Environmental Contaminants & Obesity

Master Thesis

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Master program: Toxicology & Environmental Health
Master thesis
November 2011 – February 2012

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Presentation: Health Council of the Netherlands
Committee 'Risks of prenatal exposure to hazardous substances'
February 14, 2012

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Introduction

The percentage of people with obesity is doubled compared to 20 years ago. In the Netherlands, 12 percent of the humans were obese in 2009 (CBS). Obesity is a rising problem concerning the general health. It is related to diabetes mellitus and cardiovascular diseases[1,2]. Previously it was thought that only an excess of caloric intake was the cause for obesity, but recently other insights arise. This is due to the fact that obesity becomes an epidemic in developing countries as well. For example, exposure to environmental chemicals might be related to obesity[3]. Especially endocrine disrupting chemicals (EDCs)[4] and persistent organic pollutants[5] are mentioned in literature.

Major organic pollutants are related to the prevalence of metabolic disorders like diabetes[6]. EDCs can alter gene expressions and affect offspring in that way[7]. It is known that malnourished mothers gave rise to obese children[8]. And mothers with diabetes resulted in a higher risk for metabolic diseases in their offspring[8]. Certain environmental influences on the mother can cause epigenetic adaptation of the child and result in diseases like obesity[9].

So better understanding of the link between environmental chemicals and obesity[7] and *in utero* exposure is necessary [8].

Because the link between environmental contaminants and obesity or metabolic disorders is a new field of research, most studies have been published in the last few years. Taken together, questions arise about the potential mechanisms by which environmental chemicals can affect the metabolic system.

In this literature study, the link between environmental contaminants and obesity will be described by their potential mechanisms of action. This study will focus on the environmental contaminants; bisphenol A, phthalates, brominated flame retardants, dioxins and perfluorinated compounds.

Metabolic system

Different organs and mechanism are involved in food metabolism. For example the brain, intestines, pancreas, liver, muscles and adipose tissue play an important role in the metabolic homeostasis. When food is taken in, the intestines digest the food and the nutrients are taken up into the portal vein.[12]

Brain

Regulation of food intake takes place via the satiety centre in the brain, which regulates the feeling of hunger or saturation. This feeling is mediated by hypothalamic neurons[13] (see Figure 1).

When the stomach is empty it secretes the hormone ghrelin that causes the hunger feeling. After a meal, the small intestine secret the hormone PYY and the pancreas secretes insulin that suppresses the hunger feeling. Additionally, adipose tissue secretes the hormone leptin that suppresses appetite as well. When adipose tissue declines, leptin levels will fall and the satiety feeling is no longer repressed.[12]

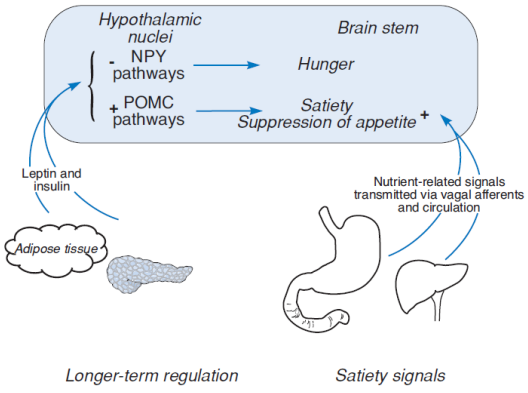

Figure 1 Schematic view of the regulation of appetite[14]

Blood

Glucose metabolism

After a meal, blood glucose levels rise. Glucose is one of the main energy sources for peripheral cells. The glucose blood concentration varies around the set point of 90 mg glucose/100 ml blood[12]. This homeostasis is regulated by the feedback mechanism of the pancreatic hormones insulin and glucagon.

When blood glucose levels rise, the beta cells of the pancreas secrete insulin into the blood stream. Insulin stimulates the transport of glucose into peripheral organs, like the muscles and liver. This causes a drop in the blood glucose level and the alpha cells of the pancreas secrete glucagon. Glucagon stimulates the breakdown of glycogen, stored in liver and muscle, into glucose by the liver. Glucose will be secreted by the liver and muscles and blood glucose levels will rise again.

Pancreas

Figure 2 gives an overview of the glucose stimulated release of insulin in pancreatic beta cells. At high blood glucose levels, glucose is transported into the beta cells by GLUT2[2]. ATP is generated from glucose and causes the depolarization of the cell membrane. The intracellular calcium concentration will increase and insulin will be secreted. On cellular level, the release is dependent on an intracellular increase of the calcium concentration which changes the membrane potential.[13,14] The alpha and beta cells have membrane estrogen receptors (ncmERs).

Circulating estradiol (E2) can bind to this receptor and can trigger a change in membrane potential. In beta cells, activation of the ncmER will result in insulin release and in alpha cells of inhibition of glucagon release.[13]

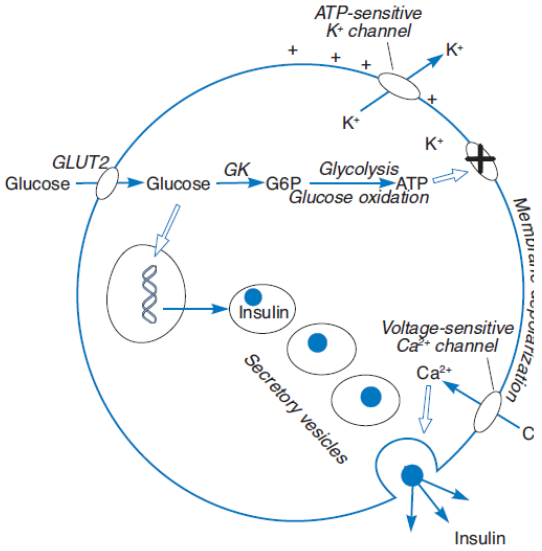

Figure 2 Release of insulin by pancreatic beta cells[14]

Liver

Nutrients are absorbed by the intestines and transported to the liver via the portal vein. The liver processes these nutrients and provides other organs with glucose (Figure 3). The liver itself uses mostly energy generated from fatty acids and amino acids.

Glucose metabolism

The glucose transporter 2 (GLUT2) transports glucose into liver cells. This uptake is dependent on the glucose concentration difference between the liver cell and blood.

Glucose is stored as glycogen or transformed to acetyl-CoA. Acetyl-CoA can be used as substrate for the tricarboxylic acid cycle (TCA cycle) to generate energy or for lipogenesis. The liver is also able to free glucose from stored glycogen and generates glucose from non-carbohydrates like lactate, fatty acids and glycerol (gluconeogenesis).

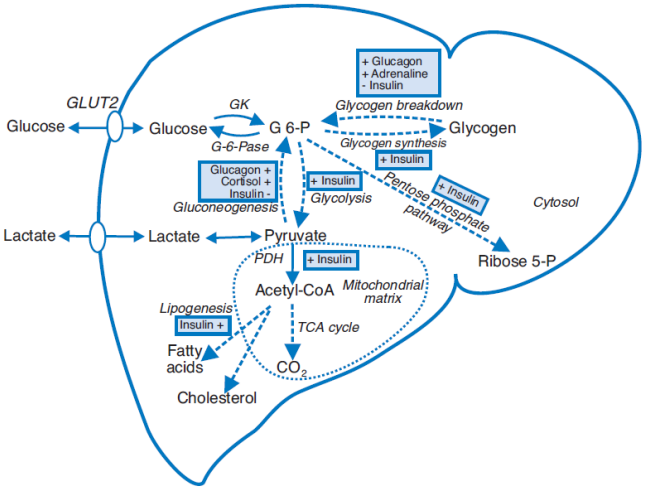

Figure 3 Outline of glucose metabolism and its hormonal regulation in the liver[14]

Fatty acid metabolism

Figure 4 gives an overview of fatty acid metabolism in the liver. Fatty acids taken up from the blood are converted into fatty acyl-Co-A. Glucagon stimulates the transformation of fatty acyl-Co-A into acyl carnitine and this metabolite can be oxidized in the mitochondria by Carnitine palmitoyltransferase I (CPT1). CTP1 is a rate limited enzyme in beta-oxidation[15]. In this process ketone bodies are formed which are a source of energy for other organs. Energy generated by oxidation of FA is mainly used for gluconeogenesis. Under the influence of insulin, fatty acyl-Co-A is transformed into triacylglycerol (TAG). TAG can be stored in the liver or transported as very low density lipoprotein (VLDL) to adipose tissue.

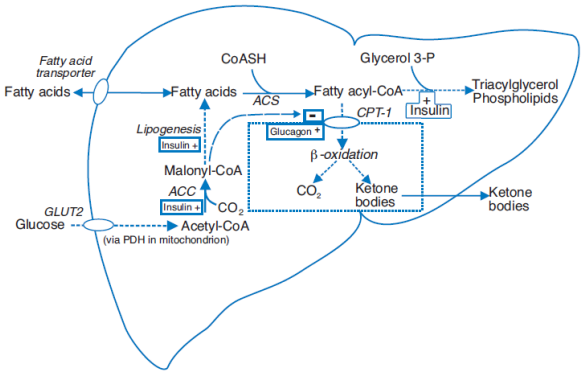

Figure 4 Fatty acid metabolism in liver[14]

Muscle

Muscles generate energy by producing ATP from glucose and FA from blood or glycogen and TAG from local storage (Figure 5). Glucose enters the cell via GLUT4, stimulated by insulin. The expression of GLUT4 transporter is regulated by the activation of the estrogens receptor.[13]

Adipose tissue

Adipose tissue consists of adipocytes, fat cells. The main function of adipose tissue is to store excess energy and free energy when necessary. This involves transport, synthesis, storage and mobilization of lipids[16]. Adipose tissue mass is determined by the amount and volume of adipocytes[17]. The number of adipocytes is set during childhood and adolescence and stable during adulthood. In this stable state, the annual turnover of adipocytes is ten percent[18]. So, an increase in adipose tissue during adulthood is the result of the filling of adipocytes and not an increase in number of adipocytes. Adipocytes are created from multi potent stem cells. The first step is the predisposition of these cells towards preadipocytes. The next step is adipogenesis, the differentiation of preadipocytes into adipocytes[17].

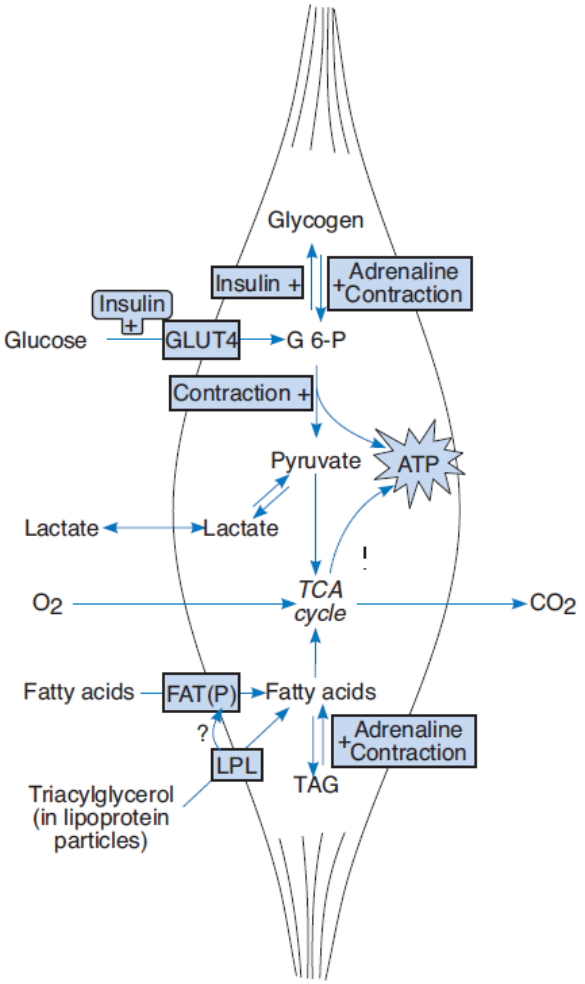

Figure 5 Generation of ATP in muscle[14]

Lipid metabolism

Figure 6 shows the lipid metabolism in adipose tissue. Excess energy is stored in adipose tissue in the form of triacylglycerol (TAG) by lipoprotein lipase (LPL). Insulin regulates the transcription factor SREBP-1c which increases gene expression involved in lipid production, like LPL[2]. To free the energy stored in adipocytes, hormone sensitive lipase (HSL) has to hydrolyze TAG. Insulin inhibits HSL levels. So, when glucose levels are high, fatty acids are not released into the blood stream.

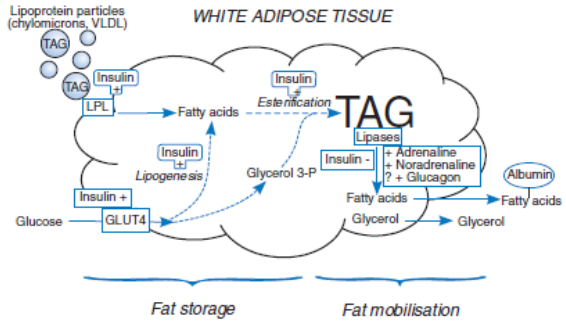

Figure 6 Lipid storage and mobilization in adipose tissue[14]

Metabolic disorders

Loss of control of metabolic homeostasis is called a metabolic disorder. The combination of certain metabolic disorders that give rise to a greater risk for cardiovascular diseases is called Syndrome X or Metabolic Syndrome. There are different definitions of disorders that contribute to a greater risk for cardiovascular diseases. Examples of risk factors are overweight or obesity, abnormal serum lipid levels and insulin resistance.[2,19] The metabolic syndrome is also related to diabetes type 2.[2]

Obesity

The world health organization states overweight and obesity as an excess of accumulated fat that presents a risk to health. To asses if people have a healthy weight, the so called body mass index (BMI) can be calculated (the weight in kilograms divided by the square of the height in meters). A BMI of 25 kg/m^2 is called overweight and a BMI of 30 kg/m^2 and above is called obese (WHO).

The onset of obesity is a positive energy balance over a longer period resulting in an excess of adipose tissue. Next to storage in adipose tissue, lipids can be stored in organs like liver and muscle[20]. From an evolutionary point of view, it is likely that people are prone to store fat, when able to.[21].

An increase in weight gain can be, next to an excessive caloric intake, caused by a disruption of energy metabolism. These disruptions can result from a genetic defect, but monogenetic mutations resulting in obesity are rare. Mutations in genes that control or influence the behavior or satiety centre are found. Mutations in leptin, pro-opiomelanocortin, melanocortin, beta3 adrenocepor, peroxisome proliferator-activator receptors (PPARs), adiponectin and FTO are also associated with obesity.[1,2] Rankinen *et al* have mapped all the known genes associated with obesity[22].

Fat is accumulated in adipose tissue. Adipose tissue is determined by the number and size of the adipocytes. It is known that obese people have more adipocytes then lean people. And because adipocytes must be filled to a minimum[17], the onset of obesity may lay in the creation of adipocytes in childhood.

An excess of body fat results in a greater release of fatty acids into the blood stream. Obese people have elevated fatty acid serum levels and this can result in certain health effects. For example, insulin resistance and diabetes type 2 are related to high FA acid serum levels[1, 2, 20].

Diabetes Mellitus

Diabetes Mellitus type 2 is an endocrine disorder determined by a lack of insulin responsiveness. It is caused by a loss of beta cell function in the pancreas and a reduced responsiveness of target cells to insulin. Glucose uptake in peripheral cells is dependent on the insulin stimulated glucose receptors. Because peripheral cells do not respond to insulin, glucose cannot be taken up by the cells and circulation glucose levels are high

(hyperglycemia). Over time, beta cells secrete less insulin and the disease will worsen.[2]

The base of insulin resistance or glucose intolerance can be a genetic defect. These defects can occur in genes related to the uptake of glucose by beta cells of the pancreas (GLUT2) or peripheral tissue. For example, defects in GLUT4[23], the insulin receptor, peroxisome proliferator- activator receptor y and adipocin can result in insulin resistance[1, 2].

A decrease in insulin secretion can also be the result of apoptosis of beta cells in the pancreas. This can be caused by an alteration in mitochondrial function of the beta cells, which increases reactive oxygen species (ROS) formation. ROS can damage DNA and can result in apoptosis.[1]

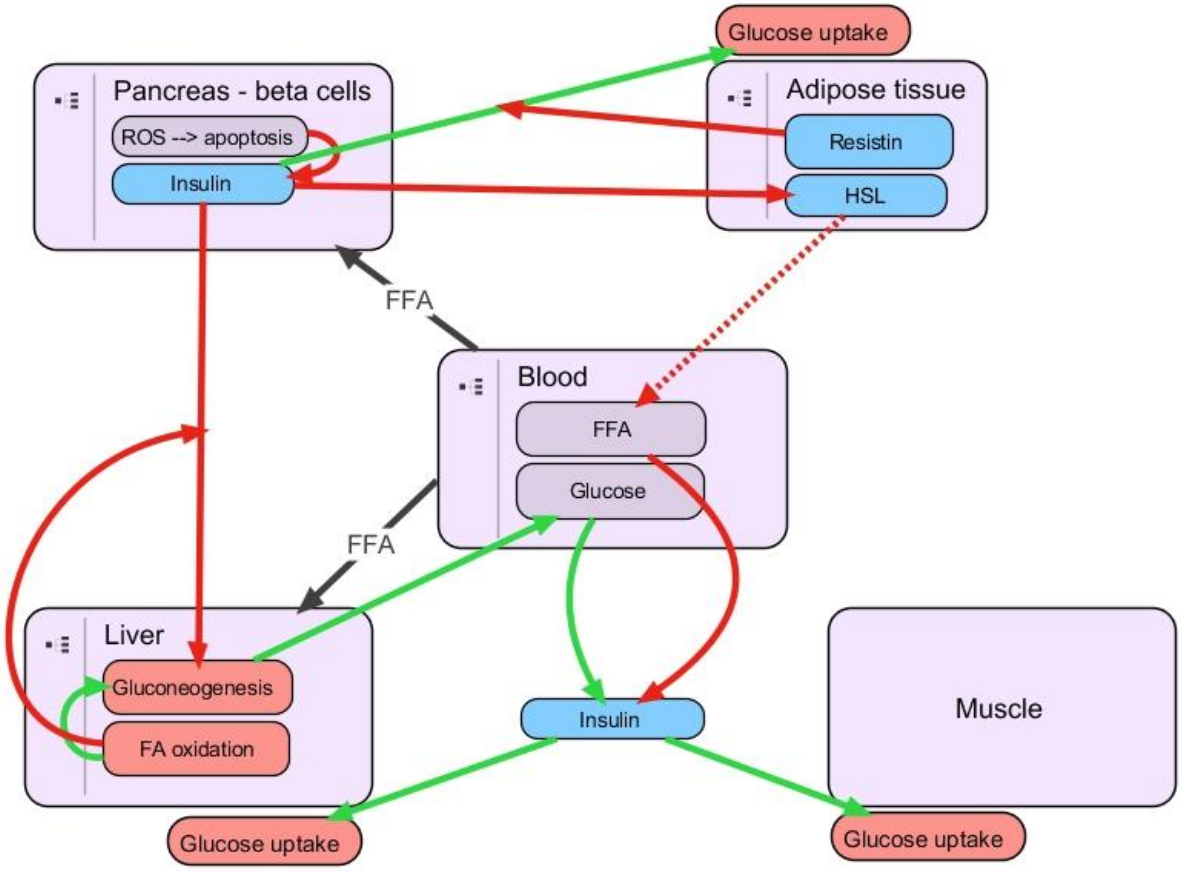

Figure 7 Effect of free fatty acids (FFA) on insulin resistance and metabolism

The onset of diabetes can be linked to obesity.[1,2] Figure 7 gives a schematic view of the effects of obesity, like high free fatty acids levels, on different organs described below.

Blood

An excess of body fat results in more release of fatty acids into the blood stream. Obese people have elevated fatty acid serum levels.

Pancreas

High exposure of beta cells to fatty acid can result in increase of lipid storage in beta cells of the pancreas. This can generate ROS and peroxidation of lipids. ROS can eventually lead to apoptosis of beta cells. So, high circulation levels of lipids can result in loss of pancreatic beta cells and inhibit the secretion of insulin.[1]

Liver

Abdominal adipose tissue releases fatty acids into the portal vein. So the liver will take up FA from circulation and portal vein. This can influence the hepatic energy metabolism[1]. Lipid metabolism and glucose metabolism are competing and an imbalance in glucose and fatty acid cycle will occur. In the fatty acid metabolism the FA metabolites diacylglycerol and ceramide are increased. These can interfere with insulin signaling pathway by phosporylation of certain proteins, resulting for instance in decrease of translocation of GLUT4, necessary for glucose transport. Next to that, an increase in FA oxidation results in more energy for gluconeogenesis and inhibition of insulin suppression on the gluconeogenesis.[1]

Muscle

In the muscle, just like the liver an increase in FFA will disturb the FA-glucose cycle. The result is a decrease in insulin mediated glucose uptake.

Adipose tissue

Obese people have more adipose tissue and store fat around the abdomen. When more lipids are stored in adipose tissue, the adipocytes grow and become hypertrophic. Large adipocytes are less sensitive to insulin, HSL will no longer be suppressed by insulin and more FA will be released into the circulation (Figure 6). The free fatty acid blood concentration rises.[1]

Next to that, adipose tissue secretes different compounds which can affect the metabolism. Examples are adiponectin and resistin that which are increased in hypertrophic adipocytes, while adipokine levels are decreased. Adiponectin increases the FA β-oxidation in adipose tissue[23] and is correlated to obesity [24]. Resistin decreases insulin mediated glucose uptake by adipocytes. Adipokine is a cytokine that inhibits insulin resistance.[1]

Environmental contaminants

In the previous section, the onset of obesity due to (epi)genetic defects was discussed. An arising question is the potential effect of environmental contaminants on the onset of obesity. Especially endocrine disrupting chemicals (EDCs) are thought to be related to the onset or maintenance of obesity. Grün and Blumberg were the first to name these EDCs as obesogens.[25]

EDCs are well known environmental contaminants to disturb the endocrine system. Because obesity is a metabolic disease, it is likely that EDCs can interfere with it. Increasingly more studies are done to link the possible effects of EDC to the metabolic system[26].

In the next section the selected EDCs, bisphenol A, phthalates, brominated flame retardants, dioxins and perfluorinated compounds are described in context with studies on the metabolic system.

Bisphenol A

Bisphenol A (BPA) is a monomer used in plastics, like baby bottles and food containers. In that way it can be released into the fluid or food.[27] Bisphenol A can act as an estrogen and bind to the estrogen receptor[28,29]. Acting through the estrogen receptor it can stimulate glycolysis[23]. It can also compete with glucocorticoids for the glucocorticoid receptor[30] and inhibit the thyroid receptor[27-29].

Epidemiological and experimental studies show a relation between BPA levels and bodyweight or diabetes.[31] For example, a human cross sectional study showed a positive relation between urinary BPA concentrations and diabetes, based on glucose and insulin measurements. No relations were found between BPA levels and LDL or triglycerides levels [32].

Pups of rats and mice prenatally exposed to low BPA levels showed an increased bodyweight[31] and to high BPA levels a decreased bodyweight[28]. Wei *et al* exposed rats to different concentrations of BPA and fed the offspring a normal or high fat diet. The low exposed pups showed an increase in bodyweight, blood insulin levels and impaired glucose tolerance later in life. Higher exposures showed no effect. The pups fed on a high fat diet showed also obesity, hyperglycemia, hyperinsulinemia and glucose intolerance.[33] Other i*n vitro* studies show an increase in weight of mice embryos[21]. Next to that, BPA induced adipocyte differentiation and glucose transport[21,28,31], by increasing the activity of GLUT4 protein *ex vivo*[28]. In combination with insulin, LPL activity and TAG storage is increased in 3T3L1 fibroblasts[31]. Perinatal exposure of BPA is also related to increased levels of thyroxine (T4)[27].

Phthalates

Phthalates are used to make polymers flexible. They are widely found in the indoor environment and food components. In contrast to other persistent organic pollutants, phthalates do not bioaccumulate[34]. The effects of diethylhexyl phthalate (DEHP) on human health have been widely studied.[27] Phthalates can bind to both estrogen receptors[35] and PPARγ[17,21,35,36]. The

phthalate metabolite DEPH can activate the constitutive androstane receptor (CAR), the pregnane X receptor (PXR)[36] and PPARα [21, 35, 36].

In humans, exposure to phthalates has been associated with obesity and insulin resistance[21,27]. Experimental rodent studies showed a decreased or no effect on bodyweight after DEHP exposure[21]. However, mice with a humanized PPARα receptor showed an increase after DEHP exposure[21]. No experimental developmental studies are done on the effect of phthalates on metabolism. Rat studies show a decrease of serum insulin levels and glycogen levels and an increase in glucose and thyroid hormones levels[37]. *In vitro* studies with 3T3L1 show a stimulating effect of phthalates on differentiation of adipocytes[21].

Brominated Flame Retardants

Brominated flame retardants (BFRs) are mainly used in the indoor environment. Although polybrominated diphenyl ethers (PBDEs) are banned, these are still commonly found in the environment. BFRs can act either as an agonist or antagonist of estrogen receptor and PBDEs are also known to bind to the thyroid receptor and pregnane X receptor (PXR).[27]

Not much research is done on the effects of BFRs on the metabolic system. Only the have been PBDEs studied in this context. Higher PBDE levels in women appear to be related to a higher body weight[21,27]. Next to that, PBDEs are associated with diabetes and the metabolic syndrome, but only few epidemiologic studies are available[21]. Experimental studies with these compounds show an increase in lipolysis in rat adipocytes[21]. Exposure to PBDEs resulted in an increase in lipolysis and decrease in glucose oxidation in rats, but did not affect the bodyweight[27].

Dioxins

Dioxins are environmental contaminants formed during combustion and industrial processes and also occur naturally in low concentrations. Examples of persistent dioxin-like compounds are polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and some planar polychlorinated biphenyls (PCBs). Since dioxins are lipophilic compounds these are stored in fatty tissue and accumulate in the food chain. Adverse effects of dioxins can be acute or chronic[38]. Depending on the cell type, dioxins can have estrogenic or anti-estrogenic effects[39]. Dioxins act predominantly through the aryl hydrocarbon receptor (AhR)[38]. PCBs are also able to act as an agonist or antagonist for the thyroid receptor (TR)[29].

Dioxin exposure can affect the energy balance [40]. Dioxin exposure is related to metabolic disorders (e.g. PCDDs), obesity and diabetes type 2 (e.g. PCBs) in adults according to some epidemiological studies[21,27]. An adult mice exposure study showed an increase in body weight and adipocyte hypertrophy when exposed to PCBs[18]. Developmental studies show that maternal PCB exposure correlated with a higher body fat mass in their children[41] or adolescence[42]. Next to a relation with body weight, PCBs in the blood cord

of human resulted in lower levels of T4 in the infant. The same was found in exposure studies in rats. However, there was no difference in body weight between the exposed and non exposed pups.[29] Dioxins in breast milk[43] and PCB serum levels[11] were not related to thyroid function or disease. Adipocyte differentiation is induced at low TCDD exposures and suppressed at high doses[21].

Perfluorinated compounds

Polyfluoroalkyl compounds (PFCs) are persistent organic pollutants[27]. Examples are perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) and the latter is able to bind to the PPARα and PPARγ[21].

Cross sectional studies show a relation between human PFC blood levels and hyperglycemia and cholesterol (HDL), but no relation with diabetes and the metabolic syndrome. The birth weight of children was negatively related to PFCs in cord blood. This is according the results of in vivo experiments where rodents were prenatally exposed to PFOS and PFOA. The exposed pups show a decreased body weight. Additionally, an increase in serum estradiol and insulin and a reduction of cholesterol and triglyceride was observed in exposed rodents.[27] In contrast, La Merill *et al* mention an increased body weight or no effect of PFO exposure in adult human and mice[21].

Nuclear receptors

The effect of environmental contaminants on the metabolic system might be because they can act through nuclear receptors. In this section the following nuclear receptors (NRs) are discussed; Estrogen Receptor (ER), Glucocorticoid Receptor (GR), Thyroid Receptor (TR), Acyl hydrocarbon Receptor (AhR), Pregnane X Receptor (PXR), Constitutive Androstane Receptor (CAR) and the Peroxisome Proliferator Activated Receptors (PPARs).

These NRs, their ligands and crosstalk with other nuclear receptors will be mentioned. Figure 8 gives an overview of the interaction of different nuclear receptors with environmental contaminants and other nuclear receptors.

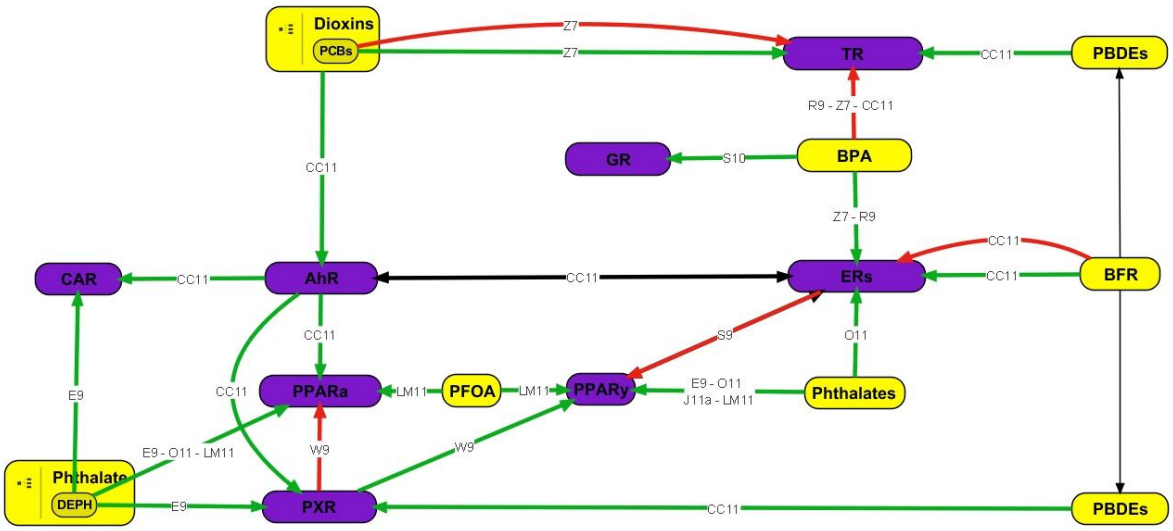

Figure 8 Interaction of environmental contaminants(yellow) with nuclear receptors (purple). Green lines show an agonistic or stimulating effect and the red lines an antagonistic or inhibitory effect. The text inside the lines is the abbreviation of the references (see appendix I).

Estrogen receptors

The estrogens receptors (ERs) are widely expressed throughout the body, like adipose tissue[28], pancreas and liver. ERα and ERβ are nuclear receptors. Activation of these receptors results in the transcription of estrogen responsive genes. Membranes can also contain ERs (mERs).

ERs can influence the regulation of different mechanisms in the body, e.g. the reproductive system and the metabolic system.

The natural ligands of the ERs are estrogens, mainly estradiol. Estrogens are important in the regulation of energy metabolism[44] and this is partly due to activation of the ERs.

The ERs pathways crosstalk with other nuclear receptor pathways. For example, it is well known that the ERs crosstalk with the AhR. Activation of the AhR can have an estrogenic or anti-estrogenic effect, depending on the cell type[39]. Regulation of ER and PPARγ responsive genes are influenced by the crosstalk of ER and PPARγ[13]. For example, ERβ is able to inhibit the PPARγ/retinoid X receptor (RXR) complex[45].

EDCs are able to interfere with the ER pathway in several ways. For example, phthalates have a low affinity for the ERs[46]. Next to that, BPA is similar to estrogens and can bind to nuclear and membrane ERs[29]. BPA is similar to estrogens, but have less affinity to the serum binding proteins and are therefore able to act more directly on the tissue[28]. Prenatal BPA exposed rats showed an altered ERβ and ERα (increased) gene expression[28]. Even BRFs and their metabolites can have an agonistic or antagonistic effect on the ERs[27]. Dioxins can have an estrogenic or anti-estrogenic effect because they act through the AhR. ERs are also involved in the increase and decrease of adipocin secretion of adipocytes, resulting in insulin resistance. Preadipocyte differentiation can be inhibited by over expression of ERs.[45]

Glucocorticoid receptor

The glucocorticoid receptor (GR) is located in the cytosol in almost all cells. The precise mechanism how the activated GR act is different per cell type. The natural ligands for the GR are glucocorticoids and cortisol, the so-called stress hormones. EDCs, like BPA, are able to compete with the natural ligand of the GR for binding sites.[30]

Thyroid hormone receptor

The thyroid hormone receptor has a few forms, namely TR alpha and TR beta and these are expressed through-out the whole body. TRα-1 is mainly present in brain and adipose tissue and TRβ mainly in the liver[16]. The natural ligands for this receptor are the thyroid hormones triiodothyronine (T3) and thyroxine (T4). These hormones will mainly act through the TRs. TR and RXR are known to cross talk on lipid metabolism genes and other signaling systems[47].

BPA can bind to the TR [28] and is able to dissociate T3 from the TR [29]). BPA exposure in dams resulted in lower T4 levels in dams and higher levels in the offspring[28]. BPA can be a TR antagonist and PBDEs bind and activate the TR[27].

Aryl hydrocarbon receptor

The aryl hydrocarbon receptor (AhR) is expressed in mainly expressed in the liver. Ligand activation of AhR results in expression of genes of the liver metabolism. AhR mediated gene expression can lead to energy metabolism alterations[48]. The AhR can induce the expression of PPARγ, CAR and PXR and crosstalks with the ER.[27]

Dioxins are high affinity binding ligands for the AhR and TCDD has the highest affinity. PBDE binds to the AhR but does not activate it.[27]

Pregnane X receptor

The nuclear pregnane X receptor (PXR) is among other located in the liver as part of the xenobiotic metabolism system. It binds to the RXR and regulates the gene expressions via the xenobiotic responsive element. PXR can inhibit PPARα and stimulate PPARγ.[15] PXR can be activated by PBDEs[27] and phthalates[36].

Constitutive androstane receptor

The constitutive androstane receptor (CAR) is a nuclear receptor also located in the liver. It is responsible for the xenobiotic metabolism via binding to the RXR and regulation of gene expressions via the xenobiotic responsive element. CAR competes with PPARα for the binding site in the 3-hydroxyacyl-CoA dehydrogenase gene promoter[15]. DEHP and phthalates can activate CAR.[36]

Peroxisome Proliferator Activated Receptors

The peroxisome proliferator activated receptors (PPARs) are ligand gated transcription factors. They were first discovered in rodents where they stimulated peroxisome proliferation. Although humans have PPARs, these are not able to proliferate peroxisomes. The three isomers, alpha, beta and gamma are mostly expressed at different sites with different functions. PPARα is mainly expressed in liver, heart and muscle. PPARβ is ubiquitous expressed and PPARγ plays a major role in adipose tissue formation.[27]

The PPARs have a large binding domain for lipid-like ligands[27]. PPARα and PPARβ can be activated by native fatty acids whereas PPARγ can be activated by FA derivates[20]. Upon binding a ligand, the PPAR forms a dimer with RXR and stimulates gene expression[27].

Phthalates are able to bind and activate all three isomers[21, 27, 36]. PFOA can also bind and activate the PPARs[21, 27]. The BPA derivate BADGE is a PPAR ligand[28], however no PPAR activation is noticed.

Nuclear receptors and the metabolic system

In the previous section, the different EDCs and their possible relation to metabolic disorders were discussed.

Following this, these EDCs were mentioned as ligands of certain nuclear receptors. The following part will address the effects of the activation of these nuclear receptors on the metabolic system. Parts of the glucose metabolism, lipid metabolism and adipogenesis will be discussed further. Figure 9 shows a schematic view of a few mechanisms involved in the metabolic system.

Estrogen receptors

Estrogens act mainly through the ERs. Low levels of estrogens and therefore less activation of the ERs result in an obese phenotype. This is observed in menopausal woman and ERα knock-out mice[27].

Hypothalamic neurons are involved in the satiety centre in the brain. Activation of membrane estrogen receptors (mER) in dopamine and pro-opiomelanocortin (POMC) hypothalamic neurons can desensitize $GABA_B$ receptors, so satiety is no longer suppressed[13].

Activation of ERα causes an increase of the insulin content in pancreatic beta cells[45]. This increase can also be stimulated by BPA via the ERs[28]. Next to mERs, ER-alpha is also located in the pancreas cells. It has been found that ER-alpha can protect the beta cells from apoptosis induced by oxidative stress.[13]

Especially in ER knockout mouse models the mechanisms of ERs on the metabolic system are revealed. It showed that ERα as well as ERβ can influence the glucose metabolism. For example, estradiol and the ERs are involved in the regulation of GLUT2. Besides that, ERα induces GLUT4 expression whereas ERβ represses this expression. It is also thought that the ERα/ERβ ratio plays a role as well. Activation of the ERs (by estrogens) resulted in an increase in glucose uptake, glycolysis and lipolysis in skeletal muscle i.e. an increase in generation and use of energy.[23]

In adipocytes, ERs are involved in the stimulation of lipolysis and insulin mediated glucose oxidation (glycolysis). ERs are also involved in the increase of adipocin secretion of adipocytes, resulting in insulin resistance.[23] Preadipocyte differentiation can be inhibited by over expression of ERs[45].

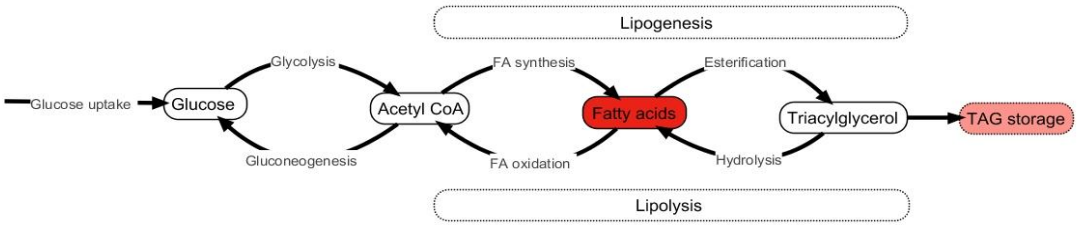

Figure 9 A schematic overview of the different mechanisms in the metabolic system. Note that gluconeogenesis takes only place in the liver.

Glucocorticoid receptor

Activation of the GR results in an increase in blood glucose levels by inducing gluconeogenesis and beta oxidation of FA[27]. Peckett *et al* preformed a literature study on the influence of glucocorticoids (GCs) on the metabolism. There are some contradictory outcomes, but the genomic effect of glucocorticoid exposure is an increase in lipogenesis[49]. During adipogenesis, stimulation of the GR results in an increase of lipid uptake[27] and therefore maturation of the preadipocyte.

Thyroid receptor

High levels of thyroid hormones are related to weight loss and increased lipolysis. The opposite is initiated with low thyroid levels.[2716, 27] In the liver T3 induces expression of lipolysis related genes like LPL, fatty acid transporter and fatty acid binding protein. These mechanisms are regulated by the thyroid receptor. In adipose tissue, T3 increases lipolysis as well and is responsible for maintaining adipocyte functions. TRα-1 plays a part in adipogenesis.[16]

Aryl hydrocarbon receptor

Some studies show a relation between the AhR and its effect on the metabolic system, but the precise mechanism is not clear. It is only known that the AhR can inhibit adipocyte differentiation by inhibiting PPARγ and crosstalk with the ER.

Pregnane X receptor

Activation of PXR can lead to stimulation of lipogenesis and inhibition of beta-oxidation and gluconeogenesis in the liver. PXR is able to stimulate the lipogenesis by up regulating the gene expression of fatty acid transporters alone or via PPARγ and certain enzymes. This results in an accumulation of fatty acids and lipogenesis in the liver. Next to that, PXR is involved in inhibition of the beta oxidation of FA by down regulating the expression of, for example, PPARα. Interaction of PXP with e.g. CREB, a positive regulator of gluconeogenesis results in an inhibition of the glucogenesis.[15]

Constitutive androstane receptor

Activation of the constitutive androstane receptor (CAR) can result in the stimulation of lipogenesis and inhibition of gluconeogenesis in the liver. The protein SRBP1 is indirectly activated and therefore stimulation of lipogenesis occurs. The CPT1 enzyme is involved in beta oxidation of fatty acids in the liver (see Figure 4). Transcription of CPT1 can be inhibited by CAR. Furthermore, competition of PPARα for the 3-hydroxyacyl-CoA dehydrogenase gene promoter stimulates lipogenesis.[15]

Peroxisome proliferator activator receptors

The PPARs play a major role in energy metabolism and especially in lipid metabolism[21, 50].

The isoform PPARα plays an important part in FA oxidation and is mainly expressed in liver, heart and muscle. The PPARα stimulates the gene expression of an enzyme in the TCA cycle and therefore stimulates energy production in the liver[15]. Target genes for PPARγ are for example GLUT4 and LPL[20]. The most important isoform in energy metabolism is PPARγ. It has a role in lipid storage, insulin sensitivity and adipogenesis[27]. The PPAR can stimulate the GLUT2 promoter in mice liver cells[13, 23], so glucose uptake increases in the liver[13]. Activation of PPARγ in adipocytes stimulates FA uptake and storage[17]. In preadipocytes this can result in the maturation into adipocytes. Therefore, PPARγ is known as the main regulator in adipogenesis[17,20]. PPARγ is mentioned as protector from insulin resistance by enhancing the uptake of free fatty acids and mutations in PPARγ are related to insulin resistance.[20]

Organs, nuclear receptors and the metabolic system

Liver

Figure 10 gives an overview of the possible stimulatory or inhibitory effect of the ER, PPARγ, GR, PXR and CAR on mechanisms in the metabolic system in the liver. Glucose uptake can be stimulated by ERs[23] and PPARγ[13,23]. Lipogenesis can be stimulated by CAR[15], PXR[15] and the GR[27], which will result in an increase in fatty acids and triacylglycerol (TAG). The breakdown of fatty acids by β-oxidation can be stimulated by the ER[23] and the GR[27] resulting in an increase of Acetyl-CoA. This process is inhibited by CAR and PXR[15], which can cause an increase in fatty acid accumulation. Gluconeogenesis is also inhibited by CAR and PXR[15].

Muscle

As seen in the liver, ER[23]and PPARγ[20] can also stimulate glucose uptake in muscle. Furthermore, ERs are able to stimulate the glycolysis and fatty acid oxidation, resulting in an increase of acetyl CoA and energy use[23]. See Figure 11 for a schematic view.

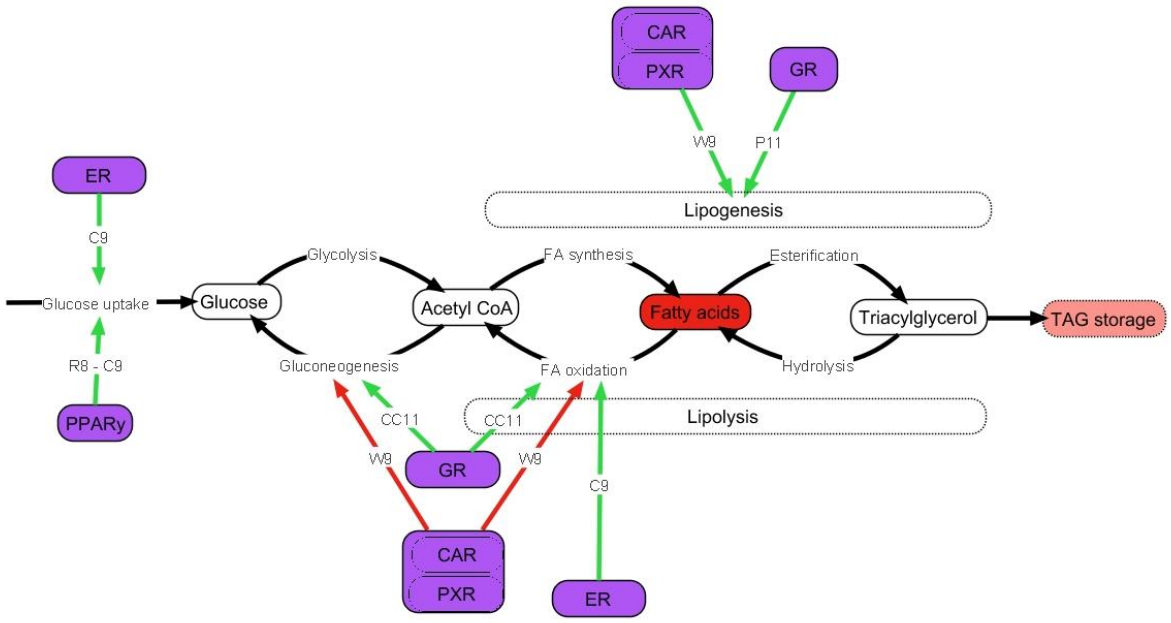

Figure 10 The effect of the nuclear receptors on the metabolic system in the liver. Green arrow is a stimulating effect and the red an inhibitory effect.

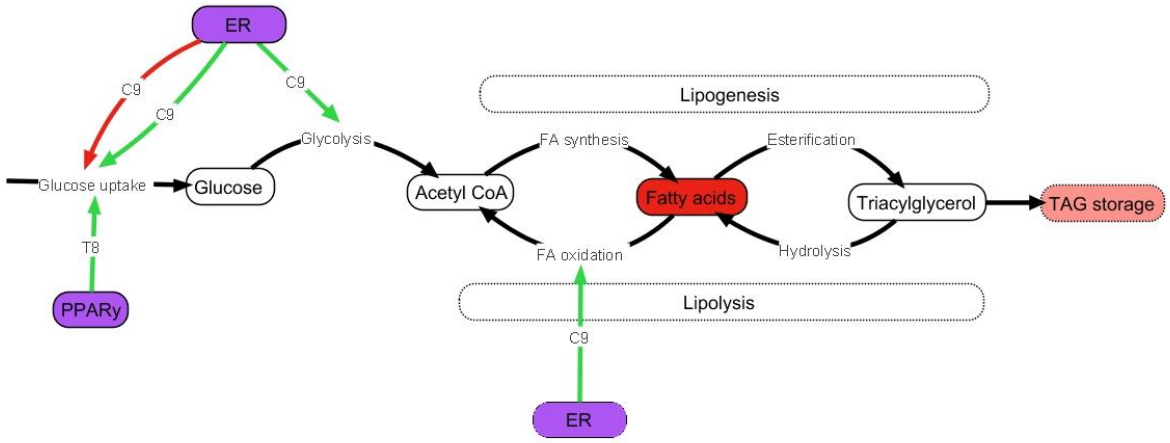

Figure 11 The effect of the nuclear receptors ER and PPARγ on the metabolic system in the muscle. Green arrow is a stimulating effect and the red an inhibitory effect.

Adipose tissue

Nuclear receptors can affect adipogenesis. Figure 12 gives a schematic view of the stimulating effect of adipogenesis by the master regulator PPARγ[17, 20], the GR[27] and the TR[16]. The ER[23] and the AhR [27] can inhibit adipogenesis.

In adipose tissue (Figure 13), ER can stimulate glucose uptake and glysolysis[23], so more acetyl-CoA is formed. The GR can stimulate the lipogenesis[49] and the PPARγ can stimulate TAG storage[17, 20]. In contrast, the GR can also stimulate FA oxidation[27]. Breakdown of lipids can be stimulated by the TR[29] and the ER[23].

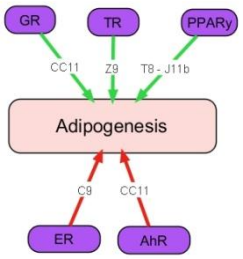

Figure 12 The effect of the nuclear receptors on adipogenesis. Green arrow is a stimulating effect and the red an inhibitory effect.

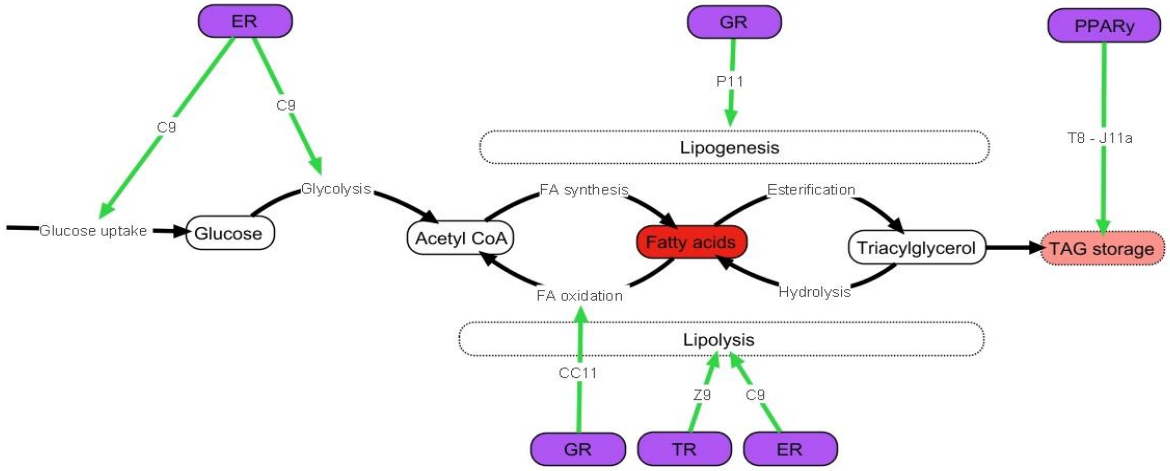

Figure 13 The effect of the nuclear receptors the metabolic system in adipose tissue. Green arrow is a stimulating effect and the red an inhibitory effect.

Discussion

This literature study focused on the potential mechanism of action of a selected group of environmental contaminants on the metabolic system, eventually to describe the biological plausibility of the link between environmental contaminants and obesity. In this study, the outcomes of several studies are put together to describe a potential mechanism. Figure 14 gives a schematic overview of the data discussed in the previous sections. It is a theoretical scheme with data from a selection of papers. It is not complete and is only meant to indicate potential modes of action.

The studies wherein the real human levels are measured and compared to a metabolic disorder can only show a relationship (correlation) and not the cause. In addition, such results are prone to confounding factors. In experimental animal studies exposure levels and effects can be closely monitored. Animals act as models for humans, but clearly, differences may exist. For example, rodents have a different use of the PPARs[51]. If experimental studies show an effect, the question should also be asked if realistic concentrations for human exposure have been used.

The metabolic system is complex and involves many mechanisms, receptors, enzymes and hormones. This provides environmental contaminants various points to interfere. The discussed environmental contaminants (bisphenol A, phthalates, brominated flame retardants, dioxins and perfluorinated compounds) can clearly act via nuclear receptors discussed in this literature study. These receptors are all linked to (disturbance of) the metabolic system providing biological plausibility for some of the experimental or epidemiological effects reported.

Thus, all the compounds discussed could theoretically be linked to metabolic disturbance via nuclear receptors. However, not all direct links between these compounds and (disturbance of) the metabolic system are experimentally established.

For instance, PFCs are associated with high FA and E2 serum levels, but no data could be found about the potential mechanistic pathways, with effects on bodyweight being controversial. Another example are the dioxin-like compounds. Many studies have been done on the toxicological effects of these chemicals, including studies trying to link these chemicals to metabolic diseases. However, various studies show different outcomes and exposure levels[21]. In contrast, BPA shows multiple links with various mechanistic pathways in the metabolic system. A relationship is found between BPA exposure and obesity in epidemiological and experimental studies. From a mechanistic point of view, the nuclear receptors, especially the ERs, which are activated by BPA can be associated to disturbance of the metabolic system. Similar mechanistic pathways can be suggested for phthalates (Figure 14). However, the experimental dose levels used were quite high[27].

Taken together, it seems biological plausible that exposure to environmental contaminants can affect the metabolic system and lead to metabolic disorders like obesity.

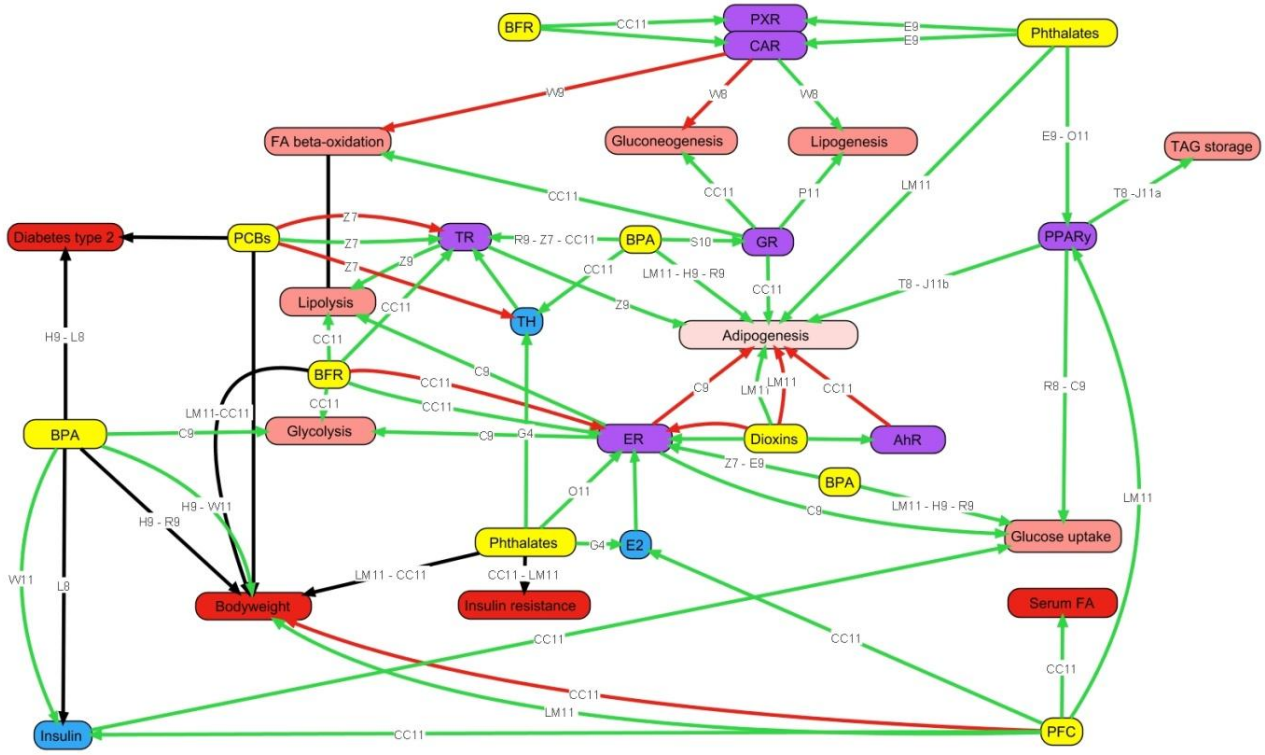

Figure 14 Schematic overview of the effects of environmental contaminants on the metabolic system.

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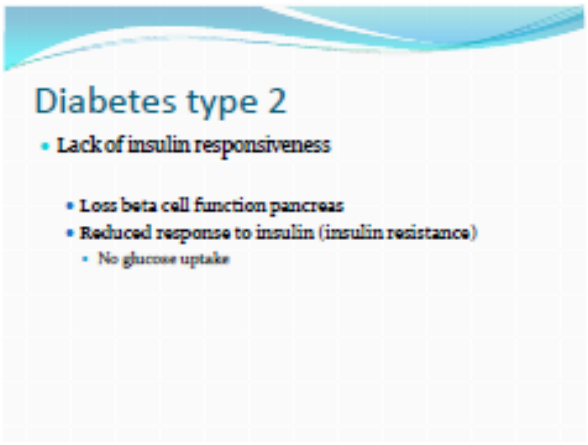
Appendix I

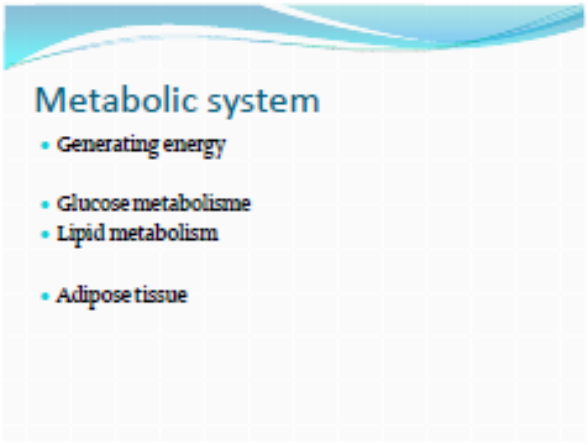
Legend figure 8, 10 - 14

Abbreviation	Author & date	Reference
C9	Chen, 2009	23
CC11	Casals-Calas, 2011	27
E9	Evaillard, 2009	36
G4	Gayathri, 2004	37
H9	Heindel, 2009	31
J11a	Janesick, 2011a	51
J11b	Janesick, 2011b	52
L8	Lang 2008	32
LM11	La Merill, 2011	21
O11	Okamoto, 2011	46
P11	Peckett, 2011	53
R8	Ropero, 2008	13
R9	Rubin, 2009	28
S10	Sargis 2010	30
S9	Swedenborg, 2009	45
T8	Tontonoz, 2008	54
W9	Wada, 2009	15
W11	Wei, 2011	33
Z7	Zoeller 2007	29
Z9	Zhu, 2009	16

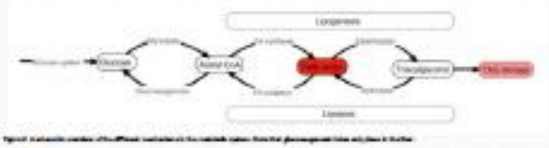
Appendix II

Handouts Presentation





Glucose/lipid metabolism



Effect of environmental contaminants?

Adipose tissue

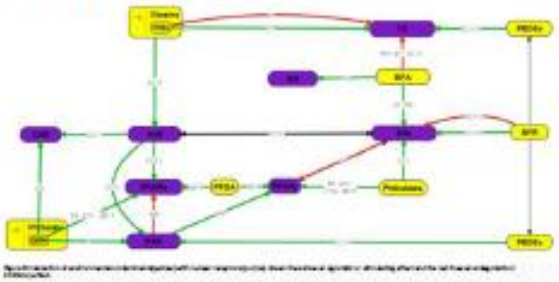
- Store excess energy (lipids)
- Amount stable in adulthood
- Amount of adipocytes set during childhood/adolescence
  - Adipogenesis

Effect of environmental contaminants?

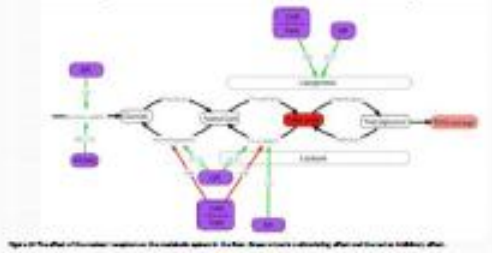
Receptors

- Estrogen receptor (ER)
- Glucocorticoid receptor (GR)
- Thyroid receptor (TR)
- Aryl hydrocarbon receptor (AhR)
- Pregnane X receptor (PXR)
- Constitutive androstane receptor (CAR)
- Peroxisome proliferator activator receptor (PPAR)

Receptors & ECs

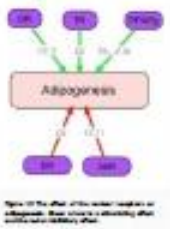


Receptors & metabolic system



Realistic concentration of compounds used?

Receptors & metabolic system



Amount adipocytes set during childhood/adolescence

Realistic concentration of compounds used?

BFRs

- Epidemiological
  - Relation with obesity, diabetes and metabolic syndrome
- Experimental
  - Increase lipolysis
  - No effect on body weight

Dioxins

- Epidemiological
  - Relation with obesity and diabetes (PCBs)
- Experimental
  - Increase body weight (PCBs)

PFCs

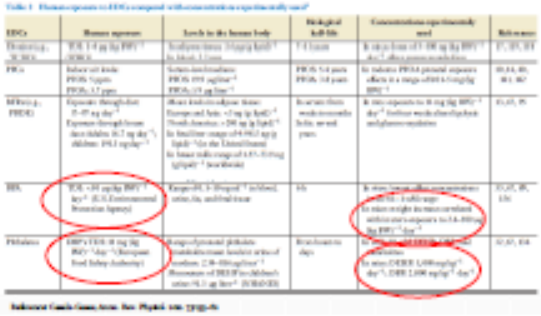
- No epidemiological data
- Experimental data ambiguous

Exposure levels - PFC

Phthalates

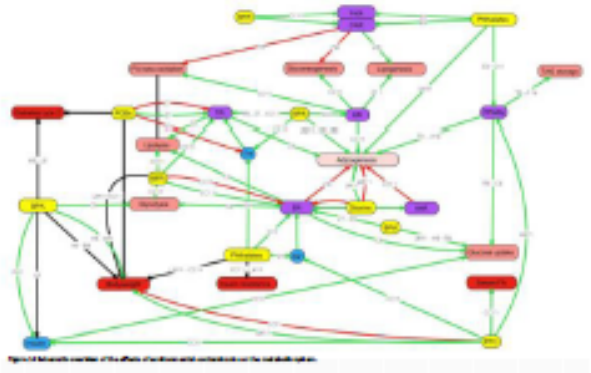
- Epidemiological
  - Relation pthalates and obesity/insulin resistance
- Experimental rodents
  - No effect on bodyweight
  - Increase with humanized PPARa
  - Decreased serum insulin levels
    - Used exposure levels high
- In vitro
  - Stimulation adipogenesis

Exposure levels



Bisphenol A

- Epidemiological
  - Relation with obesity and diabetes
- Experimental (rodents)
  - Prenatal: low BPA → increased bodyweight
  - BPA+high fat diet → increase bodyweight + insulin intolerance
- In vitro
  - Increase adipogenesis and glucose uptake



Discussion

- All ECs can theoreticly affect the metabolic system in humans
  - Epidemiolocal data → some support
  - Via nuclear receptor → biological plausible
- BFRs, Dioxins, PFCs not covered in total (yet?)
- Phthalates high experimental exposures
- BPA
  - Experimental exposures within human exposure range
  - Extra concern prenatal exposure

Conclusion

- Rol of BPA exposure in obesity seems plausible
  - Biological plausible
  - Realistic exposures
- Based on:
  - Epidemiological
  - Experimental
  - In vitro

Thank you