Environmental Research 200 (2021) 111690

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

# Prenatal air pollution exposure to diesel exhaust induces cardiometabolic disorders in adulthood in a sex-specific manner

Delphine Rousseau-Ralliard [a,b,*], Christophe Richard [a,c], Pauline Hoarau [a], Marie-Sylvie Lallemand [a], Lucie Morillon [a], Marie-Christine Aubrière [a,b], Sarah A. Valentino [a,b], Michèle Dahirel [a,b], Marine Guinot [a], Natalie Fournier [d,e], Gwendoline Morin [f], Eve Mourier [a], Sylvaine Camous [a], Rémy Slama [g], Flemming R. Cassee [h,i], Anne Couturier-Tarrade [a,b], Pascale Chavatte-Palmer [a,b,c]

[a] Université Paris-Saclay, UVSQ, INRAE, BREED, 78350, Jouy-en-Josas, France
[b] Ecole Nationale Vétérinaire d'Alfort, BREED, 94700, Maisons-Alfort, France
[c] MIMA2 Platform, INRAE, 78350, Jouy-en-Josas, France
[d] Lip(Sys)2 - EA 7357, Athérosclérose et macrophages: impact des phospholipides et des fonctions mitochondriales sur l'efflux du cholestérol, Université Paris Saclay, UFR de Pharmacie, 92290, Châtenay-Malabry, France
[e] Laboratoire de Biochimie, AP-HP (Assistance Publique-Hôpitaux de Paris), Hôpital Européen Georges Pompidou, 75015, Paris, France
[f] Université Paris-Saclay, INRAE, UE SAAJ, 78350, Jouy en Josas, France
[g] Inserm and Univ. Grenoble Alpes, U823, IAB Research Center, Team of Environmental Epidemiology Applied to Reproduction and Respiratory Health, 38700, Grenoble, France
[h] Centre for Sustainability, Environment and Health, National Institute for Public Health and the Environment, Bilthoven, Netherlands
[i] Institute of Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands

ARTICLE INFO

*Keywords:*
Gestational exposure
Fetal programming
Rabbit biomedical model
Adult offspring
Intergenerational effect

ABSTRACT

*Background:* Results from observational and experimental studies indicate that exposure to air pollutants during gestation reduces birth weight, whereas little is known on potential cardiometabolic consequences for the offspring at adulthood.
*Objectives:* Our aim was to evaluate the long-term effects of gestational exposure to diesel engine exhaust (DE) on adult offspring phenotype in a rabbit model.
*Methods:* The protocol was designed to mimic human exposure in large European cities. Females rabbits were exposed to diluted (1 mg/m$^3$) DE (exposed, n = 9) or clean air (controls, n = 7), from 3 days after mating, 2 h/d and 5 d/wk in a nose-only inhalation system throughout gestation (gestation days 3–27). After birth and weaning, 72 offspring (47 exposed and 25 controls) were raised until adulthood (7.5 months) to evaluate their cardio-metabolic status, including the monitoring of body weight and food intake, fasting biochemistry, body composition (iDXA), cardiovascular parameters and glucose tolerance. After a metabolic challenge (high fat diet in males and gestation in females), animals were euthanized for postmortem phenotyping.
*Results:* Sex-specific responses to maternal exposure were observed in adult offspring. Age-related increases in blood pressure ($p = 0.058$), glycaemia ($p = 0.029$), and perirenal fat mass ($p = 0.026$) as well as reductions in HDL-cholesterol ($p = 0.025$) and fat-to-body weight ratio ($p = 0.011$) were observed in exposed males, suggesting a metabolic syndrome. Almost only trends were observed in exposed females with higher triglycerides and decreased bone density compared to control females. Metabolic challenges triggered or amplified some biological responses, especially in females.
*Conclusions: In utero* exposure to air pollution predisposed rabbit offspring to cardiometabolic disorders in a sex-specific manner.

* Corresponding author. INRAE UMR1198 BREED, Université Paris-Saclay INRAE, ENVA, UVSQ, Biologie de la Reproduction, Environnement, Epigénétiques et Développement (BREED), Domaine de Vilvert, Bât 230-231, 78350, Jouy en Josas, France.
*E-mail address:* delphine.rousseau@inrae.fr (D. Rousseau-Ralliard).

https://doi.org/10.1016/j.envres.2021.111690
Received 23 March 2021; Received in revised form 9 July 2021; Accepted 11 July 2021
Available online 14 July 2021

## 1. Introduction

### 1.1. Background

During pollution peaks, the most sensitive people, namely children and the elderly, are invited to limit their exposure. The recommendation for pregnant women to limit their exposure to air pollutants has been widely discussed over the past 10 years (Pedersen et al., 2013). In human cohorts, exposure to fine particles from diesel exhaust during pregnancy was shown to increase risks of having a child with intra-uterine growth retardation (Pedersen et al., 2013) or small-for-gestational age (Huang et al., 2019; Slama et al., 2010). Fine particles are among the environmental factors that could disturb fetal development *via* maternal exposure by deregulating placental function, either through changes of intra-placental blood flow (Valentino et al., 2016; Weldy et al., 2014) or through immuno-inflammatory reactions and oxidative stress in placenta (Auten et al., 2012; Liu et al., 2016). Indeed, both independent epidemiological (Huang et al., 2019; Slama et al., 2010) and experimental studies, in different animal models (mice (Bolton et al., 2012) and rabbits (Valentino et al., 2016), in particular) demonstrated the fetal vulnerability to pollutants, including diesel engine exhaust. Gestational exposure could induce adverse long term consequences for the offspring, such as an increased risk of diabetes (Thiering et al., 2013), overweight or even cardiac disorders (Adar et al., 2013). These effects fall into the Developmental Origins of Health and Disease (DOHaD) (Schug et al., 2013). Indeed, pollution has been proposed as a new paradigm for the origin of metabolic disorders such as diabetes and obesity (Nappi et al., 2016). In cohort studies, however, the monitoring of individuals remains complicated over the long term and there are still very few prospective studies. In addition, children are often raised in the same polluted environment as their mothers, so these studies take into account both indirect *in utero* exposures to air pollution combined with direct postnatal exposure of these children. Thus, the use of animal models appears essential to understand the effects of exposure during *in utero* life.

### 1.2. Objectives

The primary objective of this study was to investigate the postnatal consequences, in the first generation offspring (F1), of *in utero* exposure to diesel exhaust focusing on the period from puberty to adulthood, using a previously developed rabbit model (Valentino et al., 2016). Physiological and biochemical approaches were used to evaluate sex-specific predisposition to developing cardio-metabolic pathologies at adulthood, such as hypertension, metabolic syndrome, cardiac hypertrophy, and liver disease in F1 offspring. The secondary objective of this study was to evaluate if *in utero* exposure to diesel engine exhaust could modify the response to a metabolic challenge at adulthood.

## 2. Methods

### 2.1. Ethics

The work described in the present article has been carried out in accordance with the relevant guidelines and regulations, EC Directive 86/609/EEC for animal experiments http://europa.eu.int/scadplus/leg/en/s23000.htm. All experimental protocols were approved under N°12/102 by the local ethical committee COMETHEA (“*Comité d’Ethique en Expérimentation Animale du Centre INRA de Jouy en Josas et AgroParisTech*”), referenced as N°C2EA-45 in the French National registry CNREEA (“*Comité National de Réflexion Ethique sur l’Expérimentation Animale*”).

### 2.2. Experimental design: pregnant female rabbit exposure and F1 postnatal breeding

Sixteen pregnant New-Zealand white female rabbits (INRA1077 line, 1 year old), defined as F0, were exposed by nose-only inhalation in custom-made Plexiglas tubes, to either diluted diesel engine exhaust (DE) (1 mg/m$^3$) (N = 9, exposed group) or clean purified air (N = 7, control group) for 2 h/day, 5 days/wk, from the 3rd to 27th day post-conception (dpc) (20 days altogether over a 31-day gestation). Inhalation exposure was performed 1 h in the morning and 1 h in the afternoon to mimic the daily commuting between home and work. The rest of the time, the rabbits were housed in their individual cage in a temperature, light cycle, hygrometry and air renewal controlled atmosphere. Animals had *ad libitum* access to water and food. DE exposure was performed using a 25 KVA Loxam diesel engine (Loxam, Ridderkerk, Netherlands), with a 500 nm particle filter; characteristics of the complex mixture of DE have been published previously (Valentino et al., 2016).

After 31–32 days of gestation, F0 females gave birth to 137 F1 offspring (N = 55 controls and N = 87 exposed). They were raised by their own dams until weaning, when an outbreak of diarrhea occurred, where 53% of the F1 population was lost. Some animals survived with antibiotic treatment at weaning, others without; thus, the antibiotic treatment was used as a cofactor in statistical analyzes. After weaning, the surviving offspring were maintained in standard conditions until puberty in individual cages. Play balls and toys were used to enrich their environment. Animals were accustomed to experimental procedures by everyday handling and contact with humans throughout the experimental protocol. The present experimental study was conducted on the remaining 72 pubertal animals distributed as follows: N = 12 control males (CM) and N = 13 control females (CF), N = 17 exposed males (EM) and N = 30 exposed females (EF), from 4.5 months to 7.5 months of age. The experimental protocol is presented in Fig. 1.

### 2.3. In vivo phenotyping of young adults

Classical physiological follow-up was performed to phenotype the animals in early adulthood after puberty from 4.5 to 5.5 months of age, including weekly weighting and food consumption measurement, together with fasting blood samplings in EDTA-coated tubes.

#### 2.3.1. Analysis of insulin-glucose dynamics

*2.3.1.1. Intravenous glucose tolerance test (ivGTT).* After overnight fasting, an ivGTT was performed to evaluate glucose homeostasis. Briefly, after skin application of gaultheria oil for its analgesic and vasodilatory properties, both the ear vein and artery were catheterized (Introcan, BBraun, Germany) 30 min before the beginning of the test, to be used respectively for infusion and sampling. Fasting blood glucose was first determined in all groups using a glucometer (Freestyle optium, Abbott, USA). Glucose tolerance tests were carried out using a glucose load of 0.5 g/kg (30% glucose, Braun) injected over 1 min. Samples (1 ml in dry tubes) were collected 10 min before and 5, 15, 30, 45 and 120 min after glucose infusion (t0). Glycaemia was measured using an automated analyzer (Medisense Optium Xceed). After 1 h coagulation at room temperature, the serum was separated by a 10-min centrifugation at 5500 rpm and stored at −20 °C for further insulin analyses.

*2.3.1.2. Insulin assays.* Plasma insulin concentrations were measured using a human insulin AlphaLISA immunoassay kit (PerkinElmer, USA), with standard samples of decreasing insulin concentrations prepared in inactivated and insulin-free fetal bovine serum as described elsewhere (Robles et al., 2017). Plasma samples were thawed and maintained at 4 °C during the time of assay. Four microliter samples were distributed in duplicate in opaque 96 well plates (½AreaPlateTM, PerkinElmer, USA). A 4-μL mixture of anti-insulin antibody (1 nM final concentration)

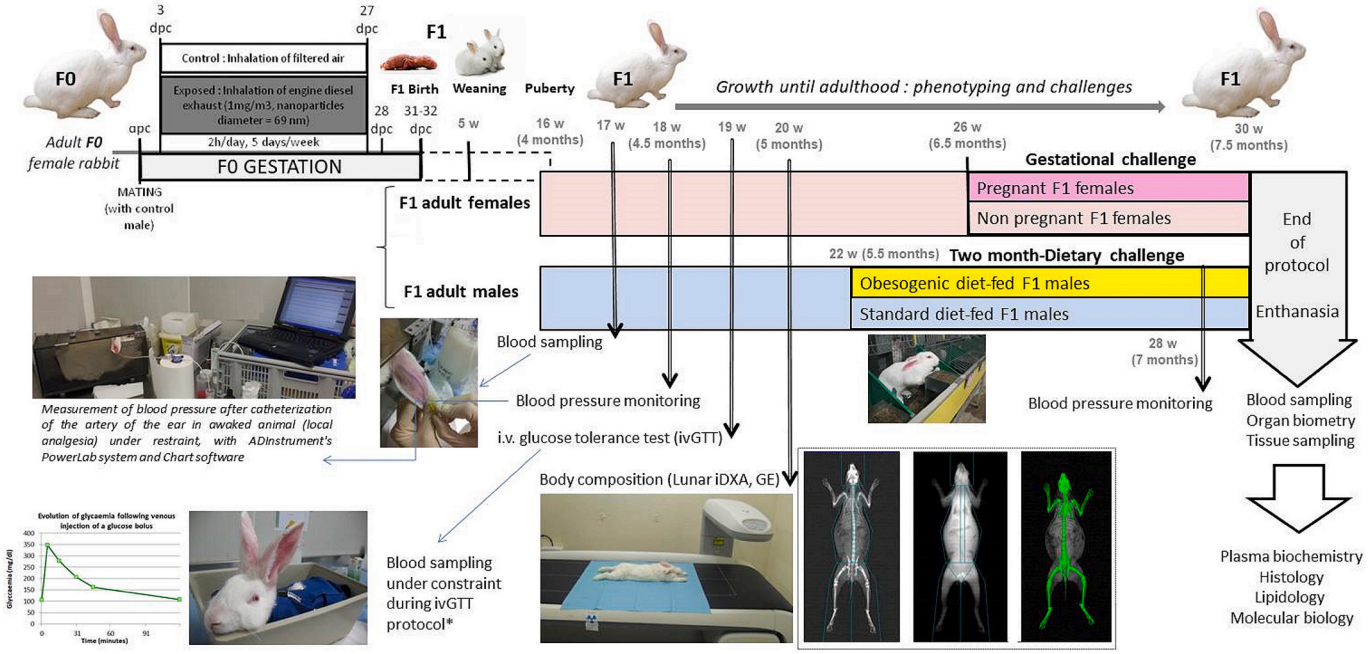


**Fig. 1.** Experimental protocol. The study was designed to mimic a worst-case scenario of ambient air pollution using a well-controlled test atmosphere without interfering with the normal gas exchange function of the rabbit lungs. Sixteen pregnant New Zealand white female rabbits (INRA1077 line, 1 year old), defined as F0 were exposed by nose-only inhalation in custom-made Plexiglas tubes to either diluted diesel exhaust (DE) (1 mg/m$^3$) (N = 9, exposed group) or clean air (N = 7, control group) for 2 h/day, 5 days/wk, from the 3rd to 27th day post-conception (dpc) (20 days altogether over a 31-day gestation). They gave birth to the F1 offspring that were raised by their own mothers until weaning, after which they were maintained in standard conditions until puberty. An epidemic of diarrhea in young rabbits around weaning reduced the population of F1 offspring by 47%. This epidemic episode affected all the animals indifferently, whatever their experimental group. This epidemic event occurred at the step of the food transition of young rabbits, mainly because of their proximity since the animals are reared under the same conditions as in rabbit breeding farms, in long spans of contiguous cages. Through anatomo-pathological analyses, it was shown that these young animals presented clinical signs of acute severe generalized necrotizing entero-typhlo-colitis induced by an infection by enteropathogenic E. coli. Three 3 waves of births were carried out to allow the in vivo physiological investigations for all animals at the same age and in the same morning time slots. This epidemic episode mainly affected the animals of the 1st wave of births in the fortnight surrounding their weaning. Once the bacteria identified and the right antibiotic treatment was administered (BAYTRIL 10% oral solution, in case of symptoms) associated with the use of prebiotic as prophylaxis (Sodijet), the sick animals were saved and the others, from the two last waves, protected. The present experimental study was conducted on the remaining 72 pubertal animals distributed as follows: N = 12 control males (CM) and N = 13 control females (CF), N = 17 exposed males (EM) and N = 30 exposed females (EF), from 4.5 months to 7.5 months of age. A metabolic challenge was performed in both control (C) and exposed (E) males from 5.5 to 7.5 month of age. The animals were randomly assigned to their challenge group. They were given either an obesogenic diet (OD; N = 6 MCO and N = 6 MEO, respectively) or a standard diet (SD; N = 6 MCS, N = 10 MES, respectively) for two months. Two males (1 MCS, fed the Standard diet (S), and 1 MEO, fed the Obesogenic diet (O)) died during the experimental period. Females were kept on the control diet and were challenged with a gestation. Altogether, 6.5-month-old F1 females (N = 11 control and N = 11 in utero exposed) were mated to control males. Only 5 out of 11 control females were diagnosed as pregnant, among which one died at mid-pregnancy and another aborted; in the exposed group, 10 out of 11 were pregnant, 1 female aborted at mid-pregnancy. All females that were still pregnant (N = 3 controls, FCP, and N = 9 exposed, FEP) were euthanized at 28 dpc to collect second generation (F2) feto-placental units. The remaining non-pregnant females (N = 3 controls (FCN), N = 6 exposed (FEN) were maintained until 7.5 months in standard dietary conditions.

and acceptor beads (10 μg/ml final concentration) was then added. After 1 h of incubation at room temperature in the dark, 32 μL of streptavidin-coated donor beads (40 μg/ml final concentrations) was added. Plates were analyzed after 30-min incubation in the dark at room temperature, using the Enspire1 reader and Manager Software (PerkinElmer, USA).

*2.3.1.3. Post-assay ivGTT analyses.* The blood glucose levels were then plotted against time as blood glucose tolerance curves. The first point (zero) of these curves represents the fasting blood sugar level. Insulin levels were also determined at each time interval using the method described above from 1 mL blood samples collected on dry tubes prior to glucose load and at each sampling point. The ivGTT were analyzed by calculating the areas under the curves (AUC) for both glucose and insulin concentration curves over time. The base values was subtracted from the result values before calculating the areas under the glucose and insulin response curves (AUC) and only positive absolute values were added (Le Floch et al., 1990). The AUC, total or normalized, were calculated using the trapezium method as follows:

$$Area\ under\ the\ curve = (Concentration\ tn + Concentration\ tn + 1) / (tn + 1 - tn)$$

These AUC reflect the integrated plasma concentration after glucose administration from 0 to 120 min above the pre-infusion baseline for all positive values. Thus, the area under the glucose concentration curve accounts for glucose clearance, while the area under the insulin concentration curve accounts for insulin production by the pancreas.

*2.3.2. Analysis of cardiovascular parameters: blood pressure and heart rate*

Blood pressure and heart rate were recorded in non-anesthetized restrained rabbits using a Powerlab system (ADInstruments, Australia) and a BP sensor (Phymep, Paris, France). Briefly, a catheter was introduced into the ear artery after analgesia by a tamponade of gaultheria oil and ear rubbing. Once catheterized, the animals were left for quiet recovery for 20-min stabilization phase of cardiovascular constants, prior to recordings. Cardiovascular parameters were measured in all animals at 4.5 months of age and repeated again only in males at 7 months of age.

#### 2.3.3. Analysis of body composition: dual-energy X-ray absorptiometry (iDXA)

Whole and regional body masses of bones, fats and lean tissue were measured by spectral imaging using Lunar Dual X-ray Absorptiometry (DXA) machine (GE Healthcare, Germany) under general anesthesia induced by injection of ketamine (10 mg/kg; Imalgen 1000, Mérial, France) and medetomidine (0.17 mg/kg; Domitor, Pfizer, France) (MIMA2, INRAE, 2018, Microscopy and Imaging Facility for Microbes, Animals and Foods, https://doi.org/10.15454/1.5572348210007727E12). Although the DXA scan does not allow separating measurements of visceral and abdominal subcutaneous fat, or separate measurements of intermuscular, intramyocellular, and subcutaneous fat in the legs (Him limb), it does provide potential clinically important information from a relatively simple procedure. Trunk fat was the amount of fat measured by the DXA from below the neck to the pelvis. Trunk fat was measured as a surrogate for abdominal adiposity, as the DXA scan software does not permit measurement of abdominal fat, drowned in the middle of the viscera The Trunk-to-Leg Fat Ratio (or the opposite) is known to be highly correlated to insulin resistance (Gavi et al., 2007).

#### 2.3.4. Metabolic challenge

A metabolic challenge was performed in both control (C) and exposed (E) males from 5.5 to 7.5 month of age. The animals were randomly assigned to their challenge group. They were given, for two months, either an obesogenic diet (OD) - male controls fed OD (MCO, N = 6) and male exposed fed OD (MEO, N = 6) - or a standard diet (SD) - male controls fed SD (MCS, N = 6) and male exposed fed SD (MES, N = 10). The OD diet was a high-fat high-sugar diet with increased energy (+18% compared to the SD), with lard (+276% fats compared to the SD) and beet sugar (+269% carbohydrates compared to the SD), as described elsewhere (Hue-Beauvais et al., 2017). Two males (1 MCS and 1 MEO) died during the experimental period.

Females were kept on the control diet, but pregnancy being considered as a period of metabolic stress (Herrera and Desoye, 2016; Hill and Szlapinski, 2020; King, 2000), the gestational metabolism was explored. Altogether, 6.5-month-old F1 females (N = 11 control and N = 11 *in utero* exposed) were mated to control males. Only 5 out of 11 control females were diagnosed as pregnant, among which one died at mid-pregnancy and another aborted; in the exposed group, 10 out of 11 were pregnant, 1 female aborted at mid-pregnancy. All females that were still pregnant (N = 3 controls, FCP, and N = 9 exposed, FEP) were euthanized at 28 dpc to collect second generation (F2) feto-placental units (data not shown (Rousseau-Ralliard et al., 2019b; Valentino et al., 2016)). The remaining non-pregnant females (N = 3 controls (FCN), N = 6 exposed (FEN) were maintained until 7.5 months in standard dietary conditions (Fig. 1).

### 2.4. Postmortem phenotyping of adult F1 rabbits

All F1 offspring were euthanized at 7.5 months of age for biometrical measurements and further histological and biochemical analyses. Euthanasia was performed by exsanguination on animals previously rendered unconscious by electronarcosis. The electrical stunning (electronarcosis), using the head-only method, is used as stunning method in rabbit slaughter facilities. A rapid bleeding within 5 s is essential to avoid any return to consciousness, and for that a transverse incision of the neck was performed, by severing both carotid arteries for rapid bleeding.

#### 2.4.1. Classical biochemistry

Triglycerides, cholesterol, glycaemia, urea, creatinine, ASAT (aspartate aminotransferase, predominating in muscles and heart) and ALAT (alanine aminotransferase, predominating in liver) activities, and insulin assays were performed on EDTA-plasma using Beckman Coulter equipment.

#### 2.4.2. Fatty acid analyses

Altered fatty acid profiles in several organs such as liver or heart can be used to characterize cardiometabolic disorders. Four hundred mg samples of cardiac ventricle and liver were used for fatty acid analysis as previously described (Rousseau-Ralliard et al., 2019b). Briefly, a classical 'Folch' lipid extraction was performed. Then neutral lipids (intracellular lipid store) and phospholipids (tissue membranes) were separated on silica acid cartridges (Supelco, Bellefonte, PA). Fatty acids (FAs) were transmethylated with BF3-methanol 7% (Boron trifluoride-methanol solution, Sigma) and analyzed by gas chromatography-FID (GC 3900 Varian, France) on an Econo-Cap EC-WAX capillary column. The FA profile was established for each sample with FA expressed as a percentage of the total of FAs. Heptadecanoic acid (C17:0), introduced prior transmethylation, was used as an internal standard to obtain a relative quantification of total FAs. The analyses identified FA from C14:0 to C24:1n-9. Only a few relevant FAs are presented, but all of the identified FA were considered in totals.

#### 2.4.3. Gene expression in male adult F1 livers

Total RNA was extracted from 10 control male (MC) livers and 10 exposed male (ME) livers (5 per metabolic challenge group) as described previously (Chomczynski and Sacchi, 1987, 2006) and purified using the RNAeasy mini kit (Qiagen, Courtaboeuf, France). Reverse transcription was performed as previously described (Tarrade et al., 2013). A few genes were targeted to explore the metabolism and inflammatory status of male livers: ALB (Albumin), ORM1 (orosomucoid 1), FGB (fibrinogen), CRP (C-reactive protein), AHR (aryl hydrocarbon receptor), IL1beta (interleukin 1 beta), TGFbeta (transforming growth factor beta), COX2 (cyclooxygenase 1), CD36 (cluster of differentiation 36), TNFalpha (tumor necrosis factor), IRS1 (substrate 1 of insulin receptor), RAGE (receptor for advanced glycation endproducts). Relative quantification of these genes was performed and calculated using Applied Biosystems software, with rabbit-specific primer sequences (Supplementary Table 1). Data analyses were undertaken with biogazelle QbasePLUS software ® (www.qbaseplus.com; Biogazelle). To determine the normalized relative quantity (NRQ), eukaryotic translation initiation factor 4E family member 2 (EIFAE2), ribosomal protein L18 (RPL18), and beta 2 globulin (B2M), as selected by geNorm, were used as housekeeping genes. The NRQ value was divided by run and gene-specific calibration factor to determine the calibrated NRQ (CNRQ) as previously described (Rousseau-Ralliard et al., 2019a; Vandesompele et al., 2002).

### 2.5. Statistics

Data are expressed as the median [Q1; Q3], with the first (Q1) and third quartiles (Q3) corresponding to 25 and 75% of scores, respectively. For ivGTT, maximum plasma glucose and insulin increments at each time point and AUC for insulin and glucose were compared. To measure the effect of *in utero* exposure to diesel exhaust on pre-challenge parameters (biochemistry, body composition and cardiovascular parameters) and gene expression, statistical analyses were performed using a linear mixed effects model (nlme package, R, Pinheiro, Bates, DebRoy, Sarkar and the R Development Core Team, 2013. nlme. R package version 3.1–111; www.r-project.org/), with F0 dams as random effect and the following covariates: litter size at birth, antibiotic therapy at weaning and sex (male or female, in youth F1 adults). The statistical effect of each covariate was analyzed, when this effect was significant, the covariate was taken into account in the final adjusted model, when it was not significant, this covariate was removed from the final adjusted model, in an approach of backward selection. In addition, when no effect of exposure was observed on entire groups (including males and females), the linear model was re-applied to test the effect of exposure within each group, according to its sex, that is to say in groups of males on one side and then in groups of females, in order to point out a sex-specific response to this exposure. This effect is presented in the last

column entitled "adjusted statistical effect per sex" in Tables 1 and 2.

Then, to measure the effect of *in utero* exposure to DE on several post-challenge parameters, namely the blood pressure differential between 4.5 and 7 months of age in males, as well as the post-challenge biochemistry, biometry and fatty acid analyses in both sex groups, non-parametrical statistical analyses were applied using a Kruskal-Wallis test, with Dunn's multiple comparisons test. For lipid analyses on liver and heart samples and plasma, principal component analyses (PCA) were additionally performed using the FactomineR (R package dedicated to multivariate data analysis (Lê et al., 2008)) to analyze the biological relevance of the significant differences observed.

## 3. Results

### 3.1. Phenotype until 5.5 months

Overall data are presented in Tables 1 and 2. Exposed refers to F1 animals exposed during their intrauterine life.

#### 3.1.1. Growth and food intake

No significant change according to group or sex was observed for growth until 5.5 months of age (Supplementary Fig. 1A). Mean food intake, as measured over 2 weeks at approximately 5 months of age, was not different either (Supplementary Fig. 1B).

#### 3.1.2. Glucose homeostasis at 4.5 months

Glucose homeostasis was not affected by *in utero* exposure nor sex (Supplementary Fig. 2A and C), whereas insulin homeostasis tended to be increased in both exposed males and females, with an increased normalized insulin AUC ($p = 0.061$), mostly in males, during the ivGTT (Table 1; Supplementary Fig. 2B and D).

#### 3.1.3. Body composition at 5 months

A few differences were observed according to group at 5 months of age, with sex-specific responses (Table 1). *In utero* exposure to diesel exhaust induced a non-significant decrease in lean body mass ($p = 0.089$) and significantly reduced hind limb fat mass percentage ($p = 0.011$), namely peripheral fats, in males, whilst in females, exposure tended to reduce trunk bone density ($p = 0.084$) and hind limb lean mass ($p = 0.080$). The trunk fat-to-hindlimb fat mass ratio was increased in exposed animals, and particularly in males.

#### 3.1.4. Plasma biochemistry

At 5-months of age, ALAT ($p = 0.023$) was increased whereas HDL cholesterol ($p = 0.042$) was decreased in exposed males, whilst females tended to have increased triglyceridemia ($p = 0.078$). The triglyceride-to-HDL cholesterol ratio increased significantly in both exposed females and males ($p = 0.014$) (Table 2).

Because postnatal responses to *in utero* exposure were sex-specific, females and males were thereafter submitted to different metabolic challenges (pregnancy in females being considered as a metabolic challenge, and obesogenic diet in males) in order to explore any conditioning underlying greater adaptive capacity or sensitivity to metabolic challenge. However, since the size group is small in the challenge portion of the study, these results should be considered with caution and will have to be confirmed.

### 3.2. Post-challenge phenotype at 7.5 months

#### 3.2.1. Cardiovascular parameters

Blood pressure recordings were performed after one month of dietary challenge (7 months of age) in males. Overall, because of individual variation and because group size was divided by 2 to distribute individuals into the two dietary groups, the power of the experiment was too low to evidence differences between groups at 28 weeks. Changes in cardiovascular parameters over time, however, were calculated for each individual between 18 and 28 weeks of age, i.e., before and after dietary challenge. For males fed the standard diet, the increase in blood pressure was significantly higher ($p = 0.031$) in exposed *versus* control males, for which pressure was more stable over time (Fig. 2). When animals were

**Table 1**
Clinical biology in control (C) and *in utero*-exposed (E) F1 male and female rabbits maintained under standard conditions up to five months of age.

| Blood parameters | C males N = 10 | E males N = 16 | C females N = 13 | E females N = 28 | Exposure effect | Sex effect | Adj stat per sex |
|---|---|---|---|---|---|---|---|
| Blood glucose (mmol/L) | 6.9 [6.5; 7.3] | 7.3 [6.9; 7.8] | 6.8 [6.7; 7.0] | 6.9 [6.6; 7.6] | 0.472 | ns | – |
| Insulin (mUI/L) | 1.9 [1.5; 2.8] | 2.0 [0.9; 3.6] | 0.6 [0.4; 2.4] | 2.0 [0.6; 2.8] | 0.187 | ns | – |
| Cholesterol (mmol/L) | 0.89 [0.80; 0.92] | 0.75 [0.70; 0.80] | 0.83 [0.73; 0.99] | 0.87 [0.74; 1.02] | 0.855 | ns | – |
| HDL cholesterol (mmol/L) | 0.67 [0.63; 0.69] | 0.57 # [0.56; 0.63] | 0.62 [0.56; 0.80] | 0.68 [0.57; 0.71] | 0.897 | ns | 0.042 EM < CM |
| non HDL cholesterol (mmol/L) | 0.18 [0.17; 0.21] | 0.16 [0.13; 0.19] | 0.20 [0.18; 0.22] | 0.19 [0.16; 0.28] | 0.128 | ns | – |
| Triglycerides (mmol/L) | 0.26 [0.25; 0.28] | 0.28 [0.26; 0.41] | 0.23 [0.20; 0.25] | 0.31 # [0.23; 0.39] | 0.126 | ns | 0.078 EF > CF |
| ASAT (UI/L) | 18.0 [16.5; 20.0] | 17.0 [15.0; 21.5] | 20.5 [19.3; 21.8] | 16.0 [15.0; 17.8] | 0.550 | ns | – |
| ALAT (UI/L) | 19 [17; 20] | 26 # [20; 31] | 31 [24; 38] | 23 [19; 29] | 0.408 | ns | 0.023 EM > CM |
| Creatinine (μmol/L) | 78 [74; 83] | 79 [72; 96] | 86 $ [80; 96] | 88 $ [78; 97] | 0.726 | 0.003 M < F | – |
| Urea (mmol/L) | 6.6 [5.9; 7.3] | 6.6 [6.2; 7.2] | 9.2 [7.8; 9.8] | 8.2 [7.5; 9.0] | 0.561 | ns | – |
| TAG/HDL chol ratio | 0.38 [0.36; 0.40] | 0.51 * [0.44; 0.66] | 0.36 [0.33; 0.43] | 0.44 * [0.39; 0.53] | 0.014 E > C | 0.055 (t) M > F | – |
| Glucose AUC (ivGTT) | 21979 [19935; 24628] | 23644 [22476; 24998] | 22335 [21131.7; 26153] | 22130 [20282; 23437] | 0.629 | ns | – |
| Insulin AUC (ivGTT) | 947 $ [791; 1253] | 1180 $ [766; 1647] | 602 [415; 887] | 741 [529; 932] | 0.186 | 0.001 M > F | – |
| Norm glucose AUC (ivGTT) | 9664 [7798; 10916] | 10947 [10427; 12469] | 10591 [7717; 12403] | 9497 [7826; 11237] | 0.852 | ns | – |
| Norm insulin AUC (ivGTT) | 701 $ [435; 1402] | 1009 # $ [742; 1659] | 315 [315; 1290] | 460 t [460; 1122] | 0.061 (t) E > C | 0.046 M > F | 0.049 EM > CM |

Data are expressed as the median [Q1; Q3]. C: controls; E: exposed; M: males; F: females. Statistics: effect of exposure in the whole groups, including both males and females, was performed using a linear mixed effects model (R), with F0 dams as random effect and the following covariates: litter size at birth, antibiotic therapy and sex of youth F1 adults, with *: $p < 0.05$ or t: with $0.05 < p < 0.1$; $: sex-specific difference, with $p < 0.01$; when considering the effect of exposure, the gender of the individual may or may not have a significant effect. When it is significant, it should be taken into account in the final adjusted model and the p-value is provided. When it has no significant effect, this parameter is removed from the final adjusted model (the sex effect is then denoted "ns"), it is a backward selection. Adj stat per sex: statistics adjusted for each sex considered separately concerning the effect of exposure, only significant effect of exposure for males or females only is reported, if applicable, with #: $p < 0.05$.

**Table 2**
Body and regional composition (iDXA) in control (C) and *in utero*-exposed (E) five-month-old F1 rabbits maintained under standard conditions.

| | C males N = 10 | E males N = 16 | C females N = 13 | E females N = 28 | Exposure effect | Sex effect | Adj stat per sex |
|---|---|---|---|---|---|---|---|
| Whole body mass (g) | 3904 [3626; 4024] | 3623 [3520; 3881] | 3813 [3542; 4292] | 3903 [3677; 4208] | 0.575 | 0.063 M < F | – |
| Body fat (g) | 379 [327; 449] | 271 [216; 416] | 482 $ [386; 527] | 519 $ [387; 595] | 0.691 | <0.001 M < F | – |
| Lean body mass (g) | 3351 [3208; 3489] | 3242 (#) [3162; 3368] | 3241 [3210; 3507] | 3317 [3122; 3533] | 0.566 | ns | 0.089 t EM > CM |
| Percentage of fat mass (%) | 10.1 [9.0; 11.6] | 7.5 [6.1; 11.3] | 12.2 $ [10.2; 13.3] | 12.9 [10.5; 15.1] | 0.831 | ns | – |
| Body bone density (g/cm$^2$) | 0.339 [0.334; 0.348] | 0.343 [0.339; 0.349] | 0.362 [0.352; 0.371] | 0.352 [0.344; 0.358] | 0.376 | 0.074 M < F | – |
| Body trunk mass (g) | 2875 [2618; 2972] | 2631 [2568; 2888] | 2858 [2606; 3252] | 2922 [2768; 3126] | 0.973 | 0.005 M < F | – |
| Trunk fat (g) | 298 [242; 344] | 215 [166; 335] | 387 [306; 427] | 425 [295; 468] | 0.772 | ns | – |
| Lean trunk mass (g) | 2485 [2351; 2585] | 2373 [2320; 2495] | 2410 [2385; 2626] | 2495 [2359; 2662] | 0.805 | ns | – |
| Percentage of trunk fat mass (%) | 10.8 [9.2; 12.0] | 8.1 [6.4; 12.2] | 13.2 [10.5; 14.1] | 13.8 [11.0; 15.8] | 0.785 | ns | – |
| Trunk bone density (g/cm$^2$) | 0.293 [0.283; 0.305] | 0.294 [0.289; 0.298] | 0.307 [0.304; 0.317] | 0.299 (#) [0.293; 0.310] | 0.066 | ns | 0.084 t (EF < CF) |
| Hind limb mass (g) | 463 [459; 500] | 465 [448; 487] | 492 [485; 511] | 465 [451; 483] | 0.148 | ns | – |
| Hind limb fat (g) | 17 [16; 18] | 8 # [6; 11] | 23 $ [12; 28] | 19 $ [14; 23] | 0.155 | p < 0.001 M < F | 0.013 EM < CM |
| Hind limb lean mass (g) | 423 [418; 454] | 436 [419; 451] | 442 [435; 466] | 426 (#) [408; 445] | 0.238 | ns | 0.080 t (EF < CF) |
| Percentage of hind limb fat mass (%) | 3.8 [3.4; 3.9] | 1.9 # [1.3; 2.2] | 4.5 $ [3.6; 5.7] | 4.0 $ [3.1; 4.8] | 0.366 | p < 0.001 M < F | 0.011 EM < CM |
| Hind limb bone density (g/cm$^2$) | 0.332 [0.328; 0.335] | 0.327 [0.322; 0.334] | 0.351 [0.337; 0.358] | 0.335 [0.328; 0.346] | 0.146 | ns | – |
| Trunk fat to hindlimb fat mass ratio | 18.1 [15.1; 19.3] | 32.8 (#) [17.8; 45.7] | 16.8 [15.0; 19.1] | 21.7 [16.8; 28.4] | 0.049 E > C | 0.008 M > F | 0.058 EM > CM |
| Trunk fat to hindlimb % fat mass ratio | 2.97 [2.59; 3.30] | 5.76 * # [3.65; 7.49] | 2.60 [2.49; 3.56] | 3.29 * [2.94; 4.42] | 0.016 E > C | 0.007 M > F | 0.025 EM > CM |

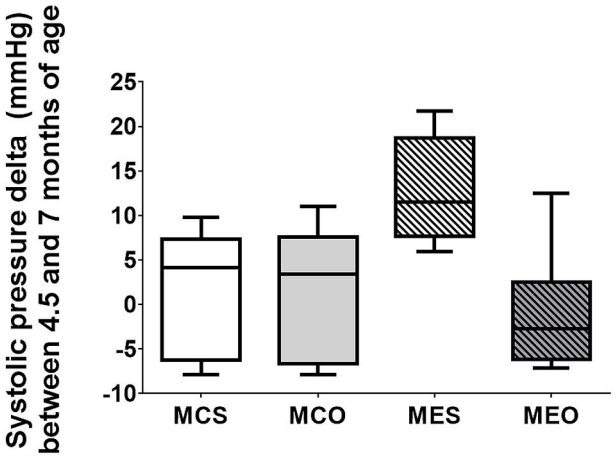


**Fig. 2.** Evolution of blood pressure over 10 weeks (from 18 to 28 weeks of age) in control (C) or exposed (E) F1 males, fed a standard (S) or obesogenic (O) diet. The animals were randomly assigned to their challenge group. They were given either an obesogenic diet (OD; N = 6 MCO and N = 6 MEO, respectively) or a standard diet (SD; N = 6 MCS, N = 10 MES, respectively). Statistics: * indicating a significant difference between MCS (n = 6) and MES (n = 10) using kruskal-Wallis test with p = 0.031.

fed the obesogenic diet, these differences were no longer present (data not shown).

### 3.2.2. Plasma biochemistry at 7.5 months of age

Clinical parameters in 7.5 months offspring are presented in Table 3. At this stage, the exposed males still had significantly reduced HDL cholesterol (p = 0.033) and hyperglycaemia (p = 0.009), regardless of the diet. Exposed males fed an obesogenic diet presented a slight not significant increase in insulinemia (p = 0.082). In exposed non-pregnant females decreased ALAT (p = 0.005) and non-significant decreased ASAT (p = 0.078) were observed. Exposed females tended to have higher plasma urea concentrations compared to their respective controls (p = 0.072), but this slight difference disappeared with gestation.

### 3.2.3. Post-mortem biometry at 7.5 months of age

Biometric data are presented in Table 4. In exposed males, regardless of the diet, perirenal fat (PRF, p = 0.078) as well as heart (p = 0.057, including atria, p = 0.058) and perirenal fat (PRF, p = 0.065) relative to body weight ratios tended to increase. Liver-to-body weight ratio was increased in SD-fed exposed males compared to its control (p = 0.026, Supplementary Table 3). Obesogenic diet, regardless of the exposure, increased body weight as well as interscapulum fat (ISF, p = 0.005) and PRF. PRF weight was more pronounced in exposed animals, regardless of the diet. In contrast, the increase in liver weight was only observed in OD-fed controls (p = 0.027. In addition, the obesogenic diet increased PRF relative to body weight ratio, regardless of the exposure, whilst exposure reduced the increase in ISF and liver relative to body weight ratios, compared to the SD-fed control group.

In females, exposure decreased body weight as well as liver (p < 0.001), lungs (p = 0.031), spleen (p = 0.040) weights in both non-pregnant and pregnant animals. In terms of ratios, only the heart relative to body weight (p = 0.039) was increased, compared to the respective controls. Gestation led to an increase in heart-to-body weight ratio (p = 0.039), while ISF-to-body weight ratio tended to decrease in controls (p = 0.052, fat mass melting). However, PRF tended to increase in pregnant exposed compared to control females (p = 0.050, Table 4, Supplementary Table 3).

### 3.2.4. Fatty acid concentration in plasma, liver and heart

Fatty acid (FA) concentrations in adult offspring tissues are reported in Table 5. In the liver, under standard diet, the concentration of fatty acids in phospholipids was increased in exposed males but not in females. The obesogenic diet led to an increase in FA concentration in phospholipids, reflecting a hepatic membrane hypertrophy, in the controls, whereas in OD-fed exposed males, no membrane hypertrophy was

**Table 3**
Clinical parameters in control (C) and *in utero-exposed* (E) F1 male and female rabbits in adulthood (7.5 months of age), maintained in standard condition or metabolically challenged (either obesogenic diet in males or gestation in females), between 5.5 and 7.5 months of age.

| Clinical parameters | Males | | | | Effect | Females | | | | Effect |
|---|---|---|---|---|---|---|---|---|---|---|
| Challenge | SD | | OD | | | Non-pregnant | | Pregnant | | |
| Exposure group | C | E | C | E | | C | E | C | E | |
| Number | N = 6 | N = 10 | N = 6 | N = 7 | | N = 4 | N = 8 | N = 3 | N = 9 | |
| Glycaemia (mmol/L) | 7.0 [6.8; 7.4] | 7.7 * [7.2; 8.1] | 7.5 [7.1; 7.6] | 8.5 * [7.9; 9.0] | E > C | 6.9 [6.6; 7.6] | 6.8 [6.5; 6.8] | 6.8 [6.7; 6.9] | 7.6 [7.3; 8.0] | – |
| Insulin (mUI/L) | 2.4 [2.3; 2.6] | 1.2 [0.9; 2.8] | 1.9 [1.5; 2.2] | 4.1 # [3.6; 10.9] | E + D ⇧ | 1.6 [0.8; 2.8] | 1.4 [0.6; 2.6] | 0.6 [0.3; 2.1] | 2.7 [1.5; 4.9] | – |
| Cholesterol (mmol/L) | 0.95 [0.81; 0.96] | 0.80 * [0.75; 0.86] | 0.93 [0.90; 1.13] | 0.85 * [0.72; 0.92] | E > C | 0.96 [0.84; 1.11] | 0.94 [0.86; 1.00] | 0.80 [0.72; 0.98] | 0.85 [0.76; 1.03] | – |
| HDL cholesterol (mmol/L) | 0.67 [0.61; 0.69] | 0.60 * [0.55; 0.63] | 0.70 [0.67; 0.86] | 0.62 * [0.53; 0.67] | E < C | 0.72 [0.62; 0.82] | 0.70 [0.65; 0.71] | 0.60 [0.55; 0.78] | 0.70 [0.63; 0.83] | – |
| non HDL cholesterol (mmol/L) | 0.24 [0.18; 0.28] | 0.20 [0.15; 0.28] | 0.25 [0.23; 0.27] | 0.20 [0.17; 0.23] | – | 0.24 [0.22; 0.29] | 0.25 [0.20; 0.31] | 0.19 [0.18; 0.21] | 0.17 [0.09; 0.20] | |
| Triglycerides (mmol/L) | 0.29 [0.27; 0.31] | 0.30 [0.28; 0.33] | 0.29 [0.28; 0.33] | 0.46 # [0.31; 0.85] | E + D ⇧ | 0.23 [0.22; 0.27] | 0.30 t [0.26; 0.42] | 0.23 [0.20; 0.25] | 0.33 t [0.28; 0.41] | t: (E > C) |
| ASAT (UI/L) | 22 [21; 24] | 21 [19; 25] | 22 [17; 28] | 19 [19; 21] | – | 22 [20; 23] | 16 * [14; 17] | 20 [19; 21] | 17 * [15; 17] | E < C |
| ALAT (UI/L) | 17 [15; 20] | 26 [17; 34] | 20 [19; 20] | 22 [19; 32] | – | 34 [31; 37] | 19 * [16; 22] | 31 [23; 39] | 28 [23; 35] | E < C |
| Creatinine (μmol/L) | 85 [85; 86] | 91 [84; 98] | 87 [83; 96] | 78 [74; 85] | – | 91 [87; 95] | 96 [91; 99] | 85 [78; 97] | 75 [63; 86] | – |
| Urea (mmol/L) | 6.7 [5.9; 7.6] | 6.7 [6.2; 7.0] | 6.5 [5.7; 6.9] | 6.1 [5.7; 6.3] | – | 7.3 [7.1; 7.8] | 8.7 [8.4; 9.1] | 9.6 $ [9.2; 10.1] | 7.7 [6.8; 8.7] | G ⇧ |

Data are expressed as the median [Q1; Q3]. Although presented together in the same table to provide a general overview of the data, data from males and females were considered and treated separately. Statistics in male and female groups: the effect of exposure combined with the challenge (nutritional in males and gestational in females), compared to standard condition, was analyzed by a non-parametric statistical test of Kruskal-Wallis test, followed by, if applicable, a pairwise comparison with Dunn's multiple comparisons test. The significant effect of exposure regardless of the challenge is noted *, with *: $p < 0.05$ (and with t: $0.05 < p < 0.1$); a specific effect of the challenge is noted $, with $: $p < 0.05$; and a specific response of exposure only when combined with the challenge is noted #, with #: $p < 0.05$. ASAT or AST: aspartate transaminase; ALAT or ALT: alanine aminotransferase; C: controls; D: Diet effect; E: in utero-exposed offspring; G: gestation effect; OD: obesogenic diet; SD: standard diet; t: tendency.

**Table 4**
Postmortem biometry: Biometry (raw data) in adult control (C) and *in utero-exposed* (E) offspring (7.5 months of age) maintained in standard conditions or metabolically challenged (obesogenic diet in males or gestation in females).

| Clinical parameters | Males | | | | Effect | Females | | | | Effect |
|---|---|---|---|---|---|---|---|---|---|---|
| Challenge | SD | | OD | | | Non-pregnant | | Pregnant | | |
| Exposure group | C | E | C | E | | C | E | C | E | |
| Number | N = 6 | N = 10 | N = 6 | N = 7 | | N = 4 | N = 8 | N = 3 | N = 9 | |
| Bodyweight (kg) | 4.3 [4.2; 4.4] | 4.2 [4.0; 4.4] | 4.6 t [4.5; 4.6] | 4.5 [4.0; 4.5] | t: D⇧ | 4.5 [4.3; 4.8] | 4.0 * [3.9; 4.1] | 5.5 [5.2; 5.7] | 4.9 * [4.7; 5.2] | E < C |
| Heart (g) | 8.6 [8.2; 9.0] | 8.5 [7.8; 9.3] | 8.4 [8.2; 8.7] | 8.7 [8.1; 9.1] | – | 8.3 [7.6; 8.9] | 7.7 [7.4; 8.1] | 11.4 $ [9.3; 11.7] | 8.4 [8.0; 8.9] | G ⇧ |
| Cardiac ventricles (g) | 7.4 [7.1; 7.9] | 7.1 [6.6; 7.9] | 7.3 [7.2; 7.5] | 7.4 [7.1; 7.8] | – | 7.0 [6.5; 7.5] | 6.5 [6.3; 6.7] | 9.3 $ [7.9; 9.8] | 7.4 # [7.0; 7.6] | G ⇧ |
| Cardiac atria (g) | 0.91 [0.91; 0.92] | 1.01 [0.90; 1.13] | 0.85 [0.76; 0.95] | 1.00 [0.90; 1.04] | – | 0.97 [0.84; 1.12] | 0.87 [0.82; 0.94] | 1.21 $ [0.92; 1.45] | 0.93 [0.79; 1.05] | G ⇧ |
| Lungs (g) | 11.4 [11.2; 11.4] | 11.6 [10.4; 12.4] | 11.3 [10.8; 12.0] | 10.6 [10.1; 11.8] | – | 11.4 [10.9; 12.2] | 10.0 * [9.7; 10.5] | 12.4 [12.2; 12.9] | 10.9 * [10.6; 11.8] | E < C |
| Liver (g) | 67.3 [63.8; 72.8] | 69.6 [63.4; 76.7] | 86.1 $ [84.5; 88.2] | 77.5 [68.5; 78.7] | E + D ⇧ | 72.7 [70.1; 81.6] | 64.7 * [62.7; 66.9] | 103 $ [94; 107] | 96 $ [91; 106] | E < CG ⇧ |
| Interscapulum fat (ISF, g) | 37.9 [30.6; 56.5] | 37.5 [31.1; 50.7] | 73.9 $ [71.7; 82.6] | 48.5 # [40.1; 51.7] | E + D ⇧ | 58.4 [51.2; 62.5] | 46.0 [32.2; 49.3] | 57.7 [53.6; 61.8] | 38.3 [32.8; 56.4] | – |
| Perirenal fat (PRF, g) | 122 [121; 133] | 152 * [118; 175] | 178 $ [166; 219] | 196 $* [142; 239] | E > CD ⇧ | 157 [153; 163] | 145 [122; 156] | 130 $ [113; 138] | 176 [127; 192] | G ⇩ |
| Kidneys (g) | 15.9 [15.7; 16.4] | 16.9 [14.5; 17.9] | 17.7 $ [16.9; 18.8] | 17.2 [15.7; 17.6] | D ⇧ | 16.2 [15.7; 16.8] | 15.0 [14.3; 15.2] | 18.5 $ [17.3; 19.2] | 17.8 $ [16.6; 19.1] | G ⇧ |
| Spleen (g) | 1.39 [1.35; 1.60] | 1.34 [1.25; 1.43] | 1.21 [1.18; 1.64] | 1.45 [1.07; 1.62] | – | 1.35 [1.28; 1.46] | 1.90 * [1.69; 2.01] | 1.94 $ [1.74; 2.22] | 1.72 $ [1.66; 1.89] | E ⇧G ⇧ |
| Adrenals (g) | 0.25 [0.22; 0.28] | 0.26 [0.24; 0.28] | 0.28 [0.27; 0.28] | 0.28 [0.25; 0.29] | – | 0.27 [0.25; 0.27] | 0.21 [0.19; 0.27] | 0.21 [0.19; 0.27] | 0.21 [0.19; 0.22] | – |

Data are expressed as the median [Q1; Q3]. Although presented together in the same table to provide a general overview of the data, data from males and females were considered and treated separately. Statistics in male and female groups: the effect of exposure combined with the challenge (nutritional in males and gestational in females), compared to standard condition, was analyzed by a non-parametric statistical test of Kruskal-Wallis test, followed by, if applicable, a pairwise comparison with Dunn's multiple comparisons test. The significant effect of exposure regardless of the challenge is noted *, with *: $p < 0.05$ (and with t: $0.05 < p < 0.1$); a specific effect of the challenge is noted $, with $: $p < 0.05$; and a specific response of exposure only when combined with the challenge is noted #, with #: $p < 0.05$. BW: body weight; C: controls; D: Diet effect; E: in utero-exposed offspring; G: gestation effect; ISF: interscapulum fat; OD: obesogenic diet; PRF: perirenal fat; SD: standard diet.

**Table 5**
Concentrations of fatty acids (in μg/g of tissue, cardiac ventricle or liver) in adult control (C) and *in utero-exposed* (E) offspring (7.5 months of age) maintained under standard conditions or metabolically challenged (obesogenic diet in males or gestation in females) at 7.5 months of age.

| Sex | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Challenge | SD | | OD | | Non-pregnant | | Pregnant | |
| Group | C | E | C | E | C | E | C | E |
| Number | N = 5 | N = 8 | N = 5 | N = 5 | N = 3 | N = 8 | N = 3 | N = 9 |
| Plasma FA | 1352 [955; 1775] | 940 [813; 1283] | 1053 [715; 1715] | 1315 [739; 1467] | 771 [720; 846] | 828 t [824; 869] | 732 [655; 893] | 762 [654; 903] |
| Ventricle NL | 9.3 [9.2; 9.4] | 12.4* [11.6; 13.1] | 12.5 [11.7; 13.8] | 14.8* [14.0; 16.0] | 20.1 [13.4; 22.8] | 27.8* [21.4; 36.5] | 30.7$ [30.4; 32.7] | 28.1# [27.2; 29.9] |
| Ventricle PL | 7.9 [7.8; 8.1] | 9.4* [8.0; 12.2] | 8.6 [8.2; 9.3] | 10.9* [10.0; 11.6] | 12.1 [10.9; 13.0] | 13.0 [10.5; 15.5] | 8.1 [7.4; 11.0] | 15.4*# [14.4; 15.7] |
| Hepatic NL | 14.7 [14.3; 15.4] | 15.1 [13.9; 16.3] | 23.2$ [21.0; 24.4] | 15.2# [15.2; 16.8] | 19.7 [19.2; 22.0] | 20.5 [19.2; 21.3] | 44.9$ [42.9; 55.0] | 108.8*$# [94.5; 127.8] |
| Hepatic PL | 15.3 [13.7; 15.8] | 20.4* [19.2; 22.3] | 21.0$ [20.7; 21.6] | 15.1# [14.7; 15.7] | 25.4 [24.3; 26.7] | 26.9 [25.9; 29.5] | 16.5$ [15.3; 17.6] | 18.5$ [16.0; 23.3] |

Data are expressed as the median [Q1; Q3], in μg/mL of plasma or mg/g of tissue. Although presented together in the same table to provide a general overview of the data, data from males and females were considered and treated separately. Statistics in male and female groups: the effect of exposure combined with the challenge (nutritional in males and gestational in females), compared to standard condition, was analyzed by a non-parametric statistical test of Kruskal-Wallis test, followed by, if applicable, a pairwise comparison with Dunn's multiple comparisons test. The significant effect of exposure regardless of the challenge is noted *, with *: $p < 0.05$ (and with t: $0.05 < p < 0.1$); a specific effect of the challenge is noted $, with $: $p < 0.05$; and a specific response of exposure only when combined with the challenge is noted #, with #: $p < 0.05$. Plasma FA concentration tend to be significantly different between non-pregnant C and E females ($p = 0.0823$). C: controls; E: in utero-exposed offspring; NL: neutral lipids (intracellular lipid stores); OD: obesogenic diet; PL: phospholipids (cell membranes); SD: standard diet.

observed. Moreover the obesogenic diet was associated to increased intracellular lipid storage in controls but not in exposed animals. In females, gestation was accompanied by decreased amount of hepatic membranes (estimated by the amount of phospholipid fatty acids per gram of liver), whereas hepatic storage lipids increased with pregnancy and even more so in pregnant exposed females that developed a fatty liver, that is, accumulation of fat inside the liver cells. These data were corroborated by histological analyzes for the liver (Supplementary Fig. 4).

In the heart, the concentration of fatty acids in phospholipids was higher in exposed males regardless of dietary challenge, while this was observed only in exposed females when they were pregnant. The increase in phospholipids reflects hypertrophy of the cardiac tissue membranes. Similarly, the concentration of fatty acids in neutral lipids, i.e. intracellular storage lipids, was higher in exposed males regardless of their diet and in exposed non-pregnant females. In pregnant exposed females, the concentration was higher than that in non-pregnant controls but was not different from pregnant controls as the concentration increased with pregnancy in both groups.

Regarding plasma, there was no statistically significant difference between the groups, except in exposed non-pregnant females that tended to have a higher FA concentration compared to controls.

#### 3.2.5. Qualitative fatty acid profiles of hepatic and cardiac (ventricles) membrane and plasma

No significant qualitative change was observed in liver membrane fatty acid profiles from exposed rabbits (Supplementary Table 4 and Supplementary Fig. 3). The differences observed were mostly related to the sex of the animals, their diet, in males, or their status, in females (pregnant or not). No significant qualitative change was induced by exposure in plasma fatty acid profiles (Supplementary Table 6 and Supplementary Fig. 4). The cardiac membrane fatty acid profiles in adult offspring are shown in Supplementary Table 5 and were analyzed by PCA in Fig. 3. These profiles differed in a sex-specific way, as shown by the confidence ellipses, more saturated, FA profiles in females presented also decreased LA while AA increased compared to males. Exposure had no significant effect on cardiac membrane fatty acid profiles when males and females were raised in standard conditions. The cardiac membrane profiles of fatty acids (phospholipids) were similar between controls and exposed SD-fed males. The two-month-dietary challenge had a strong impact on the fatty acid profiles of cardiac membranes in males. Feeding the males with an OD diet, regardless of exposure, induced a stiffening of the membranes. Indeed, the membranes were enriched in SFAs and MUFAs, to the detriment of PUFA (mostly linoleic acid), except for arachidonic acid and n-3 LCPUFA that increased compared to males fed the standard diet (Fig. 3C and D). In females, exposure induced a global significant effect on cardiac membrane fatty acid profile (Fig. 3E and F) With gestation, membranes became less saturated and more polyunsaturated (increased n-3 PUFA and arachidonic acid while linoleic acid decreased) in controls but in a lower extent in exposed females (Fig. 3E and F).

#### 3.2.6. Liver gene expression and inflammatory status in F1 males

The expression of a few genes involved in inflammation or oxidative stress, among which those coding for CRP or SAA, did not vary between the groups (Fig. 4, data not shown for SAA). In contrast, *FGB* gene expression, the protein of which indicates high inflammatory status, fibrosis or higher risk of cardiovascular disease, tended to increase with *in utero* exposure ($p = 0.056$).

The expression of the *AHR* gene, the protein of which is involved in the regulation of biological responses to polycyclic aromatic hydrocarbons, was not significantly different in males according to exposure, but this is not surprising since the offspring were no longer exposed after birth. *ORM1* gene expression was greatly reduced in exposed F1 rabbits compared to controls ($p = 0.001$). ORM1 protein expression is known for its anti-inflammatory and immunomodulatory effects. On the other hand, *ALB* gene expression tended to increase in exposed F1 rabbits ($p = 0.060$), eliminating hepatic insufficiency as potential effect. *RAGE* (receptor for advanced glycation end-products) gene expression was increased in exposed males, signifying an increase in oxidative stress ($p = 0.026$). *In utero* exposure induced long-lasting effect on the expression of target hepatic metabolic genes in adult males. The obesogenic diet increased significantly *IRS1* expression among these targeted genes in OD-fed controls only ($p = 0.050$).

## 4. Discussion

The objective of this study was to characterize the phenotype of *in utero*-exposed F1 progeny after puberty by evaluating the potential onset of cardiometabolic pathologies. Briefly sex-specific responses to maternal exposure during gestation were observed in adult offspring, corresponding to fetal metabolic programming as summarized in Fig. 5. Age-related increases in blood pressure, glycaemia, trunk-to-leg fat mass ratio and perirenal fat mass as well as reductions in HDL-cholesterol

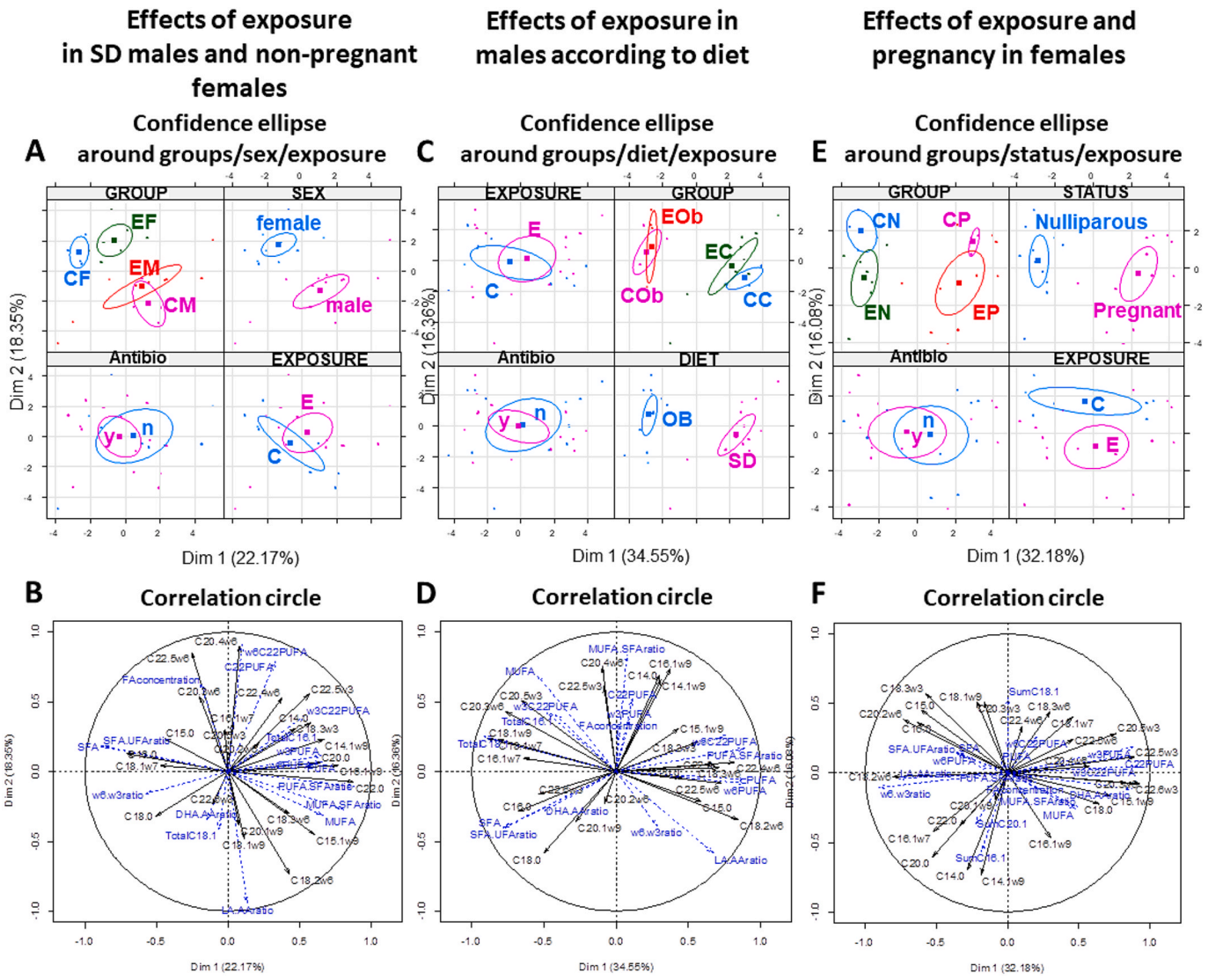


**Fig. 3.** Principal component analyses of cardiac fatty acid profiles from 7.5-month-old F1 offspring, comparing: **A-B: effects of exposure in SD males and non-pregnant females fed a standard diet; - C-D: effects of exposure in males according to diet; - E-F: effects of exposure and pregnancy in females.** Antibio: antibiotic treatment administered to offspring prior to weaning (no effect of the weaning antibiotic treatment was observed); C: control animals; CF: control females; CM: control males; CN: control nulliparous female; CP: control pregnant female; diet: metabolic nutritional challenge applied to the males (OD vs. SD); E: in utero-exposed animals; EF: Exposed females; EM: exposed males; EN: exposed nulliparous female; EP: Exposed pregnant female; n: animals not given an antibiotic; OD: obesogenic diet; SD: standard diet; y: animals given an antibiotic; status: metabolic challenge applied to the females (gestation vs. nulliparity).

were observed in adult *in utero*-exposed males, suggesting a metabolic syndrome. In adult *in utero*-exposed females, only trends were first observed, with higher triglycerides and decreased bone density compared to control females. The use of metabolic challenges in adulthood, either obesogenic diet in males and gestation in females, worsened the phenotypes in exposed animals of both sexes. Such long-term metabolic defects with sex-specificity were reported in rats whose dams were exposed to ultrafine ammonium sulfate aerosols during gestation (Wu et al., 2019).

In the present experiment, it was previously shown that *in utero* exposed rabbit fetuses were growth retarded shortly before term (Valentino et al., 2016). Then, although birthweight was still reduced in "exposed" pups (Hue-Beauvais et al., 2019), they caught up before weaning and there was no difference in weight over the duration of the present postnatal study, for the animal fed a standard diet, as recently observed in humans (Fossati et al., 2020). In mice, *in utero* exposure to pollution has been shown to increase body weight (Bolton et al., 2012) and reduce blood pressure in adulthood (Weldy et al., 2014), but these studies were performed with whole body exposure during the second half of gestation only. Contrary to nose only exposure as performed in the present study, whole body exposure allows the animals to ingest air-borne matter deposited on their fur through their licking/grooming behavior, which induces uncertainty on whether inhaled pollution or ingested pollutants are responsible for the observed effects.

Body composition analyzed by iDXA revealed an overall decrease in body lean mass in all exposed animals and a reduction in hindlimb fat in males only. In men, a loss of lean mass has been correlated with insulin resistance in both the young (Gysel et al., 2014) and the elderly (Lee et al., 2011). In addition, a reduction in the amount of peripheral fat is generally reported in insulin resistance, while abdominal fat is increased (Grundy et al., 2004). The exposed animals presented an increase of trunk-to-leg fat ratio that is known to be strongly associated to insulin resistance (Gavi et al., 2007). In humans, a correlation has been described between exposure to traffic-related air pollution in women during pregnancy and an increase in insulin resistance in their children after birth (Thiering et al., 2013). Despite an unaffected glycemic response to the glucose tolerance test, the young adult males had a higher insulin response to glucose load to maintain glucose homeostasis

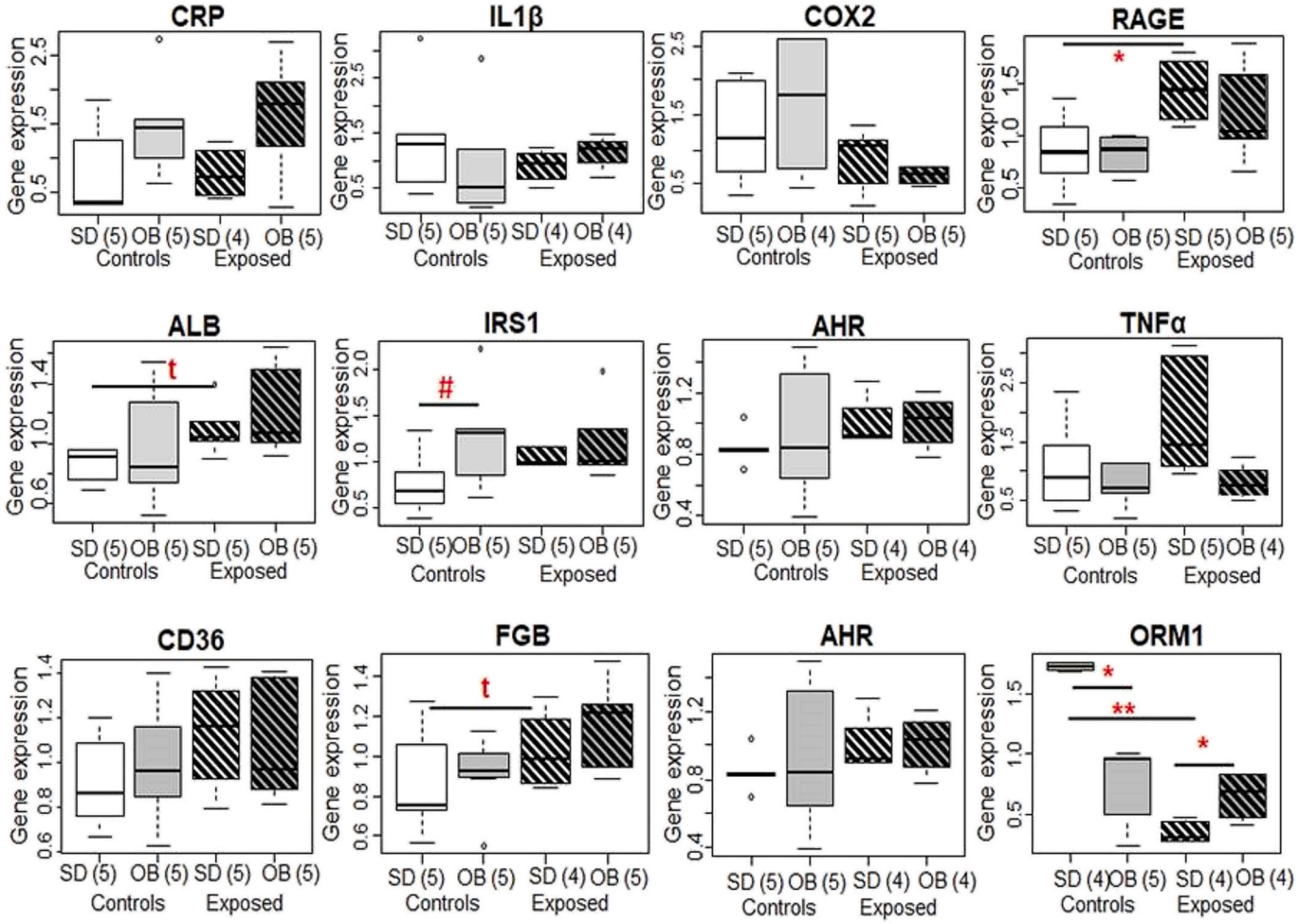


**Fig. 4.** Impact of *in utero* exposure to diesel exhaust on the expression of genes involved in inflammation, oxidative stress or metabolism in the liver of adult male offspring, after undergoing or not a two-month dietary challenge, once adult. Relative expression, obtained by RT-qPCR, of genes encoding albumin (ALB), α1 acid glycoprotein (ORM1), fibrinogen B (FGB), C-reactive protein (CRP), amyloid serum protein A (SAA), aromatic hydrocarbon receptor (AHR), interleukin 1 β (IL1β), cyclooxygenase 2 (COX2), differentiation cluster glycoprotein 36 (CD36), tumor necrosis factor α (TNFα) and insulin receptor substrate 1 (IRS1), advanced glycosylation end product-specific receptor (AGER or RAGE) in the liver of F1 male progeny in utero-exposed to diesel exhausts (exposed) or purified air (control) and subjected or not to a two-month nutritional challenge during adulthood (SD: standard diet, OD: obesogenic diet), N = 4–5 per exposed and diet group. The values were normalized by qBASE software, using 3 reference genes coding for: β2 microglobulin (B2M), 4E translation initiation factor 2 (EIF4E2) and β-actin. Statistically, a mixed-effect linear model associated with type 3 ANOVA followed by a Tukey post hoc test was performed to explore *in utero* exposure and obesogenic diet affects, with, *: $p < 0.05$, **: $p < 0.01$, t: tendency when $0.05 < p < 0.1$, and possible significant interaction, with #: $p < 0.05$. The data are represented by box plots defined by the median, the 1st and 3rd quartiles, and the min and max values.

(i.e., higher normalized insulin AUC), suggesting insulin resistance. The triglyceride-to-HDL cholesterol ratio increased in exposed animals; this ratio is described as a predictive factor of future cardiovascular events (Lee et al., 2021) and a good marker for insulin resistance in non-obese middle-aged population (Yang et al., 2021). Furthermore the young adult exposed rabbits presented an increased trunk-to-peripheral fat ratio that was shown to be predictive of a subsequent blood pressure in normal-weight pubertal boys (Kouda et al., 2020). Indeed, the time duration of the dietary challenge was sufficient to highlight a higher age-related increase in blood pressure parameters, only in exposed males fed with SD diet. Although weak this increase was however statistically significant, and relevant in the overall context of the altered cardiometabolic phenotype in males. Similarly, weak blood pressure effects of prenatal exposure to air pollution were observed in human cohorts of children and adolescents (Falkner, 2019). Cardiovascular disease, which onset is often associated to the metabolic syndrome may also be programmed through exposure to air pollution (Wallwork et al., 2017). Thus, direct exposure to diesel engine exhaust in mice was shown to elicit the production of dysfunctional HDL lipoprotein, through pro-oxidant effects (Yin et al., 2013). Humans exposed to high concentrations of traffic-related air pollutants have reduced HDL cholesterol (Li et al., 2019) and increased triglycerides (Woo et al., 2020). The lipid metabolism of mice inhaling diesel exhaust is similarly affected (Yin et al., 2019). Besides decreased HDL cholesterol, *in utero* exposure to cigarette smoke also leads to increased triglyceridemia in adult women (Cupul-Uicab et al., 2012). In the present study, gestational exposure induced sex-specific responses, with HDL cholesterol being systematically reduced in males, while triglycerides tended to increase in young F1 females, which is also a risk factor of insulin resistance and cardiovascular disease (Ginsberg et al., 2005). Increased ALAT transaminases were observed in exposed males. Reflecting liver function, an increase in ALAT is clinically associated with hepatic cytolysis (Liu et al., 2014). Conversely, in older exposed females, both ALAT and ASAT transaminase levels were reduced compared to controls. Such decreases have been associated with hepatic frailty (Liu et al., 2014).

Some studies have shown an association between direct exposure to air pollution and altered bone development in children (Calderon-Garciduenas et al., 2013; Liu et al., 2015) or bone turnover in

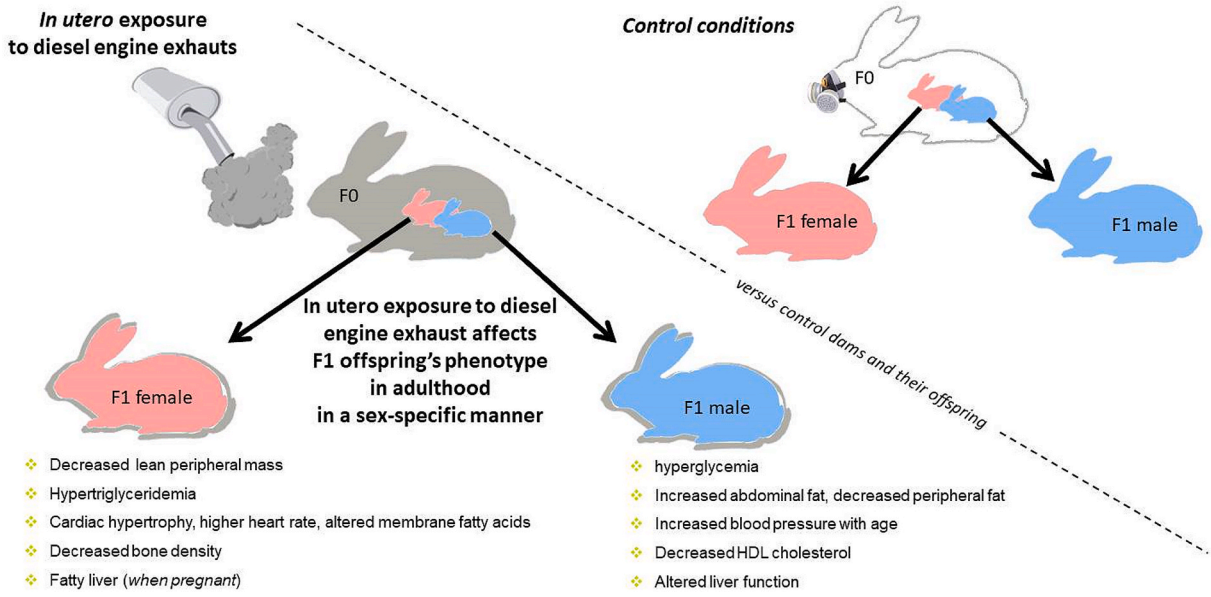


**Fig. 5.** Summary of the observed effects of *in utero* exposure to diesel engine exhaust on F1 adult offspring.

adulthood, leading to an increased risk of bone pathology in adulthood (Alvaer et al., 2007; Chang et al., 2015). Because pollution affects birth weight, special attention has been paid to bone development, and bone densitometry is thus increasingly evaluated in children living in polluted regions of the world (Calderon-Garciduenas et al., 2013). Here, the bone density of exposed females, but not males, tended to be decreased. The effects of air pollution on bone development (Chen et al., 2015) therefore deserve to be further explored. The determination of alkaline phosphatase (ALP), the elevation of which is associated with bone disorders (Lee et al., 2017) or hepatic steatosis, would be an interesting issue.

As commonly observed in transgenerational studies (Lukowicz et al., 2018), a sex-specific offspring phenotype was observed in the present study. In exposed males, the phenotype was more pronounced, the whole clinical picture underlies metabolic syndrome, as described in a dietary-induced rabbit model of metabolic syndrome (Arias-Mutis et al., 2017). In humans, metabolic syndrome is a cluster of risk factors for cardiovascular disease and type 2 diabetes mellitus (DM2) that occur together more often than by chance alone. These risk factors include: i) raised blood pressure; ii) a distinct dyslipidemic phenotype (raised triglycerides and lowered high-density lipoprotein cholesterol); iii) altered glucose metabolism and iv) central obesity. Thus, among the measured parameters in this study - a reduced muscle mass (decreased lean mass, by iDXA), increased ALAT, few markers of inflammation and oxidative stress, decreased HDL and increased blood pressure (with aging), insulin resistance - were observed. Similar cardiometabolic consequences of exposure to air pollutants in the pediatric age were recently evidenced in adolescents (Poursafa et al., 2014) as well as in the case of long-term exposure to traffic-related air pollution (Jiang et al., 2016).

Unlike exposed males, for which the early event (*in utero* exposure) was sufficient to affect the phenotype, in females, a secondary factor (described as "developer" (Hanson and Gluckman, 2014)) was necessary to induce phenotypic effects. Thus, the exposed females did not present a significantly different phenotype from that of the controls, except when they were subjected to the gestational metabolic challenge (Hill and Szlapinski, 2020), inducing dyslipidemia (tendency) together with liver steatosis (Rousseau-Ralliard et al., 2019b). In contrast, a dietary-induced metabolic challenge using an obesogenic diet (Hue--Beauvais et al., 2017) in exposed males failed to induce new metabolic perturbations, particularly on the cardiovascular parameters.

Rabbits are known to fatten when fed an obesogenic diet and develop hepatic steatosis as an early consequence (Sigrist-Flores et al., 2019). Exposed males fed either a standard or an obesogenic diet developed liver hypertrophy (increased hepatic PL), but not fatty liver (normal NL). Contrary to the hepatic steatosis developed in normal chow-fed adult male mouse offspring prenatally exposed to diesel exhaust (Wang et al., 2019), the absence of fatty liver in exposed male rabbits was confirmed by both lipid analysis (liver NL concentrations) and histological approaches (supplementary data). Here, liver hypertrophy was related to increased glycogen storage in exposed males (histological data), as described in hepatic glycogen storage diseases (Bhattacharya, 2015) but also observed in mice exposed to $PM_{2.5}$ (Zheng et al., 2013). Such excess hepatic glycogen storage might be caused either by an alteration of glucose 6-phosphatase as observed in rat offspring exposed *in utero* to hypoxia (Osumek et al., 2014). It has also been reported in humans as a complication of poorly controlled type 1 diabetes mellitus (Lombardo et al., 2019) or an early manifestation of type 1 diabetes mellitus together with aminotransferase abnormalities (Carcione et al., 2003; Chatila and West, 1996). Exposed males were hyperglycemic, had elevated hepatic transaminases, hyperlipidemia and kidney hypertrophy in addition to liver hypertrophy. Liver glycogenosis reflects the need for better control of glucose metabolism (Chatila and West, 1996). Thus, similarly, it was shown that when a hypercaloric diet is applied in adult male rats, insulin resistance increases even more if they have been exposed to PM2.5 (Yan et al., 2011).

The analysis of the expression of a few target genes in the liver of males supported the physiological observations. Fibrinogen is a large protein synthesized by the liver, representing 2–3% of proteins in blood plasma. Its increase here may reflect increased blood clotting, inflammation, platelet aggregation and atherosclerosis, all of which are involved in the metabolic syndrome (Targher et al., 2009). Orosomucoid-1 (ORM1), also known as α1-acid glycoprotein, was characterized as the major biomarker for liver regeneration and is mainly expressed by hepatocytes under stress conditions (Qin et al., 2017). In the present study, the hepatic expression of ORM1 was decreased in exposed males, potentially reflecting a pathological evolution in the liver. Orosomucoid-1 is bimodal and also exerts immunomodulatory and anti-inflammatory properties (Ligresti et al., 2012). Such a decreased expression of ORM1 in exposed males might illustrate a downregulation of the anti-inflammatory system. Finally the expression of RAGE (receptor of advanced glycation end products) was increased in exposed males, as observed in mice exposed to persistent

organic pollutants, promoting cardiovascular remodeling (Coole et al., 2019). An increased expression of RAGE is also used as a biomarker of non-alcoholic steatohepatitis (NASH) in human (Ge et al., 2018; Mehta et al., 2018). Thus, this study provides new insights concerning the sexually dimorphic effect of an *in utero* exposure to air pollution, with liver disorders in male offspring as evidenced with other kind of exposures, such as pesticides (Lukowicz et al., 2018) or high fat diet during gestation (Tarrade et al., 2013).

## 5. Conclusion

Exposure to diluted diesel engine exhaust, during gestation only, modified the phenotype of offspring in adulthood, with sex-specific responses (Fig. 5). Exposed offspring appeared to be at higher risk for insulin resistance and cardiovascular disease, but with different clinical patterns depending on sex. The pathological phenotype was first more pronounced in males than in females, with males presenting a set of symptoms reminiscent of the human metabolic syndrome, in addition to early signs of liver disease. Non-pregnant females tended to develop hypertriglyceridemia. Pregnancy, however, acted as a metabolic trigger leading to liver steatosis in F1 exposed female late-pregnancy. This study provides important insights into the pathophysiological effects in adulthood of indirect exposure to air pollution in offspring during their intrauterine life. These data could be used to support the records on the health risks of populations and fragile populations in particular, living in large polluted urban agglomerations. Indeed, outdoor air pollution is thought to be an underappreciated linkage between urbanization and the emergence of cardiometabolic diseases, with a focus on type 2 diabetes mellitus and insulin resistance (Rajagopalan and Brook, 2012; Zheng et al., 2013).

## Credit author statement

Delphine Rousseau-Ralliard, Pascale Chavatte-Palmer: Conceptualization; Flemming R. Cassee: Methodology (maternal exposure to diesel engine exhaust); Delphine Rousseau-Ralliard, Christophe Richard, Pauline Hoarau, Marie-Sylvie Lallemand, Sarah A. Valentino, Marine Guinot-Lesage and Gwendoline Morin: *in vivo* investigations; Delphine Rousseau-Ralliard, Christophe Richard, Pauline Hoarau, Marie-Sylvie Lallemand, Sarah A. Valentino, Marine Guinot-Lesage, Eve Mourier, Anne Couturier-Tarrade, Marie-Christine Aubrière, Sylvaine Camous and Michèle Dahirel: sample collection; Delphine Rousseau-Ralliard, Pauline Hoarau, Marie-Sylvie Lallemand, Marine Guinot-Lesage, Marie-Christine Aubrière, Lucie Morillon, Natalie Fournier: investigations (on collected samples, through molecular biology, biochemical or histological approaches); Delphine Rousseau-Ralliard, Pauline Hoarau, Sarah A. Valentino: Data curation; Sarah A. Valentino, Rémy Slama (statistical model) and Delphine Rousseau-Ralliard: Formal analyses; Pascale Chavatte-Palmer and Rémy Slama: Funding acquisition; Delphine Rousseau-Ralliard, Pascale Chavatte-Palmer, Anne Couturier-Tarrade, Sarah A. Valentino: Validation; Delphine Rousseau-Ralliard: Roles/Writing - original draft; Delphine Rousseau-Ralliard, Sarah A. Valentino, Pascale Chavatte-Palmer, Flemming R. Cassee and Anne Couturier-Tarrade: Writing - review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors are grateful to the staff of the UE 1298 SAAJ (Experimental unit of *Sciences de l'Animal et de l'Aliment de Jouy*) for housing the rabbits and for all the help given in the animal experimental procedures. The authors thank the MIMA2 platform (Microscopy and Imaging Facility for Microbes, Animals and Foods platform INRAE, 2018, https://doi.org/10.15454/1.5572348210007727E12), particularly CIMA (*Chirurgie et Imagerie Médicale chez l'Animal,* https://www6.jouy.inra.fr/mima2/Equipements/Imagerie-sur-l-Animal) for access to medical imaging equipment. Moreover the authors thank all the students and persons who participated in this project from near and far, from the BREED Unit or elsewhere. The authors are members of COST Actions BM1308 "Sharing advances on large animal models (SALAAM)".

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.111690.

## Funding

This work was supported by the French National Research Agency [ANR-13-CESA-0011 EPAPP, 2012, scientific coordinator: Pascale Chavatte-Palmer] and European Union [ERC consolidator 311765 E-DOHaD, 2012, scientific coordinator: Rémy Slama].

## Approval by an appropriately constituted committee for animal research

The work described in the present article has been carried out in accordance with the relevant guidelines and regulations, EC Directive 86/609/EEC for animal experiments http://europa.eu.int/scadplus/leg/en/s23000.htm. All experimental protocols were approved under N°12/102 by the local ethical committee COMETHEA ("*Comité d'Ethique en Expérimentation Animale du Centre INRA de Jouy en Josas et AgroParisTech*"), referenced as N°C2EA-45 in the French National registry CNREEA ("*Comité National de Réflexion Ethique sur l'Expérimentation Animale*").

## References

Adar, S.D., et al., 2013. Fine particulate air pollution and the progression of carotid intima-medial thickness: a prospective cohort study from the multi-ethnic study of atherosclerosis and air pollution. PLoS Med. 10, e1001430.
Alvaer, K., et al., 2007. Outdoor air pollution and bone mineral density in elderly men - the Oslo Health Study. Osteoporos. Int. 18, 1669–1674.
Arias-Mutis, O.J., et al., 2017. Development and characterization of an experimental model of diet-induced metabolic syndrome in rabbit. PloS One 12, e0178315.
Auten, R.L., et al., 2012. Maternal diesel inhalation increases airway hyperreactivity in ozone-exposed offspring. Am. J. Respir. Cell Mol. Biol. 46, 454–460.
Bhattacharya, K., 2015. Investigation and management of the hepatic glycogen storage diseases. Transl. Pediatr. 4, 240–248.
Bolton, J.L., et al., 2012. Prenatal air pollution exposure induces neuroinflammation and predisposes offspring to weight gain in adulthood in a sex-specific manner. Faseb. J. 26, 4743–4754.
Calderon-Garciduenas, L., et al., 2013. Exposure to urban air pollution and bone health in clinically healthy six-year-old children. Arh. Hig. Rada. Toksikol. 64, 23–34.
Carcione, L., et al., 2003. Liver glycogenosis as early manifestation in type 1 diabetes mellitus. Diab. Nutr. Metab. 16, 182–184.
Chang, K.H., et al., 2015. Exposure to air pollution increases the risk of osteoporosis: a nationwide longitudinal study. Medicine (Baltim.) 94, e733.
Chatila, R., West, A.B., 1996. Hepatomegaly and abnormal liver tests due to glycogenosis in adults with diabetes. Medicine (Baltim.) 75, 327–333.
Chen, Z., et al., 2015. Living near a freeway is associated with lower bone mineral density among Mexican Americans. Osteoporos. Int. 26, 1713–1721.
Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159.
Chomczynski, P., Sacchi, N., 2006. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nat. Protoc. 1, 581–585.
Coole, J.B., et al., 2019. Persistent organic pollutants (POPs) increase rage signaling to promote downstream cardiovascular remodeling. Environ. Toxicol. 34, 1149–1159.
Cupul-Uicab, L.A., et al., 2012. Exposure to tobacco smoke in utero and subsequent plasma lipids, ApoB, and CRP among adult women in the MoBa cohort. Environ. Health Perspect. 120, 1532–1537.
Falkner, B., 2019. Maternal and gestational influences on childhood blood pressure. Pediatr. Nephrol. 35 (8), 1409–1418. https://doi.org/10.1007/s00467-019-4201-x.

Fossati, S., et al., 2020. Prenatal air pollution exposure and growth and cardio-metabolic risk in preschoolers. Environ. Int. 138, 105619 https://doi.org/10.1016/j.envint.2020.105619. In this issue.
Gavi, S., et al., 2007. Limb fat to trunk fat ratio in elderly persons is a strong determinant of insulin resistance and adiponectin levels. J. Gerontol. A Biol. Sci. Med. Sci. 62, 997–1001.
Ge, X., et al., 2018. High mobility group box-1 drives fibrosis progression signaling via the receptor for advanced glycation end products in mice. Hepatology 68, 2380–2404.
Ginsberg, H.N., et al., 2005. Regulation of plasma triglycerides in insulin resistance and diabetes. Arch. Med. Res. 36, 232–240.
Grundy, S.M., et al., 2004. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Arterioscler. Thromb. Vasc. Biol. 24, e13–e18.
Gysel, T., et al., 2014. Association between insulin resistance, lean mass and muscle torque/force in proximal versus distal body parts in healthy young men. J. Musculoskelet. Neuronal Interact. 14, 41–49.
Hanson, M.A., Gluckman, P.D., 2014. Early developmental conditioning of later health and disease: physiology or pathophysiology? Physiol. Rev. 94, 1027–1076.
Herrera, E., Desoye, G., 2016. Maternal and fetal lipid metabolism under normal and gestational diabetic conditions. Horm. Mol. Biol. Clin. Invest. 26, 109–127.
Hill, D., Szlapinski, S.K., 2020. Metabolic adaptations to pregnancy in healthy and gestational diabetic pregnancies: the pancreas - placenta Axis. Curr. Vasc. Pharmacol. 19 (2), 141–153. https://doi.org/10.2174/1570161118666200320111209.
Hue-Beauvais, C., et al., 2019. Impact of exposure to diesel exhaust during pregnancy on mammary gland development and milk composition in the rabbit. PloS One 14, e0212132.
Huang, C.Y., et al., 2019. Maternal exposure to air pollution and the risk of small for gestational age in offspring: a population-based study in Taiwan. Pediatr. Neonatol. 61 (2), 231–237. https://doi.org/10.1016/j.pedneo.2019.11.008.
Hue-Beauvais, C., et al., 2017. Diet-induced modifications to milk composition have long-term effects on offspring growth in rabbits. J. Anim. Sci. 95, 761–770.
Jiang, S., et al., 2016. Traffic-related air pollution is associated with cardio-metabolic biomarkers in general residents. Int. Arch. Occup. Environ. Health 89, 911–921.
King, J.C., 2000. Physiology of pregnancy and nutrient metabolism. Am. J. Clin. Nutr. 71, 1218S.
Kouda, K., et al., 2020. Trunk-to-peripheral fat ratio predicts a subsequent blood pressure in normal-weight pubertal boys: a 3-year follow-up of the Kitakata Kids Health Study. Environ. Health Prev. Med. 25, 41.
Le Floch, J.P., et al., 1990. Blood glucose area under the curve. Methodological aspects. Diabetes Care 13, 172–175.
Lê, S., et al., 2008. FactoMineR: an R package for multivariate analysis. J. Stat. Software 25, 18.
Lee, C.G., et al., 2011. Association between insulin resistance and lean mass loss and fat mass gain in older men without diabetes mellitus. J. Am. Geriatr. Soc. 59, 1217–1224.
Lee, H.R., et al., 2021. Compared to serum triglyceride alone, the association between serum triglyceride to high-density lipoprotein cholesterol ratio and 10-year cardiovascular disease risk as determined by Framingham risk scores in a large Korean cohort. Clin. Chim. Acta 520, 29–33.
Lee, J., et al., 2017. Bone mineral density according to dual energy X-ray Absorptiometry is associated with serial serum alkaline phosphatase level in extremely low birth weight infants at discharge. Pediatr. Neonatol. 58, 251–257.
Li, J., et al., 2019. Ambient air pollution is associated with HDL (High-Density lipoprotein) dysfunction in healthy adults. Arterioscler. Thromb. Vasc. Biol. 39, 513–522.
Ligresti, G., et al., 2012. The acute phase reactant orosomucoid-1 is a bimodal regulator of angiogenesis with time- and context-dependent inhibitory and stimulatory properties. PloS One 7, e41387.
Liu, C., et al., 2015. Associations between ambient air pollution and bone turnover markers in 10-year old children: results from the GINIplus and LISAplus studies. Int. J. Hyg Environ. Health 218, 58–65.
Liu, Y., et al., 2016. Effect of fine particulate matter (PM2.5) on rat placenta pathology and perinatal outcomes. Med. Sci. Monit. 22, 3274–3280.
Liu, Z., et al., 2014. Alanine aminotransferase-old biomarker and new concept: a review. Int. J. Med. Sci. 11, 925–935.
Lombardo, F., et al., 2019. Hepatomegaly and type 1 diabetes: a clinical case of Mauriac's syndrome. Ital. J. Pediatr. 45, 3.
Lukowicz, C., et al., 2018. Metabolic effects of a chronic dietary exposure to a low-dose pesticide cocktail in mice: sexual dimorphism and role of the constitutive androstane receptor. Environ. Health Perspect. 126, 067007.
Mehta, R., et al., 2018. Polymorphisms in the receptor for advanced glycation end-products (RAGE) gene and circulating RAGE levels as a susceptibility factor for non-alcoholic steatohepatitis (NASH). PloS One 13, e0199294.
Nappi, F., et al., 2016. Endocrine aspects of environmental "obesogen" pollutants. Int. J. Environ. Res. Publ. Health 13.
Osumek, J.E., et al., 2014. Enhanced trimethylation of histone h3 mediates impaired expression of hepatic glucose 6-phosphatase expression in offspring from rat dams exposed to hypoxia during pregnancy. Reprod. Sci. 21, 112–121.
Pedersen, M., et al., 2013. Ambient air pollution and low birthweight: a European cohort study (ESCAPE). Lancet Respir Med 1, 695–704.
Poursafa, P., et al., 2014. Is air quality index associated with cardiometabolic risk factors in adolescents? The CASPIAN-III Study. Environ. Res. 134, 105–109.
Qin, X.Y., et al., 2017. Transcriptome analysis uncovers a growth-promoting activity of orosomucoid-1 on hepatocytes. EBioMedicine 24, 257–266.
Rajagopalan, S., Brook, R.D., 2012. Air pollution and type 2 diabetes: mechanistic insights. Diabetes 61, 3037–3045.
Robles, M., et al., 2017. Maternal nutrition during pregnancy affects testicular and bone development, glucose metabolism and response to overnutrition in weaned horses up to two years. PloS One 12, e0169295.
Rousseau-Ralliard, D., et al., 2019a. A short periconceptional exposure to maternal type-1 diabetes is sufficient to disrupt the feto-placental phenotype in a rabbit model. Mol. Cell. Endocrinol. 480, 42–53.
Rousseau-Ralliard, D., et al., 2019b. Effects of first-generation in utero exposure to diesel engine exhaust on second-generation placental function, fatty acid profiles and foetal metabolism in rabbits: preliminary results. Sci. Rep. 9, 9710.
Schug, T.T., et al., 2013. PPTOX III: environmental stressors in the developmental origins of disease–evidence and mechanisms. Toxicol. Sci. 131, 343–350.
Sigrist-Flores, S.C., et al., 2019. Chronic intake of moderate fat-enriched diet induces fatty liver and low-grade inflammation without obesity in rabbits. Chem. Biol. Interact. 300, 56–62.
Slama, R., et al., 2010. Maternal fine particulate matter exposure, polymorphism in xenobiotic-metabolizing genes and offspring birth weight. Reprod. Toxicol. 30, 600–612.
Targher, G., et al., 2009. Nonalcoholic fatty liver disease as a contributor to hypercoagulation and thrombophilia in the metabolic syndrome. Semin. Thromb. Hemost. 35, 277–287.
Tarrade, A., et al., 2013. Sexual dimorphism of the feto-placental phenotype in response to a high fat and control maternal diets in a rabbit model. PloS One 8, e83458.
Thiering, E., et al., 2013. Long-term exposure to traffic-related air pollution and insulin resistance in children: results from the GINIplus and LISAplus birth cohorts. Diabetologia 56, 1696–1704.
Valentino, S.A., et al., 2016. Maternal exposure to diluted diesel engine exhaust alters placental function and induces intergenerational effects in rabbits. Part. Fibre Toxicol. 13, 39.
Vandesompele, J., et al., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3. RESEARCH0034.
Wallwork, R.S., et al., 2017. Ambient fine particulate matter, outdoor temperature, and risk of metabolic syndrome. Am. J. Epidemiol. 185, 30–39.
Wang, X., et al., 2019. Prenatal exposure to diesel exhaust PM2.5 programmed non-alcoholic fatty liver disease differently in adult male offspring of mice fed normal chow and a high-fat diet. Environ. Pollut. 255, 113366.
Weldy, C.S., et al., 2014. In utero exposure to diesel exhaust air pollution promotes adverse intrauterine conditions, resulting in weight gain, altered blood pressure, and increased susceptibility to heart failure in adult mice. PloS One 9, e88582.
Woo, K.S., et al., 2020. The impact of particulate matter air pollution (PM2.5) on atherosclerosis in modernizing China: a report from the CATHAY study. Int. J. Epidemiol. 50 (2), 578–588. https://doi.org/10.1093/ije/dyaa235.
Wu, G., et al., 2019. Adverse organogenesis and predisposed long-term metabolic syndrome from prenatal exposure to fine particulate matter. Proc. Natl. Acad. Sci. U. S. A. 116, 11590–11595.
Yan, Y.H., et al., 2011. Enhanced insulin resistance in diet-induced obese rats exposed to fine particles by instillation. Inhal. Toxicol. 23, 507–519.
Yang, Y., et al., 2021. Triglycerides to high-density lipoprotein cholesterol ratio is the best surrogate marker for insulin resistance in nonobese middle-aged and elderly population: a cross-sectional study. Int. J. Endocrinol. 2021, 6676569.
Yin, F., et al., 2019. Diesel exhaust induces mitochondrial dysfunction, hyperlipidemia, and liver steatosis. Arterioscler. Thromb. Vasc. Biol. 39, 1776–1786.
Yin, F., et al., 2013. Diesel exhaust induces systemic lipid peroxidation and development of dysfunctional pro-oxidant and pro-inflammatory high-density lipoprotein. Arterioscler. Thromb. Vasc. Biol. 33, 1153–1161.
Zheng, Z., et al., 2013. Exposure to ambient particulate matter induces a NASH-like phenotype and impairs hepatic glucose metabolism in an animal model. J. Hepatol. 58, 148–154.