Environmental Science Advances

ROYAL SOCIETY OF CHEMISTRY

CRITICAL REVIEW

View Article Online
View Journal | View Issue

Cite this: *Environ. Sci.: Adv.*, 2024, **3**, 1679

Di(2-ethylhexyl)phthalate and type 2 diabetes

Sebolaishi Doris Makhubela, *[a] Ananias Hodi Kgopa, [a] Matlou Phineas Mokgotho[b] and Leshweni Jerry Shai [b]

Received 17th April 2024
Accepted 27th September 2024

DOI: 10.1039/d4va00121d

rsc.li/esadvances

The prevalence of type 2 diabetes is escalating worldwide and it has been suggested that exposure to endocrine disrupting chemicals, such as phthalates, contributes to the alarming increase. Di(2-ethylhexyl) phthalate (DEHP) is used as a plasticizer in a variety of everyday products; thus humans are constantly exposed to it. Animal studies have associated DEHP with adverse health effects such as reproduction and developmental toxicity, carcinogenicity and metabolic disruption. Concerns over the potential for similar adverse effects in humans are mounting. Recent reviews have reported the link between exposure to a broad set of phthalates and diabetes as well as diabetes-related metabolic conditions. This review evaluates the available information in the literature regarding the association between DEHP exposure and type 2 diabetes and related metabolic conditions, specifically insulin resistance and obesity.

Environmental significance

Phthalates are ubiquitous environmental contaminants and exposure to them has been associated with adverse health effects. This review highlights almost two decades of epidemiological and experimental data associating phthalate exposure, specifically di(2-ethylhexyl)phthalate (DEHP), with diabetes and related adverse metabolic effects. While some associations are clear, some are inconsistent. Nonetheless, as a precaution, DEHP exposure should be minimized. Further studies using models relevant to humans and at environmentally relevant doses could provide a better understanding of the association between DEHP and type 2 diabetes in humans.

1 Introduction

Diabetes is a chronic metabolic disease characterized by elevated blood sugar levels.[1,2] Type 2 diabetes (T2D) is caused by a combination of genetic and lifestyle factors[1] and accounts for about 90% of diabetic cases.[2] It is characterized by insulin resistance in target tissues and β cell dysfunction.[2] About 537 million adults were living with diabetes in 2021, a number that is estimated to increase to 643 million by 2030.[3] The burden due to diabetes on the global health-care system and the wider global economy is unavoidable.[1] Some studies have suggested that the increase in environmental exposure to endocrine-disrupting chemicals, such as phthalates, might also contribute to the alarming increase in the prevalence of diabetes.[4,5] Di(2-ethylhexyl)phthalate (DEHP) is one of the most widely used high molecular weight phthalates, with a production of several million tons per year worldwide.[6] DEHP is mainly used as a plasticizer to make polyvinyl chloride (PVC) more flexible in products such as toys and medical devices. It is also used in food packaging, waterproof clothing, wall coverings and automobile upholstery.[7,8] DEHP is non-covalently attached to PVC and can leach from PVC-laden items into the environment.[6–8] The widespread use of DEHP gives rise to many possible opportunities for human exposure, which in the general population mainly occurs through ingestion of contaminated food.[9,10] A review of seventeen food monitoring surveys conducted in North America, Europe and Asia found that poultry, fats and oils as well as dairy products (cream and cheese) contained high concentrations of DEHP and were likely the main contributors to exposure.[11] In another study, DEHP levels ranging from 0.02 to 2685 mg kg^{-1} were detected in various commercial food samples.[12] People may also be exposed to DEHP *via* ingestion of contaminated indoor dust that has adhered to food or objects[9,13] and through medical tubing during medical procedures.[8,14–16] DEHP exposure in the general population of the United States is estimated to be 1 to 30 μg per kg of body weight per day.[17] However, individuals undergoing chronic treatment such as dialysis and prolonged intensive care treatment[16] and neonates in intensive care units[14,15] can be exposed to higher levels of DEHP. As a result, some governments have recommended restricting the use of DEHP in medical devices.[18] Once it enters the body, DEHP is metabolized by lipases to produce the primary metabolite mono(2-ethylhexyl)phthalate (MEHP) as well as 2-ethylhexanol.[19] MEHP is further converted to secondary metabolites which are excreted in urine in their unconjugated form or as glucuronide conjugates, depending on the species.[20,21] In humans, 67% of

[a]*Department of Biochemistry and Biotechnology, Sefako Makgatho Health Sciences University, Pretoria, South Africa. E-mail: Sebolaishi.kolobe@smu.ac.za*

[b]*Department of Biomedical Sciences, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa*

orally administered DEHP is excreted in urine after 24 hours while 75% is eliminated from the body after 2 days.[22] Furthermore, MEHP contributes only 5.9% of the excreted urinary DEHP metabolites while the secondary oxidized metabolites mono(2-ethyl-5-hydroxyhexyl)phthalate (5OH-MEHP/MEHHP), mono(2-ethyl-5-oxo-hexyl)phthalate (5oxo-MEHP/MEOHP), mono(2-ethyl-5-carboxypentyl)phthalate (5cx-MEPP/MECPP) and mono(2-carboxymethylhexyl)phthalate (2cx-MMHP/MCMHP) together contribute 61%. Due to their longer elimination half-lives, MECPP and MCMHP are considered suitable indicators of chronic exposure to DEHP while the short elimination half-lives of MEHP, MEHHP and MEOHP are more indicative of short-term exposure.[22,23] DEHP metabolites have been detected in more than 90% of urine samples in exposure assessment studies of different populations[24–30] and to a lesser extent in breast milk[31] and amniotic fluid.[32] This level of exposure has raised concerns about its effects on human health, prompting the European Union,[33] USA[34] and Canada[35] to restrict the use of DEHP in certain products. Although the use of DEHP is on the decline in these countries,[25,36] DEHP usage is still unregulated in other parts of the world.[37] Studies have associated DEHP exposure with adverse health effects such as reproduction and developmental toxicity as well as carcinogenicity.[18,38] Moreover, the literature has reviewed the association between exposure to a broad set of phthalates and metabolic conditions such as diabetes,[39,40] insulin resistance[41,42] and obesity.[43,44] This review documents the association between DEHP exposure and T2D and its related metabolic conditions.

2 Epidemiological studies

Most of the epidemiological studies relating DEHP exposure to diabetes and related effects were conducted in countries such as Canada,[45] USA[46] and Korea[47] utilizing biomonitoring data collected from national surveys. Such data, to the best of our knowledge, are lacking in South Africa and Africa as a whole and collecting these data might be beneficial for disease preventative efforts in the African continent, especially because diabetes prevalence has risen faster in middle- and low-income countries than high-income countries.[1] Furthermore, the International Diabetes Federation estimates that 3 in 4 adults with diabetes live in low- and middle-income countries.[3]

2.1 Association between DEHP and diabetes

There is growing evidence that exposure to DEHP may contribute to the development of disorders related to glucose and lipid metabolism. Several epidemiological studies have assessed the relationship between the levels of DEHP metabolite and diabetes. Svensson *et al.*[48] reported a correlation between urinary levels of DEHP metabolites (MEHHP and MEOHP) and the occurrence of T2D in a case–control study involving 221 Mexican women, with 17.4% of the women having self-reported diabetes. A case–control study on USA nurses reported a relationship between MECPP and the sum of DEHP metabolites (ΣDEHPm) and T2D in middle aged women.[49] In another study, data from 2350 women participants in the 2001–2008 National Health and Nutrition Examination Survey (NHANES) indicated that ΣDEHPm (MEHP, MEHHP, and MEOHP) was associated with prevalent diabetes.[46] Similarly, a positive association was reported between DEHP metabolites (MEHHP, MEOHP and MECPP) and self-reported diabetes in a study conducted in Shanghai involving 2330 adult participants, while MEOHP, MEHHP, MECPP, MCMHP and ΣDEHPox (sum of oxidative metabolites) were associated with self-reported diabetes only in males.[29] One study assessed the relationship between serum levels of MEHP and the prevalence of diabetes and found no correlation between the two in an elderly population sample in Sweden,[50] which is contrary to the positive relation observed between urinary MEHP concentration and T2D in a Chinese adult population sample.[51] In the same case–control study, Duan *et al.*[51] also observed a positive relationship between MEHHP, MEOHP and ΣDEHPm and T2D but an inverse association with MECPP and MCMHP. Studies based on the 2015–2017 Korean National Environmental Health Survey (KoNEHS)[52] and 2017–2018 NHANES data[53] reported that MEHHP, MEOHP, MECPP and ΣDEHPm levels were related to higher prevalence of diabetes or risk of T2D in adults.

Overall, although some inconsistent findings were reported for associations of MEHP, MECPP and MCMHP with diabetes, most of the studies reported a positive relationship between DEHP exposure and diabetes. Sex-specific associations have been found in some studies[29] but not in others[52] while age-specific associations were also observed.[49,51]

2.2 Association between DEHP and insulin resistance

T2D is associated with insulin resistance and hyperglycemia. The data correlating the levels of DEHP metabolites to both these parameters are conflicting. MEHHP and MEOHP were not found to be associated with insulin resistance in the male subgroup of the 1999–2002 NHANES,[54] in adolescents and young adults from the young Taiwanese cohort study[55] and in a population of Korean women.[56] The two oxidative metabolites were also not associated with fasting insulin levels[55,56] and glucose levels.[55] Dirinck *et al.*[57] observed no correlation between DEHP metabolites (MEHP, MEHHP, MEOHP and MECPP) and glycated hemoglobin (HbA1c), fasting glucose, fasting insulin levels, the homeostasis model assessment of insulin resistance index (HOMA-IR) and the homeostasis model assessment of β-cell function (HOMA-β) in an obese Belgian adult cohort. Kim *et al.*[58] found no association between DEHP metabolites (MEHP, MEHHP, MEOHP and MECPP) and insulin resistance in a population of Korean girls but reported a positive association with MEHHP%, a marker reflecting MEHHP metabolism. A study based on the women subset of the 2001–2008 NHANES also found no association between ΣDEHPm (MEHP, MEHHP and MEOHP) and HbA1c but reported a positive association with HOMA-IR.[46] However, another study reported a positive association between MEHP and MEHHP and HbA1c levels in the 2009–2011 Canadian health survey and further observed that ΣDEHPm (MEHP, MEHHP, and MEOHP) was associated with fasting glucose, fasting insulin, HOMA-IR and HOMA-β.[45] Duan *et al.*[51] found a positive association between MEHHP and HbA1c

levels as well as MEHP and fasting glucose levels in a Chinese adult population. A study based on the 2015–2017 KoNEHS data found positive associations between ΣDEHPm and glycated hemoglobin in males while a negative association was observed with MECPP and ΣDEHPm (MEHHP, MEOHP and MECPP) in females (depending on the urinary adjustment method).[47] Positive associations between DEHP metabolites (MEHP, MEHHP, MEOHP and MECPP) and insulin resistance have been recorded in adolescents, based on NHANES data covering the period from 2003 to 2012.[59,60] Another study reported a positive relationship between MEHP and increased insulin levels and HOMA-IR in a cohort of young Taiwanese adults but not adolescents.[55] However, using the same data, Lin *et al.*[61] found a positive correlation between MEHP levels and higher insulin levels, HOMA-IR and HOMA-β in adolescents and young adults. Huang *et al.*[62] reported a positive association between ΣDEHPm (MEHP, MEHHP and MEOHP) and fasting blood glucose, fasting insulin and insulin resistance in both men and women using NHANES data of 2001–2008. James-Todd *et al.*[63] found associations between ΣDEHPm (MEHP, MEHHP, and MEOHP) and hyperglycemia in a cross-sectional NHANES study (2001–2010) of 2719 adults. A study involving Korean females also reported positive associations between ΣDEHPm (MEHHP, MEOHP, MECPP and MCMHP) and fasting glucose.[64] A recent NHANES study (2017–2018) also found that MEHHP, MEOHP, MECPP and their sum are related to fasting insulin levels and HOMA-IR.[53]

Oxidative stress plays a major role in insulin resistance.[65,66] Studies have investigated the link between DEHP exposure and oxidative stress markers, some of which results from the interaction of reactive oxygen species (ROS) with DNA or fatty acids.[67] There are reports on the relationship between DEHP metabolites and markers such as serum gamma glutamyltransferase (GGT) and urinary malondialdehyde (MDA), F2-isoprostane, and 8-hydroxydeoxyguanosine (8-OHdG). MEHHP and MEOHP were correlated with MDA and 8-OHdG in a Korean adult population sample; MEHHP also had a positive association with fasting glucose levels.[68] Using the 1999–2006 NHANES data, Ferguson *et al.*[69] found a positive association between MEHP and GGT but an inverse association with oxidative DEHP metabolites (MEHHP, MEOHP, and MECPP). DEHP metabolite (MEHP, MEHHP, MEOHP, MECPP and MCMHP) levels were associated with increased levels of F2-isoprostane in a small cohort of adolescents in New York but no associations with insulin resistance were found.[70] One study observed an association between DEHP metabolites (MEHP, MEHHP, MEOHP and MECPP) and higher levels of 8-OHdG and 8-isoprostane during pregnancy in a nested case–control study in Boston, USA.[67] However, the same DEHP metabolites were not associated with 8-isoprostane during pregnancy in a rural Mexican American population; instead, a marginal association was observed with ΣDEHP.[71] DEHP metabolites (MEHHP, MEOHP, MECPP and MCMHP) were correlated with 8-OHdG in a Saudi Arabian population sample[72,73] while MEOHP was associated with MDA levels in children.[73] Rocha *et al.*[74] observed a positive relationship between MECPP, MCMHP and ΣDEHPox with 8-OHd G levels in a Brazilian children population. A longitudinal study examined the relationship between prenatal DEHP exposure in early and late pregnancy and 8-isoprostane levels in children at 5, 9 and 14 years of age in a population of obese/overweight Mexican American children.[75] The study found that maternal MEHP, MECPP and ΣDEHP concentrations measured in early pregnancy were associated with 8-isoprostane in children at 14 years, with borderline associations observed for MEHHP and MEOHP. Kim *et al.*[76] investigated the relationship between DEHP exposure, oxidative stress and insulin resistance in an elderly Korean population. ΣDEHPm (MHEHHP and MEOHP) was associated with fasting glucose, insulin levels, insulin resistance and MDA levels in participants with a previous history of diabetes. The study further established a link between insulin resistance and higher levels of MDA and concluded that DEHP might contribute to insulin resistance by inducing oxidative stress.

Inflammation is another key factor in the development of insulin resistance and it has been suggested that oxidative stress can trigger inflammation response involved in insulin resistance.[65,66] One study examined the relationship between DEHP exposure in diabetic patients and oxidative stress, adiponectin, and inflammatory cytokines levels.[77] Adiponectin has anti-inflammatory properties and its low levels are associated with insulin resistance and T2D while the expression of the inflammatory cytokine tumour necrosis factor α (TNF-α) is associated with insulin resistance.[78] In their analysis, Duan *et al.*[77] reported a positive association between DEHP metabolites (MECPP, MEHHP, MEOHP and MCMHP) and MDA levels in participants of the study. MEHP and ΣDEHPm were also associated with MDA levels in different subgroups of participants, but this association was not observed in the larger BMI subgroup. Oxidative DEHP metabolites were positively associated with serum adiponectin levels while ΣDEHPm (MEHP, MEHHP, MEOHP, MECPP and MCMHP) and MEHP showed inverse associations with adiponectin levels in the larger BMI subgroup. Oxidative DEHP metabolite levels were negatively associated with TNF-α levels while ΣDEHPm and MEHP exhibited a positive relationship with TNF-α in the larger BMI subgroup. The positive association with TNF-α levels was attributed to phthalates contributing to insulin resistance by increasing TNF-α levels while the negative association was attributed to the lower toxicity of oxidative metabolites compared to MEHP. Lee *et al.*[64] also reported a positive association between ΣDEHPm (MEHHP, MEOHP, MECPP and MCMHP) and serum adiponectin levels and surprisingly, they found a similar association with fasting glucose levels in a Korean female study population, leading to the suggestion that DEHP possibly affects fasting blood glucose levels *via* a mechanism that does not involve adiponectin. A recent study focusing on a different age group to Duan *et al.*[77] reported a negative correlation between urinary levels of MEHP and adiponectin levels along with a positive association between MEHP and insulin resistance in a Taiwanese study involving adolescents and young adults.[61] Inconsistencies in epidemiological studies regarding DEHP exposure and adiponectin levels have been observed.[61,64]

While some studies suggest that DEHP exposure is associated with insulin resistance, there are also data suggesting otherwise. Differences in study populations may account for some of the observed inconsistencies in these associations[71] but other sources of variability need to be examined.

2.3 Association between DEHP and obesity

While obesity is a risk factor for diabetes, accounting for about 70% of the risk associated with T2D, the two can exist independently of one another.[79] Obesity is associated with the development of insulin resistance which in turn predisposes individuals to T2D.[80] Parameters such as body mass index (BMI) and waist circumference are used to predict obesity.[81] Several studies have examined the link between DEHP exposure and obesity. A positive correlation was found between MEHHP and MEOHP and waist circumference in men in the 2001–2002 NHANES.[54] Similarly, a positive association of MEHHP and MEOHP with BMI and waist circumference was reported in men but an inverse association between MEHP and these parameters in females based on NHANES data from 1999 to 2002.[82] Interestingly, using the same NHANES data for the women subset (but up to 2004), another study found that MEHP was positively associated with BMI and additionally reported that the ratio of MEHP : MEHHP was positively associated with BMI and waist circumference.[83] These contradictory findings regarding MEHP association were attributed to differences in data analysis methods.[83] An NHANES study (2001–2010) found associations between higher concentrations of ΣDEHPm and central obesity in male subjects, and further found associations with metabolic syndrome in men as well as in women less than 50 years of age.[63] Furthermore, using NHANES data from 2007–2010, positive associations were observed between DEHP metabolites (MEHP MEHHP, MEOHP and MECPP) and an increased risk of obesity in adults.[84] Similar associations were observed with BMI in a premenopausal American cohort[85] and with central obesity in females less than 45 years of age and adults in a Chinese population (MEHHP and MECPP).[86] However, DEHP metabolites (MEHHP, MEOHP, and MECPP) were not correlated with general obesity/BMI and/or abdominal obesity/waist circumference in American women in a nurse's health study[87] and in Korean adult cohorts.[56,88] A study on obese participants in Belgium reported an inverse association between MEOHP and BMI[57] while another study found a positive correlation in an elderly population of Anhui province of China, with a stronger association observed in males.[89] Van der Meer *et al.*[90] observed a correlation between MECPP and adiposity-related traits (BMI and waist circumference) in a study population of adults in North Netherlands. A positive relation was observed between MEHHP and ΣDEHPm (MEHHP, MEOHP and MECPP) and obesity in adult participants in the 2015–2017 KoNEHS; however, the association was dependent on the urinary dilution adjustment method.[47]

Such disparities in the association between DEHP metabolites and obesity have also been documented in children. An NHANES study (1999–2002) found an inverse association between MEHP and BMI and waist circumference in adolescent girls.[82] Other studies found no associations between ΣDEHPm/DEHP metabolites (MEHP, MEHHP, MEOHP and MECPP) and body mass outcomes (BMI *z*-score, overweight and obesity) in children and adolescents based on the NHANES data,[84,91] in China[92,93] and in Korea[58] but reported that MEHHP% was positively associated with obesity.[58] On the other hand, Wang *et al.*[94] documented a positive association between MEHP and BMI and waist circumference in a Shanghai children cohort. MEHP, MEHHP and ΣDEHPm were negatively associated with obesity in girls in a Shanghai study[95] while Amin *et al.*[96] observed a positive association of MEHP and MEHHP with obesity as well as a low to moderate positive association between DEHP metabolites (MEHP, MEHHP and MEOHP) and BMI in an Iranian children and adolescent population. Recently, Seo *et al.*[97] found a positive association between MECPP and obesity in children using KoNEHS data (2015–2017). Similarly, a prospective case–control study reported a correlation between higher levels of DEHP metabolites (MEHP, MEHHP and MEOHP) and BMI *z*-scores and higher risk of obesity in a Chinese children population.[98]

Longitudinal studies have mostly found no associations between ΣDEHPm/DEHP metabolite (MEHP, MEHHP, MEOHP and MECPP) levels and obesity parameters determined in a follow-up period of a year in New York children,[99] up to six years in girl participants from three centers in USA[100] and up to 24 years in preschool children from a Swedish birth cohort.[101] One study assessed the relationship between serum MEHP levels and different obesity indices as determined by dual-energy X-ray absorptiometry and abdominal magnetic resonance imaging in a Swedish elderly population and found no correlation in a two year follow-up period.[50]

Different DEHP exposure patterns in different study populations may account for some of the disparities observed in the studies above.[90] Additionally, studies found that associations between concentrations of urinary DEHP metabolites and obesity vary based on sex,[63,82,86,89,94] age,[63,82,84,86] race/ethnicity[91] and differences in the rate of DEHP metabolism due to genetic variations.[61] However, other studies did not find sex[88] or age-related differences[83] in the associations. Other sources of inconsistencies in the studies are the use of different exposure metrics/exposure assessments; these include urinary dilution adjustment/corrections for dilution and whether individual or summed metabolites are presented.[43,102] Recently Lee *et al.*[47] demonstrated that the method used to correct urinary phthalate concentrations for urinary dilution could influence the associations between DEHP metabolites and obesity. Differences in adjustment for confounding factors,[83] varying statistical analysis[86] and different standards/parameters being used to determine obesity (such as BMI and percentage body fat)[95] may also contribute to the inconsistencies.

2.4 Variability of DEHP metabolite levels over time

The use of cross-sectional data to conclude on associations between DEHP metabolites with short half-lives and chronic diseases has been highlighted as a shortfall of epidemiological observations.[103] Furthermore, most of the cross-sectional

studies involve single urine measurements of phthalates which are thought to reflect recent/acute exposure levels.[104,105] Thus, it is suggested that these levels do not reflect long-term exposure or exposure throughout disease development.[63,64,80] As such, several studies have assessed whether the DEHP metabolite levels recorded in single urine samples represent an individual's DEHP exposure over time, primarily based on assessment of the intraclass correlation coefficient of single metabolites or ΣDEHPm. Assessment of DEHP metabolite levels (MEHP, MEHHP, MEOHP and MECPP) in spot urine samples obtained during varying periods of pregnancy in various pregnant women populations showed low within-person reproducibility[105–114] and fair reproducibility of MEHP and MECPP levels.[115] Analysis of DEHP metabolites levels (MEHHP, MEOHP, MECPP and MCMHP) in spot, first morning and 24 hour urine samples collected from men and women for a period of days up to several months showed high intraday and interday within-person variability.[104,116–121] A similar trend was observed in children for urine samples collected over periods ranging from six months to years.[122,123] Other studies have however suggested that single metabolite measurements are representative/predictive of DEHP exposure over time and have reported moderate reproducibility[124] and good correlation[76] of DEHP secondary metabolite (MEOHP, MEHHP and MECPP) levels from multiple urine samples obtained over 3 years. Another study reported fair reproducibility in samples collected from children over periods ranging from days to a year, based on pooled samples.[115]

Reports regarding the consistency of MEHP levels in first morning/spot urine samples collected over time are inconsistent. One study found consistencies in MEHP levels from urine samples provided over 2 days.[125] Another study found that the participant's MEHP levels were more consistent in morning spot urine samples collected 2 to 8 days apart compared to first morning and 24-hour samples.[117] Casas *et al.*[115] found good reliability of MEHP levels in pooled urine samples collected over a period ranging from days to a year. Hauser *et al.*[126] reported within-subject variability in MEHP levels but moderate sensitivity of a single sample to predict a 3-month average exposure. Another study found that MEHP levels measured in a single sample were predictive of an individual's 6-month average concentration.[122] Other studies have however recorded within-person variability in samples collected over several days,[118,123] 1 to 4 months[104,120] and even years.[123,124]

The discrepancies in the findings above may be due to differences in study design, phthalate exposure patterns and study populations,[104,106,118] variations in an individual's food consumption patterns[116,126] and changes in phthalate metabolism due to physiological changes during/after pregnancy.[106,107] On the other hand, it is suggested that the small variability in phthalate levels over time may be a result of an individual's consistent daily time–activity patterns and stable microenvironmental phthalate concentrations[106] or consistent long-term diet patterns.[124]

The above studies were conducted on specific populations, with only a few studies reporting large and diverse cohorts; therefore studies that are representative of the general population are necessary. The intraday variability of metabolite levels has also highlighted the need to factor in the time of sample collection during the day when carrying out such studies.[116,119] Moreover, according to Preau *et al.*[116] the short elimination time associated with phthalates implies that determining exposure to phthalates over weeks or months may require multiple urine measurements. Therefore, longitudinal studies, with multiple urine measurements of DEHP metabolites at different time points, are warranted to confirm the exposure–health outcome associations from cross-sectional studies. This would be especially relevant for diseases that take time to manifest, such as T2D. Although multiple sample measurements are associated with logistical and analysis cost implications,[116,127] it appears that they are critical for reliable results in epidemiological studies involving nonpersistent chemicals.[128]

3 Experimental studies

Epidemiological studies have a limitation of failing to establish a cause-and-effect relationship.[61,69] To address this shortcoming, experimental studies have been conducted and a growing body of experimental data suggests a link between DEHP exposure and diabetes and its risk factors.[129–135] Several modes of action have been proposed for DEHP and these can assist in explaining the association between DEHP/DEHP metabolites and diabetes. These include activation of peroxisome proliferator-activated receptors (PPARs), induction of oxidative stress, impairment of β-cell function and impairment of adiponectin function.[40]

3.1 Peroxisome proliferator-activated receptor activation

PPARs are nuclear receptors which form heterodimers with the retinoid X receptor (RXR) and bind to peroxisomal proliferator response element in the promoter regions of their target genes.[136] The PPAR–RXR heterodimer can function as a transcriptional factor, activating or repressing gene expression, depending on the presence of co-activators or co-repressors.[137] The PPAR family of transcription factors comprises isoforms PPARα, PPARγ and PPARβ/δ. PPARα is predominantly found in the liver and plays a crucial role in fatty acid catabolism.[138] PPARγ is predominantly found in adipose tissue; it regulates adipogenesis and plays a role in insulin sensitivity.[137] Studies have alluded that DEHP/MEHP exerts its effects through a mechanism that is mediated by PPARγ[134,136] and that MEHP binds to the ligand binding domain of PPARγ.[139,140] While there are inconsistent findings on the ability of the parent compound DEHP to activate PPARs *in vitro*,[136,141–145] trans-activation assays have shown that MEHP is able to activate the three PPAR isoforms and further studies in intact cells show that MEHP activates biological processes that are mediated by PPARs. MEHP activated both mouse[136,141,142,146] and human PPARα,[141,142] with mouse PPARα being activated at lower MEHP concentrations than human PPARα.[142,146] Interestingly, MEHP was found to be a more potent activator of PPARα than more prevalent monoesters such as monobenzyl phthalate and monobutyl phthalate

whose urinary levels were reported to be higher than that of MEHP.[146–150] With regard to the stimulation of PPARα target genes, promoter–reporter gene assays showed that MEHP (250 μM) activated PPARα-dependent transcription of the rat acyl-CoA oxidase (ACOX) gene with both mouse and human PPARα. Human ACOX was not responsive with either PPARα, prompting the authors to conclude that DEHP does not pose a health risk to humans.[147] Similarly, MEHP concentrations of 10–100 μM stimulated the expression of PPARα target genes ACOX and cytochrome P450 4A in rat liver FAO cells but not in the human HepG2 cells.[146] This is contrary to another study where the FAO cell line was not responsive to similar concentrations of MEHP in inducing ACOX gene expression.[142] It was also highlighted that MEHP activation of human PPARα would possibly be relevant in exceptional cases of high DEHP exposure since the concentration of MEHP required for PPARα activation in *in vitro* studies is higher than the maximum serum levels of MEHP detected in the general population.[146]

In vitro studies have reported that MEHP can also activate mouse and human PPARγ with no species differences in PPAR responsiveness to MEHP.[136,141,142,146] MEHP (1 to 50 μM) further induced PPARγ dependent adipogenesis in 3T3-L1 cells,[136,142,146,148] with significant upregulation of PPARγ adipogenic target genes at concentrations ranging from 10 to 100 μM.[148]

Another study found that during differentiation, 3T3-L1 cells treated with MEHP at concentrations of 10–300 μM had reduced gene expression of PPARγ, enhanced expression of some adipogenic markers while the expression of other markers was not affected.[145] In addition, the cells accumulated MEHP and had enhanced lipolysis and glucose uptake. Treatment of murine mesenchymal stem cells with 100 μM DEHP induced adipogenesis and upregulated gene transcription of adipogenic markers (PPARγ$_2$, adiponectin, fatty acid binding protein fatty 4 (FABP4) and lipoprotein lipase).[143] While a human adipocyte cell line was not included in these studies for comparison, few studies assessed the ability of DEHP/MEHP to activate PPAR and further induce target genes in human cell models. Human subcutaneous pre-adipocytes were differentiated *in vitro,* and on day 11, the cells (30% undifferentiated and 70% differentiated) were treated with 100 μM MEHP for 4 to 48 h[149] MEHP induced differentiation of the remaining preadipocytes and upregulated transcription of genes involved in the PPARγ signaling pathway, triglyceride synthesis and storage as well as glyceroneogenesis in mature adipocytes. It is thus possible that by inducing adipogenesis and lipid accumulation, MEHP could promote obesity[150] which could possibly lead to insulin resistance. Schaedlich *et al.*[151] assessed the effect of DEHP, at a concentration similar to that detected in blood bags (50 μg ml^{-1} or 128 μM), on the differentiation of Simpson–Golabi–Behmel syndrome human preadipocytes. They however reported downregulation of certain adipogenic marker expressions, reduced triacylglyceride content and decreased accumulation of lipid droplets. On the other hand, a study on human liposarcoma SW 872 preadipocytes found that treatment with 10 μM MEHP from day 5 to 11 of adipogenesis increased mRNA levels of PPAR-α, decreased PPAR-γ mRNA levels, but had no effect on triglyceride content. The lack of MEHP effect on triglyceride content was however attributed to the low lipid content of the cell line used.[152] Schaedlich *et al.*[151] highlighted how differences in cell cultures and study designs in *in vitro* studies on human cell models can complicate comparisons.

In vivo, DEHP is converted to MEHP and animal studies investigating exposure to DEHP have reported contradictory data pertaining to the induction of adipogenic PPARγ target genes. Feige *et al.*[150] reported that DEHP (500 mg per kg per day) does not activate PPARγ and adipogenesis, based on the inability of DEHP to induce the expression of adipogenic PPARγ target genes in adipose tissue in mice. Instead, DEHP exposure induced PPARα mediated fatty acid catabolism in the liver which protected the mice against diet-induced obesity. Interestingly, DEHP did not protect engineered mouse models expressing the human PPARα from diet-induced obesity, highlighting species differences in DEHP activated biological processes. However it has been suggested that the differences in PPARα between mice and humans are responsible for the differences in response to DEHP in the two species,[150] others have alluded to differences in the PPARα target genes promoters and co-activators as the source of these differences since some PPARα target genes are not activated by either mouse or human PPARα in mouse models.[147] From their findings, Feige *et al.*[150] suggested that exposure to DEHP in humans may result in weight gain/obesity possibly *via* decreased PPARα mediated fatty acid oxidation in the liver. This effect could in turn possibly lead to insulin resistance. However, the authors cautioned that this scenario may apply to individuals exposed to high concentrations of DEHP such as those undergoing dialysis or frequent blood transfusions. The lack of adipogenesis activation by DEHP/MEHP was attributed to insufficient MEHP uptake by the adipocytes and MEHP being a selective activator of PPARγ; they observed that agonists of PPARγ promote adipogenesis and weight gain[136,150] and display different coregulator recruitment patterns to target gene promoters.[136] Lv *et al.*[153] also observed upregulated mRNA levels of β-oxidation, lipid uptake and lipolysis proteins in the white adipose tissue of male C3H/He mice exposed to 50 and 200 mg per kg body weight per day DEHP for 5 weeks while lower DEHP levels (0.5 mg per kg body weight per day) downregulated the expression of these genes. However, the authors not only reported increased adipogenesis but also found a decline in mRNA levels of key proteins involved in lipogenesis.[153]

In contrast, adult mice administered 0.5 mg kg^{-1} of body weight of DEHP or MEHP for 24 hours had increased levels of epididymal adipose and increased expression of PPARγ target genes in epididymal adipose and liver tissues.[144,148] DEHP also upregulated the expression of adipogenic transcriptional factors PPARγ, CCAAT/enhancer-binding protein (C/EBP) alpha and sterol regulatory element binding factor 1 (Srebf1) in the liver.[144] Schmidt *et al.*[154] observed that mice exposed to 0.05 mg per kg body weight per day DEHP had increased levels of FABP4 gene expression; FABP4 is involved in fatty acid uptake, transport, and metabolism.[151] In another study, DEHP exposure (50 mg per kg per day DEHP) in rats upregulated and activated the Janus-activated kinase (JAK)/signal transducer and activator

of transcription (STAT) pathway and upregulated fatty acid metabolism-related genes (FABP4, Acox, and fatty acid synthetase) and leptin expression in adipose tissue.[155] Interestingly, the expression of JAK and STAT in the liver was upregulated but the pathway was inhibited. Furthermore, expression of the same fatty acid metabolism-related genes was downregulated in the liver, prompting the authors to conclude that lipid synthesis and lipolysis in the liver were reduced by DEHP. The authors suggested that the increased fatty acid synthesis and leptin levels are associated with DEHP exposure promoting lipid accumulation; however they also pointed out that this association was based on very high DEHP levels to which humans may not be exposed.[155]

Consequently, due to the discrepancies in PPARγ activation *in vivo*, there are inconsistent reports regarding the effects of DEHP exposure on fat and body mass and whether there are sex differences in these effects. In one study, DEHP exposure (1000 mg per kg body mass per day) in mice resulted in a decrease in body mass and fat mass.[150] Hayashi *et al.*[156] found that exposure of adult mice to DEHP at 10 to 140 mg per kg body weight per day for four weeks did not affect body weight. Other studies have however found an increase in body weight and fat mass in female mice while male mice were unaffected following feeding supplemented with 0.05 mg per kg body weight per day.[157] Schmidt *et al.*[154] also reported an increase in body weight and visceral fat tissue in mice exposed to 0.05 to 500 mg per kg per day DEHP, although the weight gain was associated with increased food intake. Male mice exposed to DEHP at 0.5, 5, 50 and 200 mg per kg body weight per day for 5 weeks experienced increased body weight and fat mass, as well as increased food intake.[154] Rats administered DEHP (5, 50 or 500 mg per kg per day) for four weeks displayed increased body weight[155] but in another study, the same DEHP levels had no effect on body weight in adolescent rats following a 28 day exposure period despite increased food intake.[158] Long-term exposure (29 weeks) of adult female mice on a high fat diet to 0.05 mg per kg body weight per day DEHP increased body weight and visceral white adipose tissue[159] whereas exposure of male rats to 600 mg per kg per day for 12 weeks had no effect on body weight.[160] Investigations into perinatal DEHP/MEHP exposure have observed an increase in body mass and fat mass in male mouse offsprings but not in females perinatally exposed only to low levels of MEHP (0.05 mg per kg per day) until postnatal day 7 while these parameters were unaffected by exposure to 0.25 and 0.5 mg per kg body weight per day MEHP.[148] The same authors reported an increase in body weight in both male and female mice perinatally exposed to DEHP at 0.25 mg per kg body weight, but not at 0.05 and 0.5 mg per kg body weight until postnatal day 7.[144] Perinatal exposure of mice to DEHP at 0.05 and 5 mg per kg body weight per day until weaning (postnatal day 21) resulted in increased body weight in both male and female offsprings. The offsprings' body weight increased 9 weeks after weaning, with female mice having more fat storage.[154] Rajesh and Balasubramanian[161] found that offsprings of mice treated with DEHP (10 and 100 mg per kg per day) from gestation day 9 to 21 had increased fat mass at postnatal day 60 while the authors reported reduced body weight in a separate study.[132] DEHP exposure during gestation and lactation at 1.25 and 6.25 mg per kg per day[129] and 1, 10 and 100 mg per kg per day[162] also reduced body weight in offsprings but increased body weight at puberty at a dosage of 700 mg per kg per day.[163] Adult male rats exposed to 7 and 75 mg per kg per day DEHP during lactation or puberty did not display changes in body weight.[164] Others have attributed the differences in DEHP/MEHP effects at low and high concentrations on bodyweight/fat mass to the inverted U-shaped dose–response curve for DEHP,[148] however, some studies involving similar concentrations of DEHP/MEHP have yielded contradictory findings.[153–156,158] Some inconsistencies reported here may be due to the differences in the study design,[157,159] DEHP/MEHP dosage and timing of exposure as well as the mode of DEHP/MEHP administration and exposure duration.[148] Nonetheless, there seem to be discrepancies regarding whether DEHP exposure promotes body weight gain *in vivo*.

Inconsistencies between *in vitro* and *in vivo* data reported in the abovementioned studies regarding DEHP exposure and adipogenesis require further investigations. These studies also highlight the importance of *in vivo* studies, where all tissues expressing the different PPAR isoforms are present, especially for metabolites such as MEHP that can activate all PPAR isoforms. In addition, the ability of compounds to elicit species specific biological effects necessitates the need for additional experiments in suitable human cell models.

3.2 DEHP and β-cell dysfunction

In T2D, pancreatic β-cells accelerate the production and secretion of insulin in an attempt to compensate for insulin resistance in target cells.[54] While this effect initially results in an increase in insulin levels, it eventually places a burden on the β-cells, ultimately leading to reduced insulin secretion.[165] Oxidative stress and endoplasmic reticulum (ER) stress have been associated with β-cell dysfunction that eventually leads to diabetes.[166] Oxidative damage in β-cells can impair insulin production/secretion or induce apoptosis.[66]

DEHP exposure during pregnancy can be a risk factor for the development of diabetes in offsprings. For instance, administration of DEHP at 1.25 and 6.25 mg per kg per day[129] or 1, 10 and 100 mg per kg per day[132] during pregnancy and lactation impaired the expression of genes involved in β cell development and function in the offsprings. DEHP downregulated the expression of transcription factors critical for insulin synthesis, reduced insulin content and upregulated genes involved in ER stress in pancreatic cells in the offsprings at weaning[129] and adulthood.[132] Furthermore, adult offsprings displayed elevated fasting blood glucose and reduced serum insulin levels, reduced expression of glucose sensors, impaired insulin signaling, and glucose and insulin intolerance along with reduced *ex vivo* insulin secretion upon glucose stimulation.[132] Additionally, DEHP exposure increased the level of global hypermethylation, prompting the authors to suggest that DEHP-induced hypermethylation is a possible mechanism for β-cell dysfunction. Global DNA methylation has been associated with increased risk of insulin resistance, although this

observation was made in peripheral blood leukocytes and not diabetes-affected organs.[167] Lin *et al.*[129] made similar observations of fasting hyperglycemia, hypoinsulinemia and glucose intolerance but only in adult female offsprings and further noted reduced β-cell mass and insulin content as well as reduced *in vivo* and *ex vivo* insulin secretion upon glucose stimulation. The male adult offsprings displayed fasting glucose levels comparable to the control group and increased fasting serum insulin levels along with increased *in vivo* and *ex vivo* insulin secretion. However, another study found lower glucose stimulated insulin secretion *ex vivo* in isolated islets of adult male offsprings exposed to DEHP during lactation, with discrepancies in findings attributed to the dose and timing of DEHP exposure.[164] When comparing the effects of DEHP on glucose homeostasis at different stages of development, adult offsprings exposed to DEHP during lactation had reduced insulin sensitivity and were more sensitive to DEHP than those exposed at puberty.[168] DEHP exposure during lactation and puberty resulted in elevated fasting blood glucose; however no effect on serum insulin levels was observed. Unlike Lin *et al.*[129] and Rajesh and Balasubramanian,[132] another study found a non-significant reduction in pancreatic and duodenal homeobox 1 (PDX-1) protein levels in male and female offsprings upon exposure to 7, 70 and 700 mg per kg DEHP during utero and lactation periods.[163] The offsprings in the high DEHP group displayed higher fasting blood glucose levels and reduced insulin sensitivity, with decreased *ex vivo* glucose stimulated insulin secretion in male offsprings. In another study, 3-week-old male rats administered DEHP (750 mg kg^{-1}) for 8 weeks had elevated fasting glucose levels, reduced HOMA-β, damaged pancreatic tissue and increased MDA levels while superoxide dismutase (SOD) activity remained unaffected.[168] Inhibition of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway was also observed, along with pancreatic cell apoptosis.

In vitro studies assessing the direct effects of DEHP and its metabolites on pancreatic β-cells have shown that DEHP exposure affects the ability of these cells to produce and secrete insulin. In evaluating the suitability of the rat pancreatic β-cell line INS-1 (subclone 832/13) as a screening system for the assessment of DEHP effects on β cell function, one study found that exposure of cells to 100 μM DEHP for 2 hours increased basal insulin secretion but did not affect glucose stimulated insulin secretion.[169] Sun *et al.*[133] reported that INS-1 cells exposed to DEHP (5–625 μM) displayed an increase in ROS and a decrease in the cells' nuclear factor erythroid 2-related factor 2 (Nrf2)-dependent antioxidant response at higher DEHP doses; however lower doses of DEHP (5 μM) activated the antioxidant response. This effect was coupled to decreased insulin mRNA and protein levels at higher DEHP doses, thus affecting the amount of insulin released. In addition, DEHP exposure induced ER stress-mediated apoptosis by activating the protein kinase RNA-like endoplasmic reticulum kinase/activating transcription factor 4/C/EBP homologous protein (PERK-ATF4-CHOP) ER signaling pathway.[133] A more recent study found that DEHP (30 μM) induced ROS and caused apoptosis in INS-1 cells *via* inhibition of the PI3K/Akt/Bcl-2 signaling pathway, which resulted in reduced insulin secretion due to a reduction in the number of cells.[170] RIN-5F cells treated with 625 μM DEHP displayed elevated ROS production and lipid peroxidation and reduced enzymatic antioxidant levels.[171] DEHP also reduced insulin secretion and the protein levels of insulin receptor (IR), insulin receptor substrate-1 (IRS-1) and glucose transporter 2 (GLUT2) and induced apoptotic changes in the cells. Although She *et al.*[172] did not assess the effects of DEHP exposure on insulin-related parameters, they observed that DEHP (200 and 400 μM) caused INS-1 cell dysfunction *via* oxidative stress marked by elevated ROS generation, reduced glutathione levels and SOD activity and increased MDA levels; these effects were reduced in the presence of the antioxidant compound pyrroloquinoline quinone. DEHP also increased lysosomal membrane permeability, decreased mitochondrial membrane potential and induced DNA damage. Contrary to She *et al.*,[172] a recent study reported that MEHP (0.001–1000 μM) did not affect ROS and MDA levels or mitochondrial membrane potential and lysosomal membrane permeability in 1.1B4 pancreatic cells.[173] Instead, MEHP induced ER stress and inflammation (at low MEHP levels), downregulating PPARα and PPARγ expression. Low levels of MEHP decreased insulin secretion at low glucose concentrations but increased the levels of proteins involved in β cell function (GLUT1, musculoaponeurotic fibrosarcoma homolog A (MafA), PDX-1 and glucokinase), while lower glucokinase and GLUT1 protein levels were observed at high DEHP levels (1000 μM).[173] While the authors attributed inconsistent findings to the use of DEHP instead of MEHP, a different study observed increased ROS generation and decreased antioxidants in INS-1 cells treated with MEHP (0.1 to 10 μM) for 24 to 72 hours.[135] Additionally, there was downregulation of the expression of insulin genes 1 and 2 and PDX-1 genes, along with decreased insulin secretion at normal glucose stimulation (5.5 mM). Another study found that a 72 hour treatment of INS-1E cells with MEHP (500 μM) resulted in reduced insulin secretion following glucose stimulation, an effect that was attributed to cell death due to MEHP-induced toxicity.[174] Interestingly, an increase in insulin secretion, was observed following simultaneous exposure of the cells to 100 μM MEHP and normal levels of glucose (6.7 mM) for 2 hours.

A recent study found that MEHP (400 μM) caused nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3)-dependent pyroptosis, an inflammatory programmed cell death, and affected autophagy in INS-1 cells.[175] In other cell lines, treatment of mouse MIN6 cells with DEHP (100 pM to 10 μM) for 24 hours impaired glucose stimulated insulin release, with decreased insulin content only observed at 1 μM DEHP. The expression of the GLUT2 gene was upregulated at 10 μM DEHP while that of PDX-1 and MafA was unaffected by all DEHP concentrations.[176] Given the limited studies on human β cell models, one study evaluated the use of the human pancreatic β-cell line Endo C-βH1 as a model for the assessment of DEHP exposure.[176] Decreased insulin secretion by the cells was only observed after 7 days of DEHP treatment (1–100 nM) and was associated with a decrease in insulin content at 10 and 100 nM DEHP. Interestingly, a concentration of 1 μM DEHP increased

insulin secretion by the cells, prompting the authors to conclude that the cells exhibit a non-monotonic dose response to DEHP exposure.

The studies above show that DEHP/MEHP increases generation of ROS in β cells *in vitro*, weakens the β-cell antioxidant system and impairs insulin production/secretion. In some cases, the oxidative stress was associated with apoptosis (Fig. 1 top panel). Furthermore, these studies suggest that *in utero* DEHP exposure causes disturbances in β-cell development and function as well as glucose homeostasis in the offsprings in early life or in the long term. As evidenced by the reviewed studies, most of the data on the effect of DEHP on β-cell function were gathered using mouse cell lines while *in vivo* studies were conducted in animals such as rats. Since it has been argued that findings from animal studies are not easily extrapolated to humans due to species differences,[38] the recent findings by Al-Abdulla *et al.*[176] which demonstrate that DEHP concentrations relevant to human exposure impair insulin secretion in the human pancreatic β-cell line is a step in the right direction in advancing our understanding of the association between DEHP exposure and diabetes.

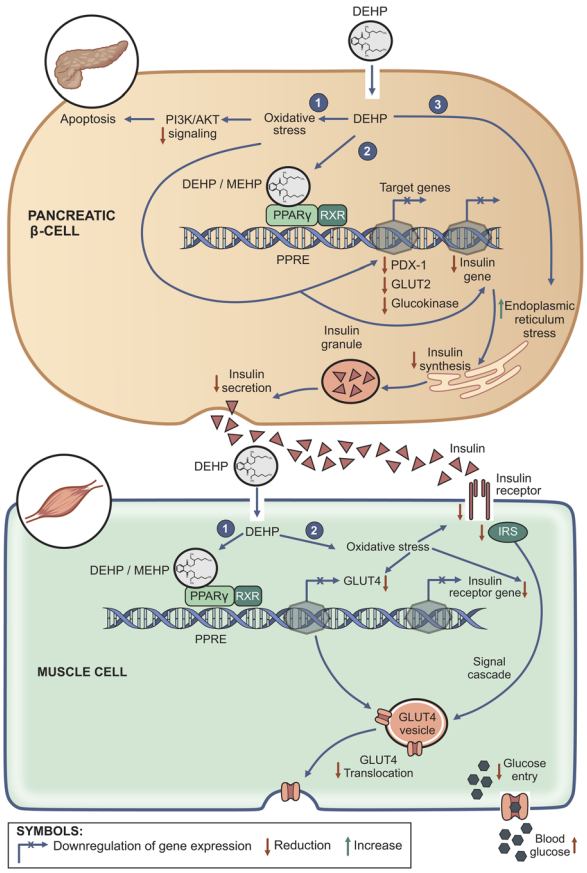

Fig. 1 Overview of some of the proposed mechanisms for the DEHP-induced metabolic disruption. In the pancreatic β cells (top panel), DEHP induces oxidative stress-mediated apoptosis (1) and endoplasmic reticulum stress (3). Oxidative stress also downregulates PDX-1 (a transcription factor involved in insulin synthesis) and insulin gene expression. DEHP/MEHP also binds to PPARγ which together with RXR binds to PPAR response elements (PPRE) in the promoter regions of genes involved in insulin synthesis (PDX-1) and glucose sensing (GLUT2 and glucokinase genes) and downregulates their expression (2). A combination of these factors ultimately impairs insulin synthesis and secretion. In the muscle cells (bottom panel) DEHP binds to PPARγ and the PPARγ–RXR heterodimer binds to PPRE in the promoter region of the GLUT4 gene and downregulates its expression (1). DEHP induces oxidative stress, downregulating the expression of GLUT4 and insulin receptor genes (2). Oxidative stress also reduces the levels of key molecules in the insulin signaling process (2), ultimately reducing translocation of GLUT4 to the cell membrane. A combination of these factors results in a decrease in glucose uptake, hence hyperglycemia.

3.3 DEHP and insulin resistance

Some studies investigating the effects of developmental DEHP exposure on rat offsprings found that DEHP impairs insulin signaling in the muscles and liver.[161,162] Pregnant rats were administered DEHP (1, 10 and 100 mg per kg body weight per day) and analysis of adult offsprings showed high fasting blood glucose levels, lower glycogen levels, impaired glucose and insulin tolerance in the muscles[161] and liver.[162] Further investigations revealed downregulation in the expression of key genes involved in insulin signaling, along with altered phosphorylation of key insulin signaling molecules.[161,162] In addition, increased methylation in the GLUT4 promoter region contributed to the downregulation of this gene in DEHP-exposed offsprings, resulting in a decline in GLUT4 cytosolic protein levels.[161] Furthermore, the offsprings had reduced testosterone and estradiol levels and displayed liver and kidney damage.[162] DEHP had opposing effects on fasting serum insulin levels in the two studies.[161,162] In a separate study, DEHP exposure in pregnant rats resulted in hypermethylation of the liver IR and GLUT2 gene promoters and reduced transcription of the corresponding genes, along with reduced glucose uptake and oxidation in adult offsprings.[177] Adolescent rats administered 50 and 500 mg per kg per day DEHP for 28 days displayed higher fasting blood glucose, serum leptin and serum insulin levels as well as insulin resistance.[158] These observations were associated with reduced leptin receptor and IR protein levels (500 mg per kg per day DEHP group) in the liver. DEHP further interfered with the Janus-activated kinase 2/signal transducer and activator of transcription 3/suppressor of cytokine signaling 3 (JAK2/STAT3/SOCS3) pathway. Exposure of 3-week-old male rats to 750 mg per kg DEHP for 8 weeks elevated fasting glucose levels, reduced muscle GLUT4 mRNA and protein levels, reduced insulin sensitivity but had no effect on HOMA-IR.[168]

Some of the earliest *in vitro* studies documenting the effect of DEHP on glucose metabolism in a liver cell line originated from a study in which Chang liver cells treated with 200 and 400 μM DEHP for 24 hour showed reduced IR levels and glucose oxidation.[178] Moreover, experimental data support the proposed mechanism of DEHP inducing insulin resistance *via* oxidative stress in insulin target cells. Treatment of L6 myotubes with 100 μM DEHP[179] or buffalo rat liver cells with 5, 500 and 50 000 nM DEHP[180] for 24 hours induced oxidative stress and apoptosis, along with reduced IR and GLUT4 gene expression and protein content, leading to impaired signaling and reduced glucose uptake and oxidation. Treatment of a human preadipocyte cell line with 50 μg per ml DEHP (128 μM) increased ROS levels but

had no effect on the protein levels of enzyme antioxidants and GLUT 4 expression.[151]

In vivo studies found that treatment of adolescent male mice with 180 mg per kg per day DEHP for 3 weeks elevated fasting blood glucose levels and increased levels of HbA1c but did not change HOMA-IR levels.[181] Reduced liver IR, IRS-1 and GLUT4 protein levels, decreased levels of proteins that regulate lipid metabolism homeostasis and increased serum triacylglycerol were also observed. In a similar study, female mice exhibited decreased liver antioxidant enzyme activity and increased MDA levels.[182] These observations were associated with increased levels of HbA1c and GLUT4 protein, decreased IR and IRS-1 protein levels, while having no effect on fasting glucose, fasting insulin and HOMA-IR levels. Hepatic cellular self-repair was the suggested reason for the increased liver GLUT4 protein levels. In both studies, the DEHP effects were aggravated in adolescent T2D mice compared to normal rats, with the female mice being more sensitive to DEHP that the male mice.[181,182] Rats treated with DEHP (10 and 100 mg per kg body weight) for 30 days displayed increased ROS production in adipose[131] and muscles tissue,[130] resulting in impaired insulin signaling and GLUT4 translocation to the plasma membrane, subsequently leading to decreased glucose uptake and oxidation along with elevated blood glucose levels. Reduced glycogen concentration was also observed.[130] Vitamin E and C were able to prevent most of the DEHP-induced effects, confirming the involvement of DEHP in inducing oxidative stress.[130,131] In another study, Balb/c mice given 50 and 250 mg per kg per day DEHP for 56 days had increased liver ROS and MDA levels; however, the authors did not further determine whether the oxidative damage was related to insulin resistance.[183] A study on metabolomics and transcriptomics analysis of the long-term exposure (12 weeks) exposure effects of 600 mg per kg per day DEHP on the rat liver reported oxidative stress induction, mitochondrial dysfunction, increased gluconeogenesis but inhibited glycolysis, disturbed insulin signaling and insulin resistance pathways, increased expression of apoptotic genes and decreased expression of proliferative genes.[160]

Zhang *et al.*[134] studied the effect of DEHP on the rat liver (0.05, 5 and 500 mg kg^{-1}) and human hepatocytes cell line L02 (5–100 μmol l^{-1}). In both the rat liver (15 week examination) and L02 cells, DEHP induced oxidative stress (*via* increasing MDA levels and decreasing SOD activity), increased PPARγ protein expression and reduced IR and GLUT4 protein levels. The rats also exhibited elevated blood glucose, insulin resistance and liver damage while L02 cells showed reduced glucose uptake and translocation of the GLUT4 transporter to the cell membrane. The PPARγ antagonist GW9662 was effective in reducing some of the DEHP-induced effects in L02 cells and the authors concluded that the DEHP-induced oxidative stress and GLUT4-associated changes occurred *via* a PPARγ-mediated mechanism. Transfections studies conducted in isolated rat adipocytes and CHO-K1 fibroblasts showed that ligand-free PPARγ (in complex with RXR) represses the activity of the GLUT4 gene by binding to its promoter and further suggested that exogenous ligands alleviate this effect while endogenous ligands enhance it.[184] However, since the DEHP metabolite MEHP activates PPARγ[142,146] it would seem that DEHP exposure in the above studies further enhanced PPARγ's ability to downregulate GLUT4 expression. Thus Zhang *et al.*[134] suggested the decline in the levels of GLUT4 protein following DEHP treatment in their study to be due to an increase in PPARγ expression, since DEHP and MEHP upregulates the expression of PPARγ at both mRNA and protein levels *in vitro*.[185] Conflicting results have since been reported regarding the effect of DEHP/MEHP on PPARγ expression;[145,151,152,154,157] however, most of the studies did not examine GLUT4 expression. The same DEHP dose exerted similar[144,148] or differential patterns[155] of PPARγ gene expression in different tissues, or exerted different effects on PPARγ gene and protein expression in the same tissue.[134,154,157]

Disturbances in insulin signaling and reduction in the expression of GLUT4 are some of the proposed mechanisms through which oxidative stress leads to insulin resistance in peripheral tissues.[66] It would seem that DEHP could downregulate GLUT4 expression *via* PPARγ-mediated binding to its promoter region, thus leading to insulin resistance.[134] While it is generally accepted that PPARγ ligands induce cellular changes *via* binding to PPARγ, it has been proposed that the ligands may also act *via* mechanisms that are receptor independent.[166] Oxidative stress seems to be a common feature through which DEHP induces its effects in insulin target cells. Therefore, based on the experimental data, it is possible that the induction of oxidative stress and subsequent impaired insulin signaling[134] and reduction in the expression of GLUT4 may represent some of mechanisms through which DEHP could contribute to insulin resistance and subsequently, the development of T2D (Fig. 1 bottom panel). While some of the details of the mechanisms involved in DEHP-induced insulin resistance have been elucidated, further studies are warranted for a full comprehension of these mechanisms.

3.4 DEHP and impaired adiponectin expression

Adiponectin is synthesized by adipose tissue and it modulates glucose levels, lipid metabolism and insulin sensitivity in insulin target organs.[186,187] Low levels of adiponectin are associated with insulin resistance, obesity and T2D.[187–189] Downregulation of adiponectin gene expression, along with increased leptin expression and an increase in body weight and visceral fat tissue, was observed in pregnant mice that were exposed to DEHP.[158] Jia *et al.*[155] found that higher leptin and lower adiponectin serum levels in rats exposed to 500 mg per kg per day DEHP were associated with an increase in the body mass index and insulin insensitivity. In another study, DEHP exposure to male and female mice for 10 weeks did not affect glucose tolerance, fasting glucose and insulin levels or cause insulin resistance (based on the HOMA-IR parameter).[157] However, impaired insulin tolerance in female mice, based on insulin tolerance test, was reported along with a decrease in circulating adiponectin levels and reduced levels of adiponectin protein in subcutaneous adipose tissue. The authors suggested that DEHP induces adipose tissue dysfunction, which was reflected by the lowered adiponectin levels, and subsequently impaired insulin

sensitivity.[157] Mice exposed to DEHP in combination with a high fat diet displayed increased fasting insulin levels and low adiponectin levels, which were suggested to contribute to glucose intolerance. The increased levels of PPARγ phosphorylation at serine 273 in adipose tissue observed in the study were suggested to be the reason for the downregulated adiponectin expression.[159]

In vitro studies showed that 3T3-L1 cells treated with 0.01% DEHP for 48 hours had decreased adiponectin mRNA and protein levels which were associated with reduced lipid content and impaired insulin stimulated glucose uptake.[157] Exposure of a human preadipocyte cell line to environmentally relevant levels of DEHP resulted in reduced adiponectin and increased leptin secretion but did not affect GLUT4 mRNA levels[151,190] and GLUT4 translocation to the cell membrane.[151] This effect was also associated with reduced triacylglyceride content and reduced accumulation of lipid droplets.[151] The lack of DEHP's effect on GLUT4 expression and translocation somewhat contradicts the observations that adiponectin protects against insulin resistance and the findings in rat muscle cells where adiponectin enhanced GLUT4 translocation.[191] It would also seem that a decline in adiponectin levels is associated with reduced lipid content in these *in vitro* studies but an increased body weight/mass in the *in vivo* studies.

In contrast to epidemiological findings, it seems that experimental studies agree that DEHP reduces adiponectin levels. The observation that lowered adiponectin levels did not affect GLUT4 gene expression and GLUT4 translocation to the cell membrane in human preadipocytes is surprising since one would expect an associated decline in these processes. This is an aspect that can perhaps be clarified with more studies in human models. According to a recent study, the mechanism through which DEHP exposure reduced adiponectin concentrations is still being elucidated.[61]

4 Conclusions

DEHP and its metabolites have relatively short half-lives in the body and thus it is suggested that they do not bioaccumulate. However, DEHP is ubiquitous in the environment and thus prolonged constant exposure, through the use of everyday products, warrants concern about its effects to human health. Experimental data in animals suggest that exposure to DEHP may be an added risk factor for developing diabetes. Furthermore, animal studies on developmental DEHP exposure suggest that DEHP may cause long term imbalances in glucose metabolism that could potentially lead to the development of diabetes. The question of whether DEHP exposure results in adverse effects in humans remains controversial in the scientific community. With that being said, it seems that the approach should be to err on the side of caution and minimize exposure to this chemical. This could involve the development of biomonitoring surveys in developing countries for environmental chemical exposure monitoring, which could then possibly inform regulatory restrictions regarding the use of DEHP. In addition, the reasons for disparities in the associations of DEHP metabolites with insulin resistance and obesity need to be investigated. There is also a need to strengthen efforts in DEHP/DEHP metabolites and diabetes-related research in areas such as longitudinal epidemiological studies and experimental studies in cell models relevant to humans, at environmentally relevant doses.

Data availability

No primary research results have been included and no new data were generated or analysed as part of this review.

Author contributions

Sebolaishi Doris Makhubela: conceptualization, writing – original draft. Ananias Hodi Kgopa: writing – review and editing. Matlou Phineas Mokgotho: writing – review and editing. Leshweni Jerry Shai: conceptualization, writing – review and editing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was funded by the Department of Higher Education and Training (South Africa) through the University Capacity Development Grant (UCDG-D428) and the National Research Foundation of South Africa (grant 129079). We would like to thank Marinda Pretorius from the Medical Illustrations and Audio-visual Services Department (Sefako Makgatho Health Sciences University) for her assistance with the illustrations.

References

1 World Health Organization, *WHO Global Report on Diabetes*, 2016, available from, **https://www.who.int/publications/i/item/9789241565257**, accessed 26 March 2020.
2 A. T. Kharroubi and H. M. Darwish, Diabetes mellitus: the epidemic of the century, *World J. Diabetes*, 2015, **6**(6), 850–867.
3 Federation I, *IDF Diabetes Atlas, Tenth*, International Diabetes, 2021, available at, **https://diabetesatlas.org/**, accessed 23 January 2024.
4 S. M. Regnier and R. M. Sargis, Adipocytes under assault: environmental disruption of adipose physiology, *Biochim. Biophys. Acta*, 2014, **1842**(3), 520–533.
5 N. Chevalier and P. Fénichel, Endocrine disruptors: new players in the pathophysiology of type 2 diabetes?, *Diabetes Metab.*, 2015, **41**(2), 107–115.
6 C. Casals-Casas and B. Desvergne, Endocrine disruptors: from endocrine to metabolic disruption, *Annu. Rev. Physiol.*, 2011, **73**, 135–162.
7 Agency for Toxic Substances and Disease Registry (ATSDR), *Toxicological profile for di-(2-ethylhexyl)phthalate*, U.S. Department of Health and Human Services, 2002, available from, **https://www.atsdr.cdc.gov/ToxProfiles/tp9.pdf**, accessed 6 September 2020.

8 R. Kavlock, K. Boekelheide, R. Chapin, M. Cunningham, E. Faustman, P. Foster, M. Golub, R. Henderson, I. Hinberg, R. Little, J. Seed, K. Shea, S. Tabacova, R. Tyl, P. Williams and T. Zacharewski, NTP Center for the Evaluation of Risks to Human Reproduction: phthalates expert panel report on the reproductive and developmental toxicity of di(2-ethylhexyl) phthalate, *Reprod. Toxicol.*, 2002, **16**(5), 529–653.

9 M. Wormuth, M. Scheringer, M. Vollenweider and K. Hungerbühler, What are the sources of exposure to eight frequently used phthalic acid esters in Europeans?, *Risk Anal.*, 2006, **26**(3), 803–824.

10 H. M. Koch, M. Lorber, K. L. Christensen, C. Pälmke, S. Koslitz and T. Brüning, Identifying sources of phthalate exposure with human biomonitoring: results of a 48h fasting study with urine collection and personal activity patterns, *Int. J. Hyg Environ. Health*, 2013, **216**(6), 672–681.

11 S. E. Serrano, J. Braun, L. Trasande, R. Dills and S. Sathyanarayana, Phthalates and diet: a review of the food monitoring and epidemiology data, *Environ. Health*, 2014, **13**(1), 43.

12 D. Xu, X. Deng, E. Fang, X. Zheng, Y. Zhou, L. Lin, L. Chen, M. Wu and Z. Huang, Determination of 23 phthalic acid esters in food by liquid chromatography tandem mass spectrometry, *J. Chromatogr. A*, 2014, **1324**, 49–56.

13 C. Bi, J. P. Maestre, H. Li, G. Zhang, R. Givehchi, A. Mahdavi, K. A. Kinney, J. Siegel, S. D. Horner and Y. Xu, Phthalates and organophosphates in settled dust and HVAC filter dust of U.S. low-income homes: association with season, building characteristics, and childhood asthma, *Environ. Int.*, 2018, **121**(pt 1), 916–930.

14 A. M. Calafat, L. L. Needham, M. J. Silva and G. Lambert, Exposure to di-(2-ethylhexyl) phthalate among premature neonates in a neonatal intensive care unit, *Pediatrics*, 2004, **113**(5), e429–e434.

15 J. Weuve, B. N. Sánchez, A. M. Calafat, T. Schettler, R. A. Green, H. Hu and R. Hauser, Exposure to phthalates in neonatal intensive care unit infants: urinary concentrations of monoesters and oxidative metabolites, *Environ. Health Perspect.*, 2006, **114**(9), 1424–1431.

16 E. Testai, P. Hartemann, S. C. Rastogi, U. Bernauer, A. Piersma, W. De Jong, H. Gulliksson, R. Sharpe, D. Schubert, E. Rodríguez-Farre and Ms Scientific Committee SCENIHR, The safety of medical devices containing DEHP plasticized PVC or other plasticizers on neonates and other groups possibly at risk (2015 update), *Regul. Toxicol. Pharmacol.*, 2016, **76**, 209–210.

17 M. D. Shelby, NTP-CERHR monograph on the potential human reproductive and developmental effects of di(2-ethylhexyl)phthalate (DEHP), *NTP CERHR Mon.*, 2006, vol. 18.

18 Y. Ito, M. Kamijima and T. Nakajima, Di(2-ethylhexyl) phthalate-induced toxicity and peroxisome proliferator-activated receptor alpha: a review, *Environ. Health Prev. Med.*, 2019, **24**(1), 47.

19 Y. Ito, H. Yokota, R. Wang, O. Yamanoshita, G. Ichihara, H. Wang, Y. Kurata, K. Takagi and T. Nakajima, Species differences in the metabolism of di(2-ethylhexyl) phthalate (DEHP) in several organs of mice, rats, and marmosets, *Arch. Toxicol.*, 2005, **79**(3), 147–154.

20 K. Kato, M. J. Silva, J. A. Reidy, D. Hurtz 3rd, N. A. Malek, L. L. Needham, H. Nakazawa, D. B. Barr and A. M. Calafat, Mono(2-ethyl-5-hydroxyhexyl) phthalate and mono-(2-ethyl-5-oxohexyl) phthalate as biomarkers for human exposure assessment to di-(2-ethylhexyl) phthalate, *Environ. Health Perspect.*, 2004, **112**(3), 327–330.

21 Y. Kurata, F. Makinodan, N. Shimamura and M. Katoh, Metabolism of di (2-ethylhexyl) phthalate (DEHP): comparative study in juvenile and fetal marmosets and rats, *J. Toxicol. Sci.*, 2012, **37**(1), 33–49.

22 H. M. Koch, H. M. Bolt, R. Preuss and J. Angerer, New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP, *Arch. Toxicol.*, 2005, **79**(7), 367–376.

23 H. M. Koch, R. Preuss and J. Angerer, Di(2-ethylhexyl) phthalate (DEHP): human metabolism and internal exposure- an update and latest results, *Int. J. Androl.*, 2006, **29**(1), 155–165.

24 G. Saravanabhavan, M. Guay, É. Langlois, S. Giroux, J. Murray and D. Haines, Biomonitoring of phthalate metabolites in the Canadian population through the Canadian Health Measures Survey (2007-2009), *Int. J. Hyg Environ. Health*, 2013, **216**(6), 652–661.

25 A. R. Zota, A. M. Calafat and T. J. Woodruff, Temporal trends in phthalate exposures: findings from the National Health and Nutrition Examination Survey, 2001-2010, *Environ. Health Perspect.*, 2014, **122**(3), 235–241.

26 W. Choi, S. Kim, Y. W. Baek, K. Choi, K. Lee, S. Kim, S. D. Yu and K. Choi, Exposure to environmental chemicals among Korean adults-updates from the second Korean National Environmental Health Survey (2012-2014), *Int. J. Hyg Environ. Health*, 2017, **220**(2 pt A), 29–35.

27 A. T. Mannetje, J. Coakley and J. Douwes, Levels and determinants of urinary phthalate metabolites in New Zealand children and adults, *Int. J. Hyg Environ. Health*, 2021, **238**, 113853.

28 G. Schwedler, E. Rucic, R. Lange, A. Conrad, H. M. Koch, C. Pälmke, T. Brüning, C. Schulz, M. I. H. Schmied-Tobies, A. Daniels and M. Kolossa-Gehring, Phthalate metabolites in urine of children and adolescents in Germany. Human biomonitoring results of the German Environmental Survey GerES V, 2014-2017, *Int. J. Hyg Environ. Health*, 2020, **225**, 113444.

29 R. Dong, S. Zhao, H. Zhang, J. Chen, M. Zhang, M. Wang, M. Wu, S. Li and B. Chen, Sex Differences in the Association of Urinary Concentrations of Phthalates Metabolites with Self-Reported Diabetes and Cardiovascular Diseases in Shanghai Adults, *Int. J. Environ. Res. Public Health*, 2017, **14**(6), 598.

30 I. Lee, Y. J. Park, M. J. Kim, S. Kim, S. Choi, J. Park, Y. H. Cho, S. Hong, J. Yoo, H. Park, G. J. Cheon, K. Choi and M. K. Moon, Associations of urinary concentrations of phthalate metabolites, bisphenol A, and parabens with obesity and diabetes mellitus in a Korean adult

population: Korean National Environmental Health Survey (KoNEHS) 2015-2017, *Environ. Int.*, 2021, **146**, 106227.

31 K. M. Main, G. K. Mortensen, M. M. Kaleva, K. A. Boisen, I. N. Damgaard, M. Chellakooty, I. M. Schmidt, A. M. Suomi, H. E. Virtanen, D. V. Petersen, A. M. Andersson, J. Toppari and N. E. Skakkebaek, Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age, *Environ. Health Perspect.*, 2006, **114**(2), 270–276.

32 M. J. Silva, J. A. Reidy, A. R. Herbert, J. L. Preau Jr, L. L. Needham and A. M. Calafat, Detection of phthalate metabolites in human amniotic fluid, *Bull. Environ. Contam. Toxicol.*, 2004, **72**(6), 1226–1231.

33 European Commission, *1999/815/EC: Commission Decision of 7 December 1999 adopting measures prohibiting the placing on the market of toys and childcare articles intended to be placed in the mouth by children under three years of age made of soft PVC containing one or more of the substances di-iso-nonyl phthalate (DINP), di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), di-iso-decyl phthalate (DIDP), di-n-octyl phthalate (DNOP), and butylbenzyl phthalate (BBP) (notified under document number C (1999) 4436) (text with EEA relevance)*, available from, **https://data.europa.eu/eli/dec/1999/815/oj**, accessed 25 May, 2021.

34 Consumer Product Safety Improvement Act (CPSIA) Public Law 110–314, 122 STAT 3016–3077, 2008, available from, **https://www.cpsc.gov/s3fs-public/cpsia.pdf**, accessed 25 May 2021.

35 Canadian Consumer Product Safety Act, *Phthalates Regulations SOR/2010-298*, 2010, available from, **https://laws-lois.justice.gc.ca/eng/regulations/SOR-2010-298/20110620/P1TT3xt3.html**, accessed 10 June 2021.

36 G. Tranfo, L. Caporossi, D. Pigini, S. Capanna, B. Papaleo and E. Paci, Temporal Trends of Urinary Phthalate Concentrations in Two Populations: Effects of REACH Authorization after Five Years, *Int. J. Environ. Res. Public Health*, 2018, **15**(9), 1950.

37 Y. Wang, H. Zhu and K. Kannan, A Review of Biomonitoring of Phthalate Exposures, *Toxics*, 2019, **7**(2), 21.

38 S. S. S. Rowdhwal and J. Chen, Toxic Effects of Di-2-ethylhexyl Phthalate: An Overview, *BioMed Res. Int.*, 2018, **2018**, 1750368.

39 H. Zhang, Y. Ben, Y. Han, Y. Zhang, Y. Li and X. Chen, Phthalate exposure and risk of diabetes mellitus: implications from a systematic review and meta-analysis, *Environ. Res.*, 2022, **204**(pt B), 112109.

40 M. Mariana and E. Cairrao, The Relationship between Phthalates and Diabetes: A Review, *Metabolites*, 2023, **13**(6), 746.

41 E. G. Radke, A. Galizia, K. A. Thayer and G. S. Cooper, Phthalate exposure and metabolic effects: a systematic review of the human epidemiological evidence, *Environ. Int.*, 2019, **132**, 104768.

42 H. Gao, D. Chen and M. Zang, Association between phthalate exposure and insulin resistance: a systematic review and meta-analysis update, *Environ. Sci. Pollut. Res. Int.*, 2021, **28**(40), 55967–55980.

43 M. Goodman, J. S. Lakind and D. R. Mattison, Do phthalates act as obesogens in humans? a systematic review of the epidemiological literature, *Crit. Rev. Toxicol.*, 2014, **44**(2), 151–175.

44 C. Ribeiro, V. Mendes, B. Peleteiro, I. Delgado, J. Araújo, M. Aggerbeck, I. Annesi-Maesano, D. Sarigiannis and E. Ramos, Association between the exposure to phthalates and adiposity: a meta-analysis in children and adults, *Environ. Res.*, 2019, **179**(pt A), 108780.

45 R. E. Dales, L. M. Kauri and S. Cakmak, The associations between phthalate exposure and insulin resistance, β-cell function and blood glucose control in a population-based sample, *Sci. Total Environ.*, 2018, **612**, 1287–1292.

46 T. James-Todd, R. Stahlhut, J. D. Meeker, S. G. Powell, R. Hauser, T. Huang and J. Rich-Edwards, Urinary phthalate metabolite concentrations and diabetes among women in the National Health and Nutrition Examination Survey (NHANES) 2001-2008, *Environ. Health Perspect.*, 2012, **120**(9), 1307–1313.

47 I. Lee, Y. J. Park, M. J. Kim, S. Kim, S. Choi, J. Park, Y. H. Cho, S. Hong, J. Yoo, H. Park, G. J. Cheon, K. Choi and M. K. Moon, Associations of urinary concentrations of phthalate metabolites, bisphenol A, and parabens with obesity and diabetes mellitus in a Korean adult population: Korean National Environmental Health Survey (KoNEHS) 2015-2017, *Environ. Int.*, 2021, **146**, 106227.

48 K. Svensson, R. U. Hernández-Ramírez, A. Burguete-García, M. E. Cebrián, A. M. Calafat, L. L. Needham, L. Claudio and L. López-Carrillo, Phthalate exposure associated with self-reported diabetes among Mexican women, *Environ. Res.*, 2011, **111**(6), 792–796.

49 Q. Sun, M. C. Cornelis, M. K. Townsend, D. K. Tobias, A. H. Eliassen, A. A. Franke, R. Hauser and F. B. Hu, Association of urinary concentrations of bisphenol A and phthalate metabolites with risk of type 2 diabetes: a prospective investigation in the Nurses' Health Study (NHS) and NHSII cohorts, *Environ. Health Perspect.*, 2014, **122**(6), 616–623.

50 P. M. Lind, B. Zethelius and L. Lind, Circulating levels of phthalate metabolites are associated with prevalent diabetes in the elderly, *Diabetes Care*, 2012, **35**(7), 1519–1524.

51 Y. Duan, H. Sun, L. Han and L. Chen, Association between phthalate exposure and glycosylated hemoglobin, fasting glucose, and type 2 diabetes mellitus: a case-control study in China, *Sci. Total Environ.*, 2019, **670**, 41–49.

52 D. J. Nam, Y. Kim, E. H. Yang, H. C. Lee and J. H. Ryoo, Relationship between urinary phthalate metabolites and diabetes: Korean National Environmental Health Survey (KoNEHS) cycle 3 (2015-2017), *Ann. Occup. Environ. Med.*, 2020, **32**, e34.

53 X. K. Liu, S. W. Si, Y. Ye, J. Y. Li, H. H. Lyu, Y. M. Ma, C. Y. Zou, H. J. Sun, L. Xue, W. Xu, H. F. Geng and J. Liang, The Link between Exposure to Phthalates and

Type 2 Diabetes Mellitus: A Study Based on NHANES Data and Bioinformatic Analysis, *Biomed. Environ. Sci.*, 2023, **36**(9), 892–896.

54 R. W. Stahlhut, E. van Wijngaarden, T. D. Dye, S. Cook and S. H. Swan, Concentrations of urinary phthalate metabolites are associated with increased waist circumference and insulin resistance in adult U.S. males, *Environ. Health Perspect.*, 2007, **115**(6), 876–882.

55 S. Y. Chen, J. S. Hwang, F. C. Sung, C. Y. Lin, C. J. Hsieh, P. C. Chen and T. C. Su, Mono-2-ethylhexyl phthalate associated with insulin resistance and lower testosterone levels in a young population, *Environ. Pollut.*, 2017, **225**, 112–117.

56 S. H. Hong, Y. A. Sung, Y. S. Hong, E. Ha, K. Jeong, H. Chung and H. Lee, Urinary bisphenol A is associated with insulin resistance and obesity in reproductive-aged women, *Clin. Endocrinol.*, 2017, **86**(4), 506–512.

57 E. Dirinck, A. C. Dirtu, T. Geens, A. Covaci, L. Van Gaal and P. G. Jorens, Urinary phthalate metabolites are associated with insulin resistance in obese subjects, *Environ. Res.*, 2015, **137**, 419–423.

58 S. H. Kim, J. W. On, H. Pyo, K. S. Ko, J. C. Won, J. Yang and M. J. Park, Percentage fractions of urinary di(2-ethylhexyl) phthalate metabolites: association with obesity and insulin resistance in Korean girls, *PLoS One*, 2018, **13**(11), e0208081.

59 L. Trasande, A. J. Spanier, S. Sathyanarayana, T. M. Attina and J. Blustein, Urinary phthalates and increased insulin resistance in adolescents, *Pediatrics*, 2013, **132**(3), e646–e655.

60 T. M. Attina and L. Trasande, Association of Exposure to Di-2-Ethylhexylphthalate Replacements With Increased Insulin Resistance in Adolescents From NHANES 2009-2012, *J. Clin. Endocrinol. Metabol.*, 2015, **100**(7), 2640–2650.

61 C. Y. Lin, H. L. Lee, C. W. Chen, C. Wang, F. C. Sung and T. C. Su, The role of angiotensin I-converting enzyme gene polymorphism and global DNA methylation in the negative associations between urine di-(2-ethylhexyl) phthalate metabolites and serum adiponectin in a young Taiwanese population, *Clin. Epigenet.*, 2023, **15**(1), 87.

62 T. Huang, A. R. Saxena, E. Isganaitis and T. James-Todd, Gender and racial/ethnic differences in the associations of urinary phthalate metabolites with markers of diabetes risk: National Health and Nutrition Examination Survey 2001-2008, *Environ. Health*, 2014, **13**(1), 6.

63 T. M. James-Todd, T. Huang, E. W. Seely and A. R. Saxena, The association between phthalates and metabolic syndrome: the National Health and Nutrition Examination Survey 2001-2010, *Environ. Health*, 2016, **15**, 52.

64 I. Lee, S. Kim, S. Park, S. Mok, Y. Jeong, H. B. Moon, J. Lee, S. Kim, H. J. Kim, G. Choi, S. Choi, S. Y. Kim, A. Lee, J. Park and K. Choi, Association of urinary phthalate metabolites and phenolics with adipokines and insulin resistance related markers among women of reproductive age, *Sci. Total Environ.*, 2019, **688**, 1319–1326.

65 K. N. Keane, V. F. Cruzat, R. Carlessi, P. I. de Bittencourt Jr and P. Newsholme, Molecular Events Linking Oxidative Stress and Inflammation to Insulin Resistance and β-Cell Dysfunction, *Oxid. Med. Cell. Longev.*, 2015, **2015**, 181643.

66 H. Yaribeygi, T. Sathyapalan, S. L. Atkin and A. Sahebkar, Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus, *Oxid. Med. Cell. Longev.*, 2020, **2020**, 8609213.

67 K. K. Ferguson, T. F. McElrath, Y. H. Chen, B. Mukherjee and J. D. Meeker, Urinary phthalate metabolites and biomarkers of oxidative stress in pregnant women: a repeated measures analysis, *Environ. Health Perspect.*, 2015, **123**(3), 210–216.

68 Y. C. Hong, E. Y. Park, M. S. Park, J. A. Ko, S. Y. Oh, H. Kim, K. H. Lee, J. H. Leem and E. H. Ha, Community level exposure to chemicals and oxidative stress in adult population, *Toxicol. Lett.*, 2009, **184**(2), 139–144.

69 K. K. Ferguson, R. Loch-Caruso and J. D. Meeker, Urinary phthalate metabolites in relation to biomarkers of inflammation and oxidative stress: NHANES 1999-2006, *Environ. Res.*, 2011, **111**(5), 718–726.

70 A. Kataria, D. Levine, S. Wertenteil, S. Vento, J. Xue, K. Rajendiran, K. Kannan, J. M. Thurman, D. Morrison, R. Brody, E. Urbina, T. Attina, L. Trasande and H. Trachtman, Exposure to bisphenols and phthalates and association with oxidant stress, insulin resistance, and endothelial dysfunction in children, *Pediatr. Res.*, 2017, **81**(6), 857–864.

71 N. Holland, K. Huen, V. Tran, K. Street, B. Nguyen, A. Bradman and B. Eskenazi, Urinary Phthalate Metabolites and Biomarkers of Oxidative Stress in a Mexican-American Cohort: Variability in Early and Late Pregnancy, *Toxics*, 2016, **4**(1), 7.

72 A. G. Asimakopoulos, J. Xue, B. P. De Carvalho, A. Iyer, K. O. Abualnaja, S. S. Yaghmoor, T. A. Kumosani and K. Kannan, Urinary biomarkers of exposure to 57 xenobiotics and its association with oxidative stress in a population in Jeddah, Saudi Arabia, *Environ. Res.*, 2016, **150**, 573–581.

73 I. Lee, R. Alakeel, S. Kim, Y. A. Al-Sheikh, H. Al-Mandeel, A. A. Alyousef, Y. Kho and K. Choi, Urinary phthalate metabolites among children in Saudi Arabia: occurrences, risks, and their association with oxidative stress markers, *Sci. Total Environ.*, 2019, **654**, 1350–1357.

74 B. A. Rocha, A. G. Asimakopoulos, F. Barbosa Jr and K. Kannan, Urinary concentrations of 25 phthalate metabolites in Brazilian children and their association with oxidative DNA damage, *Sci. Total Environ.*, 2017, **586**, 152–162.

75 V. Tran, G. Tindula, K. Huen, A. Bradman, K. Harley, K. Kogut, A. M. Calafat, B. Nguyen, K. Parra, X. Ye, B. Eskenazi and N. Holland, Prenatal phthalate exposure and 8-isoprostane among Mexican-American children with high prevalence of obesity, *J. Dev. Origins Health Dis.*, 2017, **8**(2), 196–205.

76 J. H. Kim, H. Y. Park, S. Bae, Y. H. Lim and Y. C. Hong, Diethylhexyl phthalates is associated with insulin

resistance via oxidative stress in the elderly: a panel study, *PLoS One*, 2013, **8**(8), e71392.

77 Y. Duan, L. Wang, L. Han, B. Wang, H. Sun, L. Chen, L. Zhu and Y. Luo, Exposure to phthalates in patients with diabetes and its association with oxidative stress, adiponectin, and inflammatory cytokines, *Environ. Int.*, 2017, **109**, 53–63.

78 T. Kadowaki, T. Yamauchi, N. Kubota, K. Hara, K. Ueki and K. Tobe, Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome, *J. Clin. Invest.*, 2006, **116**(7), 1784–1792.

79 J. J. Heindel, B. Blumberg, M. Cave, R. Machtinger, A. Mantovani, M. A. Mendez, A. Nadal, P. Palanza, G. Panzica, R. Sargis, L. N. Vandenberg and F. Vom Saal, Metabolism disrupting chemicals and metabolic disorders, *Reprod. Toxicol.*, 2017, **68**, 3–33.

80 B. A. Neel and R. M. Sargis, The paradox of progress: environmental disruption of metabolism and the diabetes epidemic, *Diabetes*, 2011, **60**(7), 1838–1848.

81 J. W. Hou, C. L. Lin, Y. A. Tsai, C. H. Chang, K. W. Liao, C. J. Yu, W. Yang, M. J. Lee, P. C. Huang, C. W. Sun, Y. H. Wang, F. R. Lin, W. C. Wu, M. C. Lee, W. H. Pan, B. H. Chen, M. T. Wu, C. C. Chen, S. L. Wang, C. C. Lee, C. A. Hsiung and M. L. Chen, The effects of phthalate and nonylphenol exposure on body size and secondary sexual characteristics during puberty, *Int. J. Hyg Environ. Health*, 2015, **218**(7), 603–615.

82 E. E. Hatch, J. W. Nelson, M. M. Qureshi, J. Weinberg, L. L. Moore, M. Singer and T. F. Webster, Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999-2002, *Environ. Health*, 2008, **7**, 27.

83 L. Yaghjyan, S. Sites, Y. Ruan, S.-H. Chang, L. Yaghjyan, S. Sites, Y. Ruan and S. H. Chang, Associations of urinary phthalates with body mass index, waist circumference and serum lipids among females: National Health and Nutrition Examination Survey 1999-2004, *Int. J. Obes.*, 2015, **39**(6), 994–1000.

84 M. C. Buser, H. E. Murray and F. Scinicariello, Age and sex differences in childhood and adulthood obesity association with phthalates: analyses of NHANES 2007-2010, *Int. J. Hyg Environ. Health*, 2014, **217**(6), 687–694.

85 M. V. D. Santana, S. E. Hankinson, C. Bigelow, S. R. Sturgeon, R. T. Zoeller, L. Tinker, J. A. E. Manson, A. M. Calafat, J. R. Meliker and K. W. Reeves, Urinary concentrations of phthalate biomarkers and weight change among postmenopausal women: a prospective cohort study, *Environ. Health*, 2019, **18**(1), 20.

86 R. Dong, T. Zhou, J. Chen, M. Zhang, H. Zhang, M. Wu, S. Li, L. Zhang and B. Chen, Gender- and Age-Specific Relationships Between Phthalate Exposures and Obesity in Shanghai Adults, *Arch. Environ. Contam. Toxicol.*, 2017, **73**(3), 431–441.

87 Y. Song, R. Hauser, F. B. Hu, A. A. Franke, S. Liu and Q. Sun, Urinary concentrations of bisphenol A and phthalate metabolites and weight change: a prospective investigation in US women, *Int. J. Obes.*, 2014, **38**(12), 1532–1537.

88 J. E. Lim, B. Choi and S. H. Jee, Urinary bisphenol A, phthalate metabolites, and obesity: do gender and menopausal status matter?, *Environ. Sci. Pollut. Res. Int.*, 2020, **27**(27), 34300–34310.

89 Y. L. Li, J. Lv, Z. P. Du, S. Feng, J. Sheng, Z. X. Jin, K. Y. Liu, H. Gao, X. D. Li, H. J. Cao, L. S. Yang, D. X. Xu, F. B. Tao and Q. N. Wang, The levels of phthalate exposure and associations with obesity in an elderly population in China, *Ecotoxicol. Environ. Saf.*, 2020, **201**, 110749.

90 T. P. van der Meer, M. van Faassen, A. P. van Beek, H. Snieder, I. P. Kema, B. H. R. Wolffenbuttel and J. V. van Vliet-Ostaptchouk, Exposure to Endocrine Disrupting Chemicals in the Dutch general population is associated with adiposity-related traits, *Sci. Rep.*, 2020, **10**(1), 9311.

91 L. Trasande, T. M. Attina, S. Sathyanarayana, A. J. Spanier and J. Blustein, Race/ethnicity-specific associations of urinary phthalates with childhood body mass in a nationally representative sample, *Environ. Health Perspect.*, 2013, **121**(4), 501–506.

92 Z. H. Wang, D. Gao and Z. Y. Zou, The association of phthalate metabolites with childhood waist circumference and abdominal obesity, *Eur. J. Pediatr.*, 2023, **182**(2), 803–812.

93 B. Xia, Q. Zhu, Y. Zhao, W. Ge, Y. Zhao, Q. Song, Y. Zhou, H. Shi and Y. Zhang, Phthalate exposure and childhood overweight and obesity: urinary metabolomic evidence, *Environ. Int.*, 2018, **121**(pt 1), 159–168.

94 H. Wang, Y. Zhou, C. Tang, Y. He, J. Wu, Y. Chen and Q. Jiang, Urinary phthalate metabolites are associated with body mass index and waist circumference in Chinese school children, *PLoS One*, 2013, **8**(2), e56800.

95 Y. Zhang, X. Meng, L. Chen, D. Li, L. Zhao, Y. Zhao, L. Li and H. Shi, Age and sex-specific relationships between phthalate exposures and obesity in Chinese children at puberty, *PLoS One*, 2014, **9**(8), e104852.

96 M. M. Amin, S. Parastar, K. Ebrahimpour, B. Shoshtari-Yeganeh, M. Hashemi, M. Mansourian and R. Kelishadi, Association of urinary phthalate metabolites concentrations with body mass index and waist circumference, *Environ. Sci. Pollut. Res. Int.*, 2018, **25**(11), 11143–11151.

97 M. Y. Seo, S. Moon, S. H. Kim and M. J. Park, Associations of Phthalate Metabolites and Bisphenol A Levels with Obesity in Children: The Korean National Environmental Health Survey (KoNEHS) 2015 to 2017, *Endocrinol. Metabol.*, 2022, **37**(2), 249–260.

98 Y. Dong, D. Gao, Y. Li, Z. Yang, X. Wang, M. Chen, Z. Wang, Y. Song, Z. Zou and J. Ma, Effect of childhood phthalates exposure on the risk of overweight and obesity: a nested case-control study in China, *Environ. Int.*, 2022, **158**, 106886.

99 S. L. Teitelbaum, N. Mervish, E. L. Moshier, N. Vangeepuram, M. P. Galvez, A. M. Calafat, M. J. Silva, B. L. Brenner and M. S. Wolff, Associations between

phthalate metabolite urinary concentrations and body size measures in New York City children, *Environ. Res.*, 2012, **112**, 186–193.

100 A. L. Deierlein, M. S. Wolff, A. Pajak, S. M. Pinney, G. C. Windham, M. P. Galvez, M. J. Silva, A. M. Calafat, L. H. Kushi, F. M. Biro, S. L. Teitelbaum and Breast Cancer and Environment Research Program, Longitudinal Associations of Phthalate Exposures During Childhood and Body Size Measurements in Young Girls, *Epidemiology*, 2016, **27**(4), 492–499.

101 A. Zettergren, N. Andersson, K. Larsson, I. Kull, E. Melén, A. Georgelis, M. Berglund, C. Lindh and A. Bergström, Exposure to environmental phthalates during preschool age and obesity from childhood to young adulthood, *Environ. Res.*, 2021, **192**, 110249.

102 K. Christensen, J. Sobus, M. Phillips, T. Blessinger, M. Lorber and Y. M. Tan, Changes in epidemiologic associations with different exposure metrics: a case study of phthalate exposure associations with body mass index and waist circumference, *Environ. Int.*, 2014, **73**, 66–76.

103 J. S. LaKind, M. Goodman and D. Q. Naiman, Use of NHANES data to link chemical exposures to chronic diseases: a cautionary tale, *PLoS One*, 2012, **7**(12), e51086.

104 J. D. Peck, A. M. Sweeney, E. Symanski, J. Gardiner, M. J. Silva, A. M. Calafat and S. L. Schantz, Intra- and inter-individual variability of urinary phthalate metabolite concentrations in Hmong women of reproductive age, *J. Expo. Sci. Environ. Epidemiol.*, 2010, **20**(1), 90–100.

105 J. Li, W. Xia, C. Wu, H. Zhao, Y. Zhou, J. Wei, F. Ji, H. Luan, S. Xu and Z. Cai, Variations of phthalate exposure and metabolism over three trimesters, *Environ. Pollut.*, 2019, **251**, 137–145.

106 J. D. Meeker, S. Sathyanarayana and S. H. Swan, Phthalates and other additives in plastics: human exposure and associated health outcomes, *Philos. Trans. R. Soc. London, Ser. B*, 2009, **364**(1526), 2097–2113.

107 J. J. Adibi, R. M. Whyatt, P. L. Williams, A. M. Calafat, D. Camann, R. Herrick, H. Nelson, H. K. Bhat, F. P. Perera, M. J. Silva and R. Hauser, Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples, *Environ. Health Perspect.*, 2008, **116**(4), 467–473.

108 J. M. Braun, K. W. Smith, P. L. Williams, A. M. Calafat, K. Berry, S. Ehrlich and R. Hauser, Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy, *Environ. Health Perspect.*, 2012, **120**(5), 739–745.

109 D. E. Cantonwine, J. F. Cordero, L. O. Rivera-González, L. V. Anzalota Del Toro, K. K. Ferguson, B. Mukherjee, A. M. Calafat, N. Crespo, B. Jiménez-Vélez, I. Y. Padilla, A. N. Alshawabkeh and J. D. Meeker, Urinary phthalate metabolite concentrations among pregnant women in Northern Puerto Rico: distribution, temporal variability, and predictors, *Environ. Int.*, 2014, **62**, 1–11.

110 K. K. Ferguson, T. F. McElrath, Y. A. Ko, B. Mukherjee and J. D. Meeker, Variability in urinary phthalate metabolite levels across pregnancy and sensitive windows of exposure for the risk of preterm birth, *Environ. Int.*, 2014, **70**, 118–124.

111 M. Fisher, T. E. Arbuckle, R. Mallick, A. LeBlanc, R. Hauser, M. Feeley, D. Koniecki, T. Ramsay, G. Provencher, R. Bérubé and M. Walker, Bisphenol A and phthalate metabolite urinary concentrations: daily and across pregnancy variability, *J. Expo. Sci. Environ. Epidemiol.*, 2015, **25**(3), 231–239.

112 D. Valvi, N. Monfort, R. Ventura, M. Casas, L. Casas, J. Sunyer and M. Vrijheid, Variability and predictors of urinary phthalate metabolites in Spanish pregnant women, *Int. J. Hyg Environ. Health*, 2015, **218**(2), 220–231.

113 T. M. James-Todd, J. D. Meeker, T. Huang, R. Hauser, E. W. Seely, K. K. Ferguson, J. W. Rich-Edwards and T. F. McElrath, Racial and ethnic variations in phthalate metabolite concentration changes across full-term pregnancies, *J. Expo. Sci. Environ. Epidemiol.*, 2017, **27**(2), 160–166.

114 S. Siddiq, A. M. Clemons, J. D. Meeker, C. Gennings, V. Rauh, S. H. Leisher, A. A. M. Llanos, J. A. McDonald, B. J. Wylie and P. Factor-Litvak, Predictors of Phthalate Metabolites Exposure among Healthy Pregnant Women in the United States, 2010-2015, *Int. J. Environ. Res. Public Health*, 2023, **20**(23), 7104.

115 M. Casas, X. Basagaña, A. K. Sakhi, L. S. Haug, C. Philippat, B. Granum, C. B. Manzano-Salgado, C. Brochot, F. Zeman, J. de Bont, S. Andrusaityte, L. Chatzi, D. Donaire-Gonzalez, L. Giorgis-Allemand, J. R. Gonzalez, E. Gracia-Lavedan, R. Grazuleviciene, M. Kampouri, S. Lyon-Caen, P. Pañella, I. Petraviciene, O. Robinson, J. Urquiza, M. Vafeiadi, C. Vernet, D. Waiblinger, J. Wright, C. Thomsen, R. Slama and M. Vrijheid, Variability of urinary concentrations of non-persistent chemicals in pregnant women and school-aged children, *Environ. Int.*, 2018, **121**(pt 1), 561–573.

116 J. L. Preau Jr, L. Y. Wong, M. J. Silva, L. L. Needham and A. M. Calafat, Variability over 1 week in the urinary concentrations of metabolites of diethyl phthalate and di(2-ethylhexyl) phthalate among eight adults: an observational study, *Environ. Health Perspect.*, 2010, **118**(12), 1748–1754.

117 H. Frederiksen, S. K. Kranich, N. Jørgensen, O. Taboureau, J. H. Petersen and A. M. Andersson, Temporal variability in urinary phthalate metabolite excretion based on spot, morning, and 24-h urine samples: considerations for epidemiological studies, *Environ. Sci. Technol.*, 2013, **47**(2), 958–967.

118 H. Fromme, G. Bolte, H. M. Koch, J. Angerer, S. Boehmer, H. Drexler, R. Mayer and B. Liebl, Occurrence and daily variation of phthalate metabolites in the urine of an adult population, *Int. J. Hyg Environ. Health*, 2007, **210**(1), 21–33.

119 J. D. Meeker, A. M. Calafat and R. Hauser, Urinary phthalate metabolites and their biotransformation products: predictors and temporal variability among men and women, *J. Expo. Sci. Environ. Epidemiol.*, 2012, **22**(4), 376–385.

120 L. Dewalque, C. Pirard, S. Vandepaer and C. Charlier, Temporal variability of urinary concentrations of

phthalate metabolites, parabens and benzophenone-3 in a Belgian adult population, *Environ. Res.*, 2015, **142**, 414–423.

121 M. Bastiaensen, G. Malarvannan, C. Gys, Y. A. Bamai, A. Araki and A. Covaci, Between- and within-individual variability of urinary phthalate and alternative plasticizer metabolites in spot, morning void and 24-h pooled urine samples, *Environ. Res.*, 2020, **191**, 110248.

122 S. L. Teitelbaum, J. A. Britton, A. M. Calafat, X. Ye, M. J. Silva, J. A. Reidy, M. P. Galvez, B. L. Brenner and M. S. Wolff, Temporal variability in urinary concentrations of phthalate metabolites, phytoestrogens and phenols among minority children in the United States, *Environ. Res.*, 2008, **106**(2), 257–269.

123 D. J. Watkins, M. Eliot, S. Sathyanarayana, A. M. Calafat, K. Yolton, B. P. Lanphear and J. M. Braun, Variability and predictors of urinary concentrations of phthalate metabolites during early childhood, *Environ. Sci. Technol.*, 2014, **48**(15), 8881–8890.

124 M. K. Townsend, A. A. Franke, X. Li, F. B. Hu and A. H. Eliassen, Within-person reproducibility of urinary bisphenol A and phthalate metabolites over a 1 to 3 year period among women in the Nurses' Health Studies: a prospective cohort study, *Environ. Health*, 2013, **12**(1), 80.

125 J. A. Hoppin, J. W. Brock, B. J. Davis and D. D. Baird, Reproducibility of urinary phthalate metabolites in first morning urine samples, *Environ. Health Perspect.*, 2002, **110**(5), 515–518.

126 R. Hauser, J. D. Meeker, S. Park, M. J. Silva and A. M. Calafat, Temporal variability of urinary phthalate metabolite levels in men of reproductive age, *Environ. Health Perspect.*, 2004, **112**(17), 1734–1740.

127 M. M. Yazdy, B. A. Coull, J. C. Gardiner, A. Aguiar, A. M. Calafat, X. Ye, S. L. Schantz and S. A. Korrick, A possible approach to improving the reproducibility of urinary concentrations of phthalate metabolites and phenols during pregnancy, *J. Expo. Sci. Environ. Epidemiol.*, 2018, **28**(5), 448–460.

128 J. S. LaKind, F. Idri, D. Q. Naiman and M. A. Verner, Biomonitoring and Nonpersistent Chemicals-Understanding and Addressing Variability and Exposure Misclassification, *Curr. Environ. Health Rep.*, 20193, **6**(1), 16–21.

129 Y. Lin, J. Wei, Y. Li, J. Chen, Z. Zhou, L. Song, Z. Wei, Z. Lv, X. Chen, W. Xia and S. Xu, Developmental exposure to di(2-ethylhexyl) phthalate impairs endocrine pancreas and leads to long-term adverse effects on glucose homeostasis in the rat, *Am. J. Physiol. Endocrinol. Metab.*, 2011, **301**(3), E527–E538.

130 C. Srinivasan, A. I. Khan, V. Balaji, J. Selvaraj and K. Balasubramanian, Diethyl hexyl phthalate-induced changes in insulin signaling molecules and the protective role of antioxidant vitamins in gastrocnemius muscle of adult male rat, *Toxicol. Appl. Pharmacol.*, 2011, **257**(2), 155–164.

131 P. Rajesh, S. Sathish, C. Srinivasan, J. Selvaraj and K. Balasubramanian, Phthalate is associated with insulin resistance in adipose tissue of male rat: role of antioxidant vitamins, *J. Cell. Biochem.*, 2013, **114**(3), 558–569.

132 P. Rajesh and K. Balasubramanian, Gestational exposure to di(2-ethylhexyl) phthalate (DEHP) impairs pancreatic β-cell function in F1 rat offspring, *Toxicol. Lett.*, 2015, **232**(1), 46–57.

133 X. Sun, Y. Lin, Q. Huang, J. Shi, L. Qiu, M. Kang, Y. Chen, C. Fang, T. Ye and S. Dong, Di(2-ethylhexyl) phthalate-induced apoptosis in rat INS-1 cells is dependent on activation of endoplasmic reticulum stress and suppression of antioxidant protection, *J. Cell Mol. Med.*, 2015, **19**(3), 581–594.

134 W. Zhang, X. Y. Shen, W. W. Zhang, H. Chen, W. P. Xu and W. Wei, Di-(2-ethylhexyl) phthalate could disrupt the insulin signaling pathway in liver of SD rats and L02 cells via PPARγ, *Toxicol. Appl. Pharmacol.*, 2017, **316**, 17–26.

135 G. Karabulut and N. Barlas, The possible effects of mono butyl phthalate (MBP) and mono (2-ethylhexyl) phthalate (MEHP) on INS-1 pancreatic beta cells, *Toxicol. Res.*, 2021, **10**(3), 601–612.

136 J. N. Feige, L. Gelman, D. Rossi, V. Zoete, R. Métivier, C. Tudor, S. I. Anghel, A. Grosdidier, C. Lathion, Y. Engelborghs, O. Michielin, W. Wahli and B. Desvergne, The endocrine disruptor monoethyl-hexyl-phthalate is a selective peroxisome proliferator-activated receptor gamma modulator that promotes adipogenesis, *J. Biol. Chem.*, 2007, **282**(26), 19152–19166.

137 M. Lehrke and M. A. Lazar, The many faces of PPARgamma, *Cell*, 2005, **123**(6), 993–999.

138 J. N. Feige, L. Gelman, L. Michalik, B. Desvergne and W. Wahli, From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions, *Prog. Lipid Res.*, 2006, **45**(2), 120–159.

139 I. Kratochvil, T. Hofmann, S. Rother, R. Schlichting, R. Moretti, D. Scharnweber, V. Hintze, B. I. Escher, J. Meiler, S. Kalkhof and M. von Bergen, Mono(2-ethylhexyl) phthalate (MEHP) and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP) but not di(2-ethylhexyl) phthalate (DEHP) bind productively to the peroxisome proliferator-activated receptor γ, *Rapid Commun. Mass Spectrom.*, 2019, **33**(suppl. 1), 75–85.

140 A. Useini, F. Engelberger, G. Künze and N. Sträter, Structural basis of the activation of PPARγ by the plasticizer metabolites MEHP and MINCH, *Environ. Int.*, 2023, **173**, 107822.

141 A. Lampen, S. Zimnik and H. Nau, Teratogenic phthalate esters and metabolites activate the nuclear receptors PPARs and induce differentiation of F9 cells, *Toxicol. Appl. Pharmacol.*, 2003, **188**(1), 14–23.

142 C. H. Hurst and D. J. Waxman, Activation of PPARalpha and PPARgamma by environmental phthalate monoesters, *Toxicol. Sci.*, 2003, **74**(2), 297–308.

143 R. Biemann, A. Navarrete Santos, A. Navarrete Santos, D. Riemann, J. Knelangen, M. Blüher, H. Koch and B. Fischer, Endocrine disrupting chemicals affect the

adipogenic differentiation of mesenchymal stem cells in distinct ontogenetic windows, *Biochem. Biophys. Res. Commun.*, 2012, **417**(2), 747–752.

144 C. Hao, X. Cheng, J. Guo, H. Xia and X. Ma, Perinatal exposure to diethyl-hexyl-phthalate induces obesity in mice, *Front. Biosci.*, 2013, **5**(2), 725–733.

145 H. C. Chiang, Y. T. Kuo, C. C. Shen, Y. H. Lin, S. L. Wang and T. C. Tsou, Mono(2-ethylhexyl) phthalate accumulation disturbs energy metabolism of fat cells, *Arch. Toxicol.*, 2016, **90**(3), 589–601.

146 M. T. Bility, J. T. Thompson, R. H. McKee, R. M. David, J. H. Butala, J. P. Vanden Heuvel and J. M. Peters, Activation of mouse and human peroxisome proliferator-activated receptors (PPARs) by phthalate monoesters, *Toxicol. Sci.*, 2004, **82**(1), 170–182.

147 S. C. Hasmall, N. H. James, N. Macdonald, A. R. Soames and R. A. Roberts, Species differences in response to diethylhexylphthalate: suppression of apoptosis, induction of DNA synthesis and peroxisome proliferator activated receptor alpha-mediated gene expression, *Arch. Toxicol.*, 2000, **74**(2), 85–91.

148 C. Hao, X. Cheng, H. Xia and X. Ma, The endocrine disruptor mono-(2-ethylhexyl) phthalate promotes adipocyte differentiation and induces obesity in mice, *Biosci. Rep.*, 2012, **32**(6), 619–629.

149 S. Ellero-Simatos, S. P. Claus, C. Benelli, C. Forest, F. Letourneur, N. Cagnard, P. H. Beaune and I. de Waziers, Combined transcriptomic-(1)H NMR metabonomic study reveals that monoethylhexyl phthalate stimulates adipogenesis and glyceroneogenesis in human adipocytes, *J. Proteome Res.*, 2011, **10**(12), 5493–5502.

150 J. N. Feige, A. Gerber, C. Casals-Casas, Q. Yang, C. Winkler, E. Bedu, M. Bueno, L. Gelman, J. Auwerx, F. J. Gonzalez and B. Desvergne, The pollutant diethylhexyl phthalate regulates hepatic energy metabolism via species-specific PPARalpha-dependent mechanisms, *Environ. Health Perspect.*, 2010, **118**(2), 234–241.

151 K. Schaedlich, S. Gebauer, L. Hunger, L. S. Beier, H. M. Koch, M. Wabitsch, B. Fischer and J. Ernst, DEHP deregulates adipokine levels and impairs fatty acid storage in human SGBS-adipocytes, *Sci. Rep.*, 2018, **8**(1), 3447.

152 E. Campioli, A. Batarseh, J. Li and V. Papadopoulos, The endocrine disruptor mono-(2-ethylhexyl) phthalate affects the differentiation of human liposarcoma cells (SW 872), *PLoS One*, 2011, **6**(12), e28750.

153 Z. Lv, J. Cheng, S. Huang, Y. Zhang, S. Wu, Y. Qiu, Y. Geng, Q. Zhang, G. Huang, Q. Ma, X. Xie, S. Zhou, T. Wu and Y. Ke, DEHP induces obesity and hypothyroidism through both central and peripheral pathways in C3H/He mice, *Obesity*, 2016, **24**(2), 368–378.

154 J. S. Schmidt, K. Schaedlich, N. Fiandanese, P. Pocar and B. Fischer, Effects of di(2-ethylhexyl) phthalate (DEHP) on female fertility and adipogenesis in C3H/N mice, *Environ. Health Perspect.*, 2012, **120**(8), 1123–1129.

155 Y. Jia, T. Liu, L. Zhou, J. Zhu, J. Wu, D. Sun, J. Xu, Q. Wang, H. Chen, F. Xu, Y. Zhang, T. Zhang, H. Liu and L. Ye, Effects of Di-(2-ethylhexyl) Phthalate on Lipid Metabolism by the JAK/STAT Pathway in Rats, *Int. J. Environ. Res. Public Health*, 2016, **13**(11), 1085.

156 Y. Hayashi, Y. Ito, N. Yamagishi, Y. Yanagiba, H. Tamada, D. Wang, D. H. Ramdhan, H. Naito, Y. Harada, M. Kamijima, F. J. Gonzales and T. Nakajima, Hepatic peroxisome proliferator-activated receptor α may have an important role in the toxic effects of di(2-ethylhexyl) phthalate on offspring of mice, *Toxicology*, 2011, **289**(1), 1–10.

157 N. Klöting, N. Hesselbarth, M. Gericke, A. Kunath, R. Biemann, R. Chakaroun, J. Kosacka, P. Kovacs, M. Kern, M. Stumvoll, B. Fischer, U. Rolle-Kampczyk, R. Feltens, W. Otto, D. K. Wissenbach, M. von Bergen and M. Blüher, Di-(2-Ethylhexyl)-Phthalate (DEHP) Causes Impaired Adipocyte Function and Alters Serum Metabolites, *PLoS One*, 2015, **10**(12), e0143190.

158 J. Xu, L. Zhou, S. Wang, J. Zhu, T. Liu, Y. Jia, D. Sun, H. Chen, Q. Wang, F. Xu, Y. Zhang, H. Liu, T. Zhang and L. Ye, Di-(2-ethylhexyl)-phthalate induces glucose metabolic disorder in adolescent rats, *Environ. Sci. Pollut. Res. Int.*, 2018, **25**(4), 3596–3607.

159 Y. Zhang, H. Feng, A. Tian, C. Zhang, F. Song, T. Zeng and X. Zhao, Long-term exposure to low-dose Di(2-ethylhexyl) phthalate aggravated high fat diet-induced obesity in female mice, *Ecotoxicol. Environ. Saf.*, 2023, **253**, 114679.

160 G. Li, C. Y. Zhao, Q. Wu, S. Y. Guan, H. W. Jin, X. L. Na and Y. B. Zhang, Integrated metabolomics and transcriptomics reveal di(2-ethylhexyl) phthalate-induced mitochondrial dysfunction and glucose metabolism disorder through oxidative stress in rat liver, *Ecotoxicol. Environ. Saf.*, 2021, **228**, 112988.

161 P. Rajesh and K. Balasubramanian, Phthalate exposure in utero causes epigenetic changes and impairs insulin signalling, *J. Endocrinol.*, 2014, **223**(1), 47–66.

162 G. Rajagopal, R. S. Bhaskaran and B. Karundevi, Maternal di-(2-ethylhexyl) phthalate exposure alters hepatic insulin signal transduction and glucoregulatory events in rat F1 male offspring, *J. Appl. Toxicol.*, 2019, **39**(5), 751–763.

163 A. C. Venturelli, K. B. Meyer, S. V. Fischer, D. H. Kita, R. A. Philipsen, R. N. Morais and A. J. M. Andrade, Effects of in utero and lactational exposure to phthalates on reproductive development and glycemic homeostasis in rats, *Toxicology*, 2019, **421**, 30–40.

164 A. C. Venturelli, S. V. Fischer, R. N. de Morais, S. Grassiolli and A. J. M. Andrade, Effects of exposure to Di-(2-ethylhexyl) phthalate (DEHP) during lactation and puberty on sexual maturation and glycemic homeostasis in male rats, *Clin. Nutr. ESPEN*, 2015, **10**(1), e5–e12.

165 K. Aston-Mourney, J. Proietto, G. Morahan and S. Andrikopoulos, Too much of a good thing: why it is bad to stimulate the beta cell to secrete insulin, *Diabetologia*, 2008, **51**(4), 540–545.

166 D. Gupta, T. Kono and C. Evans-Molina, The role of peroxisome proliferator-activated receptor γ in pancreatic

β cell function and survival: therapeutic implications for the treatment of type 2 diabetes mellitus, *Diabetes Obes. Metabol.*, 2010, **12**(12), 1036–1047.

167 J. Zhao, J. Goldberg, J. D. Bremner and V. Vaccarino, Global DNA methylation is associated with insulin resistance: a monozygotic twin study, *Diabetes*, 2012, **61**(2), 542–546.

168 L. Li, L. Huang, R. Lei, P. Zhang, Y. Yang, H. Liu and Y. Zhang, DEHP and DBP, common phthalates, induce glucose metabolism disorders in rats via oxidative damage of PI3K/Akt/GLUT4 signaling, *Environ. Pollut.*, 2024, **341**, 122948.

169 T. L. Hectors, C. Vanparys, A. Pereira-Fernandes, G. A. Martens and R. Blust, Evaluation of the INS-1 832/13 cell line as a beta-cell based screening system to assess pollutant effects on beta-cell function, *PLoS One*, 2013, **8**(3), e60030.

170 L. Li, F. Wang, J. Zhang, K. Wang, X. De, L. Li and Y. Zhang, Typical phthalic acid esters induce apoptosis by regulating the PI3K/Akt/Bcl-2 signaling pathway in rat insulinoma cells, *Ecotoxicol. Environ. Saf.*, 2021, **208**, 111461.

171 V. Ramachandran, M. A. V. Anand, E. David, K. Venkatachalam, S. Vijayakumar, V. Sankaran, A. Balupillai, C. C. Sangeetha, K. M. Gothandam, V. S. Kotakadi, A. Ghidan, T. Al Antary and B. Xu, Antidiabetic Activity of Gold Nanoparticles Synthesized Using Wedelolactone in RIN-5F Cell Line, *Antioxidants*, 2019, **9**(1), 8.

172 Y. She, L. Jiang, L. Zheng, H. Zuo, M. Chen, X. Sun, Q. Li, C. Geng, G. Yang, L. Jiang and X. Liu, The role of oxidative stress in DNA damage in pancreatic β cells induced by di-(2-ethylhexyl) phthalate, *Chem. Biol. Interact.*, 2017, **265**, 8–15.

173 F. D. Yöntem, S. Ayaz, Ş. Bulut, E. H. Aldoğan and M. A. Ahbab, Endoplasmic reticulum stress and pro-inflammatory responses induced by phthalate metabolites monoethylhexyl phthalate and monobutyl phthalate in 1.1B4 pancreatic beta cells, *Toxicology*, 2024, **501**, 153695.

174 N. M. Weldingh, L. Jørgensen-Kaur, R. Becher, J. A. Holme, J. Bodin, U. C. Nygaard and A. K. Bølling, Bisphenol A is More Potent than Phthalate Metabolites in Reducing Pancreatic β-Cell Function, *BioMed Res. Int.*, 2017, **2017**, 4614379.

175 L. Jiang, T. Qiu, X. Yao, H. Chen, K. Yao, X. Sun, G. Yang, L. Jiang, C. Zhang, N. Wang, H. Zhang and X. Liu, MEHP induces pyroptosis and autophagy alternation by cathepsin B activation in INS-1 cells, *Environ. Sci. Pollut. Res. Int.*, 2021, **28**(47), 66628–66642.

176 R. Al-Abdulla, H. Ferrero, S. Soriano, T. Boronat-Belda and P. Alonso-Magdalena, Screening of Relevant Metabolism-Disrupting Chemicals on Pancreatic β-Cells: Evaluation of Murine and Human In Vitro Models, *Int. J. Mol. Sci.*, 2022, **23**(8), 4182.

177 G. Rajagopal, R. S. Bhaskaran and B. Karundevi, Developmental exposure to DEHP alters hepatic glucose uptake and transcriptional regulation of GLUT2 in rat male offspring, *Toxicology*, 2019, **413**, 56–64.

178 S. Rengarajan, C. Parthasarathy, M. Anitha and K. Balasubramanian, Diethylhexyl phthalate impairs insulin binding and glucose oxidation in Chang liver cells, *Toxicol. In Vitro*, 2007, **21**(1), 99–102.

179 P. Rajesh and K. Balasubramanian, Di(2-ethylhexyl) phthalate exposure impairs insulin receptor and glucose transporter 4 gene expression in L6 myotubes, *Hum. Exp. Toxicol.*, 2014, **33**(7), 685–700.

180 Y. Ding, Y. Liu, F. Fei, L. Yang, G. Mao, T. Zhao, Z. Zhang, M. Yan, W. Feng and X. Wu, Study on the metabolism toxicity, susceptibility and mechanism of di-(2-ethylhexyl) phthalate on rat liver BRL cells with insulin resistance in vitro, *Toxicology*, 2019, **422**, 102–120.

181 Y. Ding, K. Gao, Y. Liu, G. Mao, K. Chen, X. Qiu, T. Zhao, L. Yang, W. Feng and X. Wu, Transcriptome analysis revealed the mechanism of the metabolic toxicity and susceptibility of di-(2-ethylhexyl)phthalate on adolescent male ICR mice with type 2 diabetes mellitus, *Arch. Toxicol.*, 2019, **93**(11), 3183–3206.

182 Y. Ding, T. Xu, G. Mao, Y. Chen, X. Qiu, L. Yang, T. Zhao, X. Xu, W. Feng and X. Wu, Di-(2-ethylhexyl) phthalate-induced hepatotoxicity exacerbated type 2 diabetes mellitus (T2DM) in female pubertal T2DM mice, *Food Chem. Toxicol.*, 2021, **149**, 112003.

183 J. Wang, J. Li, K. R. Zahid, K. Wang, Y. Qian, P. Ma, S. Ding, X. Yang and X. Wang, Adverse effect of DEHP exposure on the serum insulin level of Balb/c mice, *Mol. Cell. Toxicol.*, 2016, **12**, 83–91.

184 M. Armoni, N. Kritz, C. Harel, F. Bar-Yoseph, H. Chen, M. J. Quon and E. Karnieli, Peroxisome proliferator-activated receptor-gamma represses GLUT4 promoter activity in primary adipocytes, and rosiglitazone alleviates this effect, *J. Biol. Chem.*, 2003, **278**(33), 30614–30623.

185 Y. Xu, T. J. Cook and G. T. Knipp, Effects of di-(2-ethylhexyl)- phthalate (DEHP) and its metabolites on fatty acid homeostasis regulating proteins in rat placental HRP-1 trophoblast cells, *Toxicol. Sci.*, 2005, **84**(2), 287–300.

186 K. N. Keane, V. F. Cruzat, R. Carlessi, P. I. de Bittencourt Jr and P. Newsholme, Molecular Events Linking Oxidative Stress and Inflammation to Insulin Resistance and β-Cell Dysfunction, *Oxid. Med. Cell. Longev.*, 2015, **2015**, 181643.

187 T. M. D. Nguyen, Adiponectin: Role in Physiology and Pathophysiology, *Int. J. Prev. Med.*, 2020, **11**, 136.

188 E. E. Kershaw and J. S. Flier, Adipose tissue as an endocrine organ, *J. Clin. Endocrinol. Metab.*, 2004, **89**(6), 2548–2556.

189 M. E. Trujillo and P. E. Scherer, Adiponectin–journey from an adipocyte secretory protein to biomarker of the metabolic syndrome, *J. Intern. Med.*, 2005, **257**(2), 167–175.

190 J. Ernst, U. Grabiec, K. Falk, F. Dehghani and K. Schaedlich, The endocrine disruptor DEHP and the ECS: analysis of a possible crosstalk, *Endocr. Connect.*, 2020, **9**(2), 101–110.

191 R. B. Ceddia, R. Somwar, A. Maida, X. Fang, G. Bikopoulos and G. Sweeney, Globular adiponectin increases GLUT4 translocation and glucose uptake but reduces glycogen synthesis in rat skeletal muscle cells, *Diabetologia*, 2005, **48**(1), 132–139.