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RESEARCH ARTICLE

The Effects of Iodine Deficiency on Pulmonary Xenobiotic Metabolizing Enzymes in Rats

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SUMMARY

The objective of this study was to investigate the effects of iodine deficiency on pulmonary xenobiotic metabolizing enzyme systems in rats. Three-week old male Wistar rats were fed for seven weeks. Iodine deficiency was produced by sodium perchlorate containing drinking water. The total microsomal cytochrome P450 (CYP450) and cytochrome b5 (CYP b5) levels; activities of microsomal NADPH-cytochrome P450 reductase (P450R), microsomal aniline hydroxylase (CYP2E1), microsomal ethoxyresorufin O-deethylase (EROD), microsomal pentoxyresorufin O-depenthylase (PROD) and cytosolic glutathione S-transferase (GST) were determined. Significant decreases in the level of total CYP450; in the activities of P450R, CYP2E1 and GST; increases in the level of CYP b5 and the activities of EROD and PROD were observed. Overall results of this study have indicated the occurrence of significant alterations in pulmonary xenobiotic metabolism in iodine deficient pubertal rats.

Key Words: *Iodine deficiency, xenobiotic metabolizing enzymes, cytochrome P450, cytochrome b5, NADPH-cytochrome P450 reductase, aniline hydroxylase.*

ÖZET

Bu çalışmanın amacı sıçanlarda iyot eksikliğinin pulmoner ksenobiyotik metabolize edici enzim sistemleri üzerindeki etkisini incelemekti. Üç haftalık erkek Wistar sıçanlar yedi hafta boyunca beslendi. İyot eksikliği sodium perklorat içeren içme suyu verilerek oluşturuldu. Total sitokrom P450 (CYP450) ve sitokrom b5 (CYP b5) düzeyleri; mikrozomal NADPH-sitokrom P450 redüktaz (P450R), mikrozomal aniline hidroksilaz (CYP2E1), mikrozomal etoksirezorufin O-deetilaz (EROD), mikrozomal pentoksirezorufin O-depentilaz (PROD) ve sitozolik glutatyon S-transferaz (GST) aktiviteleri ölçüldü. İyot eksikliği grubunda CYP450 düzeyi ve P450R, CYP2E1 ve GST aktivitelerinde önemli azalma; CYP b5 içeriği ve EROD ve PROD aktivitelerinde önemli artış olduğu gözlendi. Bu çalışmanın sonuçları iyot eksikliği olan sıçanlarda akciğer ksenobiyotik metabolizmasında önemli değişikler olduğunu gösterdi.

Anahtar Kelimeler: *İyot eksikliği, ksenobiyotik metabolize edici enzimler, sitokrom P450, sitokrom b5, NADPH-sitokrom P450 redüktaz, aniline hidroksilaz.*

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INTRODUCTION

The lungs are directly exposed to a wide variety of inhaled drugs as well as environmental toxicants and carcinogens (Zhang et al., 2016). In the pathogenesis of lung diseases, such as chronic obstructive pulmonary disease and lung cancer, a role for environmental toxicants has been shown (Pahwa et al., 2012). Lung contains considerable amount of phase I and phase II enzymes (Zhang et al., 2016), and some lung cells such as the nonciliated bronchiolar epithelial cells in the terminal airways (Clara cells) contain relatively large amounts of cytochrome P450 enzyme (CYP450) dependent monooxygenases (Smith and Brian, 1991). Thus, lung has xenobiotic metabolizing capability.

CYP450s are the major enzymes involved in drug metabolism and bioactivation, accounting for about 75% of the total number of different metabolic reactions, and exist in hepatic and extrahepatic tissues (Guengerich, 2008). The human CYP450s metabolize a wide variety of xenobiotics, including drugs to pharmacologically inactive metabolites and occasionally to toxicologically active metabolites (Park et al., 1995). Many of the compounds associated with pulmonary diseases require enzymatic activation to exert their deleterious effects (Hukkanen et al., 2002). Although xenobiotic metabolizing enzymes in lung share many common features with those present in other tissues such as liver, kidney and gut, there are some distinctive differences. It is evident from the studies carried out to date that CYP1A1, 1B1, 2A13, 2F1, 2S1 and 4B1 are preferentially expressed in lung together with CYP2E1 and 3A5 (Pavek and Dvorak, 2008; Zhang et al., 2016). Several CYPs present in lung, like CYP1A1 and CYP1B1, are involved in metabolic activation and detoxification of polycyclic aromatic hydrocarbons (PAHs). Glutathione S-transferases (GSTs) are also expressed in animal and human lung tissues.

Experiments in rodents have shown that most constitutive hepatic P450 enzymes are subject to hormonal regulation (Anttila et al., 2011). Among other hormones, iodothyronines are particularly important. As shown in rats, thyroid hormones contribute to the regulation of constitutive CYP450s (Chen et al. 1999; Liddle et al., 1998). The results of several clinical studies have suggested that oxidative metabolism of drugs and endogenous steroids, is perturbed in disease states in association with altered concentrations of circulating iodothyronines (Fishman et al., 1965; Ram and Waxman, 1990). It has been demonstrated that iodothyronines act directly on human hepatocytes to regulate the expression of several CYP450s at a pre-translational level (Ip and Lisk, 1997). Thyroid hormones were also shown to regulate the expression of the flavoprotein NADPH cytochrome P450 reductase (P450R). The activity of this particular enzyme was also shown to alter by thyroid hormone status in extrahepatic tissues, for instance, showed 30-50% decrease in kidney of hypothyroid rats (Ram and Waxman, 1992).

As a structural component of thyroid hormones, iodine is the primary requirement for the thyroid function. Iodine deficiency is known to induce a variety of disorders of thyroid function including endemic goiter, considered as the greatest cause of preventable brain damage and mental retardation, and more than two billions of people are reported to be at the risk of iodine deficiency disorders throughout the world (World Health Organization, 2007). It has also been suggested that pulmonary functions are affected in sub-clinical hypothyrodism (Cakmak et al., 2011).

Based on this background, this study was designed to investigate the effects of iodine deficiency on pulmonary CYP450 and cytocrome b5 (CYP b5) levels and activities of P450R and xenobiotic-metabolizing enzymes such as CYP2E1, CYP1A1, CYP2B1/2 and cytosolic glutathione-S-transferase (GST). Considering the frequency of inadequate iodine intake among human populations, it was expected to obtain data contributing to envisage the alteration of extrahepatic drug metabolism in iodine deficient subjects.

METHODS AND MATERIALS

Chemicals

All chemicals, including potassium chloride, Tris HCl, ethylenediaminetetraacetic acid (EDTA), sucrose, resorufin, aniline, cytochrome c, 1-chloro-2,4 dinitrobenzene (CDNB) and NADPH were from Sigma-Aldrich (St.Louis, MO, USA).

Animals

Three-week old, male Wistar rats, obtained from Hacettepe University Experimental Animals Laboratory were used in the experiments. The standard rat chow was also obtained from Hacettepe University Experimental Animals Laboratory. The animals were divided randomly in two groups and each group was housed in plastic cages with stainless-steel grid tops. The cages were placed in a room with controlled temperature (23°C), humidity (50%) and a 12-hour light-dark cycle. Body weights (bw) were monitored weekly. Feeding period was 7 weeks. Nine animals were used for each group and received *ad libitum* diet and water. The animals were treated humanely and with regard for alleviation of suffering, and the study was approved by Hacettepe University

Ethical Committee.

Experimental Groups

(1) Control group **(C)**, was fed regular rat chow and drinking water; **(2)** Iodine deficient group **(ID)** was fed with regular rat chow and received 1% sodium perchlorate containing drinking water.

After the feeding period, animals were weighed, and sacrificed by decapitation under thiopental anesthesia. Lungs were rapidly removed, weighed and frozen at -80°C until the preparation of and cytosolic and microsomal fractions.

Preparation of Cytosolic and Microsomal Fractions

The homogenization of the lung tissue was carried out in a Teflon-glass homogenizer in a volume of potassium chloride-Tris buffer (0.154 M potassium chloride and 50 mM Tris HCl, pH 7.4) to obtain a 3 g/ml liver homogenate. Following centrifugation at 2500 x *g* for 10 min, separated supernatant was further centrifuged at 10.000 x *g* for 10 min. The supernatant was centrifuged at 105.000 x *g* for 60 min for 2 times and cytosolic supernatant was collected and used for the measurement of GST activity.

The microsomal pellet was re-suspended in a buffer containing Tris- EDTA- sucrose (20 mM Tris, 5 mM EDTA and 0.25 M sucrose; pH 7.4; 1 g lung tissue/ml). Microsomal and cytosolic fractions were aliquoted and stored in a freezer at -80°C until analysis.

Measured Parameters and Methods

All spectrophotometric and spectrofluorometric measurements were performed by using a Shimadzu spectrophotometer/spectrofluorometer (Kyoto, Japan).

CYP450 and CYP b5 Levels

CYP450 and cytochrome b5 (CYP b5) levels in lung microsomes were determined according to the method of Omura and Sato (1964) with extinction coefficients of 91 $mM^{-1}cm^{-1}$ (between 490 and 450 nm) and 185 $mM^{-1}cm^{-1}$ (between 424 and 409 nm), respectively.

NADPH-Cytochrome Reductase Activity

P450R activity was measured at 550 nm and 37°C by monitoring the reduction of cytochrome C in the presence of NADPH (Phillips and Langdon, 1962).

Xenobiotic Metabolizing Enzyme Activities

Microsomal aniline hydroxylase (CYP2E1) activity was determined by measuring p-aminophenol production according to the method of Imai et al. (1966). Microsomal 7-ethoxyresorufin O-deethylase (EROD) as a measure of CYP1A1, and 7-penthoxyresorufin O-deethylase (PROD) as a measure of CYP2B1/2 activities were determined spectrofluorometrically from the amount of resorufin produced using ethoxyresorufin and penthoxyresorufin as substrates, respectively, at excitation and emission wavelengths of 530 and 585 nm (Prough et al., 1978).

Cytosolic glutathione-S-transferase (GST) activity was determined spectrophotometrically by using CDNB as a substrate, according to the method of Habig et al. (1974).

Protein Determination

Protein concentrations were determined by the standard method of Lowry et al. (1951).

Statistical Analysis

Experimental data were analyzed with one-way analysis of variance (ANOVA) followed by the Student's t-test using a Statistical Package for Social Sciences Program (SPSS) 17.0 for windows packed program. The p values <0.05 were considered significant. Values are given as mean ± SEM.

RESULTS

Body and Lung Weights

Body weight, lung weight and relative lung weight showed slight but statistically significant decreases in ID rats vs. control (9%, 17%, 10%, respectively, $p<0.05$) as shown in Table 1.

Table 1. Effects of Iodine Deficiency on Body Weight, Lung Weight and Relative Lung Weight in the Study Groups.

	Body Weight (g)	Lung Weight (g)	Relative Lung Weight (g/100 g bw)
C (n=6)	239.80±5.70	0.974±0.120	0.435±0.010
ID (n=6)	215.40±8.30*	0.804±0.102*	0.393±0.012*

CYP450 and CYP b5 Levels and P450R activity

Pulmonary CYP450 level and P450R activity decreased significantly (24% and 33%, respectively, $p<0.05$) in ID group while CYP b5 levels increased markedly (33%, $p<0.05$) vs. control (Figure 1).

Pulmonary Xenobiotic-Metabolizing Enzyme Assessments

CYP2E1 and GST activities significantly decreased (21% and 59%, respectively, $p<0.05$) in ID animals vs. control. However, both EROD and PROD activities increased significantly (24% and 11%, respectively, $p<0.05$) in ID group in comparison to control animals (Table 2).

Table 2. Activities of Pulmonary Drug-Metabolizing Enzymes in the Study Groups.

	CYP2E1 (pmol/mg protein/min)	EROD (pmol resorufin/ mg protein/min)	PROD (pmol resorufin/ mg protein/min)	GST (μmol mg protein/min)
C (n=9)	0.507±0.002	2.364±0.239	0.702±0.014	0.087±0.001
ID (n=9)	0.401±0.004*	3.106±0.224*	0.790±0.021*	0.036±0.001*

Control group **(C)**, was fed regular rat chow and drinking water; Iodine deficient group **(ID)** was fed with regular rat chow and received 1% sodium perchlorate containing drinking water.

Values are given as mean ± SEM. *Significantly different from control ($p<0.05$).

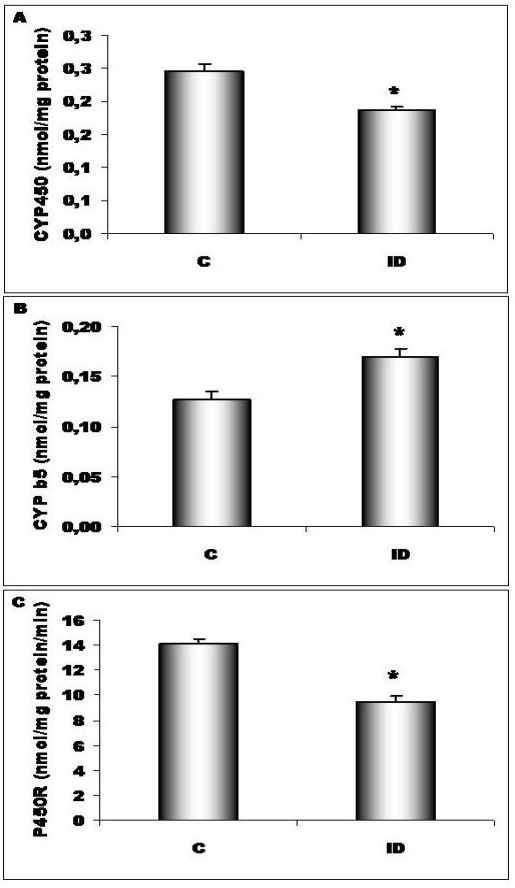

Figure 1. Pulmonary CYP450 and CYP b5 Levels and P450R Activity in the Study Groups.

DISCUSSION

Iodine is the integral component of thyroid hormones, and adequate intake of iodine is essential for thyroid function. Prolonged iodine deficiency is the primary cause of hypothyroidism in which thyroid stimulating hormone (TSH) secretion is elevated, thyroid function is disturbed, and eventually goiter formation is promoted (Laurberg et al., 2010). In the present study, perchlorate was used to induce iodine deficiency, since it blocks effectively the uptake of iodine by the thyroid gland (Chow and Woodbury, 1970) by competing with iodide at the sodium-iodide symporter, the transport system that transports iodine into the thyroid (Tran et al., 2008; Yamada et al., 1967). As we reported earlier in detail, iodine deficiency and resulting hypothyroidism was evident in the ID group by high TSH and low plasma TT_4 and TT_3 levels (Erkekoglu et al., 2012).

Thyroid hormones regulate the basal rate of all cells and thereby modulate their functions. Convincing results from different studies have shown that thyroid hormones control xenobiotic-metabolizing enzymes in liver as well as in other organs and tissues (Brtko and Dvorak, 2011; Erkekoglu et al., 2012; Malik and Hodgson, 2002). Thus, high level of thyroid hormones, as well as their low concentrations might be the underlying factors in the alterations of hepatic and extrahepatic xenobiotic metabolism.

In the present study, we observed slight but significant decreases in both lung and relative lung weight in ID rats. This is in accordance with the results of a recent study which demonstrated that prenatal iodine deficiency resulted in structurally and functionally immature lungs in neonatal rats (Carlson, 2008). In addition, our results showing significant decreases in the levels of total CYP450 and P450R, in the activities of CYP2E1 and GST, along with increases in the level of CYP b5 and the activities of EROD and PROD, indicated the occurrence of significant changes in pulmonary xenobiotic metabolism in iodine deficient pubertal rats.

Besides selective tissue exposures, high tissue oxygenation and presence of bio-activating systems that can generate toxic products from initially innocuous substances are the determinant factors in pulmonary toxicity (Hoch et al., 1980). The lungs also contribute to the systematic metabolism and clearance of xenobiotics. However, although they contain most of the phase I pathways found in the liver, the amount of enzymes may be different and there exists fewer P450 isozymes than the liver. Pulmonary phase I enzymes often exhibit unique catalytic specificity relative to those in hepatic tissue (Smith and Brian, 1991).

In addition to liver, CYP b5 and P450R, the electron donors of CYP450 enzymes are expressed

in extrahepatic tissues, including lung (Pahwa et al., 2010; Smith and Brian, 1991; Zhang et al., 2006). Any changes in CYP b5 and P450R may cause alterations in the reaction rates of CYP450-catalyzed reactions. Therefore their level and activity are of great importance for CYP450-catalyzed xenobiotic metabolism as well as metabolism of endogenous substances. In previous reports, elevation of hepatic microsomal CYP b5 levels was noted in hypothyroidism (Erkekoglu et al., 2012; Hoch et al., 1980). The results presented herein for pulmonary CYP b5 were also in the same line. Previous studies on hypothyroid rats have indicated that thyroid hormones are required for full expression of P450R in liver tissue (Li et al., 2001; Smith and Brian, 1991). Ram and Waxman (1990) also observed a decrease in P450R activity in the lung microsomes of rats treated with methamizole. It was suggested that P450R may show decreases because thyroid hormones are translational or post-translational regulatory factors in the expression of this particular co-factor (Li et al., 2001; Liu et al., 2002). Our results showing reduced pulmonary P450R activity in hypothyroid rats were again in the same line. In addition, we previously observed the same trend of changes including the same level of decreases in total CYP450 level in the liver of hypothyroid rats (Erkekoglu et al., 2012).

CYP2E1 has a ubiquitous role in the metabolism and activation of an array of solvents and several anesthetics (halothane, isoflurane, enflurane), as well as acetaminophen, dapsone, and theophylline (Parkinson, 2001). Since lung is the major target organ for all inhaled toxic chemicals, the decrease we observed in pulmonary CYP2E1 activity in ID rats might suggest a decrease in the metabolism of such chemicals and drugs.

EROD activity in rats is an indicator of CYP1A1/A2 activity and CYP1A1 enzyme is the principal enzyme metabolizing PAHs, and its activity is induced by smoking (Bouchardy et al., 2001; Parkinson, 2001). CYP1A1 plays a dual role in PAH metabolism. It is considered as the first enzyme in the initial conversion of benzo[a]pyrene to 7,8-diol and the subsequent metabolic activation or detoxification (Anttila et al., 1991; Shimada et al., 2006). CYP1A1 is expressed in human lung principally in peripheral airway epithelium and also in bronchial epithelium to some extent (Willey et al., 1996). On the other hand, PROD, the indicator of CYP2B1/B2 activity, is also responsible for the metabolism of environmental chemicals such as nicotine, arenes, arylamines, PAHs and nitrosamines (Anttila et al., 1991) and it could play a significant role in the activation of PAHs, at least in such cell types as alveolar macrophages where CYP1A1 is not expressed. Therefore the increased activation of EROD and PROD as we observed in the present study is of importance in the exposure of such toxicants, as causing acceleration in the metabolic activation of the substrates of CYP1A1/A2 and CYP2B1/2.

In conclusion, we can imply that there are few studies showing the effects of thyroid hormones on both hepatic and extrahepatic xenobiotic metabolizing enzyme systems (27, 30). To our knowledge, this is the first study in literature showing the effects of iodine deficiency on pulmonary xenobiotic metabolism. The data presented herein show the thyroidal influence on pulmonary xenobiotic metabolizing enzyme systems in rats and emphasize the importance of the iodine status. In iodine deficiency, pathways resulting in metabolic activations or removing potentially reactive intermediates might be altered in the lung leading altered effects of environmental xenobiotics and drugs. The impact of thyroid hormones on xenobiotic-metabolizing enzymes in different tissues is not fully elaborated. Further animal and human studies on a wider spectrum would provide more specific information and comprehension on the effects of iodine deficiency upon extrahepatic xenobiotic metabolism.

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REFERENCES

Anttila S, Hietanen E, Vainio H, Camus AM, Gelboin HV, Park SS, Heikkilä L, Karjalainen A, Bartsch H. Smoking and peripheral type of cancer are related to high levels of pulmonary cytochrome P450IA in lung cancer patients. *Int J Cancer* 47: 681-685, 1991.

Anttila S, Raunio H, Hakkola J. Cytochrome P450-mediated pulmonary metabolism of carcinogens: regulation and cross-talk in lung carcinogenesis. *Am J Respir Cell Mol Biol* 44: 583-590, 2011.

Bouchardy C, Benhamou S, Jourenkova N, Dayer P, Hirvonen A. Metabolic genetic polymorphisms and susceptibility to lung cancer. *Lung Cancer* 32: 109-112, 2001.

Brtko J, Dvorak Z. Role of retinoids, rexinoids and thyroid hormone in the expression of cytochrome p450 enzymes. *Curr Drug Metab* 12: 71-88, 2011.

Cakmak G, Saler T, Saglam ZA, Yenigun M, Ataoglu E, Demir T, Temiz LU. Pulmonary functions in patients with subclinical hypothyroidism. *J Pak Med Assoc* 61: 951-953, 2011.

Carlson GP. Critical appraisal of the expression of cytochrome P450 enzymes in human lung and evaluation of the possibility that such expression provides evidence of potential styrene tumorigenicity in humans. *Toxicology* 254: 1-10, 2008.

Chen GF, Ronis MJ, Ingelman-Sundberg M, Badger TM. Hormonal regulation of microsomal cytochrome P4502E1 and P450 reductase in rat liver and kidney. *Xenobiotica* 29: 437-451, 1999.

Chow SY, Woodbury DM. Kinetics of distribution of radioactive perchlorate in rat and guinea-pig thyroid glands. *J Endocrinol* 47: 207–218, 1970.

Erkekoglu P, Giray BK, Caglayan A, Hincal F. Selenium and/or iodine deficiency alters hepatic xenobiotic metabolizing enzyme activities in rats. *J Trace Elem Med Biol* 26: 36-41, 2012.

Fishman J, Hellman L, Zumoff B, Gallagher TF. Effect of thyroid on hydroxylation of estrogen in man. *J Clin Endocrinol Metab* 25: 365–368, 1965.

Godbole MM, Rao G, Paul BN, Mohan V, Singh P, Khare D, Babu S, Nath A, Singh PK, Tiwari S. Prenatal iodine deficiency results in structurally and functionally immature lungs in neonatal rats. Am J Physiol Lung *Cell Mol Physiol* 302: L1037-L1043, 2012.

Guengerich FP. Cytochrome Pp450 and chemical toxicology. *Chem Res Toxicol* 21: 70-83, 2008.

Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases, the first enzymatic step in mercapturic acid formation. *J Biol Chem* 249: 7130–7139, 1974.

Hoch FL, Depierre JW, Ernster L. Thyroid control over biomembranes. Liver-microsomal cytochrome b5 in hypothyroidism. *Eur J Biochem* 109: 301-306, 1980.

Hukkanen J, Pelkonen O, Hakkola J, Raunio H. Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. *Crit Rev Toxicol* 32: 391-411, 2002.

Imai Y, Ito A, Sato R. Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. *J Biochem* 60: 417–428, 1966.

Ip C, Lisk DJ. Modulation of phase I and phase II xenobiotic-metabolizing enzymes by selenium-enriched garlic in rats. *Nutr Cancer* 28: 184–1888, 1997.

Laurberg P, Cerqueira C, Ovesen L, Rasmussen LB, Perrild H, Andersen S, Pedersen IB, Carlé A. Iodine intake as a determinant of thyroid disorders in populations. *Best Pract Res Clin Endocrinol Metab* 24: 13-27, 2010.

Li HC, Liu D, Waxman DJ. Transcriptional induction of hepatic NADPH: cytochrome P450 oxidoreductase by thyroid hormone. *Mol Pharmacol* 59:987-995, 2001.

Liddle C, Goodwin B, George J, Tapner M, Farrell GC. Separate and Interactive Regulation of Cytochrome P450 3A4 by Triiodothyronine, Dexamethasone, and Growth Hormone in Cultured Hepatocytes. *J Clin Endoc Met* 3: 2411-2416, 1998.

Liu D, Waxman DJ. Post-transcriptional regulation of hepatic NADPH-cytochrome P450 reductase by thyroid hormone: independent effects on poly(A) tail length and mRNA stability. *Mol Pharmacol* 61: 1089-1096, 2002.

Lowry OH, Rosebrough NJ, Faar AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265–275, 1951.

Malik R, Hodgson H. The relationship between the thyroid gland and the liver. *QJM* 95: 559–569, 2002.

Omura T, Sato R. The carbonmonoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* 239: 2370–2378, 1964.

Pahwa P, Karunanayake CP, Dosman JA. Association between pulmonary dysfunction as a result of occupational exposures and risk of developing cancer. J *Occup Environ Med* 54: 1471-1480, 2012.

Park BK, Pirmohamed M, Kitteringham NR. The role of cytochrome P450 enzymes in hepatic and extrahepatic human drug toxicity. *Pharmacol Ther* 68: 385-424, 1995.

Parkinson A. Biotransformation of xenobiotics. In: Klaassen CD Ed. Casarett and Doull's Toxicology. The basic science of poisons. North America: McGraw Comp, 2001. p. 133–224.

Pavek P, Dvorak Z. Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the cytochrome P450 superfamily in human extrahepatic tissues. *Curr Drug Metab* 9: 129-143, 2008.

Phillips AH, Langdon RG. Hepatic triphosphopyridine nucleotide-cytochrome c reductase: isolation, characterization and kinetic studies. *J Biol Chem* 237: 2652–2660, 1962.

Prough RA, Burke MD, Mayer RT. Direct fluorometric methods for measuring mixed function oxidase activity. *Methods Enzymol* 52: 372–377, 1978.

Ram PA, Waxman DJ. Pretranslational control by thyroid hormone of rat liver steroid 5ع-reductase and comparison to the thyroid dependence of two growth hormone-regulated CYP2C mRNAs. *J Biol Chem* 265: 19223–19229, 1990.

Ram PA, Waxman DJ. Thyroid hormone stimulation of NADPH P450 reductase expression in liver and extrahepatic tissues. Regulation by multiple mechanisms. *J Biol Chem* 267: 3294-3301, 1992.

Shimada T, Guengerich FP. Inhibition of human cytochrome P450 1A1-, 1A2-, and 1B1-mediated activation of procarcinogens to genotoxic metabolites by polycyclic aromatic hydrocarbons. *Chem Res Toxicol* 19: 288-294, 2006.

Smith BR, Brian WR. The role of metabolism in chemical-induced pulmonary toxicity. *Toxicol Pathol* 19: 470-481, 1991.

Tran N, Valentín-Blasini L, Blount BC, McCuistion CG, Fenton MS, Gin E, et al.. Thyroid-stimulating hormone increases active transport of perchlorate into thyroid cells. *Am J Physiol Endocrinol Metab* 294: E802–E806, 2008.

Willey JC, Coy E, Brolly C, Utell MJ, Frampton MW, Hammersley J, Thilly WG, Olson D, Cairns K. Xenobiotic metabolism enzyme gene expression in human bronchial epithelial and alveolar macrophage cells. *Am J Respir Cell Mol Biol* 14: 262-271, 1996.

World Health Organization (WHO). Iodine Deficiency in Europe: A continuing public health problem. Anderson M, de Benoist B, Darnton-Hill I(eds). 2007. Available at: http://www.who.int/nutrition/publications/VMNIS_Iodine_deficiency_in_Europe.pdf.

Yamada T. Effects of perchlorate and other anions on thyroxine metabolism in the rat. *Endocrinology* 81: 1285–1290, 1967.

Zhang JY, Wang Y, Prakash C. Xenobiotic-metabolizing enzymes in human lung. *Curr Drug Metab* 7: 939-948, 2006.