Invited Review

Emerging Roles of Cadmium and Heme Oxygenase in Type-2 Diabetes and Cancer Susceptibility

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Many decades after an outbreak of severe cadmium poisoning, known as Itai-itai disease, cadmium continues to pose a significant threat to human health worldwide. This review provides an update on the effects of this environmental toxicant cadmium, observed in numerous populations despite modest exposure levels. In addition, it describes the current knowledge on the link between heme catabolism and glycolysis. It examines novel functions of heme oxygenase-2 (HO-2) that protect against type 2-diabetes and obesity, which have emerged from diabetic/obese phenotypes of the HO-2 knockout mouse model. Increased cancer susceptibility in type-2 diabetes has been noted in several large cohorts. This is a cause for concern, given the high prevalence of type-2 diabetes worldwide. A lifetime exposure to cadmium is associated with pre-diabetes, diabetes, and overall cancer mortality with sex-related differences in specific types of cancer. Liver and kidney are target organs for the toxic effects of cadmium. These two organs are central to the maintenance of blood glucose levels. Further, inhibition of gluconeogenesis is a known effect of heme, while cadmium has the propensity to alter heme catabolism. This raises the possibility that cadmium may mimic certain HO-2 deficiency conditions, resulting in diabetic symptoms. Intriguingly, evidence has emerged from a recent study to suggest the potential interaction and co-regulation of HO-2 with the key regulator of glycolysis: 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4). HO-2 could thus be critical to a metabolic switch to cancer-prone cells because the enzyme PFKFB and glycolysis are metabolic requirements for cell proliferation and resistance to apoptosis.

Keywords: cadmium; cancer; epigenetics; heme oxygenases; type-2 diabetes

Environmental exposure constitutes 70 to 90% of the risks of developing chronic diseases such as adult-onset type-2 diabetes and cancer (Rappaport and Smith 2010; Hou et al. 2012; Stein 2012). Suspected causes of the current epidemic of type-2 diabetes include a wide range of chemicals of environmental origin (Patel et al. 2010; Thayer et al. 2012). Examples of suspected environmental diabetogens are arsenic, persistent organic pollutants, cigarette smoke, nicotine, cadmium, organotins, phthalates, bisphenol A, and pesticides (Schwartz et al. 2003; Patel et al. 2010; Satarug 2012; Thayer et al. 2012). Among the suspected environmental causes of type-2 diabetes and cancer, exposure to cadmium, especially of dietary origin, is perhaps one of the least expected and the least recognized although environmental exposure to cadmium is widespread globally. This review focuses on the metal cadmium in an effort to call for public measures to minimize population exposure to this insidious toxicant, given its diverse toxic effects and global health threats observed in numerous population studies worldwide. This challenges the previous perception that the kidney is a principal target of cadmium toxic effects.

In the first part, issues around cadmium exposure, and levels of accumulation in humans, are discussed together with global health threats and an update of adverse health effects that have emerged from the studies of representative populations in the United States of America (USA), known as the National Health and Nutrition Examination Surveys (NHANES). In the second part, functions of heme oxygenase-1 (HO-1) and heme oxygenase-2 (HO-2) are highlighted along with the expression of diabetic and obese phenotypes under HO-2 deficient conditions. In addition, the potential role for HO-2 in a control of glucose utilization via glycolysis, seen in a recent study by Li and co-workers (2012) is discussed. In the third part, this review discusses epigenomic and metabolic effects of cadmium in
liver that may explain association between cadmium and type-2 diabetes and increased liver cancer susceptibility. This review concludes with a research gap analysis and future perspectives.

**Multiple exposure sources, diverse toxic effects and global health threats**

Fig. 1 provides a summary of cadmium sources and global health threats. Cadmium in soils is taken up by plant-food crops (vegetables, grains, tubers, soybeans), which are consumed by humans and animals. Tobacco plant can concentrate cadmium from soils and deposit the metal in its leaves. Each cigarette may contain up to 2.7 μg of cadmium, depending on soil cadmium concentrations (O’Connor et al. 2010). Smoking thus causes a substantial cadmium exposure because of high pulmonary uptake rates. Many tobacco-associated diseases are mediated by cadmium. Cadmium causes distinct pathological changes in a range of tissues and organs in which it deposits and accumulates. Environmental exposure to cadmium in the general population is associated with a range of chronic ailments and various types of cancers. Associations of cadmium exposure and increased all-cause mortality and cancer mortality were observed (Menke et al. 2009; Adams et al. 2012; Tellez-Plaza et al. 2012b).

**Salient features of cadmium**

Cadmium is a cumulative toxicant because of a lack of an active biochemical mechanism for its elimination from the body. In consequence, extremely small amounts of cadmium are excreted — only about 0.001% of total body burden is excreted daily mostly in urine (Kjellström and Nordberg 1978; Slob and Krajnc 1993; Choudhury et al. 2001; Satarug et al. 2002). In a 20-yr Belgian population cohort, blood and urinary cadmium levels decreased slowly; a yearly decline rate at 1.8% and 3.4%, respectively (Nawrot et al. 2008). Cadmium from diet and smoking deposit and accumulate in nearly all organs and tissues, including the retinal pigment epithelial (RPE) cells in eye tissue, thyroid, breast, lung, liver, pancreas and kidney (Satarug et al. 2000b, 2002, 2003; Uetani et al. 2006; Satarug et al. 2010). Cadmium causes distinct pathological changes in these tissues. Because tissue levels of cadmium increase with age, cadmium effects are mistakenly viewed as “normal” outcomes of aging. Cadmium accumulation in RPE has been implicated in age-related macular degenera-

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**Fig. 1. The food-chain transfer of soil cadmium and population health outcomes.**

Cadmium in the environment, notably from soils, is transferred to plant-food crops (vegetables, grains, tubers) which are consumed by humans and animals. Tobacco plant can concentrate cadmium from soils into its leaves. Each cigarette of 0.5 g of dry tobacco leaves may contain cadmium up to 2.7 μg, depending on soil cadmium levels. Smoking causes a substantial cadmium exposure because of high pulmonary uptake rates. Cadmium causes distinct pathological changes in a range of tissues and organs. These changes are mistakenly viewed as “normal” aging effects because the levels of cadmium in tissues and organs increase with age. Cadmium exposure is associated with a range of chronic ailments and common human cancers, reflected by association between cadmium exposure and all-causes mortality and cancer mortality.
Cadmium Exposure and Adverse Health Effects

Cadmium in liver plus kidney comprises one third of a total amount of cadmium in the body (body burden). Urinary cadmium concentrations correlate closely with levels of cadmium in kidney and lung (Satarug et al. 2002). This allows for use of urinary cadmium concentration as a measure of cumulative lifetime exposure (Slob and Krajnc 1993; Choudhury et al. 2001). Accumulation of cadmium in both liver and kidney is a cause for concern as these two organs contribute to the maintenance of blood glucose levels (Gerich 2010; DeFronzo et al. 2012). In the post absorptive state, kidney and liver supply an equal amount of glucose into blood circulation (Stumvoll et al. 1997; Gerich 2010; DeFronzo et al. 2012). Further, cadmium accumulation in kidney and RPE could contribute to early onset of diabetic nephropathy and retinopathy as seen in the Torres Strait (Australia) population who had elevated dietary cadmium exposures (Satarug et al. 2000b; Haswell-Elkins et al. 2007a,b; Haswell-Elkins et al. 2008).

Another salient feature of cadmium is its long residence in tissues (half-life of 10-30 years, depending on tissue types) (Kjellström and Nordberg 1978; Satarug et al. 2002). Accumulating human data indicate that cadmium toxicity occurs at exposure levels lower than previously estimated. In an exposure-response analysis, Järup and Akesson (2009) noted increased risk of kidney-bone toxicities at urinary cadmium below 1 μg/g creatinine. This raises a concern that the urinary cadmium threshold of 5.24 μg/g creatinine, established by the World Health Organization (WHO 2010) does not provide sufficient health protection.

**Chronic, high-dose toxicities (Itai-itai disease)**

Chronic exposure to high-dose cadmium causes severe kidney and bone damage. Multiple bone fractures due to osteomalacia and osteoporosis are seen in the most severe form of cadmium poisoning in humans, known as Itai-itai disease. Based on historic rice cadmium content, Itai-itai disease occurs following consumption of over 200 μg cadmium per day (ranging between 146 to 259 μg cadmium per day) for over 20 yrs, equivalent to a lifetime cadmium intake of 2,600-3,300 mg (Iwata et al. 1993; Inaba et al. 2005). A lifetime cadmium intake that caused severe kidney damage in women was 1,790 mg and 2,910 mg in men, who lived in a cadmium-contaminated area in Japan (Kobayashi et al. 2009). To prevent kidney accumulation of cadmium to toxic levels, health-based risk assessment indicates that cadmium content of staple foods should be no greater than 0.1 mg/kg (Satarug et al. 2000b, 2003; Suwazono et al. 2010). This health-based level is 4-fold lower than the maximum permissible concentration of cadmium in rice set by the Codex Alimentarius Commission of the Food and Agricultural Organization/World Health Organization (FAO/WHO) at 0.4 mg/kg (Satarug and Moore 2004; Satarug et al. 2010; Uraguchi and Fujiwara 2012).

**Chronic, low-dose toxicities (chronic disease and cancer)**

Evidence for environmental cadmium exposure posing a real human health hazard, even at low levels of exposures, comes from the NHANES data. Chronic disease-cadmium associations observed in the NHANES are summarized as follows. Ferraro et al. (2010, 2011) found cadmium exposures among adult populations in the USA were associated with chronic kidney disease and kidney stones. Similar cadmium exposures were associated also with pre-diabetes and type-2 diabetes (Schwartz et al. 2003), hypertension, cardiovascular disease (Tellez-Plaza et al. 2008), all-cause mortality (Menke et al. 2009), and overall cancer mortality in both men and women, with noted sex-related differences in specific types of cancer (Adams et al. 2012). Tellez-Plaza et al. (2012b) observed cadmium exposures associated with increased risk of death from cardiovascular disease, heart disease and coronary heart disease among participants in the 1988-1994 NHANES. Choi et al. (2012) observed an association between hearing loss and environmental exposure to cadmium and lead in an adult population in the USA. Ciesielski et al. (2012) observed an association between cadmium exposure and learning disability (neurotoxicity) in a pediatric population. In the pooled 2007-2010 NHANES data, Rokadia and Agarwal (2012) have found cadmium to be a mediator of smoking effects in promoting obstructive pulmonary disease. They observed a dose-response relationship between increasing serum cadmium concentrations and worsening of lung function.

**Exposure sources: a case of food-chain transfer of soil cadmium**

Diet is a primary exposure source for non-smoking populations (Louekari et al. 1989; Satarug et al. 2000b; Amzal et al. 2009; Arnich et al. 2012; Sand and Becker 2012; Satarug 2012). For a large portion of the general population, tobacco smoke is a secondary source (Mortensen et al. 2011). As it can be expected, foods that are consumed in larger quantities such as staple foods (rice, potatoes, wheat flour) contribute the greatest dietary source. Accordingly, grains plus grain products contribute to 26.9% of cadmium in the diet of European population, while vegetables plus vegetable products and starchy roots plus tubers contribute to 16.0% and 13.2% of cadmium in the diet, respectively (EFSA 2011, 2012). To minimize dietary cadmium exposure in the general population, the issues around cadmium levels in agricultural soils and the food-chain transfer of soil cadmium need to be systematically tackled. This needs much more awareness of cadmium levels in phosphate fertilizers, and in mining waste and waste-water. Environmental management is required to minimize cadmium contamination in the environment and food-chain transfer of soil cadmium.

Arnich et al. (2012) observed increased dietary cadmium exposures in a French population when data of the
Absorption, cellular uptake and accumulation

Cadmium has no known physiological function, and no mechanism would have been expected to have evolved for its selective transport and homeostasis. It is increasingly apparent that cadmium is acquired by transport mechanisms developed for other essential metals (Thévenod 2010; Vesey 2010; Jenkitkasemwong et al. 2012). From physical and biochemical properties, those metals are most likely to be zinc (Zn\(^{2+}\)), iron (Fe\(^{2+}\)), manganese (Mn\(^{2+}\)), and calcium (Ca\(^{2+}\)). The divalent metal transporter 1 (DMT1) is the first metal transporter found to mediate cadmium uptake in mammalian cells (Garrick et al. 2003; Bressler et al. 2004). The role played by DMT1 in cadmium absorption in humans is deduced from an inverse relationship between cadmium body burden and low body iron stores and iron deficiency. Women with low body iron stores, reflected by serum ferritin < 20 \(\mu\)g/L, were found to have 3-fold greater body cadmium burden, compared with women of the same age with adequate body iron status (Satarug et al. 2004b). Similar influences of low body iron stores and iron deficiency in enhanced intestinal cadmium absorption were observed in Korean (Lee and Kim 2012) the U.S. population (Gallagher et al. 2010a) and in Norwegian women (Meltzer et al. 2010). However, the iron-cadmium relationship was not observed in a group of Japanese women (Horiguchi et al. 2004). This could reflect transporters’ saturation effects in situations of high dietary cadmium exposure.

Finley (1999) found an inverse correlation between body iron status and manganese absorption. Likewise, Meltzer et al. (2010) found low iron stores associated with higher blood concentrations of cadmium, manganese and cobalt (Co\(^{2+}\)) in non-smoking, Norwegian women. Taken together, these findings suggest intestinal absorption and cellular uptake of iron, manganese and cadmium could be mediated by the same transporter(s).

In support of this notion, Martin et al. (2006) found that manganese co-exposure reduced cadmium uptake by 80% in the human embryonic kidney HEK293 cells, possibly through the manganese vs. cadmium competition for common metal transporter(s). Likewise, Satarug et al. (2008) have shown that manganese co-exposure could prevent cellular uptake and accumulation of cadmium, evident from a reduction in its effects in a range of human cells, including the human RPE cell line (ARPE-29), human embryonic kidney HEK293, human lung cancer A549 and human breast cancer MCF-7. Girijashanker et al. (2008) and Liu et al. (2008) have shown that cellular uptake of manganese and cadmium can be mediated by zinc transporters of the Zrt-/Irt-like protein (ZIP) family, namely ZIP8 and ZIP14. Fujishiro et al. (2012) have shown that the uptake of cadmium and manganese by mouse kidney proximal tubular cells are mediated by ZIP8, ZIP14 and DMT1. Ohana et al. (2006) suggested ZnT-1 zinc transporter may play a role also in cellular cadmium uptake and accumulation.

How much cadmium is in human diet?

Analysis of duplicate diet samples consumed daily among Japanese women indicated geometric mean values for cadmium in their diet to be 24.7 \(\mu\)g and 35.7 \(\mu\)g (Ikeda et al. 2000; Shimbo et al. 2000). Reeves and Vanderpool (1997) found 36 \(\mu\)g of cadmium in duplicate food samples consumed per day among frequent consumers of sunflower kernels. Copes et al. (2008) estimated cadmium exposure from oysters among oyster growers to be 24.8 \(\mu\)g/day. Vahter et al. (1996) reported dietary cadmium exposure to be 11 \(\mu\)g/day for Swedish women consuming a mixed diet and 28 \(\mu\)g/day for those consuming diet high in shellfish.

Berglund et al. (1994) reported the average dietary cadmium exposure ranging between 5 and 38 \(\mu\)g/day among 57 non-obese, 20 to 50 years old Swedish women.

A study of 1,348 persons in Finland showed dietary cadmium exposures of 13-17 \(\mu\)g/day in men and 12-13 \(\mu\)g/day in women (Louekari et al. 1989). Dietary cadmium exposures in the USA were 15-22.4 \(\mu\)g/day in men and 13.5-16.5 \(\mu\)g/day in women (Ruiz et al. 2010). Sand and Becker (2012) observed no sex differences in dietary habits in Sweden, and they found dietary cadmium exposure for the average consumer to be 10 \(\mu\)g/day for a 70-kg person, 40-50% coming from staple foods: potatoes and wheat. For the high consumer (dietary cadmium exposure above 95th percentile), dietary exposure was 22 \(\mu\)g/day, additional cadmium coming from seafood and spinach.

How much cadmium is accumulated in human body?

In chronic, low-dose exposure situations, kidney cortex cadmium concentrations are 10 to 20 times greater than liver cadmium concentrations (Satarug et al. 2000b, 2002, 2003; Satarug and Moore 2004). For example, in the study...
of 61 Australian autopsy cases, aged 2 to 89 years, mean cadmium content in lung, liver and kidney cortex was 0.13, 0.95 and 15.45 μg/g wet tissue weight, respectively. Mean liver cadmium content in persons aged 41-50, 51-60 and > 60 years was 1.4, 0.9, and 1.5 μg/g wet weight and the corresponding value for the kidney cortex was 25.9, 22.5 and 21.3 μg/g wet weight. On average kidney cadmium content was 16 times greater than that of the liver. Of these 61 cases, 3.3% had cadmium ≥ 50 μg/g kidney cortex weight: the level considered to be at risk of toxicity. Similar result was seen in the analysis of kidney samples from 2700 persons collected nationwide over 16 years (1978-1993) in the United Kingdom (Lyon et al. 1999). Overall mean kidney cadmium was 19 μg/g wet weight with 3.9% containing cadmium ≥ 50 μg/g kidney wet weight and mean kidney cadmium content in persons aged 40-59 years (peak-age group) was 23 μg/g wet weight (Lyon et al. 1999).

In addition to differential accumulation levels in tissues, there are gender-related differences in cadmium accumulation. For instance, females in an Australian study had twice as much cadmium in the liver, but they had lower lung zinc, compared to males, while kidneys from females tended to contain more cadmium than did males (Satarug et al. 2001, 2002). Likewise, Uetani et al. (2006) observed extensive cadmium accumulation in kidney cortex and male-female differences in their study of 72 environmentally exposed subjects, 60-91 yrs, mean 74 yrs. Uetani et al. (2006) reported cadmium accumulation also in the pancreas, thyroid gland, heart, aorta and bone. Similar levels of cadmium accumulation in liver and pancreas may indicate similarity in cadmium uptake rates in these two organs. Erie et al. (2005) and Wills et al. (2008) found that RPE and chondroid from human eyes contained more cadmium than did the retina. In addition they noted that females, old age and smokers had elevated levels of cadmium accumulation in eye tissues. Accumulation of cadmium in RPE has been linked to the development of age-related macular degeneration (Erie et al. 2005, 2007; Wills et al. 2008, 2009).

Metallothionein sequestration and cadmium’s long half-life

Cadmium has been shown to cause an induction of the metal binding protein metallothionein (MT) in various tissues and organs, including the liver and peripheral leukocytes (Boonprasert et al. 2012). The MT induction by cadmium results in cadmium sequestration, which underlies the long biological half-life of cadmium. Increased liver sequestration of zinc and copper was observed in liver samples having cadmium contents of greater than 1 μg/g wet liver weight, possibly due to MT sequestration (Satarug et al. 2001). The liver levels of copper were increased by 45-50% in persons with high cadmium body burden, compared to subjects of similar ages with medium body burden of cadmium (Satarug et al. 2001). Excessive liver copper and iron accumulations are known risk factors for liver cancer. Indeed, greater copper levels with lower MT levels were noted in neoplastic liver samples, compared with surrounding non-neoplastic liver portions (Kawata et al. 2006). The MT sequestration of cadmium is considered to provide protection against acute toxicity. However, it could also provide an opportunity for toxicity to occur without additional exposure. A bolus of liver cadmium, previously bound to MT, can be displaced by nitric oxide and is released during infection and sepsis, where cellular nitric oxide synthesis is enhanced (Satarug et al. 2000a). This may explain an increase in all-cause mortality among persons who had elevated cadmium body burden with or without pre-existing signs of cadmium-related kidney toxicity (Menke et al. 2009; Adams et al. 2012; Tellez-Plaza et al. 2012b).

Metabolic and carcinogenic effects

Type-2 diabetes: In 1980, Merali and Singhal demonstrated the ability of cadmium to induce diabetic symptoms in neonatal rats. They found the liver of these cadmium-exposed neonatal rats contained low levels of glycogen and showed evidence of enhanced gluconeogenesis (increased activity of enzymes in gluconeogenesis: pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-biphosphatase and glucose-6-phosphatase). Consistent with the data from the study in neonatal rats, chronic administration of cadmium caused high blood glucose (hyperglycemia) in adult rats (Bell et al. 1990a,b). Hyperglycemia in cadmium-exposed rats occurred long before kidney toxicity developed (Edwards and Prozialeck, 2009). This suggests hyperglycemia is not a consequence of renal cadmium toxicity that causes a reduction in tubular reabsorption of glucose. Prior to the demonstration of diabetogenic effects of cadmium, Singhal et al. (1974) showed that cadmium administration to rats via intraperitoneal injection for 45 days caused an increase in the activities of the rate-limiting enzymes in gluconeogenesis in liver and in kidney cortex. Those enzymes were pyruvate carboxylase, phosphopyruvate carboxylase, hexosediphosphatase, and glucose-6-phosphatase. Cadmium treatment also caused depletion of liver glycogen. Changes in levels of the enzymes of gluconeogenesis and liver glycogen levels persisted 4 weeks after withdrawal from exposure (Singhal et al. 1974). In a recent study, liver effects of cadmium in rats were detected one year after exposure to cadmium for a period of 4 weeks (Wang et al. 2011).

Evidence for a role of cadmium exposure in the development of type-2 diabetes in humans comes from the NHANES III data which indicated that prevalence rates of pre-diabetes and type-2 diabetes increased proportionally with increasing urinary cadmium levels (Schwartz et al. 2003). The risk estimates for abnormal fasting glucose and diabetes were 1.48 and 1.24 for pre-diabetes and diabetes, respectively, when comparisons were made for urinary cadmium levels of < 1 with those between 1.00-1.99 μg/g creatinine. These increased to 2.05 and 1.45 when comparisons were for urinary cadmium < 1 μg/g creatinine with ≥ 2
In a recent study, Kawakami et al. (2010) observed abnormal differentiation of adipocytes in cadmium-exposed mice that resulted in a reduction in adiponectin secretion. Low plasma adiponectin is linked to obesity, metabolic syndrome, insulin resistance, type-2 diabetes, and inflammation as well as several types of cancers (Ziemke and Mantzoros 2010). Cadmium thus may cause insulin resistance through lowering adiponectin release from adipose tissues and thus to lower levels of adiponectin in the circulating blood. The potential role of cadmium in causing the depressed adiponectin levels and dysregulation of cellular intermediary metabolism may contribute in part to increased cancer susceptibility observed in type-2 diabetic subjects in several large longitudinal studies worldwide. These included cohorts in Japan, Korea, Canada, the U.K., Italy (Wang et al. 2012), USA (Campbell et al. 2012), Taiwan (Lee et al. 2012), Sweden (Hemminki et al. 2010), and the combined Norwegian, Swedish and Austrian populations (Borena et al. 2012).

Cancer: Cadmium is classified as a human carcinogen by the International Agency for Research on Cancer (IARC 1993) based on evidence for elevated lung cancer incidence rates in occupational exposure settings. Cadmium-cancer associations are listed in Table 1 in chronological order. In a hospital-based study on 165 postmenopausal Japanese women, mean age 59 yrs, Nagata et al. (2005) observed a 28% increase in serum testosterone (a breast cancer risk factor) among those with urinary cadmium ≥ 3 μg/g creatinine. Nawrot et al. (2006) observed a 1.7-fold rise in lung cancer risk among those with a two-fold increase in cadmium body burden. Lung cancer risk was increased by 4.2 fold and 1.57 fold among those who lived in “high” exposure area and in the areas with two-fold increase in soil cadmium content, respectively. McElroy et al. (2006), observed a dose-response relationship between breast cancer risk and cadmium exposure (p trend = 0.01), suggesting a 2.29-fold rise in breast cancer risk as urinary cadmium increased from ≤ 0.26 μg/g creatine to ≥ 0.58 μg/g creatinine. In a hospital-based study of 45 prostate cancer cases and 58 controls, Vinceti et al. (2007) found a 4.7-fold increase in prostate cancer risk as toe-nail cadmium content rose from < 0.007 μg/g to the levels > 0.03 μg/g creatinine. In a study of prostate cancer risk factors, Wijngaarden et al. (2008) found a rise in urinary cadmium of 1 μg/g creatinine associated with a 35% increase in prostate specific antigen in men whose zinc intake below an adequate intake of 15 mg/day.

In the Swedish prospective study of 30,210 women, Akesson et al. (2008) found a 2.9-fold increase in risk of endometrial cancer among those with cadmium intake above average (> 15 μg/day). In two case-control studies (100 cases and 98 controls, living on Long Island, New York, plus 92 cases and 2,884 controls in the 1999-2008 NHANES), Gallagher et al. (2010b) observed an increased odds ratio (OR) for breast cancer with increasing urinary cadmium levels in both groups. Comparing the highest quartile with the lowest, the OR for breast cancer among Long Island women was 2.69 (95% 1.07-6.78) while the OR among women in the NHANES was 2.50 (95% 1.11-5.63). In support of a causal role for cadmium exposure in breast cancer, Julin et al. (2012a) reported that dietary cadmium intake was positively associated with overall breast cancer in the 12.2-yr cohort of 55,987 postmenopausal Swedish women. Comparing the highest tertile with the lowest tertile of dietary cadmium intake, rate ratio (RR) for all-type breast cancer was 1.27 (95% CI, 1.07-1.50) and RR for ER (+) tumors was 1.25 (95% CI, 1.03-1.52). Julin et al. (2012b) observed also a positive association between dietary cadmium exposure and prostate cancer in the 11-yr cohort of 41,089 Swedish men. Based on a food frequency questionnaire, mean dietary cadmium exposure among cohort participants was 19 μg/day. Comparing the highest with the lowest tertiles of cadmium intakes, RR for localized type of prostate cancer was 1.29 (95% CI, 1.08-1.53) while RR for advanced type was 1.05 (95% CI 0.87-1.25) and RR for fatal type was 1.14 (95% CI, 0.86-1.51).

Liver cancer: Evidence for the outcome of a lifetime exposure to cadmium on liver cancer mortality risk comes from an ecological study in China by Campbell at al. (1990). Their study covered 48 survey sites in China, which showed 39-, 600- and 28-fold variation in liver cancer mortality rates, dietary aflatoxin (AFB1)-exposure levels and prevalence of hepatitis B virus surface antigen (HBsAg+) carriers, respectively. AFB1 is produced by the mold Aspergillus flavus on peanuts, corn and soy products. Campbell and co-workers found a positive correlation between liver cancer mortality and mean daily intake of cadmium from foods of plant origin. They found no association of liver cancer mortality and intakes of AFB1. Other factors associated with liver cancer mortality risk were prevalence of hepatitis B virus surface antigen (HBsAg+) carriers, plasma cholesterol and alcohol consumption.

Tumors in exposed mice: Waalkes and Rehm (1994) found carcinogenic effects of cadmium in mice to be dependent upon mouse strains, exposure routes and dose levels. In a standard two-year bioassay, Waalkes and Rehm (1994) used male mice of two strains, the DBA/2NCr (DBA) and NFS/NCr (NFS). Cadmium was administered to mice of these two strains at 8 weeks of age (adult stage for mice) by subcutaneous injection, as a single dose of 40 μmol/kg or as weekly dose of 40 μmol/kg for 16 weeks (16 × 40 μmol/kg). Thereafter, mice were observed for an additional two years. Hepatocellular carcinomas and hepatic adenomas were not detected in any of DBA mice, but they were observed in 1 of 15 control NFS mice and in 9 of 27 NFS mice with weekly repeated cadmium administrations. Sarcoma at the injection site was observed in 9 of 35 NFS mice with repeated cadmium administration. Lung cancer was observed in NFS mice given a single dose of cadmium. Incidence rates of cadmium-induced testicular tumors in the
two strains were similar. In NFS mice, cadmium caused liver cancer, sarcoma, lung cancer and testicular tumors while in DBA mice, cadmium did not cause sarcoma, nor did it cause cancer in the liver or lung, but it caused lymphoma. Cadmium carcinogenicity thus may be influenced by cadmium dose levels, route of administration, and genetic background.

Urinary bladder cancer: Bladder cancer ranks the ninth most common cancer worldwide with the highest incidence in Egypt where infection with *Schistosoma haematobium* is endemic (Parkin, 2008). In the USA, bladder cancer ranked as the sixth most common cancer. In non-endemic area of Schistosomiasis, most bladder cancer cases were transitional cell carcinoma (TCC), originating from the transitional cells of the bladder mucosal epithelium. One third of TCC cases manifest as non-papillary tumors with high invasive and metastasis potential while two thirds manifest as non-invasive, resectable papillary tumors, with recurrence rates between 30% and 70% (Pasin et al. 2008). Male to female rate ratio is 3:1 with more than a half of bladder cancer patients being 65 years or older (Parkin 2008; Pasin et al. 2008). High recurrence rates of up to 70% necessitate frequent cystoscopy and urine cytology (Pasin et al. 2008). This makes bladder cancer the fifth highest cancer treatment and the highest care cost per patient in the USA (Hong and Loughlin 2008).

In early studies, bladder cancer was found associated with workplace exposure to bladder carcinogens, such as aromatic amines, polycyclic hydrocarbons encountered in dye and wood product industries (Pasin et al. 2008). In a recent estimate, however, workplace exposure accounted for 5-15% of European male cases while cigarette smoking was identified as a predominant risk factor (Murta-Nascimento et al. 2007). In a Spanish case-control study (1,219 newly diagnosed cases and 1,271 controls), cigarette smoking accounted for nearly all excess bladder cancer risk

### Table 1. Environmental cadmium exposure and cancer risk estimates in humans.

<table>
<thead>
<tr>
<th>Cancer type/Population/Study design</th>
<th>Women with urinary cadmium ≥ 3 μg/g creatinine had 28% greater serum testosterone (a breast cancer risk factor) than did those with urinary cadmium &lt; 3 μg/g creatinine.</th>
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<tr>
<td></td>
<td>Hazard Ratio (HR) for lung cancer attributable to a two-fold increase in cadmium body burden was 1.7 (95% CI, 1.1-2.6). HR for lung cancer attributable to living in “high” exposure area was 4.2 (95% CI, 1.2-14.4). HR for lung cancer attributable to a two-fold increase in soil cadmium was 1.57 (95% CI, 1.1-2.2).</td>
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<td>OR for bladder cancer attributable to blood cadmium rising from the lowest tertile to the highest was 5.7 (95% CI 3.3-9.9), p trend &lt; 0.001. OR was adjusted for sex, age, smoking habits and occupational exposure to putative bladder carcinogens.</td>
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<td>OR for prostate cancer attributable to increased toe-nail cadmium content from the lowest quartile to the highest quartile was 4.7 (95% CI, 1.3-17.5).</td>
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<td>A 1 μg/g creatinine rise in urinary cadmium associated with a 35% increase in serum prostate specific antigen in men whose daily zinc intakes were below 12.7 mg/day.</td>
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<td>OR for endometrial cancer attributable to cadmium intake &gt; 15 μg/day was 2.9 (95% CI, 1.05-7.79) in women who did smoke and did not use postmenopausal hormone.</td>
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<td>Median urinary cadmium was 1.8 μg/L in bladder cancer cases (14 males and 8 females) and it was 0.8 μg/L in controls.</td>
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<td>Increased OR for breast cancer with urinary cadmium levels. Comparing the highest quartile with the lowest, OR for breast cancer among Long Island women was 2.69 (95% CI 1.07-6.78). OR for breast cancer among women in the NHANES was 2.50 (95% CI 1.11-5.63).</td>
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<td></td>
<td>Dietary cadmium intake associated with overall breast cancer risk, comparing the highest tertile with the lowest. Rate Ratio (RR) for all-type breast cancer was 1.27 (95% CI, 1.07-1.50).</td>
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<td>Dietary cadmium associated with increased overall prostate cancer risk, comparing the highest with the lowest tertiles. RR for localized tumor was 1.29 (95% CI, 1.08-1.53).</td>
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<td>Dietary cadmium associated with increased overall prostate cancer risk, comparing the highest with the lowest tertiles. RR for advanced tumor was 1.05 (95% CI 0.87-1.25).</td>
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<td>RR for fatal tumor was 1.14 (95% CI, 086-1.51).</td>
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Breast cancer, U. S. (McElroy et al. 2006), case-control, 246 cases, 254 controls.

Urinary bladder cancer, Belgium (Kellen et al. 2007), case-control, 172 cases, 395 controls. Mean blood cadmium for cases was 1.1 μg/L and 0.7 μg/L for controls.

Prostate cancer, Italy (Vinceti et al. 2007), case-control, 45 cases, 58 controls.

Prostate cancer risk factors, U.S. (Wijngaarden et al. 2010b), prospective, 41,089 men, 11-yrs observation, dietary cadmium from food frequency questionnaire, mean dietary cadmium exposure was 19 μg/day.

Breast cancer risk factors, Japan (Nagata et al. 005), a hospital-based study of 164 postmenopausal women.

Lung cancer, Belgium (Nawrot et al. 2006), prospective, 994 subjects from “high” and “low” cadmium exposure areas, 17.2-yr observation.

Breast cancer, Sweden (Akesson et al. 2008), prospective, 30,210 women, 16-yr observation.

Urinary bladder cancer, Germany (Wolf et al. 2009), 22 cases and 22 controls.

Breast cancer, U.S. (Gallagher et al. 2010b), case-control, 100 cases with 98 controls, living on Long Island, New York, and 92 cases with 2,884 controls in the 1999-2008 NHANES.

Breast cancer, Sweden (Julin et al. 2012a), prospective, 55,987 postmenopausal women, 12.2-yr observation.

Prostate cancer, Sweden (Julin et al. 2012b), prospective, 41,089 men, 11-yr observation, dietary cadmium was estimated from food frequency questionnaire, mean dietary cadmium exposure was 19 μg/day.

Endometrial cancer, Sweden (Akesson et al. 2008), prospective, 4,22 men, 16-yr observation.

Breast cancer, Belgium (Nawrot et al. 2008), cross sectional, 422 men.
in men (Samanic et al. 2006). Inorganic arsenic (As$^{3+}$) was associated with bladder cancer in certain regions of Taiwan and Chile where exposure to As$^{3+}$ in drinking water > 50 µg/L (Marshall et al. 2007; Chen et al. 2008). In a study in the USA, exposure to As$^{3+}$ at the levels < 50 µg/L was found associated with bladder cancer risk only in smokers (Karagas et al. 2004). This raises the potential role for elevated exposure to cadmium from smoking.

In a meta-analysis, cigarette smoking accounted for 50% and 35% of male and female bladder cancer cases (Murta-Nascimento et al. 2007). Bladder cancer risk fell by 30% in the first 4 years after smoking cessation while a further 30% reduction occurred in 20 years later (Samanic et al. 2006). Such decade-long carry over effects argue that the carcinogenic agents in tobacco such as benzo(a)pyrene, tobacco specific nitrosamines that undergo rapid clearance by cytochrome P450 enzymes (Alexandrof et al. 2010) may not play a key role, but those like cadmium that are retained in bladder tissues over an extended period may be causative. Indeed, in a 20-yr Belgian cohort, Nawrot et al. (2008) noted that blood and urinary cadmium levels fell slowly with a yearly decline rate at 1.8% and 3.4%, respectively, as environmental exposure continued. Wang et al. (2011) reported the biochemical changes in livers of rats exposed to low-dose cadmium by intraperitoneal injection in every other day for 4 weeks were detectable a year later. This indicates the presence of cadmium in livers of those exposed rats long after withdrawal of exposure.

Further evidence for cadmium being a human bladder carcinogen comes from the Belgian case-control study that found a 5.7-fold increase in bladder cancer risk as blood cadmium rose from the lowest tertile to the highest (Kellen et al. 2007). The bladder cancer risk estimate was corrected for gender, age, smoking habits and workplace exposure. The mean blood cadmium of 172 bladder cancer cases and 395 controls were 1.1 µg/L and 0.7 µg/L, respectively, as environmental exposure continued. Wang et al. (2011) reported the biochemical changes in livers of rats exposed to low-dose cadmium by intraperitoneal injection in every other day for 4 weeks were detectable a year later. This indicates the presence of cadmium in livers of those exposed rats long after withdrawal of exposure.

Cadmium induces cancer-cell transformation of human cells: Several in vitro studies have established that exposure to low-level cadmium over a long period of time (e.g., 10 weeks or longer) causes non-neoplastic human cells to transform to cancer cells. This provides compelling evidence for carcinogenicity of cadmium. Those in vitro studies showing the propensity of cadmium to induce various immortalized, non-neoplastic cells to undergo neoplastic transformation are summarized in Table 2. The human cell lines shown to be susceptible to carcinogenic effects of cadmium include the RWPE-1 prostate epithelial cells (Achanzar et al. 2001), UROtsa uroepithelial cells (Sens et al. 2004), the MCF-10A breast epithelial cells (Benbrahim-Tallaa et al. 2009), the BEAS-2B bronchial epithelial cells (Jing et al. 2012), and pancreatic ductal epithelial cells (Qu et al. 2012). Animal cell lines found to be susceptible to cadmium carcinogenicity included the rat liver epithelial TRL1215 cell line (Takiguchi et al. 2003; Qu et al. 2005).

Among these susceptible cells, the urothelial UROtsa cell line is worthy of note because of limited human cell models of urinary bladder cancer. The UROtsa cell line was derived from the epithelium of the ureter of a 12-year-old female donor, immortalized with SV40 large T-antigen (Petzoldt et al. 1995). The UROtsa cell line is non neoplastic cell line which shows phenotypic and morphologic characteristics that resemble primary transitional epithelial cells (Rossi et al. 2001). Chronic exposure to cadmium caused the UROtsa cells to undergo neoplastic transformation, expressing the phenotype characteristic of transitional cell carcinoma of the bladder (Sens et al. 2004). The UROtsa cell line thus serves as a valuable model for research into potential mechanism(s) underlying carcinogenicity and high-rate recurrence of human urinary bladder cancer.

Sens et al. (2004) reported that the UROtsa cells were transformed to cancer cells by cadmium at 1 µM concentration and they noted that this 1 µM concentration was cytotoxic to the UROtsa cells. This could suggest that human bladder epithelium is susceptible to relatively low concentrations of cadmium. In contrast to the UROtsa cells, Achanzar et al. (2001) reported that the RWPE-1 human prostate epithelial cells were transformed to cancer cells following 8 weeks of continuous exposure to cadmium at 10 µM. They also noted the 10 µM level of cadmium was not lethal to the RWPE-1 cells. Satarug et al. (2008) reported cadmium at 10 µM was not cytotoxic to human RPE (ARPE-19) cells. As with the UROtsa cells, the breast epithelial MCF-10A cells, the bronchial epithelial BESA-2B and pancreatic ductal epithelial cells were susceptible to neoplastic transformation by lower cadmium levels, compared to the RWPE-1 human prostate epithelial cells.

The reasons for differences in sensitivity to cadmium carcinogenicity remain unclear, but could be related to potential differences in uptake and excretion rates of cadmium among these cells, and cellular concentrations of protective factors such as SOD, zinc and glutathione and induction of HO-1 and MT. For instance, induction of the ZnT1 zinc transporter in TRL1215 rat liver epithelial cells by cyproterone acetate (a synthetic anti-androgen) decreased cadmium accumulation levels and showed resistance to cytotoxicity of cadmium (Takiguchi et al. 2001). Wu et al. (2012) found that Nrf2 activation prevents acute liver injury in mice exposed to cadmium. Human prostate epithelial cells (Costello and Franklin 1998; Costello et al. 2005) and human RPE (Lengyel et al. 2007; Satarug et al. 2008) are known to contain high zinc levels that may confer resistance to cadmium cytotoxicity.

Heme oxygenase-1 and heme oxygenase-2

Heme oxygenase-1 (HO-1) and heme oxygenase-2
(HO-2) are well known for their role in heme degradation to retrieve iron for reutilization. In addition to iron recycling, there is evidence for participation of HO-2 in an oxygen-sensing mechanism (Adachi et al. 2004; Shibahara et al. 2007). Intriguingly, a large body of circumstantial evidence, including human genetic variant association (Shibahara 2003; Bao et al. 2010), suggests a potential role for these enzymes of heme catabolism in the protection against the development of a range of chronic diseases, including type-2 diabetes, obesity and oxidative damage, resulting from hyperglycemic conditions, known as glucose toxicity (Goodman et al. 2006; Ndisang et al. 2009; Sodhi et al. 2009; Hosick and Stec 2011). A lack of direct evidence has made the heme catabolism-diabetes link speculative. To date, long-sought direct evidence has emerged from diabetic/obese phenotypes of the HO-2 knockout mouse model with additional evidence coming from a recent study by Li and co-workers (2012). These new lines of evidence are discussed below together with a brief overview of HO-1 and HO-2 functions.

**Known functions of HO-1 and HO-2**

**Iron recycling:** In concert with NADPH-cytochrome P450 reductase, HO-1 and HO-2 catalyse heme degradation with the resultant release of iron, carbon monoxide (CO) and biliverdin IXα (Shibahara 2003; Shibahara et al. 2007; Khan and Quigley 2011). Biliverdin IXα is converted to bilirubin almost instantly by biliverdin reductase. Degradation of heme by HO-1 and HO-2 to retrieve iron occurs at the expense of the reducing equivalent NADPH (H+). In an adult human, about 75% of the total 400 mg bilirubin produced daily is derived from hemoglobin (Khan and Quigley 2011). The economy of iron utilization requires salvaging of iron so the bulk of iron release by HO-1 and HO-2 is reutilized in the synthesis of hemoglobin and other hemoproteins, including nitric oxide synthase, various enzymes of the mitochondrial respiratory chain and

<table>
<thead>
<tr>
<th>Immortalized, non-cancer cells and experimental conditions</th>
<th>Characteristics of cadmium-transformed cells and resultant tumors in nude mice</th>
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<tr>
<td>Rat liver epithelial TRL1215 cells. TRL1215 cells were continuously exposed to cadmium at 2.5 μM for 10 weeks (Takiguchi et al. 2003) or 1 μM for 28 weeks (Qu et al. 2005).</td>
<td>Cadmium-transformed TRL1215 cells displayed typical cancer phenotypes; hyperproliferation, increased invasiveness, and decreased dependency on serum for growth.</td>
</tr>
<tr>
<td>Human prostate epithelial RWPE-1 cells. RWPE-1 cells were continuously exposed to cadmium at 10 μM for 8 weeks (Achanzar et al. 2001).</td>
<td>Cadmium-transformed cells displayed distinctive morphological changes, compared with the passage-matched control cells. Resultant tumors in nude mice were poorly differentiated adenocarcinomas. About 80% of these tumors invaded into the subdermal muscle, fat, or connective tissue with one lung metastasis.</td>
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<tr>
<td>Human urothelial UROtsa cells. The UROtsa cells were continuously exposed to cadmium at 1 μM for 8-10 weeks (Sens et al. 2004).</td>
<td>The UROtsa cells did not form any colonies in soft agar, but cadmium-transformed UROtsa cells did form colonies in soft agar. Resultant tumors in nude mice had histological phenotypes of human transitional cell carcinoma (TCC).</td>
</tr>
<tr>
<td>Human breast epithelial MCF-10A cells. MCF-10A cells were continuously exposed to cadmium at 2.5 μM for 40 weeks (Benbrahim-Tallaa et al. 2009).</td>
<td>Cadmium-transformed MCF-10A cells displayed phenotypes of basal-like breast carcinoma; estrogen receptor (ER)-alpha negative, human epidermal growth factor receptor 2 (HER2) negative, diminished expression of breast cancer susceptibility gene 1 (BRCA1), persistent cell proliferation and elevated expression of cytokeratin 5 and p63.</td>
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<td>Human bronchial epithelial BEAS-2B cells. BEAS-2B cells were continuously exposed to cadmium at 1 μM for 6 months (Jing et al. 2012). Cells were weekly passaged and passage-matched cells serves as a control.</td>
<td>Cadmium-transformed BEAS-2B cells had aberrant activation of extracellular signal-regulated kinases (ERK) and AKT signalling with elevated expression of hypoxia-inducible factor-1 (HIF-1) and VEGF. ERK, AKT, p70S6K1 activation and HIF-1α expression were attenuated by inhibition of ROS generation.</td>
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<td>Human pancreatic ductal epithelial cells (HPDE). HPDE cells were continuously exposed to cadmium at 1 μM for 29 weeks (Qu et al. 2012).</td>
<td>Cadmium-transformed HPDE cells secreted elevated levels of matrix metalloproteinase-9 (MMP-9) and over-expressed the pancreatic cancer marker S100P. In soft agar, cadmium-transformed HPDE cells produced poorly differentiated glandular-like structures.</td>
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the cytochrome P450 super family (Schultz et al. 2010). This makes heme degradation by HO-1 and HO-2 indispensable. Two other products of heme degradation, namely carbon monoxide (CO) and bilirubin, are known for their anti-inflammatory and antioxidant properties (Shibahara 2003; Shibahara et al. 2007; Khan and Quigley 2011). These add to the biological importance of the HO-1 and HO-2 enzyme system.

**Protection against obesity and oxidative stress-related diseases:** HO-1 expression levels exhibit circadian (day-night) cycles (Yin et al. 2007; Wu et al. 2009). HO-1 expression is regulated by the transcription factor network, including CLOCK, Bmal and Per, that works in concert to generate day-night cyclic expression of the genes involved in energy metabolism (Bass and Takahashi 2010; Huang et al. 2011; Dang 2012). Disruption of diurnal cycle caused obesity in mice (Sahar and Sassone-Corsi 2009). Expression levels of HO-1 are controlled by levels of its own substrate (heme) (Furuyama et al. 2007; Yin et al. 2007) and fluctuate with changes in the cellular microenvironment, such as levels of chemical and physical stressors of both endogenous and exogenous origin (Shibahara et al. 2007). HO-1 expression also depends on various conditions, including the levels of glucose, oxygen, and shear stress occurring in vascular system (Shibahara et al. 2007). Thus, HO-1 induction is viewed as the first line cellular defense mechanism the body utilizes to protect against the development of chronic oxidative stress-related diseases. This has been an area of intense research and considerable advances have been achieved, at least in experimental disease models (Goodman et al. 2006; Ndisang et al. 2009; Sodhi et al. 2009). For example, in the Goto-Kakizaki rats, a model for hyperglycemia and insulin resistance without obesity (Goto et al. 1976), induction of HO-1 causes a reduction in fasting blood glucose levels and prevents a rise in blood glucose in post absorptive state (Ndisang et al. 2009). In an obese mouse model of type-2 diabetes, induction of HO-1 prevents weight gain, decreases visceral and subcutaneous fat content, and improves both insulin sensitivity and glucose tolerance (Li et al. 2008). These data from Goto-Kakizaki rats and from an obese mouse model support a heme oxygenase-glucose link, observed earlier in humans as discussed below.

**Maintenance of blood glucose levels:** Because CO is produced exclusively by HO-1 and HO-2, levels of exhaled CO serve as a useful biomarker for heme degradation activity. In healthy individuals, levels of exhaled CO increase with increasing blood glucose and both exhaled CO and blood glucose levels return to their respective baseline values 40 minutes after glucose administration. These data suggest that levels of HO activity may influence blood glucose levels (Paredi et al. 1999). The relationship between exhaled CO and blood glucose has been observed as well in type-2 diabetic subjects. As expected, the levels of exhaled CO are greater in diabetic subjects, compared to non-diabetic controls (Paredi et al. 1999). The elevated CO exhaled in diabetic subjects is attributable to HO-1 induction in response to rising oxidative (high-glucose) stress, reflected by elevated levels of damage proteins such as glycosylated haemoglobin. The exhaled CO-blood glucose correlation implies that exhaled CO can be used in monitoring disease progression in type-2 diabetes patients (Paredi et al. 1999). Measurement of exhaled CO provides an attractive alternative to blood glucose measurement because of the non-invasive nature of sample collection. Further methodological development is needed to address the potential interference from exposure to exogenous CO from automobile exhaust, polluted air and from cigarette smoking.

**Similarities and differences between HO-1 and HO-2**

HO-1 and HO-2 are products of two different genes (Shibahara et al. 1993). The human HO-1 gene promoter is unique due to the presence of GT repeats of varying length. The repeats are not present in rats or mice (Shibahara 2003; Shibahara et al. 2007). Increased risk for type-2 diabetes is noted in humans with longer GT repeats (Bao et al. 2010). The catalytic domains of both HO-1 and HO-2 enzymes are highly homologous, sharing 93% of their amino acid sequences. HO-2, however, contains an additional domain, which has Cys-Pro dipeptide motifs, allowing for heme binding and for interactions with other proteins (McCoubrey et al. 1997).

The presence of a distinctive domain only in HO-2 has led to the hypothesis that HO-2 has an unknown regulatory function through its interaction with other protein(s) in cellular regulatory circuits. Indeed, using protein microarray, Li et al. (2012) found that HO-2 protein could interact with at least ten proteins. One of those proteins is 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase isofrom 4 (PFKFB4). There are further differences between these two proteins to suggest that each may participate in cell functions other than iron recycling. HO-1 and HO-2 both are localised to the endoplasmic reticulum, while HO-1 has also been localized to mitochondria, nucleus and plasma membrane caveolae together with nitric oxide synthase and biliverdin reductase (Khan and Quigley 2011). The presence of HO-1 in the nucleus suggests that HO-1 may also have a regulatory role on gene expression.

**Novel functions of HO-2**

**Anti-diabetogenic action of HO-2, not replaceable by HO-1:** HO-2 deficiency causes neither lethality nor infertility. A HO-2 knockout mouse reproduces offspring that undergo normal development to adulthood, but develops the symptomatic spectrum of human type-2 diabetes; hyperglycemia, increased fat deposition, insulin resistance and hypertension with aging (Goodman et al. 2006; Sodhi et al. 2009; Li et al. 2012). Normal fertility and normal development in HO-2 knockout mice suggests that HO-1 could compensate for HO-2 functions such as iron recycling, anti-inflammation and anti-oxidative stress. However, HO-1
could not compensate for anti-diabetogenic function of HO-2, thereby suggesting such function is unique to HO-2 (Li et al. 2012). Surprisingly, sparse data exist to explain how HO-2 deficiency brings about obesity and diabetic symptoms.

**PKFKB4 provides a connecting dot:** In a quest to unravel novel HO-2 functions, Li and co-workers have pursued several lines of investigation, including protein microarray technology (Li et al. 2012). Their protein microarray has given them the first clue to link HO-2 with glycolysis, seen in an interaction between HO-2 protein and PFKFB4. This is the key regulator of glycolysis in liver (Okar et al. 2001). Inhibition of PFKFB4 activity causes apoptosis in liver cancer cells (Jeon et al. 2011). Co-incidentally, this particular enzyme of the glycolytic pathway has attracted a great deal of attention in recent years following a breakthrough discovery made by several research groups on the heavy dependence of various cancer cells on PFKFB enzyme (Yalcin et al. 009; Goidts et al. 2011; Ros et al. 2012) and glycolysis for their survival (Vader Heiden et al. 2009; Ferreira 2010; Koppenol et al. 2011). Another example of the cancers whose survival depends on PFKFB/glycolysis is the glioblastoma, a common type of brain tumor in adults (Goidts et al. 2011). The metabolic requirement of cancer cells, first observed by Warburg, is now known as the Warburg effect (Warburg 1956; Wu et al. 2007; Vader et al. 2009). The Warburg hypothesis on an enhanced glycolysis and impaired mitochondria in cancer causation provides basis for the application of positron emission tomography (PET) in cancer diagnosis (Gambhir 2002).

**HO-2 and the brain:** HO-2 is expressed abundantly in the brain, of interest since the brain constitutes only 2% of body weight, but consumes up to 20% of total consumption of both oxygen and glucose (Bolaños et al. 2010). High rates of glucose and oxygen utilization by the brain have been attributable to high rates of glycolysis, particularly in astrocytes. Such high rates are achieved by high activity levels of PFKFB isoform 3 (PKFB3) which is expressed most abundantly in the astrocytes (Bolanos et al. 2010). The neurons, however, lack PFKFB3 protein expression. In consequence, glucose is shunted through the pentose phosphate pathway (Bolanos et al. 2010). The physiological significance of HO-2 expression in these two major cell types (astrocytes and neurons) in the brain requires further studies. In a recent study, Morikawa et al. (2012) have demonstrated another novel role of HO-2 in control of basal energy metabolism in the cerebral cortex region of the brain. In normal oxygen tension, the cerebral cortex of HO-2 deficient mice had higher ATP levels than in wild-type mice. In low oxygen tension (hypoxia), the cerebral cortex ATP levels were maintained in wild-type mice, but they were decreased by 50% in the HO-2 deficit mice (Morikawa et al. 2012). The reasons for impaired ability to maintain ATP levels in HO-2 deficient mice require further studies as they may provide an important clue to the understanding of the expression of obese phenotype in HO-2 knockout mice.

**HO-2 involvement in glucose sensing mechanism:** To investigate further the HO-2 and PFKFB4 interaction shown in the protein microarray experiments, Li and co-workers turned to HepG2 cells (Li et al. 2012). HepG2 cells are human hepatocellular carcinoma cells that retain crucial characteristics, especially glucose metabolism of human hepatocytes, which fitted with the aims Li and co-workers study. Under high glucose conditions, mRNA and protein levels for HO-2 and PFKFB4 in HepG2 cells are up-regulated (Li et al. 2012). This results in the stimulation of glycolysis in these cells. In the opposite direction, mRNA and protein levels of HO-1 are down-regulated by high glucose. Further support of HO-2/PFKFB4 co-regulation comes from gene knockdown experiments with small interfering RNA. In gene knockdown experiments, Li and co-workers observed a drastic reduction in PFKFB4 mRNA levels following silencing of the HO-2 gene (Li et al. 2012). There is no change in PFKFB4 mRNA levels following the HO-1 gene silencing. This supports a specificity of HO-2-PFKFB4 co-regulation mechanism.

Under glucose deprivation conditions, HO-1 up-regulation prevails, with no change in HO-2 expression, but a reduction in PFKFB4 expression. In effect, there would be low glycolysis in HepG2 cells under glucose deprivation conditions. Li and co-workers postulate that increased heme catabolism via HO-1 up-regulation blunts the rate of glycolysis in liver. Because heme is known to have inhibitory effects on glucose production (gluconeogenesis) (Yalcin et al. 009; Goidts et al. 2011; Ros et al. 2012), up-regulation of HO-1 is a logical response as it can lower intracellular heme concentrations in order to take liver glucose production for its own use and to release it into the circulation to maintain blood glucose levels. Dampening of glycolysis is required to prevent a futile cycle.

**HO-2 deficiency: a case of regulatory defect:** Changes in HO-1 and HO-2 expression levels in the HepG2 cells in response to low- and high-glucose can be translated to the equivalent in vivo conditions, namely fasting and post absorptive states. As shown in Fig. 1, lowered glycolysis with enhanced gluconeogenesis, seen in wild-type mouse liver in the fasting state, could be achieved by HO-1 up-regulation plus PFKFB4 down-regulation. In the post-absorptive state, high glycolysis with suppressed gluconeogenesis could be achieved by HO-1 down-regulation plus HO-2 and PFKFB4 up-regulation. Both HO-1 and HO-2 are required to prevent a fall or a rise in blood glucose levels during fasting and post absorptive periods, respectively. HO-2 expression ensures PFKFB4 expression.

Failure in any of these (HO-1, HO-2 and PFKFB4) causes the development of hyperglycemia and type-2 diabetes as seen in HO-2 knockout mice. Indeed, HO-1 protein expression levels fell by 35-40% in the livers of HO-2 knockout mice (Han et al. 2009; Sodhi et al. 2009). Although reduction in HO-1 protein expression could render the liver susceptible to oxidative damage, the HO-1
repression is an example of metabolic adaptation to safeguard the cellular redox state. This could be achieved by utilizing NADPH (H+) for regenerating reduced glutathione (GSH) rather than for heme catabolism. GSH recycling is a mechanism for maintenance of cell redox state. It is central to normal protein folding and cell function. PFKFB4 expression is regulated by the transcriptional factor, known as hepatocyte nuclear factor-6 (Hnf-6) (Rider et al. 2004). Hnf6-knockout caused diabetes in mice (Lannoy et al. 2002). PFKFB4 protein phosphorylation, mediated by the c-AMP dependent protein kinase A (PKA) caused a fall in fructose 2,6 bisphosphate (F-2,6-P₂) in liver, thereby increasing gluconeogenesis with concomitantly reducing glycolysis (Rider et al. 2004).

For the reasons discussed above, PFKFB4 protein detected in HO-2 knockout mouse liver samples (Li et al. 2012) requires further studies to determine if the detectable PFKFB4 protein converts fructose-6-phosphate to F-2,6-P₂. This would ensure that high glycolysis rates do occur. This may explain increased susceptibility to cancer, especially liver cancer, in diabetic subjects because active PFKFB4 and high glycolysis are known metabolic phenotypes of cell proliferation and cell survival; the characteristics of cancer-prone cells (Vader Heiden et al. 2009; Yalcin et al. 2009; Ferreira 2010; Koppenol et al. 2011; Goidts et al. 2011; Ros et al. 2012).

Metabolic effects and liver cancer susceptibility

**Effects of cadmium on cellular intermediary metabolism**

Data from cohort studies consistently identified the liver of subjects with type-2 diabetes with or without obesity to be at-risk of cancer transformation (Borena et al. 2012; Campbell et al. 2012; Wang et al. 2012). The diagram (Fig. 3) shows metabolic interrelationships between glucose, heme, and glutathione in the liver together with sites of cadmium’s effects. We indicate several sites where cadmium exerts its effects on liver intermediary metabolism. One is on heme degradation. Cadmium is a potent HO-1 inducer (Takeda et al. 1994; Satarug et al. 2008) and thus it has the propensity to impact intracellular heme concentrations and causing enhanced gluconeogenesis (Fig. 2). Cadmium increased expression of enzymes of gluconeogenesis, namely pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase, fructose1,6-biphosphatase and glucose-6-phosphatase. Cadmium increases glycogenolysis with resultant liver glycogen depletion. We thus postulate that cadmium causes over production of glucose, resulting in hyperglycaemia in fasting stage and that persistent hepatic gluconeogenesis and/or impaired ability to suppress hepatic gluconeogenesis after a meal cause fed-state hyperglycaemia in cadmium-induced diabetes.

Another site of cadmium effects is on the GSH recy-
Cadmium Exposure and Adverse Health Effects

Cadmium can increase the demand for NADPH in GSH regeneration and heme catabolism due to HO-1 induction by cadmium. Consequently, glucose is metabolized via the pentose phosphate pathway for NADPH generation rather than via mitochondrial oxidative phosphorylation for ATP production. In light of the data showing effects of cadmium on mitochondrial oxidative phosphorylation in human proximal tubular cells, leading to less ATP production (Gobe and Crane 2010), impaired ability to generate and to maintain optimal cellular ATP levels may occur in cadmium-exposed cells. Supporting the mitochondrial effects of cadmium in humans, Ellis et al. (2012) reported cadmium exposures to be associated with abnormal mitochondrial function, reflected by abnormal urinary excretion of mitochondria markers such as citrate, 3-hydroxyisovalerate, and 4-deoxyerythronic acid. Cadmium thus may mimic HO-2 deficiency in causing impaired ability to maintain cellular ATP levels as observed in the cerebral cortex of HO-2 knockout mice in hypoxia (Morikawa et al. 2012).

Liver inflammation

Work with isolated hepatocytes and human liver cell lines identified two possible routes of hepatic uptake of cadmium. One is for an unbound, free (ionic) form of cadmium and the other is for protein-bound form. Free ionic cadmium (Cd^{2+}) is probably taken up by the same metal transporters that liver cells use to acquire physiologically essential metals, notably iron, manganese, zinc and copper. Protein-bound cadmium is thought to enter liver cells by receptor-mediated endocytosis (Sabolic et al. 2010; Thevénod 2010; Vesey 2010). Following internalization, the bound protein is digested in the lysosome with the release of free ionic cadmium. In an in vitro experiment, the free ionic form of cadmium (CdCl_{2}) induced expression of cytokines (TNF-α and IL-1β) in HepG2 cells (Souza et al. 2004). These inductive effects of free cadmium on pro-inflammatory cytokines remain to be substantiated with in vivo experiments because the vast majority of cadmium in blood circulation is protein bound with extremely low concentrations of unbound form.

Sabolic and co-workers (2010) have shown that pro-
tein (metallothionein) bound cadmium, administered intravenously, does not enter hepatocytes, but it does enter the Kupffer cells. This observation raises the possibility that internalization of protein-bound cadmium by the Kupffer cells could lead to the release of various pro-inflammatory cytokines, including interleukin 6 (IL-6), and tumor necrosis factor-alpha (TNF-α). Subclinical liver inflammation may develop following cadmium exposure. Intriguingly, IL-6 has been shown to be involved in liver cancer development in a mouse model used for demonstration of increased liver cancer risk in male sex (Naugler et al. 2007). Additionally, inhibitory effects of estrogens on IL-6 production may explain lower liver cancer risks in females, compared to males. The potential role of cadmium in liver inflammation is supported by data from adult participants in the NHANES III, which showed correlations between blood cadmium and increased serum alkaline phosphatase (a marker of liver inflammation), increased prevalence of diabetes and hypercholesterolemia (Cheung et al. 2009). Lee et al. (2006) found urinary cadmium and blood lead associated with the marker of inflammation, namely serum gamma-glutamyltransferase (GGT) among 10,098 adult participants in the NHANES III after adjusting for race, sex, and age.

Epigenetic effects and metabolic adaptation

Prominent roles for cadmium in causing aberrant epigenetic alterations together with induction of cellular adaptation have emerged from molecular analysis of cadmium-induced neoplastic transformation of rat and human cell lines, listed in Table 2. A 10-fold increase in expression levels of the oncogenes c-myc and c-jun together with change in DNA methylation state was observed in the transformation of the TRL1215 rat liver epithelial cells (Takiguchi et al. 2003; Qu et al. 2005). Global DNA hypomethylation and over expression of c-myc and k-ras were observed in cadmium-induced transformation of the MCF-10 human breast epithelial cells estrogen receptor (ER)-negative to ER-positive breast cancer cells (Benbrahim-Tallaa et al. 2009). In neoplastic transformation of the human epithelial RWPE-1 cells, cadmium caused diminished expression of the tumor suppressor genes (RASSF1A and p16) while causing over expression of DNMT3b and generalized DNA hypermethylation (Benbrahim-Tallaa et al. 2007).

The epigenetic effects of cadmium in humans are supported by increased expression of miRNA-146a detected in peripheral white blood cells from workers who were exposed to cadmium by inhalation (Bollati et al. 2010). The increased expression of miRNA-146a by cadmium may represent inflammatory response because miRNA-146 expression is known to be regulated by the NF-κB transcription factor, involved in inflammation and innate immunity (Williams et al. 2008; Curtale et al. 2010). Hossain et al. (2012) showed further evidence for cadmium epigenetic effects in peripheral leucocytes from 202 non-smoking women from the northern Argentinean Andes. They found cadmium exposure associated with DNA hypomethylation of CpG islands of long interspersed nuclear element-1 (LINE-1). Further, they found that the association between cadmium and DNA hypomethylation was modified by the genotypes of DNA (cytosine-5-)methyltransferase 1 gene (DNMT1) (Hossain et al. 2012).

Hypothetical liver cell model showing molecular targets of cadmium carcinogenicity

We constructed a hypothetical liver cell model (Fig. 4) to illustrate molecular targets and metabolic impairments imposed by chronic liver exposure to cadmium. Cadmium increases expression of MT via metal response element (MRE). Cadmium induction of MT results in sequestration of cadmium, thereby preventing acute toxicity. However, zinc and copper are also sequestered along with cadmium. This can reduce the availability of zinc and copper for the antioxidant enzyme superoxide dismutase (SOD) such as Zn/Cu-SOD in the cytosol and Mn-SOD in mitochondria. Increased intracellular GSH concentration (high GSH/GSSG ratio) noted in chronic exposure conditions (Liu et al. 2009) could reflect one of the metabolic adaptations to compensate for reduced SOD activity.

Cadmium increases expression of HO-1 via the cadmium response element (CdRE), and Maf recognition antioxidant response element (MARE), also known as stress response element (StRE) (Simmons et al. 2011). Cadmium suppresses lysosomal degradation of Nrf2 (Steward et al. 2003) and causes nuclear export of the repressor Bach1, which allows transactivation of the HO-1 gene by the Nrf2/small Maf complex (Suzuki et al. 2003). HO-1 induction by cadmium via Nrf2 signalling pathway increases cellular concentrations of antioxidant (bilirubin), anti-apoptotic and anti-inflammatory agent (CO) which can reduce liver damage (Wu et al. 2012) and make the liver resistant to apoptosis.

Cadmium increases expression of pyruvate carboxylase (PC), which produces an intermediate for the generation of succinyl CoA used in heme synthesis. Cadmium affects mitochondrial ATP production with resultant increases in AMP/ATP ratios. Cadmium causes transactivation of oncogenes, which are now known to drive glycolysis and cancer-cell transformation (Dang, 2012; Johnson and Perkins 2012; Moretti et al. 2012). In addition, HO-1 induction alters expression of certain CYP isoforms. Work in our laboratory indicates cadmium affects liver expression of CYP2C9, CYP4A11, CYP2E1 and CYP2A6 (Baker et al. 2001, 2005; Satarug et al. 2004a,c, 2006a,b). CYP2A6 and CYP2E1 are involved in the activation of liver carcinogens. Induction of CYP2A6 and CYP2E1 by cadmium could be another mechanism underlying increased susceptibility to liver disease and liver cancer since a CYP-mediated reaction is a known source of free radicals that cause mitochondrial malfunction (Gobe and Crane 2010) and DNA damage (Alexandrov et al. 2010).
Research gaps and future perspectives

A large number of population-based studies presented in the first part of this review provide compelling evidence of the toxic effects of chronic exposure to cadmium 10 fold lower than the level causing a mild renal tubular impairment. Of principal concern is the significant proportion of the general population at risk of cadmium over-exposure. Satarug (2012) reported that dietary cadmium exposure in Bangkok (Thailand) resulted in urinary cadmium ≥ 1 μg/g creatinine in 22.5% of women who never smoked. In the USA, environmental exposures resulted in urinary cadmium ≥ 1 μg/g creatinine in 4.8% of non-smokers and 20.8% of smokers, although the population mean urinary cadmium was below 0.5 μg/g creatinine (Mortensen et al. 2011). These data call for measures to be implemented to minimize cadmium exposure from all sources, especially diet. Soil-cadmium contamination and high rates of soil-to-plant transfer of cadmium need to be addressed. Whether cadmium in foods is natural or anthropogenic makes no difference to its effects on human health. Research into ways of reducing cadmium accumulation in major human staple foods such as rice grains (Uraguchi and Fujiwara 2012) should be encouraged. Health-based risk assessment indicates that cadmium content of staple foods should be no greater than 0.1 mg/kg. This level is 4 fold lower than the recently established maximum permissible concentration of cadmium in rice of 0.4 mg/kg.

In 1967, Schroeder and Buckman described hypertension in rats exposed to low-level cadmium. In 1980, Merali and Singhal demonstrated diabetogenic effects of cadmium in neonatal rats. Waalkes and Rehm (1994) demonstrated cadmium carcinogenesis in mice. Almost three decades after these reports of hypertensive, diabetogenic and carcinogenic effects of cadmium in experimental animals, cadmium exposure in the general population of the USA has been found to be associated with increased risks of pre-diabetes, type-2 diabetes (Schwartz et al. 2003), hypertension (Tellez-Plaza et al. 2008) and cancer mortality (Adams et al. 2012; Tellez-Plaza et al. 2012b). In addition, cadmium exposure has been associated with learning disability in children (Ciesielski et al. 2012) and hearing disability in adolescents (Shargorodsky et al. 2011) and adults in the USA (Choi et al. 2012). These findings suggest cadmium...
has impacts on neurodevelopment and impairs function of the inner ear.

An observation made by Li et al. (2012) on an existing link between heme catabolism and glucose utilization via glycolysis provides the first biologically-plausible mechanism underlying increased cancer risk in type-2 diabetes. It is likely that similar mechanisms are operating in humans. Clearly, further research is warranted to fully characterize the heme-glucose phenomenon. It is fundamental to metabolic adaptation requirements in all nucleated cells. Such research should be directed toward identification of cellular signalling pathways responsive to changes in glucose levels, especially liver and kidney, known as glucose suppliers (Stumvoll et al. 1997; Gerich 2010; Defronzo et al. 2012), and the brain (astrocytes and neurons), all known as the principal users of glucose and oxygen. A role for Wnt/β-catenin signalling in the glucose-sensing mechanism has been shown in macrophage cells (Anagnostou and Sheperd 2008), and its connection to heme catabolism needs to be explored. Proteomic analysis identified glucose metabolism in mouse liver as the target of β-catenin signalling pathway (Chafey et al. 2009). Polymorphism in the β-catenin effector gene TCF7/L2 was associated with increased risk of type-2 diabetes (Tong et al. 2009).

Accumulating human data suggest abnormal liver and kidney metabolic function in type 2-diabetic subjects (Meyer et al. 1998; Gerich 2010; Defronzo et al. 2012). Expansion of research to other cell types, notably adipose tissues and the brain, is of particular scientific merit, in light of obese phenotype of HO-2 deficient mice and since HO-2 is expressed prominently in the brain. Both HO-1 and HO-2 may well have anti-cancer effects, independent of their heme degradation activity akin to the anti-diabetogenic action of HO-2. Data from protein microarray experiments of Li and co-workers (2012) are thus a resource awaiting scientific scrutiny.

A useful outcome from such research would be a solid conceptual framework, based on impacts of environmental chemicals on heme-glucose metabolic interrelationships. The concept can then be moved forward to test if human exposure to numerous environmental chemicals over the past two decades contributes to the current worldwide rise in the incidence of type-2 diabetes (Nolan et al. 2011; Ramachandran et al. 2012) and increased cancer risks in type-2 diabetic subjects.

Both genetic and environmental factors are involved in development of chronic diseases such as type-2 diabetes, and cancer. Environmental factors could contribute up to 70 to 90% of disease risks. A wide range of environmental chemicals including cadmium are suspected causative factors. It remains unclear how the environmental chemicals of diverse structures and properties exert their influences and yet produce similar pathologic outcomes. Rappaport and Smith (2010) have coined the term “exposome” to call for a new paradigm to assess how a lifetime of exposure to numerous environmental chemicals influences the risk of developing chronic diseases.

Evidence is accumulating to link epigenetic alterations (DNA methylation, histone modification and micro RNA) with carcinogenicity of a large group of toxicants, including cadmium, that lack an ability to cause gene mutation (Huang et al. 2008; Hou et al. 2012; Stein 2012). Abnormal methylation or one-carbon metabolism has emerged as one of the potential mechanism that could underlie cadmium epigenetic effects. This was first observed in rats chronically fed with methyl-deficient diets (Poirier and Vlasova 2002) and later seen in studies of human urinary metabolite profiles (Ellis et al. 2012). The new challenge now is to probe mechanisms of cadmium causation of epigenetic changes for which strategies for reversal of effects and prevention of effects can be developed.

Cadmium will continue to pose a real threat to human health. There is an urgent need for public measures to reduce current population exposures from both dietary and smoking sources. A lack of therapeutically effective chelating agents to enhance the body’s excretion of cadmium makes exposure prevention pivotal. Long-term management of cadmium in the environment and in agriculture is required to minimize the food-chain transfer of cadmium, notably from use of phosphate fertilizers. The indefinite persistence of cadmium in the environment requires a long-term approach to minimization of human exposure through environmental management and maintenance of lower cadmium levels wherever possible.

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Conflict of Interest

The authors declare no conflict of interest.

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