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## ***In vivo* exposures to particulate matter collected from Saudi Arabia or nickel chloride display similar dysregulation of metabolic syndrome genes**

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### **Abstract**

Particulate matter (PM) exposures have been linked to mortality, low birth weights, hospital admissions, and diseases associated with metabolic syndrome, including diabetes mellitus, cardiovascular disease, and obesity. In a previous *in vitro* and *in vivo* study, data demonstrated that PM<sub>10µm</sub> collected from Jeddah, Saudi Arabia (PM<sub>SA</sub>) altered expression of genes involved in lipid and cholesterol metabolism, as well as many other genes associated with metabolic disorders. PM<sub>SA</sub> contains a relatively high concentration of nickel (Ni), known to be linked to several metabolic disorders. In order to evaluate if Ni and PM exposures induce similar gene expression profiles, mice were exposed to 100µg/50µl PM<sub>SA</sub> (PM-100), 50µg/50µl nickel chloride (Ni-50), or 100µg/50µl nickel chloride (Ni-100) twice a week for 4 weeks and hepatic gene expression changes determined. Ultimately, 55 of the same genes were altered in all 3 exposures. However, where the two Ni groups differed markedly was in the regulation (up or down) of these genes. Ni-100 and PM-100 groups displayed similar regulations, whereby 104 of the 107 genes were similarly modulated. Many of the 107 genes involved in metabolic syndrome and include *ALDH4A1*, *BCO2*, *CYP1A*, *CYP2U*, *TOP2A*. In addition, the top affected pathways such as fatty acid  $\alpha$ -oxidation, and lipid and carbohydrate metabolism, are involved in metabolic diseases. Most notably, the top diseased outcome affected by these changes in gene expression was cardiovascular disease. Given these data, it appears that Ni and PM<sub>SA</sub> exposures display similar gene expression profiles, modulating the expression of genes involved in metabolic disorders.

### **Keywords**

gene expression; metabolic diseases; liver; cholesterol; particulate matter; nickel

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### **Conflict of Interest Statement**

None of the authors of the manuscript have a conflict of interest.

## Introduction

Particulate matter (PM) exposure has long been associated with many diseases classified as metabolic syndrome (MtS) diseases. Metabolic syndrome refers to 5 risk factors: obesity, hypertension, hyperglycemia, high triglycerides, and low high density lipoprotein (HDL). Subjects who have 3 or more of these risk factors are considered to display MtS.

Epidemiological and molecular studies demonstrated PM exposure to be associated with MtS risk factors: hypertension (Pope et al, 2015), hyperglycemia (Fleisch et al, 2014; Li et al, 2014; Wang et al, 2014; Zheng et al, 2013), high triglycerides (Rizzo et al, 2014; Yeatts et al, 2007), obesity (Roberts et al, 2014), and low HDL (Li et al, 2013). In general, PM exposures are beginning to be taken more seriously after years of research demonstrated links between PM and increased hospital admissions (Colucci et al, 2006; Vigotti et al, 2007), chronic obstructive pulmonary disease (Gan et al, 2013), lung cancer (Yanagi et al, 2012), asthma (Karakatsani et al, 2012), migraine headaches (Chiu and Yang, 2015), urticaria (Kousha and Valacchi, 2015) and cardiovascular diseases (Brook et al, 2010; Mazzoli-Rocha et al, 2010; Nishiwaki et al, 2012; Beckerman et al, 2012; Chang et al, 2013; Chiu et al, 2013). PM was categorized to be the major component of air pollution producing the most deleterious effects on human health (Colucci et al, 2006; Samet and Krewski, 2007).

Jeddah, the second largest city in the Kingdom of Saudi Arabia, contains many factors that make it a site for heavy PM exposure. There are power plants, oil refineries, industrial companies, and over 1.4 million cars, not to mention heavy dust storms. All of these factors contribute to high risk potential for PM exposures (Khodeir et al, 2012). Incidences of diabetes mellitus have significantly increased in Saudi Arabia over the past decade. Al-Rubeaan et al (2014) found in a cohort of over 5000 subjects, abnormal glucose metabolism occurred in almost 35% of subjects. An investigation performed by Al-Nozha et al (2004) noted overall prevalence for diabetes mellitus in adults in Saudi Arabia was 23.7%. Saudi Arabia is already burdened with MtS-associated diseases, and given the progressive nature of this country, PM exposures are only likely to be increased and consequently MtS-associated disease frequencies will rise (Al-Malki et al, 2003; Madani et al, 2000).

Recently, the use of gene expression profiling has increased in an attempt to more comprehensively elucidate mechanisms underlying PM-mediated adverse health effects (Huang, 2013). To investigate this ongoing issue, our lab undertook studies to characterize the influence of PM collected from Saudi Arabia using both *in vitro* and *in vivo* exposures. PM from Saudi Arabia (PM<sub>SA</sub>) was collected at the University campus and is a mixture of coarse and fine PM, with a PM<sub>2.5</sub>/PM<sub>10</sub> ratio of 0.33. *In vitro* exposure showed BEAS-2B (human bronchial epithelial cells) acutely treated with PM<sub>SA</sub> displayed dysregulation in pathways involving lipid and cholesterol metabolism (Sun et al, 2012). *In vivo* exposure revealed mice exposed to 100µg/50µl of PM<sub>SA</sub> for 24 hr displayed alterations in many genes involved in metabolic syndrome (Brocato et al, 2014).

Since genes involved in metabolic syndrome are primarily transcribed in the liver, a 4 week exposure was performed in mice using 100 µg PM<sub>SA</sub> or 50 or 100 µg of nickel chloride (NiCl). Both Ni and PM exposures are associated with MtS and Ni is present in a relatively

high concentration in the  $PM_{SA}$ . The aim of this study was to see if exposure to Ni or  $PM_{SA}$  dysregulate pathways involved in MtS in a similar manner.

## Materials and Methods

### Animals

Specific pathogen-free 8–10 week-old male FVB/N mice weighing 23–25 g were purchased from Taconic Farms (German-town, NY). All animals were housed in an approved facility at NYUSOM and acclimated for 1–2 weeks under controlled temperature ( $22 \pm 2^\circ\text{C}$ ) and relative humidity (30–50%) with a 12-hr light/dark cycle prior to use in any experiments. Mice were provided *ad libitum* access to standard lab chow and filtered water except during oropharyngeal exposures. At the time of exposures, mice were randomly assigned to each exposure group ( $n = 5$ ). All protocols were approved by the NYU School of Medicine IACUC. One out of 5 mice died in the Ni-50, PM-100, and control groups and 2 out of 5 mice died in the Ni-100 group by the end of the 4 weeks. All deaths occurred during aspiration.

### Oropharyngeal Aspirations (OPA)

Mice ( $n=5$ ) were exposed via aspiration to  $100 \mu\text{g } PM_{10}$  (3.92 mg/kg) collected from Jeddah, Saudi Arabia. The cumulative dose of PM received by the mice over the 4 week period was 39.36 mg/kg. The dose of  $PM_{2.5}$  received by each mouse was 1.29 mg/kg. The two other treatment groups received  $NiCl_2 - 50$  or  $100 \mu\text{g}$ . Control mice ( $n=5$ ) were exposed to an equivalent volume of sterile pyrogen free water. For each aspiration, mice were anesthetized in a closed container containing isoflurane (1–3% in oxygen) (Butler Schein, Dublin, OH), weighed, and aspirated with a volume of approximately  $50 \mu\text{l}$

### Animal Processing Post-Exposure

Oropharyngeal aspirated animals were euthanized 24hr post-final via intraperitoneal (ip) injection of pentobarbital (150–200 mg/kg). At necropsy, blood was drawn from the *vena cava* and serum was isolated and stored at  $-20^\circ\text{C}$ . Lungs, kidneys, spleen, and liver were collected and stored at  $-80^\circ\text{C}$  for future analyses.

### Particle Characterization

Details regarding the particle collection and extraction techniques, as well as, the components of  $PM_{SA}$  were previously described (Khodeir et al, 2012; Sun et al, 2012).  $PM_{SA}$  was analyzed by x-ray fluorescence for the concentration of 27 elements. Re-suspended soil and oil combustion contributed 82% of the mass and mixed industrial sources, traffic sources, and marine aerosols were also found to be present. The  $PM_{SA}$  was heavily concentrated with silicon, calcium, sulfur, aluminum and iron. Other metals present include nickel, vanadium, arsenic, lead, cadmium, manganese, titanium and magnesium.

### RNA extraction and microarray hybridization

Total RNA was extracted from lungs of control, Ni, and  $PM_{SA}$ -exposed mice using Trizol (Invitrogen) and further purified using RNeasy Plus Micro Kit (Qiagen). To synthesize

double-stranded cDNA (dsDNA), 100ng total RNA was used. cRNA was synthesized from dsDNA template, and subsequently used to produce sense single-stranded cDNA (ssDNA) with incorporated deoxyuridine triphosphate. The ssDNAs were fragmented, end-labeled, and hybridized to Affymetrix Mouse Gene 1.0 ST Array (Affymetrix). Hybridization and scanning of the arrays were performed using a standard procedure.

### Microarray data analysis

Microarray data analysis was performed using GeneSpring v12.0 (Agilent Technologies). All microarray data is MIAME compliant and raw data were deposited in NCBI's Gene Expression Omnibus (GEO ID: GSE38172). The expression value of each probe set was determined after quantile normalization using RMA algorithm and baseline transformation to median levels of control samples. Differentially expressed genes were identified using one-way ANOVA ( $p < 0.05$ ). Functional annotation was analyzed with the Gene Ontology (GO) classification system using DAVID software (<http://david.abcc.ncifcrf.gov/home.jsp>). Gene network and pathway analysis was performed using Ingenuity Pathway Analysis (<http://www.ingenuity.com>).

### Real time quantitative PCR

Total RNA extracted from control and treated lung tissue was converted to single stranded cDNA using Superscript® III (Invitrogen). Quantitative real-time PCR analysis was performed using SYBR green PCR system (Applied Biosystems) on ABI prism 7900HT system (Applied Biosystems). Relative gene expression levels were normalized to ACTB expression. All PCR reactions were performed in duplicate.

## Results

### Exposure to PM<sub>SA</sub> or Ni on gene expression of liver cells in vivo

Gene expression changes were investigated in livers of FVB/N mice exposed for 4 weeks via aspiration to Ni at 50 µg nickel chloride (Ni-50) or 100 µg nickel chloride (Ni-100), or 100 µg PM collected from Saudi Arabia (PM-100). Fold change analysis (one-way ANOVA, alpha level only) identified 1,054 dysregulated genes by Ni-50; 476 were down-regulated and 578 were up-regulated (Tables 1–3). Ni-100 altered the expression of 2,701 genes; 1,298 were down-regulated and 1,403 were up-regulated. PM-100 affected expression of 716 genes; 476 were down-regulated and 578 were up-regulated. The fold change of the altered genes was greater than 1.1. Tables 1–3 show the top 10 up- and down-regulated genes for each exposure group.

Figure 1 is a Venn diagram illustrating the overlap of dysregulated genes amongst the 3 exposure groups. There were 55 of the same genes altered in all 3 exposures. Between Ni exposures, regulation of similar genes varied greatly. Only 34 out of the 55 genes were altered in the same direction, up or down. Surprisingly, PM-100 and Ni-100 altered the expression of the 55 genes in a similar fashion; 52 out of the 55 genes were regulated in the same direction. The similarity in altered gene expression was further investigated between Ni-100 and PM-100 by examining all 107 genes that were changed by both exposures. Data

demonstrated that 104 out of 107 altered genes were regulated in the same direction. Tables 4 (down-regulated) and 5 (up-regulated) list the top 10 genes affected by both Ni and PM.

### Metabolic syndrome (MtS) - associated genes

To investigate the biological function of the 107 genes differentially modulated by Ni-100 and PM-100 exposures, the list of changed genes were uploaded into the Ingenuity Pathway Analysis (IPA) tool. Many of the dysregulated genes altered in the same direction by Ni-100 and PM-100 were involved in pathways associated with MtS. The top canonical pathways (Figure 2) ranked by p-value were fatty acid  $\alpha$ -oxidation, bupropion degradation, and acetone degradation. *ALDH4A1* (-1.13) and *BCO2* (-1.15) were the two major genes deregulated in the fatty acid  $\alpha$ -oxidation pathway that play a role in metabolic syndrome (Amengual et al, 2011; Pang et al, 2014; Yoon et al, 2004). *CYP1A2* (-1.12) and *CYP2U1* (-1.18) were both down-regulated in the bupropion and acetone degradation pathways and both genes encode enzymes that are involved in lipid metabolism (Chuang et al, 2004; Shertzer et al, 2004).

Network analysis of the altered genes revealed that 13 significant networks were affected by the change in expression. Several networks involved in MtS were affected including “carbohydrate metabolism, lipid metabolism, small molecule biochemistry” with 11 focus molecules and a significance score of 20 (the negative log of the p-value). Other interesting networks include “cell cycle, cellular movement, cellular assembly and organization” (score: 50); “infectious disease, cellular compromise, gastrointestinal disease” (score: 30); “behavior, nervous system development and function, tissue development” (score: 27).

The most prominent disease affected by the altered genes was cardiovascular disease with 13 affected molecules including *TOP2A*, *VEGFB*, and *IL6R*. Other affected diseases that are worth mentioning include, developmental disorders, skeletal and muscle disorders, organismal injury and abnormalities, and connective tissue disorders. *TSA* and *FoxM1* were found to be upstream regulators predicted to be activated. Both of these molecules are upstream regulators of *TOP2A*, *CCNA2*, and *PLK1*.

*CDKN1A*, which encodes p21, a very potent cyclin-dependent kinase inhibitor (Broude et al, 2007), was identified as an upstream regulator of many of MtS-associated genes that were increased by Ni-100 and PM-100. *CDKN1A* was predicted to be inhibited and this led to up-regulation of *TOP2A*, *PLK1*, *CCNA2*, and others (Figure 3). Other upstream regulators worth noting are Rb and E2F. Rb sequesters E2F in the nucleus and prevents it from acting as a transcription factor to promote cell cycle progression from G1 to S-phase (Hiebert, 1993; Hiebert et al, 1992). These proteins increased gene expression of the same genes as p21, which was expected since p21 inhibition of CDK2 and CDK4/6 leads to dephosphorylation and activation of Rb (Broude et al, 2007).

PM modulated the expression of several MtS-associated genes that was not altered by Ni. *STAT5A* (-1.17) was down-regulated and this gene encodes a protein involved in lipid storage (Teglund et al, 1998) and metabolism of fatty acids (Schirra et al, 2008). *APO4* (-1.33) encodes a protein involved in the metabolism of cholesterol (Duverger et al, 1991) and increases biosynthesis of fatty acids (Goldberg et al, 1990).

## Gene expression validation

To validate the gene expression changes observed in the microarray analysis, total RNA was extracted from livers of PM or Ni- exposed mice and purified. Several candidate genes were selected for quantitative real time PCR (RT-qPCR). Gene fold changes were compared to those obtained from microarrays. *ALDH4a4*, *CYP1a2*, *TOP2A*, *BCO2*, *CYP2U1*, *PLK1*, and *AURKB* were selected as candidate genes to validate the microarray. All of these genes were altered in the same direction in both Ni-100 and PM-100 exposed mice. Table 6 summarizes the average fold change of the selected genes from the microarray analysis, as well as corresponding RT-qPCR results.

## Discussion

### Metabolic syndrome- associated diseases are on the rise in Saudi Arabia

Cardiovascular disease and diabetes, two chronic diseases that result from the risk factors of MtS, are on the rise in the Kingdom of Saudi Arabia. Obesity has also become a major health concern in Saudi Arabia. A study by (Al-Othaimen AI, 2007) found that 38% of male and 28% female of the 19,598 Saudi Arabian citizens tested were overweight. In addition, a number of other studies highlighted the obesity problem in Saudi Arabia (Al-Malki et al, 2003; Madani et al, 2000). Studies evaluating locations with high levels of PM exposure demonstrated that PM increases the amount of hospital admissions and promotes cardiovascular disease and diabetes. Recently, (Khodeir et al, 2012) conducted a multi-week, multiple site sampling campaign to determine the source apportionment and elemental composition of PM<sub>10</sub> in Jeddah. The major source factors for PM<sub>10</sub> were soil re-suspensions, oil combustion, mixed industrial sources, traffic sources, and marine aerosols. Components of the PM<sub>10</sub> from Jeddah were characterized by Khodeir et al (2012). Jeddah consistently exceeds the 80 µg/m<sup>3</sup> PM established by the Presidency of Meteorology and Environment in Saudi Arabia.

### Top Canonical Pathways

IPA and DAVID were used to analyze the 107 genes similarly altered by both Ni-100 and PM-100 and the top canonical pathways involved in MtS are presented in Figure 2. The top canonical pathway was fatty acid oxidation where *BCO2* and *ALDH4A1* were both down-regulated. Dysregulation in fatty acid oxidation pathways are involved in insulin sensitivity (Randle et al, 1994) and obesity (Furukawa and Araki, 2013; Furukawa et al, 2004). A detailed review by Wakil and Abu-Elheiga (2009) on fatty acids and MtS discuss the key roles that fatty acids play as cellular signaling molecules and how their dysfunctions form the etiology of MtS. The gene encoding *ALDH4A1* (aldehyde dehydrogenase family 4A1) leads to production of proline and deficiency of this enzyme results in the metabolic disease hyperprolinemia (Kamoun et al, 1998; Onenli-Mungan et al, 2004). Several reports supported the notion that down-regulation of *ALDH4A1* might increase cell damage due to generation of reactive oxygen species (ROS). *ALDH4A1* blocks Nrf2, which is a transcription factor, that activates genes involved in fatty acid oxidation (FAO) (Pang et al, 2014). Thus down-regulation in expression of *ALDH4A1* may potentially increase FAO. An investigation by Yoon et al (2004) suggested that p53 might play a protective role against cell damage induced by generation of intracellular ROS, through transcriptional activation



of *ALDH4A1*. ALDH4-associated effects on p53 may be mediated by enzymatic interaction with MDM2 (Nicholson et al, 2014). Thus a down-regulation of ALDH4 might potentially lead to enhanced cell damage induced by ROS.

*BCO2* (beta-carotene oxygenase 2), which was also down-regulated in the fatty acid oxidation pathway, oxidizes beta-carotene for biosynthesis of vitamin A (Kiefer et al, 2001). Beta-carotene metabolizing enzymes are expressed in adipocytes and shown to reduce adipocyte size and body fat % in mice by regulating PPAR $\gamma$  activity (Amengual et al, 2011). Ni and PM-induced down-regulation of *BCO2* may contribute to obesity.

Most of the other canonical pathways given by IPA were involved in MtS. The second most influenced pathway according to p-value was bupropion degradation. Bupropion is better known as Wellbutrin, a type of antidepressant that is also used to treat obesity.

Dysregulation of the catabolic pathways involved in this drug's metabolism is likely to interfere with the effects of the drug. There is now a phase III clinical trial for a sustained release form of bupropion called Naproxene (Apovian et al, 2013). Studies found that the drug is a new approach to tackling the problem of obesity and may soon be a unique and valuable therapeutic option (Apovian et al, 2013; Padwal, 2009; Wadden et al, 2011).

Other canonical pathways (in order of significance) included, acetone degradation, proline degradation, and taurine biosynthesis. Acetones are produced by decarboxylation of ketone bodies. Inability to degrade acetone might result in ketoacidosis and promote diabetes (Kamel and Halperin, 2015). Interfering with taurine biosynthesis might promote liver disease and hypertension (Zhang et al., 2014). Supplementation with taurine was found to reduce oxidative stress and promote liver health in a murine model.

### Upstream Regulators

IPA found many upstream regulators that were predicted to be inhibited or activated based on the altered genes and the direction the genes changed. The most interesting of these regulators was CDKN1A (Figure 3). CDKN1A, also known as p21 is involved in the cell cycle by inhibiting cyclin dependent kinases which acts to terminate the cell cycle at the G1-S phase transition. IPA predicted p21 to be markedly inhibited based on the gene changes. All genes leading to p21 inhibition were up-regulated; *PLK1*, *TOP2A*, *KIF20*, *CCNA2*, *AURKB*, and *STMN1*. Moreover, many of these genes were also identified in MtS.

Up-regulation of *PLK1* (polo-like kinase 1) is associated with hepatocellular carcinoma. Hypomethylation of the *PLK1* gene is associated with hepatocellular carcinoma (Stefanska et al, 2011). According to [clinicaltrials.gov](http://clinicaltrials.gov), levofloxacin, a TOP2A inhibitor, is in phase IV clinical trials for the treatment of obesity in humans. Therefore, an up-regulation of TOP2A (DNA topoisomerase 2 A) as produced by Ni and PM treatment would promote obesity. *KIF20A* (kinesin family member 20 A) was found to be regulated by CLOCK in mouse livers. CLOCK is also involved in lipid metabolism (Oishi et al, 2005). Gnocchi et al (2015) indicated how the circadian clock and lipid metabolism are interconnected and further understanding of this relationship is pertinent in combatting metabolic diseases .

*CCNA2* (cyclin that regulates CDK2) promotes the G1/S and G2/M cell cycle transitions. Up-regulation enhances transitions through the cell cycle and increase proliferation. *CCNA2* expression is associated with hepatocellular carcinoma (Satow et al, 2010). *STMN1* (stathmin1) promotes disassembly of microtubules by destabilizing microtubules and up-regulation was shown to enhance expression of *VEGF* (vascular endothelial growth factor) (Tamura et al, 2013).

Two other upstream regulators worth discussing are TSA (trichostatin-A) and FOXM1 (forkhead box M1). Both of these molecules were predicted to be activated based on gene expression. Surprisingly, the same set of genes used to predict p21 inhibition was used to predict activation of these upstream molecules; *PLK1*, *TOP2A*, *KIF20A*, *CCNA2*, *STMN1*, *AURKB*. TSA is a group 1 and II HDAC (histone deacetylase) inhibitor but does not inhibit sirtuins (group 3). Tian et al (2014) demonstrated the importance of acetylation in breast cancer. Acetylation-defective mutants of Ppar $\gamma$ 1 were associated with reduced lipid synthesis in ErbB2 overexpressing breast cancer cells. Activation of FOXM1 may also contribute metabolic disorders. FOXM1 is activated in highly proliferating normal cells and cancer cells; it promotes proliferation by progressing the cell cycle via directly activating the transcription of cyclin D1 and cyclin B1. An investigation by Hu et al (2014) reported that down-regulation of FOXM1 suppressed proliferation of hepatocellular carcinoma cells.

#### **Other Ni and PM-dysregulated genes involved in metabolic syndrome**

Besides the genes listed above, several other genes were identified from our gene list that play roles in diseases associated with MtS. One of these genes was *PAX6*. *PAX6* (paired box 6) was down-regulated in both Ni and PM-treated mice and is crucial for  $\beta$ -cell function, insulin biosynthesis, and glucose-induced insulin secretion; decreased Pax6 diminishes processing of insulin (Gosmain et al, 2012b). Decreased Pax6 decreases release of glucagon (Gosmain et al, 2012a). Overexpression of *PAX6* mRNA reduced synthesis of insulin (Wolf et al, 2010). *CYP1A2* (cytochrome P450, family 1, subfamily A) mRNA was also lowered in livers of Ni or PM-treated mice. *CYP1A2* is involved in the synthesis of lipids, steroids, and cholesterol. *CYP1A2* protects against ROS production in mouse liver microsomes (Shertzer et al, 2004). *C12orf4* (chromosome 12 open reading frame 4) was partially silenced by both Ni and PM and silencing of this gene is associated with carcinoma of the liver according to the Catalogue Of Somatic Mutations In Cancer (COSMIC).

#### **Ni and PM induce similar alterations of MtS-associated genes**

The toxicity of PM depends largely on its components. Lippmann et al (2007) noted that the cardiotoxicity effects of fine PM exposures are predominantly driven by Ni. Niu et al (2013) noted that metallic components of PM may be responsible for the cardiovascular disorders produced by PM exposures. Nickel has long been reported from epidemiological and molecular studies to promote cardiovascular disease (Bell et al, 2014; Ying et al, 2013), respiratory cancers (Grimsrud and Peto, 2006), insulin resistance (Xu et al, 2012) and mortality (Laden et al, 2000). Hsu and Lippmann (2007) previously found that the levels of Ni in concentrated ambient particulate (CAP) matter PM<sub>2.5</sub> were strongly correlated to acute changes in heart rates and their variability. Xu et al (2012) noted that Ni exposure exerted effects on metabolism comparable to CAP matter PM<sub>2.5</sub>. Exposure to both Ni and PM



significantly enhanced fasting glucose and worsened insulin resistance indices, when compared with exposure to CAP matter alone. The effects of the co-exposure were thought to be mediated by AMP-activated protein kinase. Another study that co-exposed mice to nickel and CAP also found synergistic effects between the two. Tumor necrosis factor- $\alpha$  and monocyte chemoattractant protein 1 were both significantly up-regulated in co-exposed mice (Goebeler et al, 1999).

Given the numerous studies that pointed towards Ni as being a key player in the adverse health effects associated with PM exposures, it was not surprising to find that this metal affected the expression of MtS-associated genes in a similar fashion. The 3 exposure groups (Ni-50, Ni-100, and PM-100) shared 55 genes that displayed altered expression. The regulation of those genes between the two Ni groups was not similar; however, Ni-100 and PM-100 shared 52 out of 55 genes changed in the same direction. Ni-100 and PM-100 shared 107 of the same altered genes, and 104 out of 107 of those genes were regulated in the same direction. Data suggest that Ni and PM at the same dose (100  $\mu\text{g}$ ) induce similar alterations to genes involved in MtS.

The amount of Ni in the PM is 9.2  $\text{ng}/\text{m}^3$ . This is a high concentration compared to several other sites. Saudi Arabia burns a lot of oil and when oil is burned you produce Ni and vanadium. This may explain why Ni concentrations in Saudi Arabia PM are relatively high compared to other locations.

## Conclusions

Particulate matter exposures are known to be associated with a number of adverse health outcomes, hospital admissions (Colucci et al, 2006; Vigotti et al, 2007), chronic obstructive pulmonary disease (Gan et al, 2013), lung cancer (Yanagi et al, 2012), asthma (Karakatsani et al, 2012) migraine headaches (Chiu and Yang, 2015), urticarial (Kousha and Valacchi, 2015) and cardiovascular diseases (Brook et al, 2010; Mazzoli-Rocha et al, 2010; Nishiwaki et al, 2012; Beckerman et al, 2012; Chang et al, 2013; Chiu et al, 2013). Both organic and inorganic components of PM hold potential to produce problems once inhaled (Ghio et al, 2012). It is difficult to pinpoint the exact PM component that is producing damage being evaluated. Since Ni was relatively high in the PM from Saudi Arabia, it was decided to compare gene expression changes occurring after a 4 week *in vivo* exposure to PM vs. gene alterations after *in vivo* exposure to the metal. Almost all of the changes in the 107 similar genes were altered in the same direction. Many of these genes were found to play roles in development of MtS risk factors or diseases associated with this syndrome.

Based upon our findings, it appears that exposure to Ni or PM at the same dose dysregulates the same MtS-associated pathways. Further understanding of the mechanisms by which exposure to Ni or PM produces these adverse effects may provide novel prevention and therapeutic strategies for better control and treatment of metabolic disorders such as obesity, type 2 diabetes, and insulin resistance.

It is likely that given the history of Ni with metabolic disorders, Ni in PM may be contributing to many of the differentially expressed genes induced by PM. In order to

irrefutably conclude that Ni is the major culprit, an experiment needs to be conducted where all components of PM remain exactly the same, while Ni is removed completely. However, to acquire these two types of PM would be extremely difficult. Future experiments investigating PM components need to consider this limitation or try to find similar PM samples where one sample contains the component in question at low levels. Alternatively, an overexpression experiment similar to the investigation by Xu et al (2012) would also provide benefits.

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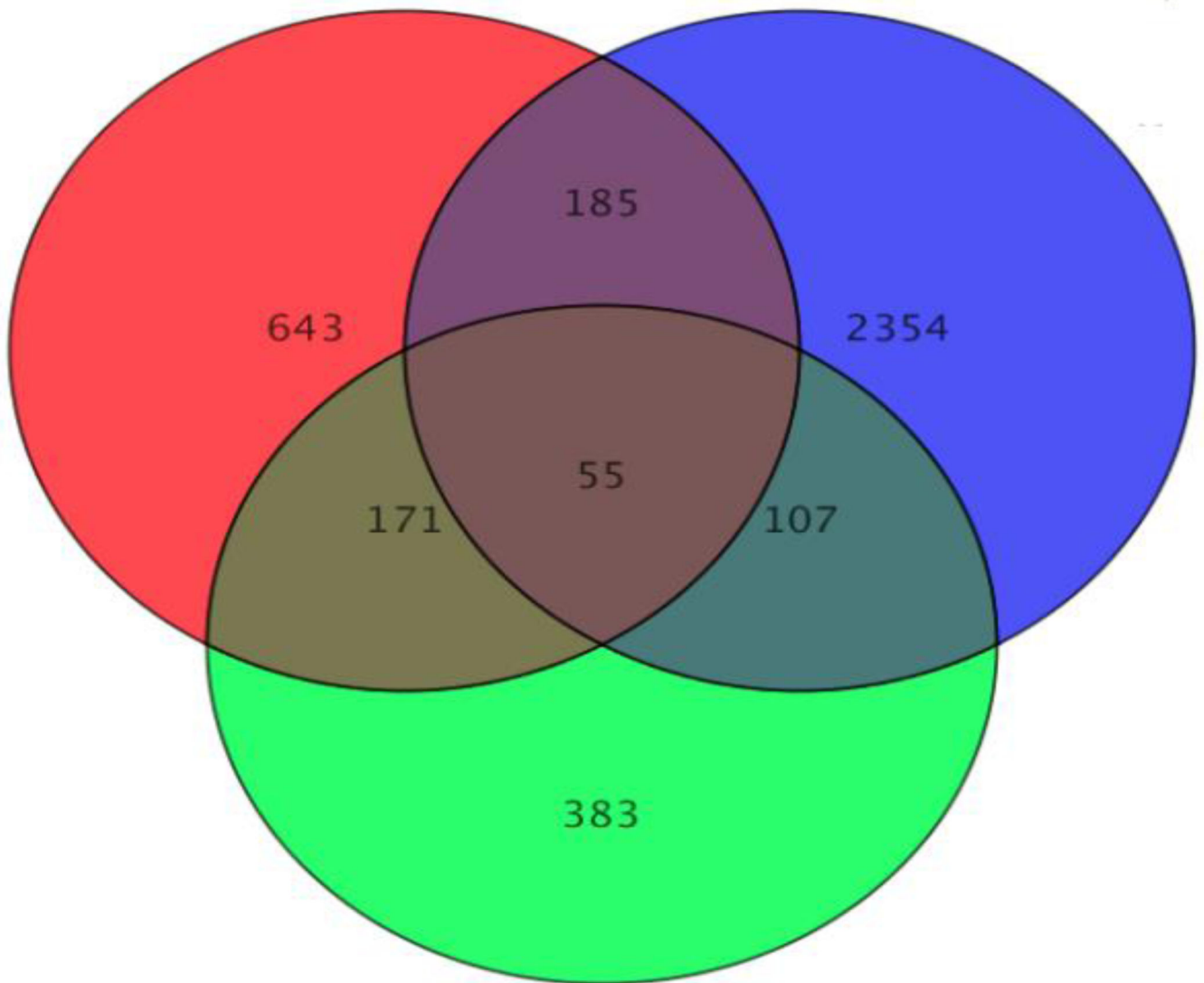
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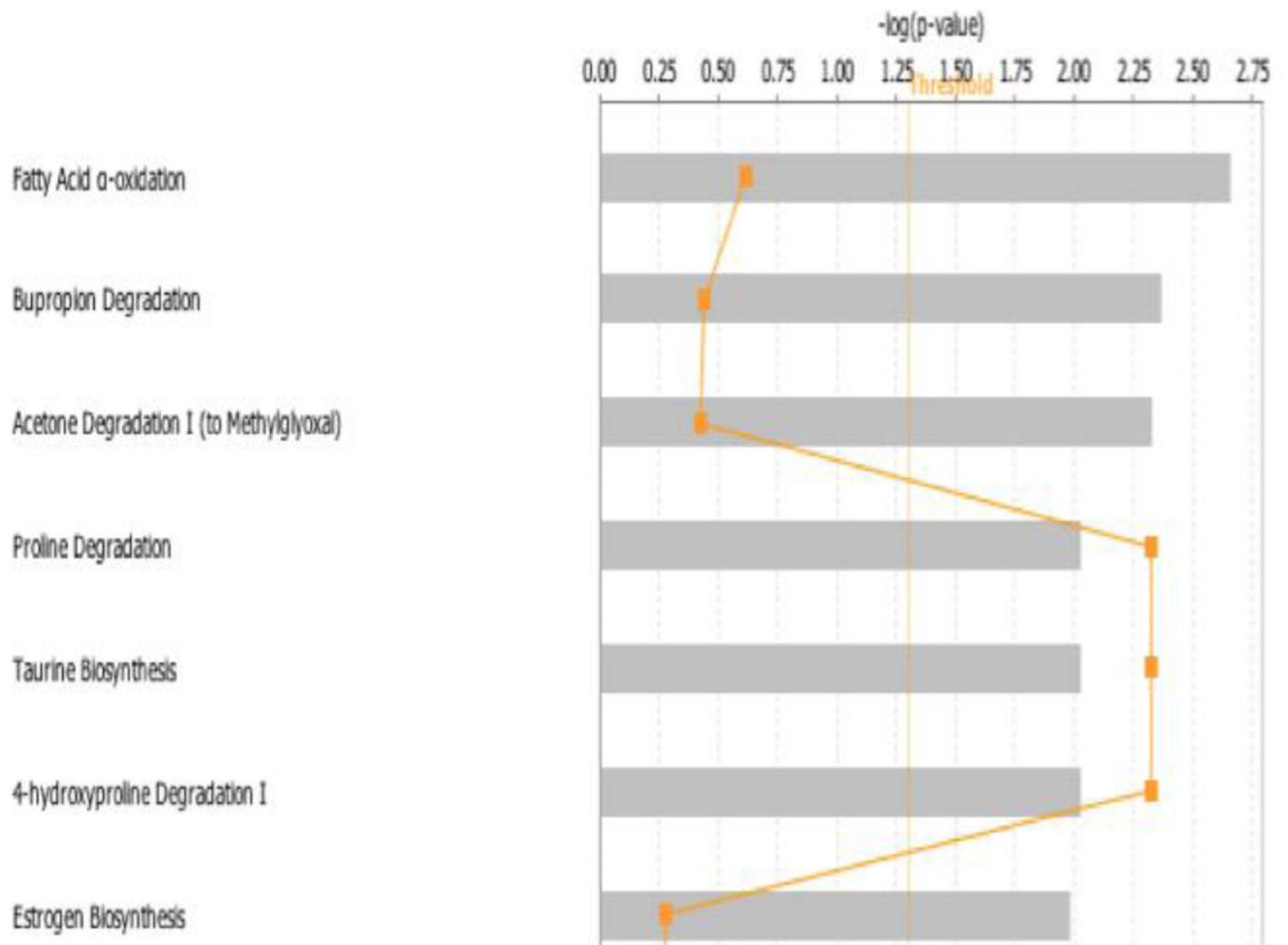
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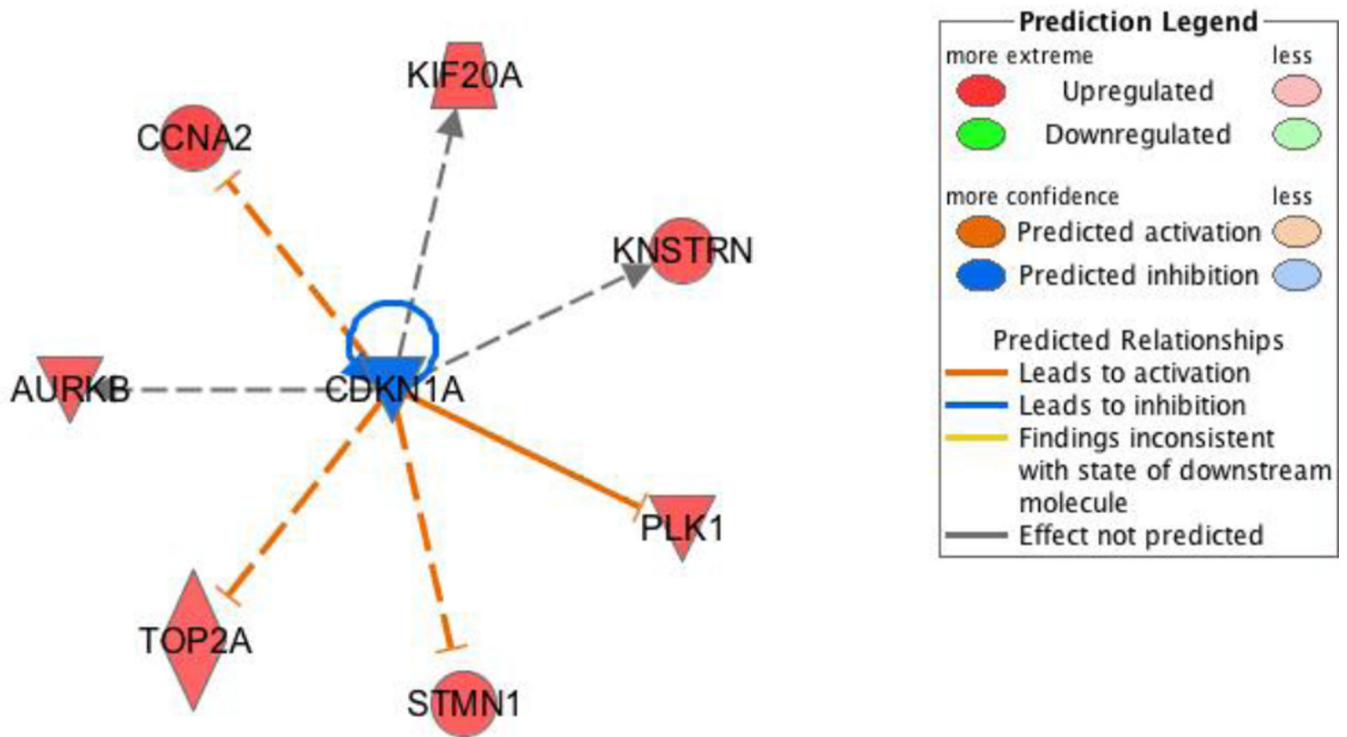
**Figure 1. Venn Diagram**

Red (50  $\mu\text{g Ni}$ ) (Ni-50); Blue (100  $\mu\text{g Ni}$ ) (Ni-100); Green (100  $\mu\text{g PM}$ ) (PM-100). Ni-50 dysregulated 1,054 genes; Ni-100 dysregulated 2,701 genes; PM-100 dysregulated 716 genes. There were 55 similar genes altered by all 3 exposure groups. The two doses of nickel only changed 34 out of the 55 genes in the same direction, while the PM-100 and the Ni-100 changed 52 out of the 55 genes in the same direction. Ni-100 and PM-100 affected 107 similar genes, 104 of which were modulated in the same direction.



**Figure 2. Top Canonical Pathways**

Top 7 canonical pathways identified by IPA from genes changed more than 1.1-fold ( $p < 0.05$ ). Bars represent  $-\log(p\text{-value})$  for significance; orange lines represent the ratio of changed genes to the total number of genes in the specific pathway.



**Figure 3. Upstream Regulator: CDKN1A**

IPA identified many upstream regulators predicted to be active or inactive based on the gene expression profile. CDKN1A, also known as p21, was predicted to be strongly inhibited based on the surrounding genes, all of which are activated. All of these genes besides one (KNSTRN) that work to inhibit CDKN1A are involved in metabolic syndrome. The figure legend describes predicted relationships of all the genes to the upstream regulator.

**Table 1**

Top ten up- and down-regulated genes for mice exposed to 50 µg of nickel chloride.

Gene Name	Gene Symbol	Fold Change
Cytochrome P450, family 4, subfamily a, polypeptide 14	Cyp4a14	2.83
Acyl-CoA thioesterase 3	Acot3	2.14
Inhibitor of DNA binding 1	Id1	1.92
Vanin 1	Vnn1	1.84
Small nucleolar RNA, C/D box 14C	Snord14c	1.79
Suppressor of cytokine signaling 2	Socs2	1.78
Cytochrome P450, family 4, subfamily a, polypeptide 31	Cyp4a31	1.77
Small nucleolar RNA, C/D box 14E	Snord14e	1.76
Cytochrome P450, family 4, subfamily a, polypeptide 10	Cyp4a10	1.71
Heat shock protein 1	Hsph1	1.66
Metallothionein 2	Mt2	-2.54
Orosomucoid 2	Orm2	-2.42
Cytochrome P450, family 2, subfamily b, polypeptide 10	Cyp2b10	-1.69
Orosomucoid 3	Orm3	-1.59
Leucine-rich repeats and transmembrane domains 1	Lrtm1	-1.53
Early growth response 1	Egr1	-1.52
Hemochromatosis type 2	Hfe2	-1.51
Lipocalin 2	Lcn2	-1.50
Acyl-CoA synthetase short-chain family member 2	Acss2	-1.45
Carboxylesterase 2C	Ces2C	-1.45

**Table 2**

Top ten up- and down-regulated genes for mice exposed to 100 µg of nickel chloride.

Gene Name	Gene Symbol	Fold Change
Cytokine inducible SH2-containing protein	Cish	3.54
Myelocytomatosis oncogene	Myc	2.85
Cytochrome P450, family 4, subfamily a, polypeptide 14	Cyp4a14	2.82
Pleckstrin homology-like domain, family A, member 1	Phlda1	2.70
Serine peptidase inhibitor, clade A, member 4	Serpina4	2.62
Forkhead box Q1	Foxq1	2.59
Solute carrier family 25, member 30	Slc25a30	2.29
Cytochrome P450, family 4, subfamily a, polypeptide 31	Cyp4a31	2.24
WD repeat and SOCS box-containing 1	Wsb1	1.85
Small nucleolar RNA, C/D box 14C	Snord14c	1.84
Uridine phosphorylase 2	Upp2	-2.07
Thyroid hormone responsive	Thrsp	-2.05
Arrestin domain containing 3	Arrdc3	-1.92
Cytochrome P450, family 7, subfamily A, polypeptide 1	Cyp7a1	-1.79
3-hydroxy-3-methylglutaryl-Coenzyme A reductase	Hmgcr	-1.70
MicroRNA 107	Mir107	-1.69
Lanosterol synthase	Lss	-1.58
Serine/arginine-rich splicing factor 3	Srsf3	-1.57
Neuregulin 4	Nrg4	-1.55
Methylsterol monooxygenase 1	Sc4mol	-1.54

**Table 3**

Top ten up- and down-regulated genes for mice exposed to 100 µg of particulate matter from Saudi Arabia

Gene Name	Gene Symbol	Fold Change
Cytochrome P450, family 4, subfamily a, polypeptide 14	Cyp4a14	2.34
Cytochrome P450, family 4, subfamily a, polypeptide 10	Cyp4a10	1.85
Cysteine-rich with EGF-like domains 2	Crel2	1.71
Heat shock protein 1	Hsph1	1.65
Serine peptidase inhibitor, clade A, member 7	Serpina7	1.63
G0/G1 switch gene 2	G0s2	1.58
Cysteine sulfinic acid decarboxylase	Csad	1.49
Acyl-CoA thioesterase 2	Acot2	1.49
Glutathione S-transferase, alpha 1	Gsta1	1.48
Jun proto-oncogene	Jun	1.47
Orosomucoid 1	Orm1	-2.42
Orosomucoid 3	Orm3	-1.89
Metallothionein 2	Mt2	-1.80
Zinc finger and BTB domain containing 16	Zbtb16	-1.54
Cytochrome P450, family 39, subfamily a, polypeptide 1	Cyp39a1	-1.45
Early growth response 1	Egr1	-1.44
Solute carrier family 8 (sodium/lithium/calcium exchanger), member B1	Slc24a6	-1.39
Solute carrier family 22 (organic anion transporter), member 7	Slc22a7	-1.38
Interleukin 1 receptor, type I	Il1r1	-1.37
PRA1 domain family 2	Praf2	-1.35



**1Table 4**

Down-regulated genes similar for mice exposed to 100 µg of particulate matter from Saudi Arabia or 100 µg of nickel chloride.

Gene Name	Gene Symbol	Regulation
Serine/arginine-rich splicing factor 3	Srsf3	Down
Cytochrome P450, family 2, subfamily u, polypeptide 1	Cyp2U1	Down
ATP synthase mitochondrial F1 complex assembly factor 1	Atpaf1	Down
Interleukin 6 receptor, alpha	Il6ra	Down
MutS homolog 2	Msh2	Down
ATP synthase mitochondrial F1 complex assembly factor 1	Atpaf1	Down
Mitogen-activated protein kinase associated protein 1	Mapkap1	Down
Stem-loop binding protein	Slbp	Down
Asparagine-linked glycosylation 14	Alg14	Down
Protein tyrosine phosphatase, receptor type, A	Ptpra	Down
Polymerase (RNA) I polypeptide D	Polr1d	Down
Protein inhibitor of activated STAT 3	Pias3	Down
Transmembrane protein 184a	Tmem184a	Down
Cysteine sulfinic acid decarboxylase	Csad	Down
Synaptotagmin	Syt1	Down

<sup>1</sup>This is not a complete list of genes down-regulated by nickel and particulate matter.

**1Table 5**

Altered genes similar for mice exposed to 100 µg of particulate matter from Saudi Arabia or 100 µg of nickel chloride.

Gene Name	Gene Symbol	Regulation
<b>Polo-like kinase 1</b>	Plk1	Up
<b>Spondin 2, extracellular matrix protein</b>	Spon2	Up
<b>Retinol saturase (all trans retinol 13,14 reductase)</b>	Retstat	Up
<b>M-phase phosphoprotein 9</b>	Mphosph9	Up
<b>Vascular endothelial growth factor B</b>	Vegfb	Up
<b>Kinesin family member 20A</b>	Kif20a	Up
<b>Proline rich 23A</b>	Prr23a	Up
<b>Scleraxis</b>	Scx	Up
<b>Serine incorporator 2</b>	Serinc2	Up
<b>Stathmin 1</b>	Stmn1	Up
<b>Olfactory receptor 485</b>	Olf485	Up
<b>Glutamate receptor, ionotropic, AMPA1 (alpha 1)</b>	Gria1	Up
<b>M-phase phosphoprotein 9</b>	Mphosph9	Up
<b>Neutral cholesterol ester hydrolase 1</b>	Nceh1	Up
<b>Neuralized homolog 1b</b>	Neurl1B	Up

<sup>1</sup>This is not a complete list of genes up-regulated by nickel and particulate matter.

**Table 6**

RT-qPCR validation.

Gene Symbol	Microarray (PM-100)	RT-qPCR <sup>I</sup> (PM-100)	Microarray (Ni-100)	RT-qPCR <sup>I</sup> (Ni-100)
<i>ALDH4a1</i>	-1.13	-1.22	-1.16	-1.38
<i>CYP1a2</i>	-1.12	-1.19	-1.14	-1.67
<i>TOP2A</i>	1.12	1.07	1.21	1.18
<i>BCO2</i>	-1.15	-1.34	-1.25	-1.22
<i>CYP2U1</i>	-1.18	-1.84	-1.41	-1.61
<i>PLK1</i>	1.20	2.65	1.29	1.72
<i>AURKB</i>	1.17	1.33	1.25	1.21

PM-100: 100 µg Particulate Matter from Jeddah, Saudi Arabia; Ni-100: 100 µg of nickel chloride

<sup>I</sup>Data is presented as a mean of duplicates.

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