasiveness and cause less secondary effects. The UV-radiation hypothesis of cataract formation states that photons penetrate through to the cornea and subsequently cause the generation of photochemically derived ROS in the aqueous humour and lens of the eye. Fittingly, cataract formation is accompanied by many signs of oxidative stress such as excessive protein glycation and lipid peroxidation, depletion of GSH and a decrease in ATP levels. *In vitro* incubation of mice and rat eye lens with pyruvate demonstrated the beneficial effect of this α-ketoacid in preventing cataract formation by scavenging ROS. These studies demonstrate the therapeutic potential of pyruvate in offsetting the cataractogenesis effects of solar radiation and other factors that act via ROS toxicity (Hegde, 2007, Hegde, 2005).

The use of α-ketoacids in the detoxification of ROS has been shown in many other cases. In numerous brain pathologies such as neurodegenerative diseases or in acute injuries such as ischemia or trauma, H_2O_2 is a suspected culprit in the development of neuropathogenesis. A study by Desagher *et al.* examined the ability of pyruvate to improve the survival of cultured striatal neurons exposed to oxidative agents. Pyruvate protected neurons against both H_2O_2 added to the external medium and $O_2^{\cdot-}$ endogenously produced through the redox cycling agent menadione. The neuroprotective effect of pyruvate appeared to result from the ability of the α-ketoacid to undergo non-enzymatic decarboxylation in the presence of ROS. In addition, several other α-ketoacids including α-ketobutyrate, which is not an energy substrate, also provided the neuroprotective effect of pyruvate. This study also showed that optimal neuroprotection was achieved with relatively low concentrations of pyruvate (>1mM) and that due to its low toxicity and its capacity to cross the blood-brain barrier, this α-ketoacid opens a new therapeutic perspective in ROS associated-brain pathologies (Desagher, 1997).

The advantages of α-ketoacid supplementation for the cardiovascular system have been demonstrated in numerous settings including cardiopulmonary bypass surgery, cardiopulmonary resuscitation, myocardial stunning, and cardiac failure. An important factor in these situations includes the trauma brought upon by myocardial ischemia. As the muscles of the heart are re-oxygenated after a prolonged anaerobic period, the tissue is faced with a depleted energy supply and a massive burst of ROS formation. Therapy with pyruvate has been shown to decrease the damage caused to the ischemic myocardium after reperfusion (Mallet *et al.*, 2005).

The clinical use of α-ketoacids along with their natural occurrence as an antioxidant shows promise of a new line of therapeutic drugs against a wide variety of diseases and dysfunctions. Since Al toxicity is linked to disease states such as obesity, chronic inflammation and anemia, α-ketoacids supplementation may perhaps offer a treatment for the effects of this ROS-forming metal toxin.

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