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# Early biomarkers of cadmium exposure and nephrotoxicity

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**Abstract** As the risks of cadmium (Cd)-induced kidney disease have become increasingly apparent, much attention has been focused on the development and use of sensitive biomarkers of Cd nephrotoxicity. The purpose of this review is to briefly summarize the current state of Cd biomarker research. The review includes overviews of the toxicokinetics of Cd, the mechanisms of Cd-induced proximal tubule injury, and mechanistic summaries of some of the biomarkers (*N*-acetyl-$\beta$-D-glucosamidase; $\beta_2$-microglubulin, metallothionein, etc.) that have been most widely used in monitoring of human populations for Cd exposure and nephrotoxicity. In addition, several novel biomarkers (kidney injury molecule-1, $\alpha$-glutathione-S-transferase and insulin) that offer the potential for improved biomonitoring of Cd-exposed populations are discussed.

**Keywords** Cadmium · Biomarkers · Kidney · Nephrotoxicity · Proximal tubule

## Cd as an environmental health problem

The importance of Cd as an environmental health problem has become increasingly apparent over the past 50 years (Jarup and Akesson 2009; Nordberg 2004, 2009). Since the beginning of the industrial revolution, large amounts of Cd have been released from the lithosphere into the environment. Cd and its compounds have been used extensively in the electroplating industry, and in the manufacturing of batteries, dyes, paints, plastics and consumer electronics (ATSDR 2008b). Cd has also been released into the environment through mining and smelting activities, the burning of refuse materials that contain Cd, and the use of Cd-contaminated sludge and phosphate salts as fertilizers (ATSDR 2008b). As a stable divalent metal, Cd is not biodegradable and it persists in the environment for long periods of time.

Humans are usually exposed to Cd in the workplace or through the ingestion of Cd-contaminated food or water (ATSDR 2008b; Jarup and Akesson 2009). In addition, tobacco contains significant amounts of Cd and smoking is one of the primary sources of Cd exposure in the general population (Mannino et al. 2004; Menke et al. 2009; Satarug and Moore 2004; Scherer and Barkemeyer 1983). Exposure to Cd can result in a variety of adverse effects. Depending on the dose, route and duration of exposure, Cd can damage various organs including the lung, liver, kidney, bone, pancreas and endocrine system (for reviews see (ATSDR 2008b; Bernard 2008; Byrne et al. 2009; Edwards and Prozialeck 2009; Jarup et al. 1998; Jarup and Akesson 2009); it has also been classified as a human carcinogen (ATSDR 2008b; IARC 1993; Joseph 2009; Waalkes 2003; Waisberg et al. 2003). Various aspects of Cd

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exposure patterns, carcinogenesis and organ toxicity are reviewed in the accompanying articles in this journal.

With the chronic, low-level patterns of exposure that are common in human populations, the kidney is the primary target of toxicity, where Cd accumulates in the epithelial cells of the proximal tubule, resulting in a generalized reabsorptive dysfunction that is characterized by polyuria, glucosuria and low molecular weight proteinuria (Gonick 2008; Jarup 2002; Kjellstrom 1986; Thevenod 2003). Recent studies have highlighted the fact that adverse renal effects of Cd may result from even low levels of exposure and that women, children and individuals with confounding health conditions, such as diabetes, may be especially susceptible (Akesson et al. 2005; ATSDR 2008b; de Burbure et al. 2006; Friedman et al. 2006; Hellstrom et al. 2001; Jarup 2002; Navas-Acien et al. 2009; Nawrot et al. 2008; Satarug et al. 2003; Thomas et al. 2009). Despite efforts by many countries and international agencies to reduce the usage and release of Cd into the environment, Cd pollution continues to represent a major public health concern in many parts of the world (Jarup and Akesson 2009; Satarug et al. 2003; Satoh et al. 2002). The problem appears to be especially serious in emerging industrial nations where environmental controls are still being developed (Bandara et al. 2008; Nordberg 2004; Satarug et al. 2003; Teeyakasem et al. 2007). In addition there is evidence that Cd exposure from natural sources such as soil and volcanic activity may represent a hazard in some regions (Haswell-Elkins et al. 2008; Moriguchi et al. 2009b; Tang et al. 2009).

## Monitoring of human populations

One of the major challenges in the fields of public health and Cd toxicology has involved the monitoring of at risk populations for early signs of exposure and toxicity (Bernard 2004; Fowler 2009; Jarup and Akesson 2009; Mueller et al. 1998; Staessen et al. 1996). Intuitively, it might seem that the most direct way to monitor levels of Cd exposure would be to simply measure blood or urinary levels of Cd. However, this issue is greatly complicated by the unique toxicokinetics of Cd in the body, where the tendency of Cd to be sequestered in organs such as liver and kidney is especially problematic. While blood levels of Cd can yield information regarding recent exposures, they often do not provide information regarding the total body burden of Cd or the severity of injury in specific target organs. Likewise, the monitoring and interpretation of data on urinary levels of Cd are not as straightforward as one might expect. With low, or even moderate, levels of exposure, any Cd that is filtered at the glomerulus is almost completely reabsorbed by epithelial cells of the proximal tubule; little or no Cd is excreted in the urine. It is only when the body burden of Cd is fairly large and/or kidney injury begins to appear that urinary excretion of Cd increases significantly. As a result of these limitations in interpreting data on blood and urinary levels of Cd, investigators have utilized various biomarkers to assess levels of Cd exposure and toxicity.

The United States National Institutes of Health have broadly defined the term "biomarker" as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Biomarkers Definition Working Group 2001). In the present discussion, we will consider the topic of Cd biomarkers from a more narrow perspective, as "any substance or molecule that can serve as a indicator of the functional state or level of toxic injury in an organism, organ, tissue or cell".

As a result of Cd's tendency to accumulate in epithelial cells of the proximal tubule, the kidney is usually the primary critical target organ of Cd toxicity in the body. The kidney is, in effect, a sentinel of Cd exposure. Consequently, many of the most useful biomarkers of Cd exposure and toxicity have been markers of the various effects of Cd in the kidney.

From a historical perspective, it is noteworthy that many of the primary advances in the biomonitoring of human populations for Cd exposure occurred in the mid 1980's–early 1990's. It was during this time frame that some of the most widely-used urinary biomarkers such as $\beta_2$-microglobulin, metallothionein, and *N*-acetyl-$\beta$-D-glucosamidase (NAG) were first characterized and validated (Kawada 1995; Koyama et al. 1992; Lauwerys et al. 1994; Nordberg 2009; Shaikh and Tohyama 1984; Shaikh and Smith 1986). These classical markers have provided essential tools for investigators to evaluate and define the levels of Cd exposure that may result in adverse

effects in human populations. However, as results of recent studies with these traditional biomarkers have shown that the problems associated with even low levels of Cd exposure may be more serious than previously thought, there has been a growing interest in the development of more sensitive markers of Cd exposure and toxicity. The purpose of this review is to briefly summarize the current state of Cd biomarker research. This is not intended to be a comprehensive review, but rather a summary of key concepts and emerging issues in this area. For more detailed discussions of specific biomarkers the reader is referred to several outstanding reviews by other authors (Bernard 2004; Fels 1999; Fowler 2009; Nordberg et al. 2005; Pinot et al. 2000; Roels et al. 1999; Staessen et al. 1996). The primary focus of the present review will be on those biomarkers that have proven to be most useful in monitoring of human populations. However, pertinent findings from experimental studies in animals will also be discussed. In addition, several novel biomarkers that offer the potential for improved biomonitoring of Cd-exposed populations will be considered.

## Cd toxicokinetics

Before discussing the specific markers of Cd-induced kidney injury, it is first necessary to consider some aspects of the toxicokinetics of Cd. This topic has previously been the subject of several excellent reviews (Jin et al. 1998; Nordberg et al. 1986; Nordberg 1984; Zalups and Ahmad 2003). Therefore, only the key aspects of Cd toxicokinetics will be mentioned here. Figure 1 summarizes some of the key features of the absorption and distribution of Cd in the body. With respiratory exposure, Cd is very efficiently absorbed from the lung; up to 40–60% of inhaled Cd reaches the systemic circulation. With oral exposure, the absorption of Cd from the gastrointestinal tract is considerably lower (only 5–10%). However, with long term exposure, even this low level of absorption from the gastrointestinal tract can lead to systemic accumulation of Cd and subsequent toxicities. It is also noteworthy that gastrointestinal absorption of Cd may be substantially higher in individuals with low body stores of iron, which is a factor that could contribute to individual variations in sensitivity to Cd exposure.

It is important to note that once Cd is absorbed into the bloodstream, whether from the lung or gastrointestinal tract, it tends to concentrate in blood cells (mainly erythrocytes, but also leucocytes); only a small percentage (<10%) remains in the plasma (Nordberg et al. 1986). For this reason, the monitoring of blood samples for levels of Cd exposure typically involves the analysis of whole blood. It is also important to note that essentially all of the Cd

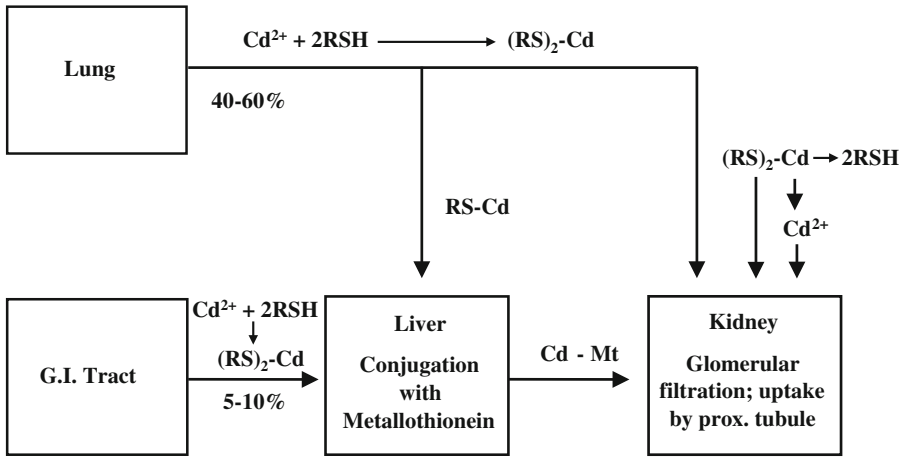


**Fig. 1** Schematic overview of absorption and distribution of Cd in the body. Cd is efficiently absorbed from the lung and to a lesser extent from the gastrointestinal tract (GI). In plasma, Cd binds to proteins and low molecular weight thiols (RSH) that deliver Cd in the form of RS-Cd to tissues. Initially, a large portion of the absorbed Cd is delivered to the liver where it induces the synthesis of metallothionein. Over time, the resulting Cd-Mt and RS-Cd are filtered at the glomeruli and taken up by epithelial cells of the proximal tubule

that is present in plasma is bound to proteins and other molecules. (Barbier et al. 2005; Bridges and Zalups 2005; Zalups and Ahmad 2003). Cd in plasma may either be tightly bound to specific metal binding proteins such as metallothionein (Klaassen et al. 2009, 1999; Klaassen and Liu 1997; Webb 1986), or may be loosely associated with molecules, such as albumin, amino acids or the sulfhydryl compounds, glutathione or cysteine. Because of the high affinity of Cd for metallothionein, Cd that is bound to metallothionein is not available for uptake by most tissues, although the Cd-metallothionein complex can be taken up by the epithelium of the proximal tubule (Klaassen et al. 2009; Squibb and Fowler 1984; Webb 1986). By contrast, the interaction of Cd with most other molecules in plasma is of a lower affinity (Fuhr and Rabenstein 1973; Rabenstein 1989; Trisak et al. 1990). Consequently, Cd that is associated with these molecules can dissociate and bind to other target molecules on cell surfaces and, in some cases, enter the cells (Barbier et al. 2005; Bridges and Zalups 2005). Recent findings suggest that metal ion transporters of the Zrt-/Irt-related protein (ZIP) family, such as the ZIP8, may play a key role in mediating the cellular uptake of Cd (He et al. 2009), although there is also evidence for the direct uptake of Cd-thiol conjugates in the kidney (Bridges and Zalups 2005; Zalups and Ahmad 2003). All of these factors need to be considered in relating the results of studies on the levels of Cd in blood to the distribution of Cd to critical target organs such as the kidney.

With regard to the distribution of Cd to tissues, following oral absorption, Cd is initially transported, via the portal circulation, to the liver where it is efficiently taken up by hepatocytes. In the hepatocytes, Cd induces the synthesis of metallothionein, which binds and sequesters Cd, thereby buffering its toxic effects in the cell. However, as the hepatocytes in which Cd is sequestered die off, either through normal turnover or as a result of Cd injury, the Cd-metallothionein complex can be released into the blood stream (Jin et al. 1998; Klaassen et al. 2009). Even though the Cd-metallothionein complex is non-toxic to most organs, it can be filtered at the glomerulus and taken up by the epithelial cells of the proximal tubule. In effect, Cd-metallothionein can have the paradoxical effect of facilitating the delivery of Cd from the liver to the kidney. Likewise, any Cd that is bound to low molecular weight molecules in plasma, such as cysteine and glutathione, can be filtered at the glomerulus, and Cd, either as the free ion ($Cd^{2+}$) or as the sulfhydryl conjugates, can be taken up by the proximal tubule epithelium. Even though it is not shown in the figure, there is evidence that the peritubular capillaries, which are fenestrated, can deliver $Cd^{2+}$ and Cd-thiol conjugates to the basolateral surface of the proximal tubule epithelial cells, from where Cd can then be taken up (Bridges and Zalups 2005).

After it is taken up by the epithelial cells of the proximal tubule, the Cd-metallothionein complex initially accumulates in lysosomes, where the complex is degraded, resulting in the release of Cd within the cell (Goyer et al. 1989; Klaassen et al. 2009; Squibb and Fowler 1984). The released $Cd^{2+}$, most likely rapidly associates with intracellular sulfhydryl groups, either on proteins or low molecular weight compounds such as glutathione. The interactions of Cd with sulfhydryl groups on proteins can result in direct alterations in protein function. These interactions, along with alterations in glutathione metabolism, can result in the induction of oxidative stress (Liu et al. 2009). The intracellular $Cd^{2+}$ also induces the synthesis of additional metallothionein (Klaassen et al. 2009), and can interfere with the actions of essential metal ions (Martelli et al. 2006) and disrupt various signaling pathways (Thevenod 2009).

With chronic exposure, the levels of Cd in the proximal tubule cells continue to increase until a critical threshold concentration of about 150–200 μg/g of tissue is reached (Jarup 2002; Roels et al. 1979). The classic view is that as this threshold concentration is approached, the cells undergo oxidative stress that leads to injury and either necrotic or apoptotic cell death (Liu et al. 2009; Shaikh et al. 1999; Tanimoto et al. 1993; Thevenod 2003). The cellulary injury causes alterations in proximal tubule function as well as the shedding of injured cells and cytosolic contents into the urine. The shedding of dead or injured cells triggers a repair process in which neighboring non-injured cells dedifferentiate in a process known as epithelial-mesenchymal transformal formation. The dedifferentiated cells migrate to the denuded area of the basement membrane and replace the injured cells (Bonventre 2003; Gobe and Endre 2003; Lieberthal et al. 1998; Racusen 1993).

## Specific biomarkers of Cd nephrotoxicity

The traditional urinary biomarkers that have been used to monitor Cd toxicity in the kidney reflect various steps in the sequence of pathologic events described in the previous section. Figure 2 summarizes the functional origins of the various urinary markers as they relate to the general pathophysiology of Cd-induced proximal tubule injury. From a functional standpoint, these traditional urinary markers of Cd nephrotoxicity can be classified into 3 broad categories: (1) Cd and Cd-binding proteins such as

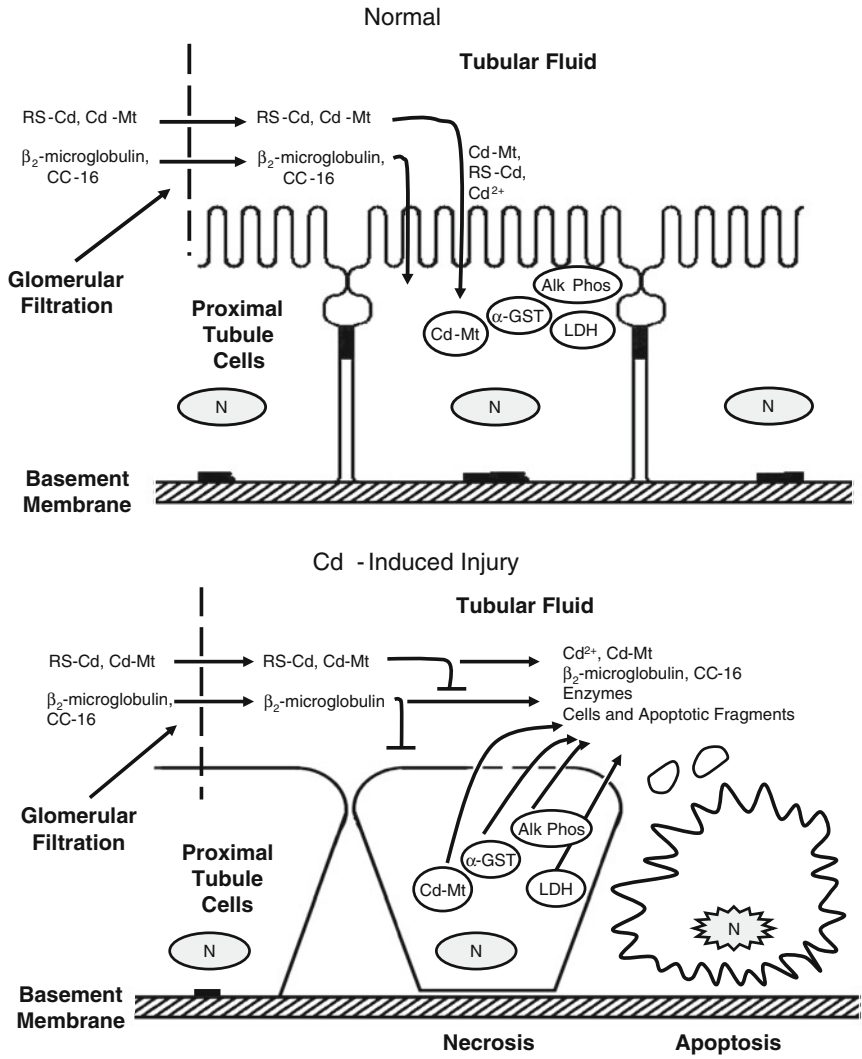


**Fig. 2** Mechanistic origins of classic biomarkers of Cd nephrotoxicity. The *top panel* illustrates how Cd that is present in the plasma and bound to either metallothionein (Cd-Mt) or low molecular thiols (RS-Cd) is filtered at the glomerulus and Cd, either in the form of Cd-Mt, $(RS)_2$-Cd or $Cd^{2+}$ is taken up by the epithelial cells of the proximal tubule. Likewise, low molecular weight plasma proteins such as $\beta_2$-microglobulin and CC-16 are filtered at the glomerulus and efficiently taken up by the proximal tubule cells. The epithelial cells also contain enzymes such as lactate dehydrogenase (LDH), alkaline phosphatase and α-glutathione-S-transferase (α-GST). At this stage of exposure, the urinary excretion of Cd, metallothionein, $\beta_2$-microglobulin and the enzymes is negligible. The *bottom panel* shows that as levels of Cd build up, the proximal tubule cells are injured and begin to die, either through necrotic or apoptotic mechanisms. These cytotoxic effects are associated with alterations in cell morphology, decreased reabsorbtive function, and shedding of cells, cell fragments and cytosolic contents into the urine. At this point, the excretion of urinary biomarkers increases markedly

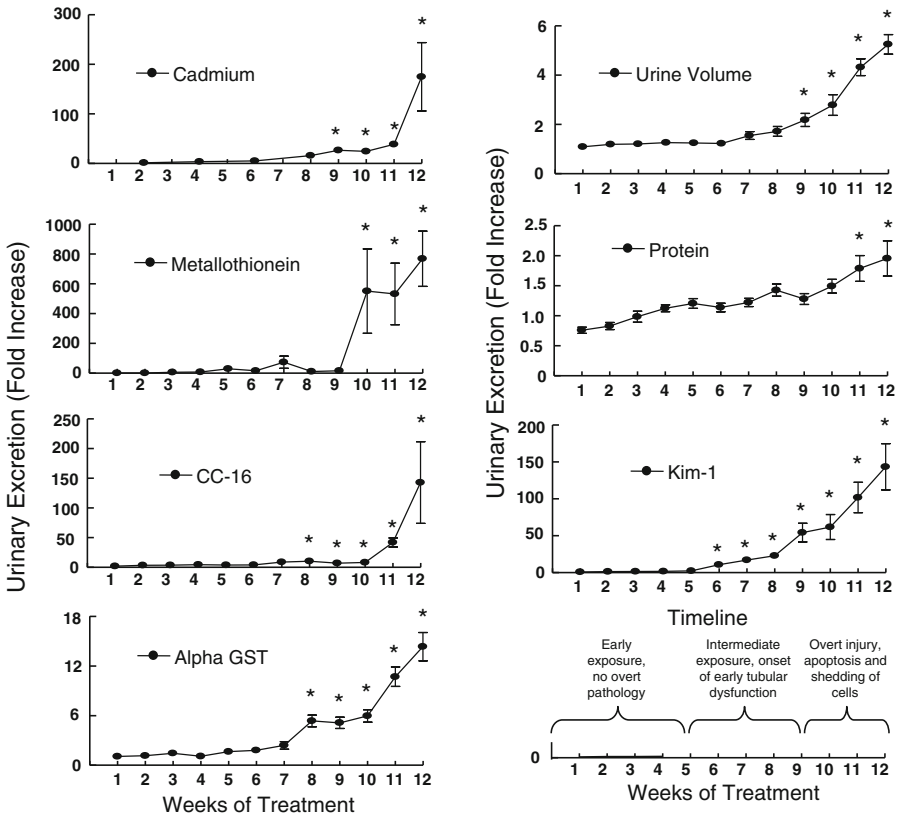


**Fig. 3** Effects of Cd on urinary excretion of Cd, Metallothionein, CC-16, α-GST, Protein and Kim-1. Data for the various parameters were taken from previously published studies (Prozialeck et al. 2007, 2009a, b) and recalculated as fold increase over time matched controls. Rats were treated with Cd (0.6 mg/kg), injected subcutaneously, 5 days per week for up to 12 weeks and weekly urine samples were analyzed for the various parameters. Values represent the mean ± SE. An * denotes statistically significant differences from week-matched controls ($P > 0.05$) as described in Prozialeck et al. (2007, 2009a, b)

metallothionein (2) low molecular weight proteins, and (3) proteins and enzymes derived from the brush border, intracellular organelles or the cytosol of proximal tubule epithelial cells. Each of these categories of markers are discussed in detail below. Figure 3 shows the general patterns for the urinary excretion of each of these 3 classes of biomarkers. The figure also includes data for a novel urinary biomarker known as kidney injury molecule-1 (Kim-1) along with a timeline describing specific events in the pathophysiologic process. Reference will be made to this figure as each of the classes of markers are discussed below.

## Cd and metallothionein

The urinary excretion of Cd and metallothionein have been used both as markers of Cd exposure and of Cd-induced proximal tubule injury (Bernard 2004; Chen et al. 2006c; Mueller et al. 1998; Shaikh and Smith 1986; Shaikh et al. 1987). The typical patterns for the urinary excretion of Cd and metallothionein are illustrated by the animal data shown in Fig. 3. The graphs show time course for the urinary excretion of the various markers in rats that were treated with Cd for 12 weeks. The key events in the pathologic process are indicated in the time line. Under normal conditions, circulating Cd which is bound to low molecular weight molecules such as metallothionein, cysteine or glutathione, is filtered at the glomerulus and taken up by the epithelial cells of the proximal tubule (Bridges and Zalups 2005; Thevenod 2003). During these early stages of exposure only extremely small amounts are excreted in the urine (Shaikh et al. 1999; Suzuki 1980). During this stage of exposure (labeled as "Early exposure" in the time line in the figure), the presence of Cd or metallothionein in the urine most likely results from the normal turnover and shedding of epithelial cells and is a reflection of the level of Cd exposure and the body burden of Cd (Jarup 2002; Shaikh et al. 1987; Suzuki 1980). However, over time, the concentration of Cd in the epithelial cells increases to the point that Cd disrupts tubular reabsorptive processes. At this stage (labeled "Intermediate exposure" in the figure) the excretion of Cd and metallothionein begin to increase in a,

more or less, linear manner. As the intracellular levels of Cd increase further, more of the epithelial cells begin to die and slough off. At this point, the urinary excretion of Cd and metallothionein increase markedly (Nakajima et al. 2005; Prozialeck et al. 2009b, a; Shaikh and Tohyama 1984; Shaikh and Smith 1986; Suzuki 1980). This surge in the urinary excretion of Cd and metallothionein coincides with the onset of polyuria and proteinuria (Prozialeck et al. 2007, 2009b, a). These findings are consistent with the hypothesis that the early, linear phases of Cd and metallothionein excretion are a reflection of Cd exposure, whereas the later rises in excretion are a reflection of Cd-induced tubular injury.

There are several aspects of the monitoring of blood and urinary Cd and metallothionein that have been problematic and controversial. The first problem involves the definition of the critical levels to identify Cd exposure and the onset of proximal tubule injury. The World Health Organization, US Environmental Protection Agency and many other agencies have established guidelines for the monitoring of populations for Cd exposure and for Cd exposure limits (ATSDR 2008a, b; Huang 2004; World Health Organization (WHO) 2000). However, there are some significant variations among the standards from these different agencies. Nevertheless, some generalizations can be made. The blood levels of Cd in non-exposed populations are typically less than 0.5 μg/l. Blood levels higher than 1.0 μg/l are generally indicative of Cd exposure; levels higher than 5 μg/l are considered hazardous. Urinary levels of Cd in non-exposed populations are usually below 0.5 μg/g creatinine; values above 1–2 μg/g are indicative of exposure or elevated body burden. The critical urinary Cd concentration that is associated with the onset of renal injury is usually about 2–10 μg/g creatinine, which corresponds to a renal cortical Cd concentration of about 150–200 μg/g tissue (Jarup 2002; Roels et al. 1979). It should be emphasized that these generalizations are derived from consensus-based standards that have been established by various regulating agencies. There is significant evidence that even lower urinary levels of Cd may be associated with adverse effects (Jarup et al. 2000; Jarup and Alfven 2004; Nawrot et al. 2008; Noonan et al. 2002; Schulz et al. 2009; Thomas et al. 2009; Uno et al. 2005). For metallothionein, the critical urinary level that is associated with the onset of overt kidney injury is approximately 300 μg/g creatinine (Shaikh and Tohyama 1984; Shaikh et al. 1987, 1990). This estimate is based on an assumed value for the urinary excretion of Cd of 3 μg/g creatinine. Other investigators have recommended significantly lower critical urinary metallothionein levels (Chen et al. 2006a, b, c).

One of the more controversial aspects of monitoring the urinary levels of Cd involve the use of chelating agents such as EDTA or DMSA to enhance the urinary excretion of Cd. These processes are referred to as "provoked urine excretion tests" and typically involve the administration of chelators such as EDTA 24 h prior to the collection of urine for the determination of urinary Cd levels (Crinnion 2009a, b; Soden et al. 2007). Such a pretreatment clearly causes at least a transient increase in the urinary excretion of Cd (Crinnion 2009a, b). However, it is not clear how this increase in excretion relates to the total burden of Cd in critical organs such as the kidney. A key problem is that chelators such as EDTA are cell membrane impermeant and do not mobilize Cd from intracellular stores. The increase in urinary Cd excretion following EDTA administration most likely represents the removal of Cd from easily accessible pools in body fluids and on cell surfaces, each of which represent only a small amount of the total body burden of Cd. As a result, the utility and interpretation of results of these provoked challenge tests remain unclear.

## Low molecular weight proteins

The second category of Cd urinary biomarkers includes a variety of low molecular weight proteins such as $\beta_2$-microglobulin, Clara-cell protein (CC-16), $\alpha_1$-microglobulin, retinol binding protein and vitamin D binding protein. These low molecular weight proteins, are present in plasma and are small enough to be easily filtered at the glomerulus. Under normal circumstances, these filtered proteins are efficiently reabsorbed by the proximal tubule and are not excreted to any great extent in the urine (Bernard et al. 1983; Bernard and Lauwerys 1995; Bernard and Hermans 1997; Bernard 2004; Bernard et al. 1987, 1994, 1983). However, as Cd accumulates in the proximal tubule, absorption of these proteins becomes impaired and the proteins begin to appear in the urine. Of these proteins, $\beta_2$-microglobin has

been most widely employed as a standard marker for monitoring for the early stages of Cd exposure and toxicity. It is also the only marker currently in use that has been related to severity of tubular dysfunction, in the absence of other disease conditions. Urinary levels of $\beta_2$-microglobulin of 1,000 μg/g creatinine (or greater) is considered to indicate irreversibility of renal effects. This level is typically associated with urinary cadmium of greater than 5 μg/g creatinine. For population monitoring, a cut-off value of 300 μg/g $\beta_2$-microgobulin creatinine has been used (Huang 2004; OSHA 1999). Other investigators have recommended lower critical exposure levels or bench mark dosage levels (BMDL) derived from mathematical models that are based on dose-response data and reflect levels of Cd exposure that do not result in adverse health effects (Chen et al. 2006c; Uno et al. 2005). Even though $\beta_2$-microglobulin has proven to be a very useful biomarker, its lack of stability in acidic urine can be problematic. As with $\beta_2$-microglobulin, increased levels of retinal binding protein (RBP) is suggestive of impairment of tubular reabsorptive function. Unlike $\beta_2$-microglobulin, however, RBP is stable in acidic urine and no special preservative or alkaline treatment is required.

## Proximal tubule-derived enzymes

Some of the most extensively used markers of Cd-induced proximal tubule injury have been enzymes, that are expressed in proximal tubule epithelial cells. A variety of enzymes including: *N*-acetyl-$\beta$-D-glucosamidase (NAG), lactate dehydrogenase (LDH), alkaline phosphatase, and more recently, alpha-glutathione-S-transferase (α-GST) have been studied in this context. The appearance of these enzymes in urine is classically thought to result from the leakage of intracellular contents when necrotic proximal tubule epithelial cells lose their membrane integrity and/or slough off into the urine (Ferguson et al. 2008; Nakajima et al. 2005; Sundberg et al. 1994; Vaidya et al. 2008). However, results of recent studies from our laboratory indicate that at the time the Cd-induced increase in the urinary excretion of α-GST and LDH occurs, there is little evidence of necrosis in the proximal tubule (Prozialeck et al. 2009b) suggesting that the Cd-induced urinary excretion of these enzymes may be due to the shedding of viable or apoptotic cells into the urine.

From a practical standpoint, NAG, has clearly proven to be especially useful in the monitoring of human populations. NAG is a lysosomal enzyme that exists as multiple isoforms. Both form A and B are expressed in kidney. However, the B form, which is abundant in proximal tubule epithelial cells, is regarded as the more sensitive and reliable marker of Cd-induced injury. However, assays that do not differentiate between the two isoforms can also yield useful results. Several epidemiologic studies over the past 15 years have shown that NAG outperforms other traditional markers (Jin et al. 1999; Moriguchi et al. 2003, 2009a, b; Noonan et al. 2002; Suwazono et al. 2006). However, it is also noteworthy that it does not perform as well in animal (rat) models of Cd nephrotoxicity (Prozialeck et al., 2009b, a). One major advantage of NAG is that it is relatively stable in non-preserved urine. This is in contrast to α-GST, which requires use of special preservatives prior to storage of samples. On the other hand, results of recent studies indicate that α-GST is an earlier marker of Cd toxicity than either NAG or LDH in the rat (Prozialeck et al. 2009b).

Recent studies suggest that α-GST may also be an especially useful early marker of Cd-induced kidney injury. Garcon et al. (2004, 2007) reported that α-GST is a sensitive indicator of kidney injury in workers who had been exposed to Pb and Cd. Results of studies from our laboratories showed that α-GST was a more sensitive markers of kidney injury than either NAG or LDH in a rat model of Cd-induced kidney injury (Prozialeck et al. 2009b, a). One particularly interesting aspect of α-GST is that its expression can be increased by oxidative stress (Casalino et al. 2006; Garcon et al. 2004). The fact that the α-GST is up-regulated by both Cd and oxidative stress could explain why it appears in urine before other cytosolic enzymes such as LDH.

Enzymes such as NAG, α-GST and LDH are attractive urinary markers because even low levels can be detected with relatively simple assays. On the other hand, enzymes can be subject to inhibition/interference with exogenous substances. For example, Hg is a powerful inhibitor of LDH (Lash and Zalups 1992) and urea can affect NAG (Mueller et al. 1989). This can create problems when using the

enzymes as markers of damage induced by toxicants, especially when multiple exposures may be involved.

### Miscellaneous markers of proximal tubule dysfunction, amino acids, glucose, $Na^+$, $K^+$ and $Ca^{2+}$

In addition to its effects on these protein biomarkers, Cd causes a generalized proximal tubule dysfunction that results in an increase in the urinary excretion of amino acids, $Na^+$, $K^+$, $PO_4^-$ and $Ca^{2+}$ (Kjellstrom 1986; Shaikh and Smith 1986). Even though these effects are characteristic of Cd nephrotoxicity, the urinary excretion of these substances can be influenced by many factors other than Cd exposure (Shaikh and Smith 1986). In general, these substances have not been widely applied in the monitoring of human populations for early signs of Cd exposure. One notable exception, however, is $Ca^{2+}$, which has been shown to be a reliable indicator of Cd-induced proximal dysfunction in exposed human subjects (Staessen et al. 1996; Wu et al. 2001).

### Markers of glomerular injury

While the proximal tubule is the primary target of Cd-induced kidney injury, there is evidence that Cd, particularly at higher levels of exposure, can also affect the glomeruli (Xiao et al. 2009). Changes in classic markers of glomerular dysfunction such as serum or urinary creatinine are generally not seen during the early or mild stages of Cd-induced kidney injury (Prozialeck and Edwards 2007; Prozialeck et al. 2009a). However, several investigators have reported associations between Cd exposure and alterations (either increased or decreased) creatinine clearance (Bernard 2004; Mueller et al. 1998; Navas-Acien et al. 2009; Trzcinka-Ochocka et al. 2004). Some studies have also shown increased urinary excretion of albumin during the early stages of Cd toxicity (Haswell-Elkins et al. 2008; Mueller 1993; Mueller et al. 1998), which is classically interpreted as a marker of glomerular damage. Navas-Acien et al. (2009) have recently reported significant alterations in glomerular filtration along with albuminuria in subjects exposed to Cd and Pb. At present, the relative contributions and relationship of glomerular injury and proximal tubule injury to these reported increases in urinary albumin excretion remain unclear.

### Novel biomarkers

While each of these traditional markers have proven to have some utility in monitoring Cd toxicity in humans and experimental animals, several problems remain. For example, urinary metallothionein and Cd are markers of Cd exposure as well as proximal tubular injury, and identifying the critical levels to indicate the onset of injury has been problematic (Chen et al. 2006b; Nakajima et al. 2005; Shaikh and Tohyama 1984; Shaikh and Smith 1986; Shaikh et al. 1990; Suwazono et al. 2006). Moreover, the urinary excretion of proteins such as $\beta_2$-microglobulin can be influenced by actions of toxicants on organs other than the kidney (Halatek et al. 2005; Hantson et al. 2008; Roels et al. 1999). Most significantly, these markers only identify later stages of Cd-induced kidney injury. By the time they appear in the urine, the injury to the kidney is usually irreversible (ATSDR 2008b; Kobayashi et al. 2006; Wu et al. 2004). Thus, there is a need for better biomarkers of Cd-nephrotoxicity.

Most of the traditional biomarkers of Cd nephrotoxicity are based on the classic sequence of pathologic events shown in Fig. 2. However, an increasing volume of evidence indicates that the early stages of Cd toxicity involve changes in proximal tubule cell adhesion and function that occur before the onset of cell death (Prozialeck et al. 2003, 2009a). In addition, several recent studies indicate that the onset of Cd-induced kidney injury may be preceded by changes in specific markers of metallothionein expression, immune function and glucose metabolism. Together, these recent findings raise the possibility of identifying more specific and earlier biomarkers of Cd exposure and toxicity. One of the more promising urinary markers that have been described recently is kidney injury molecule-1 (Kim-1).

Kim-1 is a transmembrane protein that is not detectable in normal kidney but is expressed at high levels in the proximal tubule after ischemic or toxic injury (Vaidya et al. 2008). Kim-1 acts as a regulator of cell adhesion and endocytosis in regenerating cells of the injured tubule as they reform a functional

epithelial barrier (Bailly et al. 2002; Ichimura et al. 2008). This process is associated with the proteolytic cleavage of the ectodomain of Kim-1 into the urine (Bailly et al. 2002). The ectodomain is stable in urine and has been shown to be a sensitive marker of renal injury induced by a variety of agents (Vaidya et al. 2008). The evidence for the general utility of Kim-1 as an early marker of kidney injury is so compelling that the FDA has adopted Kim-1 as a standard biomarker for the preclinical safety evaluation of drug candidates (FDA 2008). Moreover, Kim-1 technology is rapidly being developed for the monitoring and diagnosis of kidney disease in humans (Vaidya et al. 2009).

In a series of recent studies in collaboration with Drs. Vishal Vaidya and Joseph Bonventre, at Harvard University, we have shown that Kim-1 outperforms traditional markers of Cd-induced kidney injury (Prozialeck et al. 2007, 2009a, b; Vaidya et al. 2009). Results of some of these studies are summarized in Fig. 3. In these studies, rats were treated with Cd (0.6 mg/kg, 5 days per week for up to 12 weeks) and weekly urine samples were assayed for levels of Kim-1 and a panel of other markers of kidney injury. Some of the results of this study are summarized in Fig. 3. As may be seen, Kim-1 was detected in the urine 4–5 weeks before the onset of proteinuria, and 2–5 weeks before the appearance of other markers including: metallothionein and CC-16. Other studies showed that the Cd-induced increase in Kim-1 expression occurred at a time (6 weeks) when there was little or no evidence of either necrosis or apoptosis of proximal tubule epithelial cells (Prozialeck et al. 2009b). The fact that Kim-1 can be detected at a time before lethal injury to proximal tubule epithelial cells has occurred may be especially significant. Perhaps, with earlier detection via Kim-1, it may be possible to reverse, or at least more effectively treat, Cd-induced kidney injury (Hotz et al. 1999; Wu et al. 2008) In light of this possibility, studies on the utility of Kim-1 as marker of Cd toxicity in humans are certainly warranted.

## Early systemic markers of Cd toxicity

All of the biomarkers that we have described thus far reflect specific toxic actions of Cd in the kidney (loss of reabsorptive function, loss of cell membrane integrity, necrosis, apoptosis, etc.). However, results of recent studies suggest that Cd may produce very early systemic effects that may contribute to the pathophysiology of Cd-induced kidney injury. Markers of these systemic effects may provide a means of assessing risks of toxicity before the actual onset of kidney injury. They also have the potential to provide critical information regarding individual variations in the sensitivity to Cd nephrotoxicity. Two very promising lines of research center on the immunologic responses to metallothionein and polymorphisms in the patterns of expression of metallothionein.

Recent studies indicate that Cd exposure results in the production of metallothionein antibodies in blood (Nordberg 2009). In a study of Chinese smelter workers exposed to Cd, levels of plasma metallothionein antibody were significantly correlated with urinary $\beta_2$ microglobulin and NAG (Chen et al. 2006a). However, the same study showed that urinary albumin, metallothionein and Cd were not significantly correlated with plasma levels of metallothionein antibodies. In another study, diabetic patients with no history of occupational exposure to Cd were examined. Plasma metallothionein antibody levels were significantly correlated with urinary $\beta_2$ microglobulin, but not with urinary Cd (Chen et al. 2006c). In these studies, the presence of plasma metallothionein antibodies was highly variable in the populations. The investigators suggested that the metallothionein antibodies might inhibit the protective actions of metallothionein. This reported variation in metallothionein antibody production may contribute to the high degree of variation observed in individual susceptibility to Cd-induced renal dysfunction. Since the presence of plasma metallothionein antibody was correlated with only the early biomarkers of Cd exposure, and not later markers of Cd-induced renal injury, it appears plasma metallothionein antibody levels may only be transiently elevated during Cd exposure. Antibody levels may become elevated during initial exposure but then decline with progressive renal injury. Additional studies are needed to ascertain the time course and significance of the appearance of plasma metallothionein antibodies during Cd exposure.

Other researchers have begun to examine the possible relationships between individual susceptibility to Cd toxicity and metallothionein gene polymorphisms (Miura 2009). When metallothionein

expression was compared to Cd concentrations in the renal cortex in 55 autopsied individuals in Japan, two different groups were identified. One group had the expected proportional increase in expression of metallothionein with increased Cd content while a second group had no change in metallothonein expression regardless of elevated Cd levels (Yoshida et al. 1998). One explanation for this may be genetic differences in the promoter region of the methallothionein gene. In a later study examining DNA isolated from leukocytes, a single-nucleotide polymorphism in the promoter region of the methallothionein-IIA gene was found in 18% of 119 Japanese subjects (Kita et al. 2006). HEK293 cells expressing this single-nucleotide polymorphism had significantly decreased metallothionein expression in the presence of Cd (Kita et al. 2006). Interestingly, Yoshida et al. (1998) reported that there was no correlation between low metallothionein expression and renal-related cause of death or any signs of overt, renal pathology. Although this study would indicate there is no relationship between metallothionein expression and Cd-induced renal damage, there is ample experimental evidence from animal studies that correlate levels of metallothonein expression with resistance to Cd toxicity (Liu et al. 2000; Webb 1979). Furthermore, other human studies show a direct correlation with metallothionein expression and Cd exposure. In one study in Japan, the expression of a specific isoform of metallothonein primarily expressed in peripheral blood lymphocytes (metallothionein-IA) was examined. Metallothonein-IA expression was found to directly correlate with elevated blood Cd and urinary $\beta_2$ microglobulin, Cd and albumin (Chang et al. 2009).

These studies suggest a potential use of metallothionein isoform expression in determining an individual's specific sensitivity to Cd toxicity. However, this issue may not be as straightforward as it would seem to be. Metallothionein is classically thought to serve a protective role against Cd toxicity. However, it also has the paradoxical effect of delivering Cd to the kidney. This issue is further complicated by the Cd-induced synthesis of metallothionein in the kidney. Additional studies are needed to determine how the apparent genetic differences in metallothionein expression influence the accumulation of Cd in the kidney and the sensitivity to Cd-induced kidney injury.

Another line of recent research has focused on the finding that low level Cd exposure is associated with alterations in glucose levels and glucose metabolism that precede the onset of kidney injury. Within the kidney, Cd exposure alters the expression of two key enzymes involved in glucose metabolism, glucose 6-phosphate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase (Edwards et al. 2008). Furthermore, there is a growing body of literature showing significant correlations between exposure to Cd and the prevalence of diabetes in countries such as; Pakistan (Afridi et al. 2008), China (Chen et al. 2006c), USA (Schwartz et al. 2003) and Australia (Haswell-Elkins et al. 2008). Experimental studies using animal models of Cd exposure have shown Cd to have diabetogenic effects in both acute (Bell et al. 1990) and sub-chronic exposure models (Merali and Singhal 1975, 1980; Han et al. 2003; Lei et al. 2007). Our own preliminary studies using a 12 week subchronic model of Cd exposure in the rat show that Cd caused elevations in fasting blood glucose levels several weeks prior to any overt signs (polyuria or proteinuria) of Cd-induced renal dysfunction (Edwards and Prozialeck 2009). These effects were associated with a significant (50%) decrease in serum insulin levels and morphological changes within the pancreas (Edwards and Prozialeck 2009). Furthermore in an occupational study where Chinese smelter workers had been exposed to Cd, a significant dose-dependent relationship was found between lower blood insulin levels and elevated urinary Cd levels (Lei et al. 2007).

Given the well established link between diabetes and the development of kidney disease, the ability of Cd to affect levels and metabolism of glucose could have significant implications for the pathophysiology of Cd-induced kidney injury. These findings may also have implications for monitoring populations for early signs of Cd toxicity. The fact that the Cd-induced changes in glucose and insulin levels appeared before changes in indicators of renal dysfunction such as urinary $\beta_2$ microglobulin, NAG and albumin (Lei et al. 2007) indicates that the measurement of serum glucose and insulin may be more sensitive markers of Cd exposure than traditional biomarkers of renal dysfunction. Since the measurement of blood glucose and serum insulin are routinely performed, they would be quick and easy measurements to obtain in common clinical settings. One obvious caveat is that most cases of hyperglycemia and diabetes are not associated with Cd

exposure. However, blood glucose and more importantly, serum insulin may be important biomarkers in the preliminary screening of individuals who are thought to have been exposed to Cd.

## Biomarker caveats and problems

In considering the utility of any urinary biomarker of Cd exposure, it is important to keep in mind that the determination of the concentration of a marker in a single urine sample does not take into account factors such as urine volume, which can vary markedly, (either higher than normal or lower than normal) in various stages of Cd-induced renal injury. The most widely used approach for circumventing this problem has been to normalize values for the biomarkers to urinary creatinine excretion. However this, too, has its shortcomings (Barr et al. 2005; Ikeda et al. 2003; Moriguchi et al. 2003; Suwazono et al. 2005). The most effective way of standardizing values for urinary biomarkers is to utilize 24 h urine samples, with the biomarker values normalized to 24 h urinary creatinine excretion. Unfortunately this approach is rather costly and is not always feasible, especially in monitoring populations in remote under-developed regions.

Another major issue in interpreting the results of studies with many biomarkers is that they are not specific markers of Cd-induced injury; that is, their levels can be influenced by toxic substances other than Cd. For example, the expression and urinary shedding of Kim-1 can be triggered by exposure to a wide variety of proximal tubule toxicants other than Cd (Vaidya et al. 2008). In addition, urinary levels of some markers may be influenced by toxic actions of Cd on organs other than the kidney. For example, as the invariant light-chain component of class I major histocompatibility antigens, $\beta_2$ microglobulin is derived from many tissues, and serum levels of the protein can be influenced by pathologic processes in a variety of organs (Broeckaert et al. 2000; Halatek et al. 2005; Kobryn et al. 1989; Poulik et al. 1979).While an argument can be made that this lack of organ specificity could actually be desirable for biomonitoring the effects of a substance, such as Cd, that can affect multiple organ systems, this same lack of organ specificity can greatly complicate the use of the biomarker for mechanistic studies. For these reasons, any interpretation of results with any biomarkers must include some reference to levels of Cd in blood, tissues and/or urine.

A final critical issue that needs to be considered is that in many situations, populations are exposed to other toxic substances, particularly metals such as Pb, Hg and As, at the same time they are exposed to Cd. Several recent studies have highlighted the possibility that Cd, Pb, Hg and As may act synergistically to damage the kidney and thereby affect the levels of various biomarkers (Alfven et al. 2002; Buchet et al. 2003; Choudhury and Mudipalli 2008; Navas-Acien et al. 2009; Nordberg et al. 2005; Thomas et al. 2009; Wang and Fowler 2008). Most of the studies addressing this issue have utilized traditional markers of Cd exposure and toxicity. Additional studies are needed to identify the optimal markers, or combination of markers, for monitoring complex exposure to multiple metals. In addition, the possible interactions between Cd and various organic toxicants is a subject that has yet to be explored.

## Summary and perspective

Biomarkers have proven to be useful tools for evaluating Cd exposure and toxicity in human populations as well in laboratory studies on animals. However, many fundamental issues regarding the selection of markers and definition of their critical levels have yet to be fully resolved. Recent studies have provided hope that new and even more sensitive Cd biomarkers can be developed. At the same time, it is apparent that data on biomarkers are essentially meaningless if not considered in conjunction with standard blood and urinary Cd analyses. Moreover, it seems unlikely that any single biomarker will be able to provide a complete picture of the effects of Cd on an organ as complex as the kidney. Analyses of panels of markers could provide a much more comprehensive picture of the multifaceted effects of Cd on the kidney. Additional studies are needed in order to determine the optimal combination of markers for identifying the earliest and most specific stages of Cd nephrotoxicity. It is our hope that this review/commentary will help to facilitate such studies.

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## References

Afridi HI, Kazi TG, Kazi N, Jamali MK, Arain MB, Jalbani N, Baig JA, Sarfraz RA (2008) Evaluation of status of toxic metals in biological samples of diabetes mellitus patients. Diabetes Res Clin Pract 80:280–288

Akesson A, Lundh T, Vahter M, Bjellerup P, Lidfeldt J, Nerbrand C, Samsioe G, Stromberg U, Skerfving S (2005) Tubular and glomerular kidney effects in Swedish women with low environmental cadmium exposure. Environ Health Perspect 113:1627–1631

Alfven T, Jarup L, Elinder CG (2002) Cadmium and lead in blood in relation to low bone mineral density and tubular proteinuria. Environ Health Perspect 110:699–702

ATSDR (2008a) Case Studies in Environmental Medicine (CSEM) Cadmium Toxicity. http://www.atsdr.cdc gov/csem/cadmium/cdlaboratory_evaluation.html

ATSDR (2008b) Toxicological Profile for Cadmium. http://www.atsdr.cdc.gov/cercla/toxprofiles/tp5.html

Bailly V, Zhang Z, Meier W, Cate R, Sanicola M, Bonventre JV (2002) Shedding of kidney injury molecule-1, a putative adhesion protein involved in renal regeneration. J Biol Chem 277:39739–39748

Bandara JM, Senevirathna DM, Dasanayake DM, Herath V, Bandara JM, Abeysekara T, Rajapaksha KH (2008) Chronic renal failure among farm families in cascade irrigation systems in Sri Lanka associated with elevated dietary cadmium levels in rice and freshwater fish (Tilapia). Environ Geochem Health 30:465–478

Barbier O, Jacquillet G, Tauc M, Cougnon M, Poujeol P (2005) Effect of heavy metals on, and handling by, the kidney. Nephron Physiol 99:105–110

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL (2005) Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 113: 192–200

Bell RR, Early JL, Nonavinakere VK, Mallory Z (1990) Effect of cadmium on blood glucose level in the rat. Toxicol Lett 54:199–205

Bernard A (2004) Renal dysfunction induced by cadmium: biomarkers of critical effects. Biometals 17:519–523

Bernard A (2008) Cadmium & its adverse effects on human health. Indian J Med Res 128:557–564

Bernard A, Hermans C (1997) Biomonitoring of early effects on the kidney or the lung. Sci Total Environ 199:205–211

Bernard A, Lauwerys R (1995) Low-molecular-weight proteins as markers of organ toxicity with special reference to Clara cell protein. Toxicol Lett 77:145–151

Bernard A, Viau C, Lauwerys R (1983) Renal handling of human beta 2-microglobulin in normal and cadmium-poisoned rats. Arch Toxicol 53:49–57

Bernard AM, Vyskocil AA, Mahieu P, Lauwerys RR (1987) Assessment of urinary retinol-binding protein as an index of proximal tubular injury. Clin Chem 33:775–779

Bernard AM, Thielemans NO, Lauwerys RR (1994) Urinary protein 1 or Clara cell protein: a new sensitive marker of proximal tubular dysfunction. Kidney Int Suppl 47:S34–S37

Biomarkers Definition Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69:89–95

Bonventre JV (2003) Dedifferentiation and proliferation of surviving epithelial cells in acute renal failure. J Am Soc Nephrol 14(Suppl 1): S55–S61

Bridges CC, Zalups RK (2005) Molecular and ionic mimicry and the transport of toxic metals. Toxicol Appl Pharmacol 204:274–308

Broeckaert F, Clippe A, Knoops B, Hermans C, Bernard A (2000) Clara cell secretory protein (CC16): features as a peripheral lung biomarker. Ann N Y Acad Sci 923:68–77

Buchet JP, Heilier JF, Bernard A, Lison D, Jin T, Wu X, Kong Q, Nordberg G (2003) Urinary protein excretion in humans exposed to arsenic and cadmium. Int Arch Occup Environ Health 76:111–120

Byrne C, Divekar SD, Storchan GB, Parodi DA, Martin MB (2009) Cadmium–a metallohormone? Toxicol Appl Pharmacol 238:266–271

Casalino E, Sblano C, Calzaretti G, Landriscina C (2006) Acute cadmium intoxication induces alpha-class glutathione S-transferase protein synthesis and enzyme activity in rat liver. Toxicology 217:240–245

Chang X, Jin T, Chen L, Nordberg M, Lei L (2009) Metallothionein I isoform mRNA expression in peripheral lymphocytes as a biomarker for occupational cadmium exposure. Exp Biol Med (Maywood) 234:666–672

Chen L, Jin T, Huang B, Chang X, Lei L, Nordberg GF, Nordberg M (2006a) Plasma metallothionein antibody and cadmium-induced renal dysfunction in an occupational population in China. Toxicol Sci 91:104–112

Chen L, Jin T, Huang B, Nordberg G, Nordberg M (2006b) Critical exposure level of cadmium for elevated urinary metallothionein–an occupational population study in China. Toxicol Appl Pharmacol 215:93–99

Chen L, Lei L, Jin T, Nordberg M, Nordberg GF (2006c) Plasma metallothionein antibody, urinary cadmium, and renal dysfunction in a Chinese type 2 diabetic population. Diabetes Care 29:2682–2687

Choudhury H, Mudipalli A (2008) Potential considerations & concerns in the risk characterization for the interaction profiles of metals. Indian J Med Res 128:462–483

Crinnion WJ (2009a) The benefit of pre- and post-challenge urine heavy metal testing: part 2. Altern Med Rev 14: 103–108

Crinnion WJ (2009b) The benefits of pre- and post-challenge urine heavy metal testing: Part 1. Altern Med Rev 14:3–8

de Burbure C, Buchet JP, Leroyer A, Nisse C, Haguenoer JM, Mutti A, Smerhovsky Z, Cikrt M, Trzcinka-Ochocka M, Razniewska G, Jakubowski M, Bernard A (2006) Renal and neurologic effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels. Environ Health Perspect 114:584–590

Edwards JR, Prozialeck WC (2009) Cadmium, diabetes and chronic kidney disease. Toxicol Appl Pharmacol 238: 289–293

Edwards JR, Lamar PC, Prozialeck WC (2008) Upregulation of glyceraldehyde 3 phosphate dehydrogenase (GAPDH) and glucose 6 phosphate dehydrogenase (G6PDH) during the early stages of cadmium induced renal injury. The Toxicologist CD—An official Journal of the Society of Toxicology 102:Abstract #458

FDA (2008) European Medicines Agency to Consider Additional Test Results when Assessing New Drug Safety. http://www.fda.gov/bbs/topics/NEWS/2008/NEW01850.html

Fels LM (1999) Risk assessment of nephrotoxicity of cadmium. Ren Fail 21:275–281

Ferguson MA, Vaidya VS, Bonventre JV (2008) Biomarkers of nephrotoxic acute kidney injury. Toxicology 245:182–193

Fowler BA (2009) Monitoring of human populations for early markers of cadmium toxicity: a review. Toxicol Appl Pharmacol 238:294–300

Friedman LS, Lukyanova EM, Kundiev YI, Shkiryak-Nizhnyk ZA, Chislovska NV, Mucha A, Zvinchuk AV, Oliynyk I, Hryhorczuk D (2006) Anthropometric, environmental, and dietary predictors of elevated blood cadmium levels in Ukrainian children: Ukraine ELSPAC group. Environ Res 102:83–89

Fuhr BJ, Rabenstein DL (1973) Nuclear magnetic resonance studies of the solution chemistry of metal complexes. IX. The binding of cadmium, zinc, lead, and mercury by glutathione. J Am Chem Soc 95:6944–6950

Garcon G, Leleu B, Zerimech F, Marez T, Haguenoer JM, Furon D, Shirali P (2004) Biologic markers of oxidative stress and nephrotoxicity as studied in biomonitoring of adverse effects of occupational exposure to lead and cadmium. J Occup Environ Med 46:1180–1186

Garcon G, Leleu B, Marez T, Zerimech F, Haguenoer JM, Furon D, Shirali P (2007) Biomonitoring of the adverse effects induced by the chronic exposure to lead and cadmium on kidney function: usefulness of alpha-glutathione S-transferase. Sci Total Environ 377:165–172

Gobe GC, Endre ZH (2003) Cell death in toxic nephropathies. Semin Nephrol 23:416–424

Gonick HC (2008) Nephrotoxicity of cadmium & lead. Indian J Med Res 128:335–352

Goyer RA, Miller CR, Zhu SY, Victery W (1989) Non-metallothionein-bound cadmium in the pathogenesis of cadmium nephrotoxicity in the rat. Toxicol Appl Pharmacol 101:232–244

Halatek T, Gromadzinska J, Wasowicz W, Rydzynski K (2005) Serum clara-cell protein and beta2-microglobulin as early markers of occupational exposure to nitric oxides. Inhal Toxicol 17:87–97

Han JC, Park SY, Hah BG, Choi GH, Kim YK, Kwon TH, Kim EK, Lachaal M, Jung CY, Lee W (2003) Cadmium induces impaired glucose tolerance in rat by down-regulating GLUT4 expression in adipocytes. Arch Biochem Biophys 413:213–220

Hantson P, Bernard A, Hermans C (2008) Kinetics and determinants of the changes of CC16, a lung secretory protein in a rat model of toxic lung injury. Clin Toxicol (Phila) 46:230–238

Haswell-Elkins M, Satarug S, O'Rourke P, Moore M, Ng J, McGrath V, Walmby M (2008) Striking association between urinary cadmium level and albuminuria among Torres Strait Islander people with diabetes. Environ Res 106:379–383

He L, Wang B, Hay EB, Nebert DW (2009) Discovery of ZIP transporters that participate in cadmium damage to testis and kidney. Toxicol Appl Pharmacol 238:250–257

Hellstrom L, Elinder CG, Dahlberg B, Lundberg M, Jarup L, Persson B, Axelson O (2001) Cadmium exposure and end-stage renal disease. Am J Kidney Dis 38:1001–1008

Hotz P, Buchet JP, Bernard A, Lison D, Lauwerys R (1999) Renal effects of low-level environmental cadmium exposure: 5-year follow-up of a subcohort from the Cadmibel study. Lancet 354:1508–1513

Huang J (2004) Chinese National health standards for occupational exposure to cadmium and diagnostic criteria of occupational chronic cadmium poisoning. Biometals 17:511

IARC (1993) Cadmium and cadmium compounds. Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. IARC Monogr Eval Carcinog Risks Hum 58:119–237

Ichimura T, Asseldonk EJ, Humphreys BD, Gunaratnam L, Duffield JS, Bonventre JV (2008) Kidney injury molecule-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. J Clin Invest 118:1657–1668

Ikeda M, Ezaki T, Tsukahara T, Moriguchi J, Furuki K, Fukui Y, Okamoto S, Ukai H, Sakurai H (2003) Bias induced by the use of creatinine-corrected values in evaluation of beta2-microgloblin levels. Toxicol Lett 145:197–207

Jarup L (2002) Cadmium overload and toxicity. Nephrol Dial Transplant 17(Suppl 2): 35–39

Jarup L, Akesson A (2009) Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol 238:201–208

Jarup L, Alfven T (2004) Low level cadmium exposure, renal and bone effects–the OSCAR study. Biometals 17:505–509

Jarup L, Berglund M, Elinder CG, Nordberg G, Vahter M (1998) Health effects of cadmium exposure—a review of the literature and a risk estimate. Scand J Work Environ Health 24(Suppl 1): 1–51

Jarup L, Hellstrom L, Alfven T, Carlsson MD, Grubb A, Persson B, Pettersson C, Spang G, Schutz A, Elinder CG (2000) Low level exposure to cadmium and early kidney damage: the OSCAR study. Occup Environ Med 57:668–672

Jin T, Lu J, Nordberg M (1998) Toxicokinetics and biochemistry of cadmium with special emphasis on the role of metallothionein. Neurotoxicology 19:529–535

Jin T, Nordberg G, Wu X, Ye T, Kong Q, Wang Z, Zhuang F, Cai S (1999) Urinary N-acetyl-beta-D-glucosaminidase isoenzymes as biomarker of renal dysfunction caused by cadmium in a general population. Environ Res 81:167–173

Joseph P (2009) Mechanisms of cadmium carcinogenesis. Toxicol Appl Pharmacol 238:272–279

Kawada T (1995) Indicators of renal effects of exposure to cadmium: N-acetyl-beta-D-glucosaminidase and others. Sangyo Eiseigaku Zasshi 37:69–73

Kita K, Miura N, Yoshida M, Yamazaki K, Ohkubo T, Imai Y, Naganuma A (2006) Potential effect on cellular response to cadmium of a single-nucleotide A–> G polymorphism in the promoter of the human gene for metallothionein IIA. Hum Genet 120:553–560

Kjellstrom T (1986) Renal effects. In: Friberg L, Elinder C-G, Kjellstrom T, Nordberg GF (eds) Cadmium and health: a toxicological and epidemiological appraisal. CRC Press, Boca Raton, pp 21–109

Klaassen CD, Liu J (1997) Role of metallothionein in cadmium-induced hepatotoxicity and nephrotoxicity. Drug Metab Rev 29:79–102

Klaassen CD, Liu J, Choudhuri S (1999) Metallothionein: an intracellular protein to protect against cadmium toxicity. Annu Rev Pharmacol Toxicol 39:267–294

Klaassen CD, Liu J, Diwan BA (2009) Metallothionein protection of cadmium toxicity. Toxicol Appl Pharmacol 238:215–220

Kobayashi E, Suwazono Y, Uetani M, Inaba T, Oishi M, Kido T, Nishijo M, Nakagawa H, Nogawa K (2006) Estimation of benchmark dose as the threshold levels of urinary cadmium, based on excretion of total protein, beta2-microglobulin, and N-acetyl-beta-D-glucosaminidase in cadmium nonpolluted regions in Japan. Environ Res 101:401–406

Kobryn A, Pacsa A, White AG, Kumar MS, Abouna GM (1989) Are peripheral blood lymphocytes the source of elevated B-2-microglobulin in renal transplant recipients? Transplant Proc 21:302–303

Koyama H, Satoh H, Suzuki S, Tohyama C (1992) Increased urinary cadmium excretion and its relationship to urinary N-acetyl-beta-D-glucosaminidase activity in smokers. Arch Toxicol 66:598–601

Lash LH, Zalups RK (1992) Mercuric chloride-induced cytotoxicity and compensatory hypertrophy in rat kidney proximal tubular cells. J Pharmacol Exp Ther 261: 819–829

Lauwerys RR, Bernard AM, Roels HA, Buchet JP (1994) Cadmium: exposure markers as predictors of nephrotoxic effects. Clin Chem 40:1391–1394

Lei LJ, Jin TY, Zhou YF (2007) Insulin expression in rats exposed to cadmium. Biomed Environ Sci 20:295–301

Lieberthal W, Koh JS, Levine JS (1998) Necrosis and apoptosis in acute renal failure. Semin Nephrol 18:505–518

Liu Y, Liu J, Habeebu SM, Waalkes MP, Klaassen CD (2000) Metallothionein-I/II null mice are sensitive to chronic oral cadmium-induced nephrotoxicity. Toxicol Sci 57:167–176

Liu J, Qu W, Kadiiska MB (2009) Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol Appl Pharmacol 238:209–214

Mannino DM, Holguin F, Greves HM, Savage-Brown A, Stock AL, Jones RL (2004) Urinary cadmium levels predict lower lung function in current and former smokers: data from the Third National Health and Nutrition Examination Survey. Thorax 59:194–198

Martelli A, Rousselet E, Dycke C, Bouron A, Moulis JM (2006) Cadmium toxicity in animal cells by interference with essential metals. Biochimie 88:1807–1814

Menke A, Muntner P, Silbergeld EK, Platz EA, Guallar E (2009) Cadmium levels in urine and mortality among US adults. Environ Health Perspect 117:190–196

Merali Z, Singhal RL (1975) Protective effect of selenium on certain hepatotoxic and pancreotoxic manifestations of subacute cadmium administration. J Pharmacol Exp Ther 195:58–66

Merali Z, Singhal RL (1980) Diabetogenic effects of chronic oral cadmium administration to neonatal rats. Br J Pharmacol 69:151–157

Miura N (2009) Individual susceptibility to cadmium toxicity and metallothionein gene polymorphisms: with references to current status of occupational cadmium exposure. Ind Health 47:487–494

Moriguchi J, Ezaki T, Tsukahara T, Furuki K, Fukui Y, Okamoto S, Ukai H, Sakurai H, Shimbo S, Ikeda M (2003) Comparative evaluation of four urinary tubular dysfunction markers, with special references to the effects of aging and correction for creatinine concentration. Toxicol Lett 143:279–290

Moriguchi J, Inoue Y, Kamiyama S, Horiguchi M, Murata K, Sakuragi S, Fukui Y, Ohashi F, Ikeda M (2009a) N-acetyl-beta-D-glucosaminidase (NAG) as the most sensitive marker of tubular dysfunction for monitoring residents in non-polluted areas. Toxicol Lett 190:1–8

Moriguchi J, Inoue Y, Kamiyama S, Sakuragi S, Horiguchi M, Murata K, Fukui Y, Ohashi F, Ikeda M (2009b) Cadmium and tubular dysfunction marker levels in urine of residents in non-polluted areas with natural abundance of cadmium in Japan. Int Arch Occup Environ Health (Epub ahead of print)

Mueller PW (1993) Detecting the renal effects of cadmium toxicity. Clin Chem 39:743–745

Mueller PW, MacNeil ML, Steinberg KK (1989) N-acetyl-beta-D-glucosaminidase assay in urine: urea inhibition. J Anal Toxicol 13:188–190

Mueller PW, Price RG, Finn WF (1998) New approaches for detecting thresholds of human nephrotoxicity using cadmium as an example. Environ Health Perspect 106:227–230

Nakajima M, Kobayashi E, Suwazono Y, Uetani M, Oishi M, Inaba T, Kido T, Shaikh ZA, Nogawa K (2005) Excretion of urinary cadmium, copper, and zinc in cadmium-exposed and nonexposed subjects, with special reference to urinary excretion of beta2-microglobulin and metallothionein. Biol Trace Elem Res 108:17–31

Navas-Acien A, Tellez-Plaza M, Guallar E, Muntner P, Silbergeld E, Jaar B, Weaver V (2009) Blood cadmium and lead and chronic kidney disease in US adults: a joint analysis. Am J Epidemiol 170:1156–1164

Nawrot TS, Van HE, Thijs L, Richart T, Kuznetsova T, Jin Y, Vangronsveld J, Roels HA, Staessen JA (2008) Cadmium-related mortality and long-term secular trends in the cadmium body burden of an environmentally exposed population. Environ Health Perspect 116:1620–1628

Noonan CW, Sarasua SM, Campagna D, Kathman SJ, Lybarger JA, Mueller PW (2002) Effects of exposure to low levels of environmental cadmium on renal biomarkers. Environ Health Perspect 110:151–155

Nordberg M (1984) General aspects of cadmium: transport, uptake and metabolism by the kidney. Environ Health Perspect 54:13–20

Nordberg GF (2004) Cadmium and health in the 21st century–historical remarks and trends for the future. Biometals 17:485–489

Nordberg GF (2009) Historical perspectives on cadmium toxicology. Toxicol Appl Pharmacol 238:192–200

Nordberg GF, Kjellstrom T, Nordberg G (1986) Kinetics and metabolism. In: Friberg L, Elinder CG, Kjellstrom T, Nordberg GF (eds) Cadmium and health: a toxicological and epidemiological appraisal. CRC Press, Boca Raton, pp 103–178

Nordberg GF, Jin T, Hong F, Zhang A, Buchet JP, Bernard A (2005) Biomarkers of cadmium and arsenic interactions. Toxicol Appl Pharmacol 206:191–197

OSHA (1999) Substance Safety Data Sheet- Cadmium—1910. 1027 App. http://www.oshagov/pls/oshaweb/owadisp show_document?p_table = standar

Pinot F, Kreps SE, Bachelet M, Hainaut P, Bakonyi M, Polla BS (2000) Cadmium in the environment: sources, mechanisms of biotoxicity, and biomarkers. Rev Environ Health 15:299–323

Poulik MD, Gold P, Shuster J (1979) beta 2-Microglobulin: methods and clinical applications. CRC Crit Rev Clin Lab Sci 10:225–245

Prozialeck WC, Edwards JR (2007) Cell adhesion molecules in chemically-induced renal injury. Pharmacol Ther 114: 74–93

Prozialeck WC, Lamar PC, Lynch SM (2003) Cadmium alters the localization of N-cadherin, E-cadherin, and beta-catenin in the proximal tubule epithelium. Toxicol Appl Pharmacol 189:180–195

Prozialeck WC, Vaidya VS, Liu J, Waalkes MP, Edwards JR, Lamar PC, Bernard AM, Dumont X, Bonventre JV (2007) Kidney injury molecule-1 is an early biomarker of cadmium nephrotoxicity. Kidney Int 72:985–993

Prozialeck WC, Edwards JR, Lamar PC, Liu J, Vaidya VS, Bonventre JV (2009a) Expression of kidney injury molecule-1 (Kim-1) in relation to necrosis and apoptosis during the early stages of Cd-induced proximal tubule injury. Toxicol Appl Pharmacol 238:306–314

Prozialeck WC, Edwards JR, Vaidya VS, Bonventre JV (2009b) Preclinical evaluation of novel urinary biomarkers of cadmium nephrotoxicity. Toxicol Appl Pharmacol 238:301–305

Rabenstein DL (1989) Metal complexes of glutathione and their biological significance. In: Dolphin D, Auromovie O, Poulson R (eds) Glutathione: chemical, biochemical and medical aspects. Wiley, New York, pp 147–186

Racusen LC (1993) Tubular injury in human kidneys: pathologic findings and pathogenic mechanisms. Clin Investig 71:858–860

Roels H, Bernard A, Buchet JP, Goret A, Lauwerys R, Chettle DR, Harvey TC, Haddad IA (1979) Critical concentration of cadmium in renal cortex and urine. Lancet 1:221

Roels HA, Hoet P, Lison D (1999) Usefulness of biomarkers of exposure to inorganic mercury, lead, or cadmium in controlling occupational and environmental risks of nephrotoxicity. Ren Fail 21:251–262

Satarug S, Moore MR (2004) Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. Environ Health Perspect 112:1099–1103

Satarug S, Baker JR, Urbenjapol S, Haswell-Elkins M, Reilly PE, Williams DJ, Moore MR (2003) A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol Lett 137:65–83

Satoh M, Koyama H, Kaji T, Kito H, Tohyama C (2002) Perspectives on cadmium toxicity research. Tohoku J Exp Med 196:23–32

Scherer G, Barkemeyer H (1983) Cadmium concentrations in tobacco and tobacco smoke. Ecotoxicol Environ Saf 7: 71–78

Schulz C, Angerer J, Ewers U, Heudorf U, Wilhelm M (2009) Revised and new reference values for environmental pollutants in urine or blood of children in Germany derived from the German environmental survey on children 2003–2006 (GerES IV). Int J Hyg Environ Health 212:637–647

Schwartz GG, Il'yasova D, Ivanova A (2003) Urinary cadmium, impaired fasting glucose, and diabetes in the NHANES III. Diabetes Care 26:468–470

Shaikh ZA, Smith LM (1986) Biological indicators of cadmium exposure and toxicity. Exp Suppl 50:124–130

Shaikh ZA, Tohyama C (1984) Urinary metallothionein as an indicator of cadmium body burden and of cadmium-induced nephrotoxicity. Environ Health Perspect 54: 171–174

Shaikh ZA, Tohyama C, Nolan CV (1987) Occupational exposure to cadmium: effect on metallothionein and other biological indices of exposure and renal function. Arch Toxicol 59:360–364

Shaikh ZA, Ellis KJ, Subramanian KS, Greenberg A (1990) Biological monitoring for occupational cadmium exposure: the urinary metallothionein. Toxicology 63:53–62

Shaikh ZA, Vu TT, Zaman K (1999) Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants. Toxicol Appl Pharmacol 154:256–263

Soden SE, Lowry JA, Garrison CB, Wasserman GS (2007) 24-hour provoked urine excretion test for heavy metals in children with autism and typically developing controls, a pilot study. Clin Toxicol (Phila) 45:476–481

Squibb KS, Fowler BA (1984) Intracellular metabolism and effects of circulating cadmium-metallothionein in the kidney. Environ Health Perspect 54:31–35

Staessen JA, Buchet JP, Ginucchio G, Lauwerys RR, Lijnen P, Roels H, Fagard R (1996) Public health implications of environmental exposure to cadmium and lead: an overview of epidemiological studies in Belgium. Working Groups. J Cardiovasc Risk 3:26–41

Sundberg A, Appelkvist EL, Dallner G, Nilsson R (1994) Glutathione transferases in the urine: sensitive methods for detection of kidney damage induced by nephrotoxic agents in humans. Environ Health Perspect 102(Suppl 3): 293–296

Suwazono Y, Akesson A, Alfven T, Jarup L, Vahter M (2005) Creatinine versus specific gravity-adjusted urinary cadmium concentrations. Biomarkers 10:117–126

Suwazono Y, Sand S, Vahter M, Filipsson AF, Skerfving S, Lidfeldt J, Akesson A (2006) Benchmark dose for cadmium-induced renal effects in humans. Environ Health Perspect 114:1072–1076

Suzuki Y (1980) Cadmium metabolism and toxicity in rats after long-term subcutaneous administration. J Toxicol Environ Health 6:469–482

Tang J, Xiao T, Wang S, Lei J, Zhang M, Gong Y, Li H, Ning Z, He L (2009) High cadmium concentrations in areas

with endemic fluorosis: a serious hidden toxin? Chemosphere 76:300–305
Tanimoto A, Hamada T, Koide O (1993) Cell death and regeneration of renal proximal tubular cells in rats with subchronic cadmium intoxication. Toxicol Pathol 21: 341–352
Teeyakasem W, Nishijo M, Honda R, Satarug S, Swaddiwudhipong W, Ruangyuttikarn W (2007) Monitoring of cadmium toxicity in a Thai population with high-level environmental exposure. Toxicol Lett 169:185–195
Thevenod F (2003) Nephrotoxicity and the proximal tubule. Insights from cadmium. Nephron Physiol 93:87–93
Thevenod F (2009) Cadmium and cellular signaling cascades: to be or not to be? Toxicol Appl Pharmacol 238:221–239
Thomas LD, Hodgson S, Nieuwenhuijsen M, Jarup L (2009) Early kidney damage in a population exposed to cadmium and other heavy metals. Environ Health Perspect 117: 181–184
Trisak ST, Doumgdee P, Rode BM (1990) Binding of zinc and cadmium to human serum albumin. Int J Biochem 22:977–981
Trzcinka-Ochocka M, Jakubowski M, Razniewska G, Halatek T, Gazewski A (2004) The effects of environmental cadmium exposure on kidney function: the possible influence of age. Environ Res 95:143–150
Uno T, Kobayashi E, Suwazono Y, Okubo Y, Miura K, Sakata K, Okayama A, Ueshima H, Nakagawa H, Nogawa K (2005) Health effects of cadmium exposure in the general environment in Japan with special reference to the lower limit of the benchmark dose as the threshold level of urinary cadmium. Scand J Work Environ Health 31:307–315
Vaidya VS, Ferguson MA, Bonventre JV (2008) Biomarkers of acute kidney injury. Annu Rev Pharmacol Toxicol 48: 463–493
Vaidya VS, Ford GM, Waikar SS, Wang Y, Clement MB, Ramirez V, Glaab WE, Troth SP, Sistare FD, Prozialeck WC, Edwards JR, Bobadilla NA, Mefferd SC, Bonventre JV (2009) A rapid urine test for early detection of kidney injury. Kidney Int 76:108–114
Waalkes MP (2003) Cadmium carcinogenesis. Mutat Res 533:107–120
Waisberg M, Joseph P, Hale B, Beyersmann D (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 192:95–117
Wang G, Fowler BA (2008) Roles of biomarkers in evaluating interactions among mixtures of lead, cadmium and arsenic. Toxicol Appl Pharmacol 233:92–99
Webb M (1979) Functions of hepatic and renal metallothioneins in the control of the metabolism of cadmium and certain other bivalent cations. Exp Suppl 34:313–320
Webb M (1986) Role of metallothionein in cadmium metabolism. In: Foulkes EC (ed) Cadmium, hanbook of experimental pharmcology. Springer-Verlag, New York, pp 281–337
World Health Organization (WHO) (2000) Cadmium. http://www.euro.who.int/document/aiq/6_3cadmium.pdf
Wu X, Jin T, Wang Z, Ye T, Kong Q, Nordberg G (2001) Urinary calcium as a biomarker of renal dysfunction in a general population exposed to cadmium. J Occup Environ Med 43:898–904
Wu X, Su S, Zhai R, Chen K, Jin T, Huang B, Zhou Y, Ge X, Wei G, Liao R (2004) Lack of reversal effect of EDTA treatment on cadmium induced renal dysfunction: a fourteen-year follow-up. Biometals 17:435–441
Wu X, Liang Y, Jin T, Ye T, Kong Q, Wang Z, Lei L, Bergdahl IA, Nordberg GF (2008) Renal effects evolution in a Chinese population after reduction of cadmium exposure in rice. Environ Res 108:233–238
Xiao W, Liu Y, Templeton DM (2009) Pleiotropic effects of cadmium in mesangial cells. Toxicol Appl Pharmacol 238:315–326
Yoshida M, Ohta H, Yamauchi Y, Seki Y, Sagi M, Yamazaki K, Sumi Y (1998) Age-dependent changes in metallothionein levels in liver and kidney of the Japanese. Biol Trace Elem Res 63:167–175
Zalups RK, Ahmad S (2003) Molecular handling of cadmium in transporting epithelia. Toxicol Appl Pharmacol 186:163–188