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Perinatal exposure to chlorpyrifos and/or a high-fat diet is associated with liver damage in male rat offspring

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Declarations of interest: none
List of abbreviations:

1. Acaca: Acetyl-CoA carboxylase 1
2. Actb: Beta actin
3. ALAT: Alanine aminotransferase
4. ALP: Alkaline phosphatase
5. ASAT: Aspartate aminotransferase
6. BW: Body weight
7. Cd36: Fatty Acid Translocase
8. cDNA: Complementary DNA
9. Col1a1: Collagen alpha-1
10. CPF: Chlorpyrifos
11. Dgat1: Diglyceride O-acyltransferase 1
12. Dgat2: Diglyceride O-acyltransferase 2
13. DOHaD: Developmental Origins of Health and Disease
14. Fasn: Fatty acid synthase
15. Gapdh: Glyceraldehyde 3-phosphate dehydrogenase
16. G6pc: Glucose-6-phosphatase
17. HDL; High-density lipoprotein
18. HF: High-fat diet
19. Hmgcr: 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
20. Hprt1: hypoxanthine guanine phosphoribosyl transferase 1
21. IGF1: Insulin-like growth factor-1
22. IL1b: Interleukin 1 beta
23. Lp: Lipoprotein lipase
24. LDH: Lactate dehydrogenase
25. Mlxipl/Chrebp: Carbohydrate response element binding protein
26. NOAEL: No observable adverse effect level
27. OP: Organophosphate
28. PAS: Periodic acid Schiff
29. Pck1/Pepck: Phosphoenolpyruvate carboxykinase
30. PND: Postnatal day
31. Ppara: Peroxisome proliferator-activated receptor alpha
32. Pparg: Peroxisome proliferator-activated receptor gamma
Prkaa1/Ampka: AMP-activated protein kinase catalytic subunit alpha
RT-PCR: Reverse transcription polymerase chain reaction
SC: Standard chow diet
SEM: Standard error of the mean
Slc2a2/Glut2: Solute carrier family 2, facilitated glucose transporter member 2
Srebf1/Srebp1c: Sterol regulatory element binding factor 1 variant 2
TC: Total cholesterol
TG: Triglyceride
T2DM: Type 2 diabetes
Ubc: Ubiquitin C
VLDL: Very-low-density lipoprotein.
**Abstract:**

Metabolic impairments in childhood are known to promote the development of type 2 diabetes and/or obesity in adulthood. These impairments may result from perinatal exposure to harmful environmental factors, such as pesticide residues or the consumption of a “western” diet. In the present study, we sought to determine whether an obesogenic profile, metabolic disorders and liver damage in offspring (observed during young adulthood) were related to maternal exposure to the pesticide chlorpyrifos (CPF) and/or a high-fat diet (HFD) starting 4 months before conception and ending at weaning. After the end of exposure, 51 male rat pups were left to develop under normal conditions and were studied in young adulthood. Despite the absence of direct exposure to harmful factors (other than through the dam’s milk), maternal exposure to CPF or an HFD was associated with changes in the offspring’s metabolic activity in the liver in the offspring. This indirect exposure to CPF was associated with a relative reduction in the expression of genes coding for enzymes involved in lipid or glucose metabolism but did induce histopathological changes in the offspring at adulthood. Maternal exposure to an HFD alone or to CPF alone gave similar results in offspring, changes in the same direction. Exposure of the mother to HFD did not exacerbate CPF effects. Co-exposure to both CPF and HFD did not increase the observed effects compared to each factor taken separately.

**Keywords:** Chlorpyrifos; High-fat diet; Maternal exposure; Metabolic diseases; Liver; Rats
1. Introduction

The prevalence of obesity or overweight in children is a serious, growing public health concern and is associated with greater morbidity and mortality in early adulthood. There is a genetic predisposition to obesity/overweight: parental obesity doubles the risk of adult obesity in children under the age of 10 (Whitaker et al., 2009) and is therefore a major risk factor in this context. The biological mechanisms that underlie these effects have not yet been fully elucidated, although a growing body of evidence suggests that environmental factors during pregnancy may modulate foetal growth and development and thus predispose the offspring to metabolic diseases in later life (for a review, see (Sarron et al., 2020)). These environmental factors include pesticides, i.e. substances used to control organisms that are harmful for plants and animals in agriculture. Even though the use of pesticides increases yields in agriculture, it is increasingly clear that these compounds have detrimental health effects - particularly during highly sensitive periods of life. The perinatal period comprises several highly plastic developmental phases, during which sensitivity to environmental factors is at its highest. The maternal environment (including exposure to chemical agents) might therefore influence foetal growth. Most of these factors are likely to influence growth directly through changes in nutrient transfer or damage to the thyroid gland, or indirectly through foetal immunotoxicity and neurotoxicity (Kamai et al., 2019). Indeed, prenatal pesticide exposure is likely to contribute to the occurrence of obesity and type 2 diabetes later in life, exemplifying the “Developmental Origins of Health and Disease” concept. Organophosphates (OPs) are still the most widely used category of pesticides worldwide, although their known consequences on both animal and human health means that use in France and other countries is currently decreasing. The OPs’ main mechanism of action is the irreversible inhibition of acetylcholinesterase activity in both insects and mammals. However, other mechanisms are probably involved in the long-term effects of foetal or neonatal OP exposure (Slotkin, 2004). Most human exposure is due to the oral ingestion of pesticide residues present on fruits and vegetables. Since OPs and their metabolites cross the placenta easily, a foetus can be exposed throughout pregnancy (Fish, 1966). The OP chlorpyrifos-ethyl (CPF) has been widely used to protect cereal crops against pests. However, CPF’s use has been questioned because a large body of evidence shows that this compound can disrupt embryonic
development (Slotkin et al., 2005b) and affect the foetal nervous system (Hunter et al., 1999; Akhtar et al., 2006) - phenomena that have repercussions in postnatal life (Slotkin, 1999). Early-life CPF exposure alters glucose and lipid metabolism (culminating in prediabetes) (Slotkin, 2011) and induces oxidative stress and lipid peroxidation. The persistence of these alterations into adulthood suggests that adverse effects can be transgenerational (Ndonwi et al., 2019). This type of exposure is a known risk factor for obesity (Lassiter and Brimijoin, 2008) and is responsible for maternal and developmental toxicity (Maurissen et al., 2000). We have reported previously that repeated, low-level, oral maternal exposure to CPF was altering foetal development resulting in long-term neurological effects on the offspring with later consequences in adulthood (Laporte et al., 2018). We also showed that CPF exposure during gestation and development has both short- and long-term impacts on the digestive tract (Joly Condette et al., 2015). Furthermore, we suggested that various types of pre- and post-natal exposure could impair adult metabolism and biochemical status and thus predispose the individual to metabolic syndrome. This hypothesis was developed further in a recent review; the researchers hypothesized that OP exposure is hepatotoxic, induces inflammation and oxidative stress, and leads to hyperglycaemia and other impairments in glucose metabolism (including increased glycogenolysis and gluconeogenesis) thus directly associating OPs with obesity and diabetes (Czajka et al., 2019). In particular, CPF exposure was shown to impair lipid metabolism and induce histopathological changes in the liver (Elsharkawy et al., 2013).

Furthermore, the mother’s diet and body composition before pregnancy have a great influence on her child’s future health (Barker, 2003). A study of 97 overweight or obese pregnant women found the latter’s children developed fatty liver in young adulthood (Cantoral et al., 2020). This effect has also been observed in the laboratory, since long-term maternal consumption of a high-fat diet (HFD), mimicking dietary habits in Western societies (Srinivasan et al., 2006)) starting before pregnancy and lasting until weaning was associated with metabolic syndrome phenotypes in the rat offspring (Tellechea et al., 2017). Taken as a whole, these data suggest that maternal obesity and pesticide exposure are (among others) risk factors for impaired postnatal metabolism and the occurrence of obesity or type 2 diabetes in the offspring. Furthermore, neonatal parathion exposure of rats on an HFD favoured the incidence of obesity independently of dietary factors (Adigun et al., 2010). Lastly,
it has been reported that an obese phenotype can exacerbate the toxicity of CPF exposure in mice (Kondakala et al., 2017). Hence, the primary objective of the present study was to determine whether, in rats, exposure of dams to CPF and an HF diet, from 4 months before gestation to weaning, increased the occurrence of obesity, metabolic disorders and liver damage among their male offspring.
2. Methods

2.1. Animal Housing

We studied 51 male offspring of 16 female Wistar rats (Janvier laboratory - Saint Berthevin, France. All animals were pair-housed in stainless-steel, enriched cages (with a roller, a sizzle pad, and hardwood chip bedding) at a controlled temperature (23±1°C), at constant relative humidity (26%) with a 12-hour/12-hour dark/artificial light cycle (El Khayat El Sabbouri et al., 2020).

The animals’ welfare was monitored daily. All experimental protocols had been audited by the local animal care and use committee (C2EA#96, Amiens, France) and approved by the French Ministry of Research (reference: APAFIS # 8207-2016121322563594 v2). All animal experiments complied with the European Union Directive 2010/63/EU.

2.2. Animal Experiments

The male offspring of dams exposed to the different environmental conditions were monitored from birth until early adulthood, i.e. postnatal day (PND) 60. The offspring were not exposed to chemical treatments or non-standard environmental factors and had ad libitum access to a standard chow diet (SCD, Serlab 3436 maintenance chow, Serlab, Montataire, France) and water (Figure 1) throughout the study. After one week of acclimation, the 16 dams (mean ± standard error of the mean body weight: 225 ± 17 g at 8 weeks of age) were assigned at random to the two experimental groups: one group (n=8) received chlorpyrifos-ethyl (CPF, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate (LGC Standards Laboratory, Molsheim, France), 1 mg/kgBW/d per os in 1 mL/kg rapeseed oil vehicle (MP Biomedicals, Illkirch, France)) and the other group (n=8) received vehicle alone (1 mL/kg) (Figure 1).
Figure 1 – The experimental plan.

The left-hand panel shows the experiment’s timeline, and the main panel shows the groups and events for the dams and the offspring. CPF, chlorpyrifos; HF, high-fat diet; SC, standard chow diet; PND, postnatal day.

The CPF and non-CPF groups were each subdivided into an HF group (n=4, in which 60% of the energy came from fat; D12492 chow; Ssniff Spezialdiäten GmbH, Soest, Germany) or an SCD group (n=4, with Serlab 3436 maintenance chow or, during gestation and lactation, Serlab 3336 breeding chow) (Figure 1). This model has been shown to induce metabolic alterations (elevated BW, hyperinsulinemia, programming of adiposity, etc.) in the offspring at adulthood (7 months of age) (Lecoutre et al., 2016). A recent study showed that standard maintenance chow (as used in the present experiment) is a suitable control for a 16- to 18-week HFD study; a low-fat
diet is not necessarily required for effects on the phenotype (weight gain), metabolism (glucose tolerance, levels of high-density lipoprotein (HDL), triglycerides (TGs), plasma cytokines and adipocytokines) and behavioural outcomes (Almeida-Suhett et al., 2019).

Furthermore, we studied the HFD’s transgenerational influence on the offspring (all of which received a SC diet), rather than on the exposed dams. Further details of the diets are given in Table 1.

Table 1- Composition of the rodent chows used in the study.

<table>
<thead>
<tr>
<th>Crude nutrients (%)</th>
<th>HF diet</th>
<th>SC diet</th>
<th>Standard breeding chow diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product reference</strong></td>
<td>D12492</td>
<td>3436</td>
<td>3336</td>
</tr>
<tr>
<td><strong>Supplier</strong></td>
<td>Ssniff Spezialdiäten GmbH, Soest, Germany</td>
<td>Serlab, Montataire, France</td>
<td>Serlab, Montataire, France</td>
</tr>
</tbody>
</table>

Fatty acid composition of the HFD: lauric acid (0.07%), myristic acid (0.44%), palmitic acid (7.93%), stearic acid (4.37%), arachidic acid (0.11%), linolenic acid (0.49%), linoleic acid (4.64%), palmitoleic acid (0.94%), and oleic acid (13.97%)

b. Contains: wheat middling, wheat, barley, soybean meal (non-GMO), corn (non-GMO), poultry meal, wheat starch, whey powder, soybean oil (2.13%), sunflower cake, brewer’s dried yeast, minerals, vitamins, and amino acids

c. Contains: flaked oats, soybean meal (non-GMO), poultry meal, wheat middling, wheat starch, barley, herring meal, grass meal, whey powder, corn (non-GMO), soybean oil, minerals, vitamins, and amino acids
<table>
<thead>
<tr>
<th>Purpose</th>
<th>High-calorie diet</th>
<th>Maintenance diet</th>
<th>Pregnancy/lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross energy (GE) MJ/kg</td>
<td>25</td>
<td>16.1</td>
<td>17</td>
</tr>
<tr>
<td>Metabolizable energy (ME) MJ/kg</td>
<td>21.6</td>
<td>13.1</td>
<td>14.3</td>
</tr>
<tr>
<td>Metabolizable energy density (Kcal/g)</td>
<td>5.16</td>
<td>3.13</td>
<td>3.41</td>
</tr>
<tr>
<td>Major nutrients (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>24.4</td>
<td>18.5</td>
<td>23.5</td>
</tr>
<tr>
<td>Crude fat</td>
<td>34.6</td>
<td>4.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>6.0</td>
<td>4.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Crude ash</td>
<td>5.3</td>
<td>6.5</td>
<td>5.7</td>
</tr>
<tr>
<td>Starch</td>
<td>0.1</td>
<td>35</td>
<td>36</td>
</tr>
<tr>
<td>Sugar</td>
<td>9.4</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>N-free extracts</td>
<td>26.3</td>
<td>54</td>
<td>51.3</td>
</tr>
<tr>
<td>Minerals (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>0.92</td>
<td>1.05</td>
<td>1.00</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.64</td>
<td>0.80</td>
<td>0.74</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.23</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.97</td>
<td>0.78</td>
<td>0.80</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Unknown</td>
<td>0.36</td>
<td>0.33</td>
</tr>
<tr>
<td>Amino acids (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>0.95</td>
<td>1.10</td>
<td>1.50</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.02</td>
<td>1.00</td>
<td>1.40</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.86</td>
<td>0.39</td>
<td>0.60</td>
</tr>
<tr>
<td>Methionine + cysteine</td>
<td>1.31</td>
<td>0.76</td>
<td>0.60</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.33</td>
<td>0.20</td>
<td>0.27</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.07</td>
<td>0.65</td>
<td>0.87</td>
</tr>
<tr>
<td>Vitamins (/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>15,000 IU</td>
<td>14,000 IU</td>
<td>14,000 IU</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>1,500 IU</td>
<td>1,000 IU</td>
<td>1,000 IU</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>150 mg</td>
<td>110 mg</td>
<td>110</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>20 mg</td>
<td>2 mg</td>
<td>2 mg</td>
</tr>
<tr>
<td>Thiamine (B1)</td>
<td>25 mg</td>
<td>30 mg</td>
<td>32 mg</td>
</tr>
<tr>
<td>Riboflavin (B2)</td>
<td>16 mg</td>
<td>20 mg</td>
<td>21 mg</td>
</tr>
<tr>
<td>Pyridoxine (B6)</td>
<td>16 mg</td>
<td>14 mg</td>
<td>14 mg</td>
</tr>
<tr>
<td>Cobalamin (B12)</td>
<td>30 µg</td>
<td>50 µg</td>
<td>50 µg</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>47 mg</td>
<td>70 mg</td>
<td>70 mg</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>55 mg</td>
<td>33 mg</td>
<td>33 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td><strong>Folic acid</strong></td>
<td>16 mg</td>
<td>2 mg</td>
<td>2.4 mg</td>
</tr>
<tr>
<td><strong>Biotin</strong></td>
<td>300 µg</td>
<td>220 µg</td>
<td>220 µg</td>
</tr>
<tr>
<td><strong>Choline</strong></td>
<td>920 mg</td>
<td>2000 mg</td>
<td>2000 mg</td>
</tr>
<tr>
<td><strong>Vitamin C</strong></td>
<td>Unknown</td>
<td>40 mg</td>
<td>40 mg</td>
</tr>
</tbody>
</table>

**Trace elements (mg/kg)**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Iron</strong></td>
<td>168</td>
<td>250</td>
<td>200</td>
</tr>
<tr>
<td><strong>Zinc</strong></td>
<td>65</td>
<td>60</td>
<td>68</td>
</tr>
<tr>
<td><strong>Copper</strong></td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td><strong>Iodine</strong></td>
<td>1.2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Manganese</strong></td>
<td>95</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td><strong>Selenium</strong></td>
<td>0.2</td>
<td>0.3</td>
<td>0.27</td>
</tr>
</tbody>
</table>

At the age of 24 weeks (i.e. after 4 months of exposure), the 16 dams (mean body weight: 316 ± 33 g BW) were mated. Pregnancy was confirmed by the presence of spermatozoa and/or a plug in the vagina, checked twice with a smear early on the following morning. After fertilization, pregnant females were individually housed in clean plastic cages. The various treatments and diets (CPF vs. vehicle, and HF diet vs. SC diet) were continued until the end of the lactation period. PND1 was defined as the day of parturition. On average, a litter comprised 10 ± 5 pups, with 4 ± 3 males. There were no intergroup differences in the litter size. Male pups accounted for 54% of the offspring. Each pup was tattooed on the leg with a special veterinary ink (Centravet, Nancy, France) for individual identification. The pups were counted, sexed and weighed at PND3. To limit potential maternal separation stress in early life, the pups were not weighed at PND1. At weaning (PND21), the pups were separated from their mothers and were fed tap water and standard chow (Serlab 3436) *ad libitum* (Figure 1). In order to avoid potentially confounding factors (such as menstrual cycles and the impact of hormones on physiological functions), only male
pups were studied further (Figure 1) in the following four groups: (i) the ctrl-SCD group (maternal exposure to SCD and vehicle, n=16); (ii) the CPF-SCD group (maternal exposure to SCD and CPF, n=20), (iii) the ctrl-HFD group (maternal exposure to an HFD and vehicle, n=10), and (iv) the CPF-HFD group: maternal exposure to both CPF and HFD (n=5).

Due to poor fertility, the number of male pups was lower in the co-exposure group (CPF-HF) than in the other groups. Pups were weighed every 3 days until PND54 (early adulthood). Animals were fasted on the night before sacrifice. Rats were euthanized at PND60 (8 weeks of age) with a single, lethal, intraperitoneal injection of pentobarbital sodium (EXAGON®, Axience SAS, Pantin, France; 140 mg/kg - 0.35 mL/kg). Tissue samples and blood were immediately harvested under sterile conditions and stored at −80°C. For the gene expression study, liver tissue samples were harvested and dived into the Invitrogen RNALater Stabilization Solution (Life Technologies, Thermo Fisher Scientific, Courtaboeuf, France) - a non-toxic tissue storage reagent that rapidly permeates tissues to stabilize and protect cellular RNA. Plasma samples were obtained by centrifugation and immediately frozen at −80°C.

2.3. Evaluation of possible impairment of lipid and glucose metabolism in offspring exposed perinatally to CPF and/or an HF diet

2.3.1. Gene expression of proteins involved in lipid and carbohydrate metabolism, as measured in a reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was isolated from liver samples using the FastGene Basic ® RNA kit (NIPPON Genetics Europe, Dueren, Germany). The total RNA concentration was measured spectrophotometrically using a Nanodrop 1000 spectrophotometer (Life Technologies). cDNA was generated by reverse transcription of 1 µg mRNA with the High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Life Technologies), as described previously (Joly Condette et al., 2015). The expression of genes coding for proteins involved in lipid and carbohydrate metabolism was measured using RT-PCRs (Table 2).
### Table 2 – Metabolic genes assessed in the study

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Tm</th>
<th>Accession number and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HOUSEKEEPING GENES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gapdh</em></td>
<td>Glyceraldehyde 3 phosphate dehydrogenase</td>
<td>AGACAGGCC GCATCTTCT TGT</td>
<td>CTTGCCGT GGTAGA GTCAT</td>
<td>NM_01700 8.4 (Melgar-Rojas et al., 2015)</td>
</tr>
<tr>
<td><em>Actb</em></td>
<td>Beta actin</td>
<td>ACGTCGAC ATCCGCAA AGACCTC</td>
<td>TGATCTCC TTCTGCAT CCGTCA</td>
<td>60 (Li et al., 2018)</td>
</tr>
<tr>
<td><em>Hprt1</em></td>
<td>Hypoxanthine guanine phosphoribosyl transferase 1</td>
<td>TCGTACCTT TCTCACCAC AGTATCTAG</td>
<td>GAAAACTA AGACACCT CCCATCA</td>
<td>60 (Long et al., 2019)</td>
</tr>
<tr>
<td><em>Ubc</em></td>
<td>Ubiquitin C</td>
<td>CTCATGGA CTGATTATG GACAGGAC</td>
<td>GCAGGTCC AGCAAG AACTTATA GCC</td>
<td>NM_01258 3 (Hermier et al., 2017)</td>
</tr>
<tr>
<td><strong>LIPOGENESIS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>TAGCTGG AAGTGAC GGTGGT</td>
<td>NM_01148 0 (Li et al., 2018)</td>
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<tr>
<td><em>Acaca</em></td>
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<td>TGTCTATTC GGGGTTGAC TTTCG</td>
<td>CCGTTCAT TATACGCA CGTACG</td>
<td>NM_02219 3 (Ahmed et al., 2015)</td>
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<tr>
<td><em>Fasn</em></td>
<td>Fatty acid</td>
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<td>GCCGCGG</td>
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<tr>
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<td>Function</td>
<td>Synthase Sequence</td>
<td>Transcription Factor</td>
<td>Reference</td>
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<tr>
<td>Lpl</td>
<td>Lipoprotein lipase</td>
<td>GCTACGTG GCTA</td>
<td>CAAGATTCA TTTTCTGG GACTGA</td>
<td>(Ahmed et al., 2015)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>GCCACTG TGCCGTA CAGAGA</td>
<td></td>
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<td>NM_01259</td>
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<td>Mlixpl</td>
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<td>NM_13355</td>
<td>2.1 (Vida et al., 2014)</td>
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<td>Prkaa1</td>
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<td>CAAC</td>
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<td>GGAGACC TTCTGCTC AGTGG</td>
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<td>NM_01287</td>
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<td>Phosphoenol pyruvate carboxykinase</td>
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<td><strong>CHOLESTEROL SYNTHESIS</strong></td>
<td><strong>Hmgcr</strong></td>
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<td><strong>FA OXIDATION</strong></td>
<td><strong>Ppara</strong></td>
<td>Peroxisome proliferator activated receptor alpha</td>
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<td>CTCGTGT GCCCTCC CTCAAG</td>
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<td><strong>LIPID ACCUMULATION</strong></td>
<td><strong>Cd36</strong></td>
<td>Cluster of differentiation 36 also known as fatty acid translocase</td>
<td>AACCCAGA GGAAGTGG CAAAG</td>
<td>GACAGTG AAGGCTC AAAGATG G</td>
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<td></td>
<td><strong>Pparg</strong></td>
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<td><strong>Dgat1</strong></td>
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<td><strong>FIBROSIS</strong></td>
<td><strong>Col1a1</strong></td>
<td>Collagen type I alpha 1 chain</td>
<td>TACAGCAG GCTTGTGG ATGG</td>
<td>CAGATTG GGATGGA GGGAGTT</td>
</tr>
</tbody>
</table>
Primers and SYBR™ Green Master Mix were purchased from Life Technologies. PCRs were run on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Reactions were performed in duplicate. Four housekeeping genes (Gapdh, Hprt1, Ubc, and Actb) were tested under the same conditions. Expression stability was determined using RefFinder (Xie et al., 2012) BestKeeper (Pfaffl et al., 2004), Normfinder (Andersen et al., 2004) and Genorm (Vandesompele et al., 2002) programs with the help of the delta-Ct method (Silver et al., 2006). After a comparison of the results, Gapdh was selected as the best housekeeping gene (Supplementary Material 1). Levels of amplified cDNA were quantified using the $2^{-\Delta \Delta Ct}$ method ($\Delta \Delta Ct = \Delta Ct_{exposed} - \text{mean } \Delta Ct_{control}$), relative to Gapdh.

2.3.2. Assays of lipid and carbohydrate metabolic markers in plasma

Plasma glucose and lipids (TGs, total cholesterol (CT), and HDL were assayed on a Roche COBAS analyser (Roche Diagnostic, Meylan, France). The concentrations of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were calculated as VLDL = TG/2.2 and LDL = CT - HDL - VLDL. Liver enzymes: alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and lactate dehydrogenase (LDH), were also assayed on a Roche COBAS analyser. Lastly, leptin and insulin were assayed using the specific rat ELISA Kit (#KRC2281 and #ERINS; Life Technologies) with detection ranges of 62.5-4000 pg/mL and 4.69-300 µIU/mL respectively. All plasma assays were performed in the Endocrinology Laboratory at Cochin Hospital (Paris, France).
2.4. Evaluation of impairments in hepatic metabolism in offspring exposed perinatally to CPF and/or an HF diet

2.4.1. Hepatic TG levels

Hepatic TG levels were evaluated in 10 mg of liver tissue, using the PicoProbe Triglyceride Quantification Assay Kit (Fluorometric) ab178780 from Abcam, Cambridge, United Kingdom, according to the manufacturer’s instructions. Liver TG levels (expressed in mg/g liver tissue) were determined in a microplate-based fluorometric assay (excitation/emission = 535/587 nm). In order to relate the well fluorescence intensity to the TG level, a standard curve (from 0 to 4000 pmol/mL) was generated. All assays were performed in duplicate.

2.4.2. Liver tissue structure

Immediately after sacrifice, pieces of liver left lobe were fixed in Carnoy’s solution (60% absolute alcohol, 30% chloroform, and 10% acetic acid v/v) during 48 h before being embedded into xylene and then paraffin. Samples were then sliced into 5 μm sections and transferred to glass slides for staining with three different reagents. The first set of slides was stained with periodic acid Schiff (PAS) reagent (395B-1KT, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), in order to (i) highlight carbohydrates (mucins, basal lamina, glycogen…) in the liver tissue, and (ii) estimate liver glycogen levels (based on the proportion of PAS-positive hepatocytes). The second set of slides was stained with Picro Sirius Red, in order to visualize collagen type I and III fibres and evaluate fibrosis (if present). The third set of slides was treated with Masson’s trichrome stain, to reveal collagen fibres. Histological sections were observed under the light microscope (Olympus U TV1 X, Olympus, Japan) by a histopathologist who was blinded to the group assignment. Photos were taken using a camera attached to the microscope (Olympus DP-10 Digital Camera, Olympus America. New York). Staining was quantified on two fields/slide (100x magnification) with the help of ImageJ software (Schneider et al., 2012).
2.5. **Statistical analysis**

Data were expressed as the mean ± standard error of the mean (SEM) and were analysed using GraphPad Prism software (version 5.04 for Windows, GraphPad Software Inc., San Diego, CA, USA www.graphpad.com). Groups were compared using a non-parametric Kruskal-Wallis test; if the intergroup difference was statistically significant, Dunn’s post-hoc test was then applied. The threshold for statistical significance was set to p<0.05.

For additional information, please refer to the Key Resources Table.
3. Results

3.1. Perinatal exposure to CPF with an SC or an HF diet is associated with alterations in the growth rate and glycaemia but not in lipemia

3.1.1. Perinatal exposure to CPF is associated with slower pup growth from birth until weaning

Male pups from mothers exposed to CPF had a significantly lower mean ± SEM body weight immediately after birth (PND3; 7.99 g ± 0.11 g in the CPF-SC group, vs. 9.04 g ± 0.32 g in the ctrl-SC group; relative decrease: 12%; p=0.0196) (Figure 2). In contrast, perinatal exposure to an HF diet was associated with a significantly greater body weight at weaning (PND21: 73.3 g ± 3.03 g in the ctrl-HF group, vs. 55.5 g ± 1.90 g in the ctrl-SC group; relative increase: 32%; p=0.0317). At the end of the study (PND54), the body weight was greater (but not significantly) in the ctrl-HF group (322.2 g ± 12.99 g) than in the ctrl-SC group (288.8 g ± 4.36 g; relative increase: 12%; p=0.1932). Furthermore, perinatal HF diet exposure was associated with a significantly greater body weight in CPF-HF rats soon after birth (PND3; 10.09 g ± 0.13 g, vs. 7.99 g ± 0.11 g in CPF-SC; relative increase: 26%; p=0.0015), at weaning (PND21; 71.27 g ± 0.99 g in CPF-HF vs. 47.20 g ± 2.60 g in CPF-SC; relative increase: 51%; p=0.0017) and at early adulthood (PND54; 313.2 g ± 7.08 g in CPF-HF vs. 285.0 g ± 3.64 g in CPF-SC; relative increase: 10%; p=0.0351) (Figure 2).
Figure 2 – Body weight (g) at birth (PND3), after weaning (PND21) and in early adulthood (PND54) in male offspring born to mothers exposed to CPF and/or an HF diet.

Data were expressed as the mean ± SEM (n=5-20). * ** significant difference (p<0.05 and p<0.01, respectively) vs. ctrl-SC offspring. ctrl, control; CPF, chlorpyrifos; HF, high-fat diet; SC, standard chow diet; PND, postnatal day.

3.1.2. Perinatal CPF exposure alters regulation of the energy balance

The hepatic and plasma TG levels were higher (by 14% and 20%, respectively) in male offspring of dams from the CPF-SC group, relative to the ctrl-SC offspring. However, these differences were not statistically significant. Perinatal HFD exposure was also associated with a higher hepatic TG content (by 35%, vs. the ctrl-SC offspring: p=NS). There were no significant intergroup differences in the lipid profile (Table 3).

Plasma levels of insulin-like growth factor-1 (IGF1) and leptin were significantly lower in the CPF-SC offspring than in the ctrl-SC offspring (relative decreases: 49.11%, p=0.0043, and 57.80%, p=0.0321, respectively). The insulin level and glycaemia were slightly but not significantly higher (relative increases: 20% and 23%, respectively) in the CPF-SC offspring than in the ctrl-SC offspring. Perinatal HFD
exposure was associated with a slightly but not significantly higher glycaemia value (relative increase vs. ctrl-SC: 16%). However, there were no significant differences between the CPF-HF and ctrl-HF groups with regard to glycaemia or hormone levels (Table 3).

Table 3 - Lipid and carbohydrate metabolism in 8-week-old male offspring of dams exposed to CPF and/or an HFD.

Plasma samples were assayed for total cholesterol (TC), plasma and hepatic triglycerides (TGs), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and glycaemia-associated hormones. The data were quoted as the mean ± SEM (n=5-20). *,** significant difference (p<0.05 and p<0.01, respectively) vs. ctrl-SC offspring.

<table>
<thead>
<tr>
<th></th>
<th>ctrl-SC</th>
<th>CPF-SC</th>
<th>ctrl-HF</th>
<th>CPF-HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (µmol/L)</td>
<td>2214 ± 72.6</td>
<td>2252 ± 92.0</td>
<td>2064 ± 86.4</td>
<td>1987 ± 63.3</td>
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<tr>
<td>TG (µmol/L)</td>
<td>951 ± 73.7</td>
<td>1139 ± 103.1</td>
<td>960 ± 94.4</td>
<td>1463 ± 69.8</td>
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<tr>
<td>TG (mg/g liver)</td>
<td>24.21 ± 3.5</td>
<td>27.37 ± 1.4</td>
<td>32.89 ± 4.0</td>
<td>36.06 ± 8.21</td>
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<tr>
<td>HDL (µmol/L)</td>
<td>1533 ± 62.1</td>
<td>1573 ± 74.1</td>
<td>1470 ± 69.7</td>
<td>1323 ± 57.8</td>
</tr>
<tr>
<td>LDL (µmol/L)</td>
<td>478.3 ± 32.3</td>
<td>426.6 ± 25.2</td>
<td>759.7 ± 25.7</td>
<td>370.7 ± 15.8</td>
</tr>
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<td>Fasting glycaemia</td>
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<td>14.43 ± 0.8</td>
<td>13.53 ± 1.3</td>
<td>14.99 ± 2.4</td>
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<tr>
<td>(mmol/L)</td>
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<tr>
<td>Fasting leptin</td>
<td>622.3 ± 86.3</td>
<td>305.6 ± 33.6 *</td>
<td>759.7 ± 163.1</td>
<td>469.1 ± 63.9</td>
</tr>
<tr>
<td>(pg/mL)</td>
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<tr>
<td>Fasting insulin</td>
<td>10.12 ± 0.9</td>
<td>12.19 ± 1.3</td>
<td>9.73 ± 0.7</td>
<td>10.33 ± 3.8</td>
</tr>
<tr>
<td>(µU/L)</td>
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</table>
Fasting IGF1 (ng/mL) | 6.47 ± 0.33 | 3.74 ± 0.57 ** | 6.00 ± 0.50 | 4.61 ± 1.73

3.2. Perinatal exposure to CPF (but not to an HFD) is associated with significantly lower expression of genes coding for lipid metabolism.

Perinatal CPF exposure was associated with significantly lower mRNA expression of acetyl-CoA carboxylase 1 (Acaca; relative decrease vs. ctrl-SC: 58%; p=0.0019) and fatty acid synthase (Fasn, responsible for the synthesis of malonyl-CoA from acetyl-CoA) (relative decrease: 39%; p=0.0023). Exposure to both CPF and an HFD was associated with significantly greater expression of Srebf1/Srebp1c (coding for sterol regulatory element binding protein, a central regulator of lipogenesis) than for exposure to CPF alone (relative increase: 230%; p=0.0009 vs. CPF-SC; Figure 3). Furthermore, perinatal HFD exposure was associated with significantly greater expression of Acaca (relative increase vs. ctrl-SC: 90%; p<0.0001); Fasn (relative increase: 75%; p<0.0001) and Srebf1/Srebp1c (relative increase: 37%; p=0.0340). Exposure to both CPF and an HFD was associated with significantly lower expression of Srebf1/Srebp1c than for exposure to CPF alone (relative decrease vs. CPF-SC: 197%; p=0.0030). However, we did not observe any significant intergroup differences in the expression of Mlxipl/Chrebp (carbohydrate response element binding protein, another major transcription factor involved in lipid synthesis) (Figure 3).
Figure 3 – mRNA expression (RT-PCR) of lipogenesis markers in 8-week-old male offspring of dams exposed to CPF and/or an HF diet.

The mRNA gene expression levels were quantified as the fold induction over the control group (set to 1). Data were expressed as the mean ± SEM (n=5-20), using the $2^{-\Delta\Delta Ct}$ calculation method with the Gapdh housekeeping gene. *, ** and *** statistically significant difference (p<0.05, p<0.01, and p<0.001, respectively) vs. ctrl-SCD offspring. ctrl, control; CPF; chlorpyrifos; HF, high-fat diet; SC, standard chow diet; Acaca, acetyl-CoA carboxylase 1; Fasn, fatty acid synthase; Mlxipl, carbohydrate response element binding protein; Srebf1, sterol regulatory element binding factor 1 variant 2.

Perinatal CPF exposure was associated with significantly greater mRNA expression of the Pparg gene (coding for peroxisome proliferator-activated receptor gamma, a nuclear receptor involved in lipid storage; relative increase in the CPF-SC group vs. ctrl-SC: 238%; p=0.0002) and the lipoprotein lipase (Lpl) gene (relative increase: 126%; p<0.0001). This exposure was associated with significantly lower mRNA expression of the fatty acid transporter Cd36 (relative decrease vs. ctrl-SC: 38%, p=0.0015) and the diacylglycerol O-acyltransferases Dgat1 and Dgat2 involved in lipid transport (relative decreases vs. ctrl-SC: 70% and 65%, respectively; p=0.0005 and p=0.0002, respectively).

Perinatal HFD exposure was associated with significantly greater mRNA expression of the nuclear receptor Pparg (relative increase vs. ctrl-SC: 238%; p=0.0033). In
contrast, this exposure was associated significantly lower mRNA expression of \textit{Cd36} (relative decrease vs. ctrl-SC: 73%; \(p=0.0003\)), \textit{Dgat1} (relative decrease: 92%; \(p<0.0001\)) and \textit{Dgat2} (relative decrease: 76%; \(p=0.0002\)) (Figure 4).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{mRNA expression (RT-PCR) of markers of lipid accumulation in 8-week-old male offspring of dams exposed to CPF and/or an HF diet.
The mRNA gene expression levels were quantified as the fold induction, relative to the control group (set to 1). Data were expressed as the mean ± SEM (n=5-20), using the \(2^{-\Delta\Delta Ct}\) calculation method with the \textit{Gapdh} housekeeping gene. **, *** and **** statistically significant difference (\(p<0.01\), \(p<0.001\) and \(p<0.0001\), respectively) vs. ctrl-SC offspring. ctrl, control; CPF; chlorpyrifos; HF, high-fat diet; SC, standard chow diet; \textit{Cd36}; fatty acid translocase; \textit{Dgat1}; Diacylglycerol O-acyltransferase 1; \textit{Dgat2}; Diacylglycerol O-acyltransferase 2; \textit{Lpl}; lipoprotein lipase; \textit{Pparg}; peroxisome proliferator-activated receptor gamma.}
\end{figure}

Perinatal exposure to an HF diet was associated with significantly lower mRNA expression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (\textit{Hmgcr}, a major involved in lipid (cholesterol) synthesis), relative to the ctrl-SC offspring (relative decrease: 39%; \(p=0.0069\)) (Figure 5).
Figure 5 - mRNA expression (RT-PCR) of markers of cholesterol synthesis in 8-week-old male offspring of dams exposed to CPF and/or an HF diet.

The mRNA gene expression levels were quantified as the fold induction, relative to the control group (set to 1). Data were expressed as the mean ± SEM (n=5-20), using the $2^{-\Delta\Delta\text{Ct}}$ calculation method with the Gapdh housekeeping gene. ** statistically significant difference (p<0.01) vs. ctrl-SC offspring. ctrl, control; CPF; chlorpyrifos; HF, high-fat diet; SC, standard chow diet; Hmgcr, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase.

3.3. Perinatal exposure to CPF is associated with significantly lower expression of genes coding for enzymes in glucose metabolism but this influence is not exacerbated by perinatal exposure to HF diet

Perinatal CPF exposure was associated with significantly lower mRNA expression of G6pc (coding for glucose-6-phosphatase; relative decrease vs. ctrl-SC: 60%; p=0.0002) and Slc2a2/Glut2 (coding for a hepatic glucose transporter; relative decrease: 72%; p=0.0002). Perinatal HF diet exposure was associated with significantly lower mRNA expression of G6pc (relative decrease vs. ctrl-SC: 58%; p=0.0396) and Slc2a2/Glut2 (relative decrease: 87%; p=0.0014) (Figure 6).
Figure 6 - mRNA expression (RT-PCR) of markers of carbohydrate metabolism in 8-week-old male offspring of dams exposed to CPF and/or an HF diet.

The mRNA gene expression levels were quantified as the fold induction, relative to the control group (set to 1). Data were expressed as the mean ± SEM (n=5-20), using the $2^{-\Delta\Delta Ct}$ calculation method with the Gapdh housekeeping gene. *, ** and *** statistically significant difference (p<0.05, p<0.01 and p<0.001, respectively) vs. ctrl-SCD offspring. CPF; chlorpyrifos; ctrl, control; HF, high-fat diet; SC, standard chow diet; G6pc, glucose-6-phosphatase; Slc2a2, solute carrier family 2, facilitated glucose transporter member 2.

Lastly, perinatal HFD exposure was associated with significantly lower mRNA expression of $Pck1/Peck$ (coding for phosphoenolpyruvate carboxykinase; relative decrease vs ctrl-SC: 50%; $p=0.0207$). Perinatal exposure to both HFD and CPF was associated with significantly lower mRNA expressed of $Ppara$, relative to CPF exposure alone (relative decrease vs. CPF-SC: 80%; $p=0.0018$) (Figure 7).
Figure 7 - mRNA expression (RT-PCR) of markers of gluconeogenesis and fatty acid oxidation in 8-week-old male offspring of dams exposed to CPF and/or an HF diet.

The mRNA gene expression levels were quantified as the fold induction, relative to the control group (set to 1). Data were expressed as the mean ± SEM (n=5-20), using the $2^{-\Delta\Delta Ct}$ calculation method with the Gapdh housekeeping gene. * statistically significant difference (p<0.05) vs. ctrl-SCD offspring. ctrl, control; CPF; chlorpyrifos; HF; high-fat diet; SC, standard chow diet; Pck1, phosphoenolpyruvate carboxykinase; Ppara, peroxisome proliferator activated receptor alpha.

3.4. Perinatal exposure to an HF diet and/or CPF was not associated with changes in liver tissue structure

3.4.1. Perinatal exposure to CPF and/or an HF diet was not associated with histopathological changes in the offspring’s liver

In all histopathological analyses, the offspring’s liver tissues sections had a normal structure and composition (a central vein, and well-organized hepatocytes) (Figure 8 A). Indeed, no histopathological changes (portal area hypertrophy, dilatation of bile duct or the branch of the hepatic artery, mononuclear cell infiltration, vacuolization, etc.) were observed in liver sections in any of the groups studied. PAS staining of liver sections showed that exposure of CPF and/or HFD was not associated with steatosis or inflammation (Figure 8A): the liver’s tissue architecture (e.g. percentage of steatosis) was not modified, relative to ctrl-SCD offspring (data not shown). However, the hepatocyte surface area was significantly lower (by 17%; p=0.0145) in
the CPF-SCD offspring than in the ctrl-SCD offspring. No collagen fibres could be seen between the hepatocytes – suggesting that the amount of collagen was below the staining methods’ limits of detection. Therefore, we did not observe any characteristic features of hepatic fibrosis (Figure 8 B and C).

A

PAS staining

<table>
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<th>ctrl-HF</th>
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Masson’s trichrome staining

ctrl-SC  CPF-SC

ctrl-HF  CPF-HF

Sirius Red staining

ctrl-SC  CPF-SC

ctrl-HF  CPF-HF
Figure 8 – PAS, Masson’s trichrome and Sirius Red staining of liver tissue in the 8-week-old male offspring of mothers exposed to CPF and/or an HF diet.

(A) PAS staining (magnification: 100x), (B) Masson trichrome staining of collagen fibres (100x), (C) Sirius Red staining of collagen I and III fibres (100x). Data were expressed as the mean ± SEM (n=5-20): there were no significant intergroup differences. ctrl, control; CPF; chlorpyrifos; HF, high-fat diet, SC, standard chow diet; PAS; periodic acid Schiff.

3.4.2. Perinatal CPF exposure might be associated with subsequent fibrosis

Perinatal exposure of dams to CPF was associated with significantly lower mRNA expression of the *IL1b* gene (an inductor of the inflammatory response (relative decrease vs. ctrl-SC: 46%; p=0.0071) and significantly greater mRNA expression of *Col1a1* (coding for one of the two main collagen fibres; relative increase vs. ctrl-SC: 160%; p<0.0001) (Figure 9). Maternal exposure to an HFD was associated with significantly lower *IL1b* gene expression (relative decrease vs. ctrl-SC: 82%; p<0.0001) (Figure 9).
**Figure 9** - mRNA expression (RT-PCR) of markers of inflammation and fibrosis in 8-week-old male offspring of dams exposed to CPF and/or an HF diet.

The mRNA gene expression levels correspond to the fold-induction over the control group (set to 1). Data were expressed as the mean ± SEM (n=5-20), using the $2^{-\Delta\Delta Ct}$ calculation method with the Gapdh housekeeping gene. **, **** statistically significant difference (p<0.01 and p<0.0001, respectively) vs. ctrl-SC offspring. ctrl, control; CPF; chlorpyrifos; HF, high-fat diet; SC, standard chow diet; Col1a1; collagen alpha-1; IL1b; interleukin 1 beta.

The mRNA expression of Prkaa1/Ampka (coding for a sensor of the cell’s energy homeostasis) was significantly lower (by 33%) in the CPF-SC offspring than in the ctrl-SC offspring (p=0.0448). Perinatal HFD exposure was associated with significantly lower mRNA expression of Prkaa1/Ampka (relative decrease vs. ctrl-SC: 69%) (Figure 10).
3.4.3. Perinatal CPF exposure is associated with an elevation of liver damage markers

The plasma levels of liver enzymes tended to be higher in the offspring of mothers treated with CPF. This difference was significant (relative increase vs. ctrl-SC: 68%; p=0.0347) for ALP - one of the most sensitive markers of liver damage (Table 4).

Table 4 - Plasma levels of markers of hepatic metabolic activity in 8-week-old male offspring of dams exposed to CPF and/or an HF diet.

The data were expressed as the mean ± SEM (n=5-20). * statistically significant difference (p<0.05) vs. ctrl-SCD offspring. ctrl, control; CPF; chlorpyrifos; HF, high-fat diet; SC, standard chow diet; Prkaa1; AMP-activated protein kinase catalytic subunit alpha.
<table>
<thead>
<tr>
<th></th>
<th>ctrl-SC</th>
<th>CPF-SC</th>
<th>ctrl-HF</th>
<th>CPF-HF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALP (UI/L)</strong></td>
<td>94.4 ± 21.2</td>
<td>159.1 ± 7.0 *</td>
<td>126.1 ± 7.8</td>
<td>147.6 ± 22.1</td>
</tr>
<tr>
<td><strong>ALAT (UI/L)</strong></td>
<td>41.12 ± 9.7</td>
<td>71.79 ± 8.4</td>
<td>77.96 ± 10.7</td>
<td>67.52 ± 4.9</td>
</tr>
<tr>
<td><strong>ASAT (UI/L)</strong></td>
<td>116.3 ± 32.1</td>
<td>207.5 ± 35.1</td>
<td>243.0 ± 59.7</td>
<td>251.8 ± 72.0</td>
</tr>
<tr>
<td><strong>ASAT/ALAT (UI/L)</strong></td>
<td>2.51 ± 0.3</td>
<td>2.74 ± 0.2</td>
<td>2.84 ± 0.29</td>
<td>3.62 ± 0.9</td>
</tr>
<tr>
<td><strong>LDH (UI/L)</strong></td>
<td>539.9 ± 256.1</td>
<td>673.8 ± 103.6</td>
<td>585.8 ± 124.5</td>
<td>1145 ± 343.4</td>
</tr>
</tbody>
</table>
4. Discussion

Perinatal exposure to OPs might be associated with abnormalities in body weight gain and with changes in the expression of certain genes involved in the regulation of glucose and lipid homeostasis in adulthood. Here, we studied the consequences of maternal metabolic disorders and pesticide exposure during critical windows of development on the offspring’s metabolism and liver tissue architecture.

We exposed the dams to a 1 mg/kg/d dose of CPF which is the oral No Observable Adverse Effect Level in the rat for this pesticide. According to the literature, this dose can depress acetylcholinesterase activity (Cochran et al., 1995). To ensure that all the animals were exposed to the same amount of CPF and to mimic natural exposure in humans (residues on fruits and vegetables), the animals were gavaged. When performed by an experienced handler, gavaging does not generate stress or behavioural or metabolic changes in the dam or the offspring (McDonnell-Dowling et al., 2017). In the HF diet used in the present experiments, 60% of the energy content came from fat; the literature data show that this type of diet induces metabolic alterations in the offspring in adulthood (at 7 months of age) (Lecoutre et al., 2016).

We found that chronic, repeated exposure of dams to CPF was associated with developmental impairments in the male offspring, as characterized by a lower birth weight and lower plasma levels of growth factors (such as IGF1) and leptin. As leptin is produced mainly in adipose tissue, a low level of this hormone decrease might be linked to low body fat deposition or dysregulated leptin secretion in the male pups. Furthermore, CPF exposure in the dams appeared to induce intrauterine growth restriction, since we observed a significant relative reduction in birthweight. This might be due to CPF’s toxic effect on placental nutrition of the foetus, as suggested by studies of rats exposed orally to CPF during the pre- and postnatal periods (Akhter et al., 2006; Joly Condette et al., 2015, 2014; Mattsson et al., 2000; Maurissen et al., 2000). Moreover, the CPF exposure in our experiments continued after birth via lactation. It has been reported that CPF concentrates in the mother’s milk and that pups are exposed to measurable amounts of CPF during lactation (Mattsson et al., 2000). These impairments appeared to have disappeared by adulthood (PND54), when the offspring were no longer exposed to CPF. In contrast, perinatal exposure to an HFD was associated with greater body weight at weaning.
and in early adulthood, although the differences vs. control animals were not statistically significant. Our results are in line with those of some other studies (Ribaroff et al., 2017; Tellechea et al., 2017). Even though we did not observe a cumulative effect of perinatal HF diet and CPF exposures, we did find that the HF diet was associated with a significantly greater body weight from birth into young adulthood in pups exposed to CPF - as described previously by other researchers (Cortés-Álvarez et al., 2020). Indeed, the nutritional composition of breast milk is related to maternal obesity and diet during pregnancy and lactation. Ingestion of an HF diet increases the lipid concentration in the mother’s milk (Purcell et al., 2011) and modulates the metabolic defects associated with early-life exposure to various OPs. For example, neonatal exposure to parathion and an HF diet was associated with a greater incidence of obesity, diabetes, liver problems and metabolism perturbations in the rat (Adigun et al., 2010; Lassiter and Brimijoin, 2008). In our experiments, maternal CPF exposure was associated with statistically higher plasma levels of insulin and glucose and a clear trend of higher TG. These results were linked to previous studies (Slotkin, 2011; Slotkin et al., 2005a). Unexpectedly, we found that dams in the CPF-HF group were less fertile than the other dams. A recent review reported that an HF diet impairs female reproductive function by altering the hypothalamic-pituitary-ovarian axis (Hohos and Skaznik-Wikel, 2017), and a late study revealed that CPF also induces reproductive problems (changes in sperm, serum hormone levels, oxidative stress in the testis, and the activity of enzymes related to spermatogenesis) in the rat (Li et al., 2019). We did not observe such consequences in our model. Since all the animals in the present study were raised in the same environment, the observed difference can only be explained by the cumulative effect of the two harmful factors studied here. To clarify this point, the cumulative impacts of CPF and an HFD on reproductive functions (with a focus on putative follicular atresia in the dams’ ovaries) shall be investigated further.

The low weight gain described above was correlated with alterations in lipid and glucose metabolism. Perinatal exposure to CPF or an HF diet was associated with lower expression of key genes in lipogenesis, such as \textit{Acaca} (coding for an enzyme that produces malonyl-CoA for fatty acid synthesis) and \textit{Fasn} (coding for an enzyme that catalyses the last step in fatty acid biosynthesis). In contrast, expression of \textit{Srebf1/Srebp1c} (coding for the main transcription factor for \textit{Acaca} and \textit{Fasn}) was
only affected by HF diet exposure. Perinatal exposure to CPF or an HF diet also led
to a relative decrease in the expression of lipid accumulation markers such as \textit{Cd36},
\textit{Dgat} 1, and \textit{Dgat}2; the latter two enzymes catalyse the final step in TG synthesis.
Although this decrease might conceivably lead to a lower TG content in the liver, we
actually observed a slight increase. We hypothesize that fewer fatty acids were
synthesized from acetyl CoA and so fewer TGs were stored in the liver. This
difference was associated with greater expression of \textit{Lpl} following perinatal CPF
exposure. \textit{Lpl} hydrolyses TGs into fatty acids and glycerol and is also a ligand
facilitating the cellular uptake of VLDL and LDL. This could be responsible for an
increase in lipid transport. As a consequence, we could expect an increase of \textit{Cd36}
mRNA expression related to lipid-rich diets and hepatic steatosis (Miquilena-Colina et
al., 2011). But we did not notice this increase in progeny from mother exposed to
CPF or HFD, indicated a normal state of hepatocytes. Moreover, perinatal exposures
to CPF alone or an HF diet alone were both associated with greater mRNA
expression of \textit{Pparg} (a nuclear receptor and transcription factor involved in lipid
storage) in the male pups. Likewise, perinatal HF diet exposure was also associated
with lower mRNA expression of \textit{Hmgcr} - the main gene related to lipid (cholesterol)
synthesis - in these animals. The lower level of hepatic lipid storage in the offspring
exposed to CPF and HFD was probably due an overall reduction in lipid metabolism,
as suggested by the lower gene expression of key factors. However, it remains to be
seen whether protein expression and function are modified. The mechanism(s)
underlying differences in gene expression will also have to be characterized. A
change in protein levels would indicate dysregulation of the main hepatic lipogenic
pathways and thus an impairment in hepatic metabolism. In line with the literature
data, concomitant perinatal co-exposure of the dams to both CPF and HFD tended to
have the same consequences as each factor separately; although synergistic effects
were not observed (Adigun et al., 2010).

Maternal CPF exposure was associated with greater mRNA expression of \textit{Ppara} in
the pups' liver, whereas maternal HF diet exposure was associated with lower
expression of the gene in its offspring. \textit{Ppara} is involved in the inflammatory
response and in fatty acid oxidation. \textit{Ppara} targeted genes control the release of fatty
acids from TG-rich lipoproteins, chylomicrons, and VLDL. In our study, the lower
plasma TG level reflected lower hepatic synthesis and greater catabolism in the
plasma; this may have been related to regulation of the Lpl gene. This hypothesis was confirmed by the significantly greater mRNA expression of Lpl and Ppara genes and the elevation (albeit non-significant) in the plasma TG level in the offspring of CPF-exposed dams. Perinatal CPF and HF diet exposure was also associated with differences in glucose metabolism. We noted lower expression of Slc2a2/Slc2a2 (a glucose transporter) and G6pc (a major regulatory enzyme in gluconeogenesis and which catalyses the hydrolysis of glucose-6-phosphate to D-glucose). A lower level of gluconeogenesis might be related to the elevation (albeit non-significant) in glycaemia after perinatal exposure of dams to CPF or an HF diet. Moreover, insulin inhibits the transcription of Pck1/Pepck gene, which is involved in gluconeogenesis and the transformation of oxaloacetate into phosphoenolpyruvate. Perinatal HFD exposure was associated with lower Pck1/Pepck gene expression in the offspring. We hypothesize that lower glucose uptake by liver cells resulted in an elevation of glycaemia and a relative decrease in lipid synthesis in general and TG synthesis in particular.

We also looked at potential tissue changes and inflammation in the liver. The plasma levels of liver enzymes went higher after perinatal CPF exposure (relative to controls), with a relative reduction in hepatocyte size (in agreement with the literature (Mansour and Mossa, 2010)); these differences were suggestive of damage to the hepatocyte membrane. The relative increase associated with CPF exposure was statistically significant for ALP - the most sensitive marker of liver damage. No histological alterations in young adult rats were noted in any of the experimental groups. Maternal exposure to CPF was also associated with greater expression of the Col1a1 gene (coding for one of the two main components of collagen fibres); this might predict the subsequent development of fibrosis. In contrast, maternal exposure to an HF diet was not associated with any differences in markers of liver damage in the offspring, except a slight but nonsignificant elevation of plasma levels of liver enzymes. Nevertheless, concomitant maternal exposure to both an HF diet and CPF was associated with lower mRNA expression of Prkaa1/Ampka, a sensor of the cell’s energy homeostasis. This enzyme switches on fatty acid oxidation and glycolysis and switches off lipogenesis via short-term effects on regulatory protein phosphorylation and long-term effects on gene expression (Foretz et al., 2006). We expected to observe greater expression of this gene in the offspring of dams exposed to both an
HF diet and CPF; this would have explained the lower level of lipogenesis. The observed impacts might only be predictive of more severe impairments later in life; the age of 8 weeks was a probably too early to observe such metabolic effects. Even though the exact fat content of an HF diet is not correlated with any outcomes in offspring, other factors (such as the species, the effects of the interventional diet on the mother, the intervention time point, and the micronutrient and macronutrient content of the control diet) might account for the differences in outcomes between studies in which HF diets were used to induce obesity (Ribaroff et al., 2017).

The primary objective of the present study was to assess the potential impact of a harmful maternal environment (an obesogenic diet and/or exposure to the pesticide CPF) in the offspring. Even in the absence of direct contact between the offspring and the harmful factors (except through lactation), we found that exposure had an impact on metabolic activity in the liver. Indirect exposure to CPF was associated with alterations in the mRNA expression of several liver enzymes, which in turn might have reduced neoglucogenesis and de novo lipogenesis. Exposure to an HF diet alone during the perinatal period induced the same type of effect as CPF alone - showing that long-term metabolic disorders can occur in the absence of inflammatory signs (Figure 11).
Figure 11 – Hypothetical changes in the liver during the development of the male offspring of dams exposed to CPF alone and/or combined with an HFD. CPF; chlorpyrifos; HFD, high-fat diet; Acaca, acetyl-CoA carboxylase 1; Cd36; fatty acid translocase; Col1at; collagen alpha-1; Dgat1; Diacylglycerol O-acyltransferase 1; Dgat2; Diacylglycerol O-acyltransferase 2; FA, fatty acids; Fasn, fatty acid synthase; G6pc, glucose-6-phosphatase; Hmgr, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; Il1b; interleukin 1 beta; Lpl; lipoprotein lipase; Pck1, phosphoenolpyruvate carboxykinase; Ppara, peroxisome proliferator activated receptor alpha; Pparg, peroxisome proliferator activated receptor gamma; Prkaa1; AMP-activated protein kinase catalytic subunit alpha; Slc2a2, solute carrier family 2, facilitated glucose transporter member 2; Srebf1, sterol regulatory element binding factor 1 variant 2.
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parathion exposure and interactions with a high-fat diet in adulthood: Adenylyl
81, 605–612. https://doi.org/10.1016/j.brainresbull.2010.01.003
effects of Taf and Egyptian pomegranates: molecular study. Bioscience,
Biotechnology, and Biochemistry 79, 598–609.
https://doi.org/10.1080/09168451.2014.982505
Akhtar, N., Srivastava, M.K., Raizada, R.B., 2006. Transplacental disposition and
https://doi.org/10.2131/jts.31.521
Control diet in a high-fat diet study in mice: Regular chow and purified low-fat
diet have similar effects on phenotypic, metabolic, and behavioral outcomes.
https://doi.org/10.1080/1028415X.2017.1349359
Quantitative Reverse Transcription-PCR Data: A Model-Based Variance
Estimation Approach to Identify Genes Suited for Normalization, Applied to
Bladder and Colon Cancer Data Sets. Cancer Res 64, 5245–5250.
https://doi.org/10.1158/0008-5472.CAN-04-0496
18, 733–736. https://doi.org/10.1023/a:1025388901248
Cantoral, A., Montoya, A., Luna-Villa, L., Roldán-Valadez, E.A., Hernández-Avila, M.,
Kershenobich, D., Perng, W., Peterson, K.E., Hu, H., Rivera, J.A., Téllez-Rojo,
M.M., 2020. Overweight and obesity status from the prenatal period to
adolescence and its association with Non-alcoholic fatty liver disease in young
Gynaecology n/a. https://doi.org/10.1111/1471-0528.16199
Chen, T., Yao, L., Ke, D., Cao, W., Zuo, G., Zhou, L., Jiang, J., Yamahara, J., Li, Y.,
Wang, J., 2016. Treatment with Rhodiola crenulata root extract ameliorates
insulin resistance in fructose-fed rats by modulating sarcolemmal and
intracellular fatty acid translocase/CD36 redistribution in skeletal muscle. BMC
Chlorpyrifos: Hazard assessment based on a review of the effects of short-
term and long-term exposure in animals and humans. Food and Chemical
Toxicology 33, 165–172. https://doi.org/10.1016/0278-6915(94)00124-7
Cortés-Álvarez, N.Y., Vuelvas-Olmos, C.R., Pinto-González, M.F., Guzmán-Muñiz,
pregnancy impairs memory acquisition and increases leptin receptor
https://doi.org/10.1080/1028415X.2020.1728473
Czajka, M., Matysiak-Kucharek, M., Jodłowska-Jędrych, B., Sawicki, K., Fal, B.,
Drop, B., Kruszewski, M., Kapka-Skrzypczak, L., 2019. Organophosphorus
pesticides can influence the development of obesity and type 2 diabetes with
concomitant metabolic changes. Environmental Research 178, 108685.

https://doi.org/10.1016/j.envres.2019.108685

El Khayat El Sabbouri, H., Gay-Quéheillard, J., Joumaa, W.H., Delanaud, S.,
Guibourdencie, M., Darwiche, W., Djekkoun, N., Bach, V., Ramadan, W.,
2020. Does the perigestational exposure to chlorpyrifos and/or high-fat diet
affect respiratory parameters and diaphragmatic muscle contractility in young
rats? Food and Chemical Toxicology 140, 111322.

https://doi.org/10.1016/j.fct.2020.111322

Elsharkawy, E.E., Yahia, D., El-Nisr, N.A., 2013. Sub-chronic exposure to
chlorpyrifos induces hematological, metabolic disorders and oxidative stress in
rat: Attenuation by glutathione. Environmental Toxicology and Pharmacology


https://doi.org/10.1002.9378(66)90526-6

Foretz, M., Taleux, N., Guigas, B., Hormann, S., Beauloye, C., Andreelli, F., Bertrand,
nouvelle voie thérapeutique pour le traitement des maladies métaboliques et

https://doi.org/10.1051/medsci/2006224381

Hermier, D., Mathé, V., Lan, A., Santini, C., Quignard-Boulangé, A., Huneau, J.-F.,
Mariotti, F., 2017. Postprandial low-grade inflammation does not specifically
require TLR4 activation in the rat. Nutr Metab (Lond) 14, 1–7.

https://doi.org/10.1186/s12986-017-0220-4


Comparative Distribution of Trichloropyridinol in the Fetus and Dam.
Toxicology and Applied Pharmacology 158, 16–23.

https://doi.org/10.1006/taap.1999.8689

Joly Condette, C., Khorsi-Cauet, H., Morlière, P., Zabijak, L., Reygner, J., Bach, V.,
Gay-Quéheillard, J., 2014. Increased Gut Permeability and Bacterial
https://doi.org/10.1371/journal.pone.0102217

Chlorpyrifos Exposure During Perinatal Period Affects Intestinal Microbiota

https://doi.org/10.1097/MPG.0000000000000734

Kamai, E.M., McElrath, T.F., Ferguson, K.K., 2019. Fetal growth in environmental
epidemiology: mechanisms, limitations, and a review of associations with
biomarkers of non-persistent chemical exposures during pregnancy. Environ

to chlorpyrifos on cholinergic and non-cholinergic targets in normal and high-
fat fed male C57BL/6J mice. Toxicology and Applied Pharmacology 337, 67–
75. https://doi.org/10.1016/j.taap.2017.10.019

neurotoxicity in the progeny after maternal gavage with chlorpyrifos. Food and
Chemical Toxicology 113, 66–72. https://doi.org/10.1016/j.fct.2018.01.026


Agonist. J Pharmacol Exp Ther 327, 716–726. https://doi.org/10.1124/jpet.108.143271


Chlorpyrifos (CPF) gavage or vehicle

High fat diet (HFD) or standard chow diet (SCD)

PREGESTATION
4 months

GESTATION

LACTATION
PND1

WEANING
PND21

PND54

Perinatal exposure to pesticide: Growth retardation

Perinatal exposure to pesticide and/or high fat diet: Reduction in neoglucogenesis and lipogenesis de novo