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Supplemental Material

Effects of PCB126 on Adipose-to-Muscle Communication in an *in Vitro* Model

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Figure S3. Effect of direct or indirect PCB126 exposure on *Cyp1a1* mRNA expression in C2C12 myotubes. Differentiated C2C12 myotubes were exposed for the last 24hrs of differentiation (A) to different PCB126 concentrations in insulin sensitive (IS) or insulin resistant (IR) conditions or (B) to the conditioned medium (CM) of 3T3-L1 adipocytes exposed to different PCB126 concentrations in IS or IR conditions. *Cyp1a1* mRNA levels were normalized to β-actin mRNA levels and analyzed using the ΔΔCT method. Average of normalized ΔΔCT is presented relative to the vehicle (IS, no PCB) ±SEM. n=3 independent experiments, each independent experiment was done in triplicate. The exact mean and SEM values for data presented in Figure S3 can be found in Tables S33 and S34.

Figure S4. Effect of PCB126 exposure and insulin resistance on adipokine secretion in 3T3-L1 adipocytes. A. 3T3-L1 adipocytes were differentiated in insulin resistant (IR) conditions and treated for the last 24hrs of differentiation with different PCB126 concentrations. Leptin was measured in the medium using the mouse adipocyte magnetic bead panel kit (Millipore, MADCYMAG-72K-07). Leptin was only detected when IR adipocytes were treated with 100 nM PCB126. ND: not detected. B-D. Insulin sensitive (IS) and insulin resistant (IR) 3T3-L1 adipocytes were exposed to different concentrations of PCB126 for 24hrs. Interleukin 6 (IL-6) (B), adiponectin (C) and leptin (D) levels were then measured in the conditioned medium using ELISA kits. Mean ±SEM. n=3-4 independent experiments, each independent experiment was done in duplicate. *: $P<0.05$, **: $P<0.01$ compared to vehicle-treated adipocytes, ##: $P<0.01$, ###: $P<0.001$, main effect of IR conditions (2-way ANOVA with Fisher's PLSD post-hoc test). The exact mean and SEM values for data presented in Figure S4 can be found in Tables S35 to S38.

Figure S5. Insulin signaling in 3T3-L1 adipocytes exposed to PCB126 in insulin resistant (IR) conditions and in C2C12 exposed to the conditioned medium (CM) of PCB126-treated IR adipocytes. A. 3T3-L1 adipocytes were differentiated in IR conditions and treated for the last 24hrs of differentiation with different PCB126 concentrations and cells were subsequently treated with ±100 nM insulin. Top panel: quantification of p-IRS1/α-tubulin, p-Akt/Akt and p-AS160/AS160 by density analysis. Bottom panel: representative western blots. Tubulin was used as the loading control. B. Differentiated C2C12 myotubes were exposed for the last 24hrs of differentiation to the CM of PCB126-treated IR adipocytes and cells were subsequently treated with ±100 nM insulin. Top panel: quantification of p-Akt/Akt and p-GSK3/GSK3 by density analysis. Bottom panel: representative western blots. GAPDH was used as the loading control. Data are presented relative to the vehicle as mean ±SEM. n=3 independent experiments. The exact mean and SEM values for data presented in Figure S5 can be found in Tables S39 and S40.

Figure S6. Glucose uptake in C2C12 or mouse primary myotubes directly exposed to PCB126 in insulin sensitive (IS) and insulin resistant (IR) conditions. Differentiated C2C12 myotubes (A) or mouse primary myotubes (B,C) were exposed for the last 24hrs of differentiation to different PCB126 concentrations in IS or IR conditions (A and B) or to the CM of PCB126-treated IR adipocytes for the last 24hrs of differentiation (C). After differentiation and treatments, cells were subsequently treated with ±100 nM insulin for 20 min and exposed to 10 μM 2-deoxy-glucose and 0.5 μCi/mL [^{3}H]2-deoxyglucose for 10 min. Data are presented relative to the vehicle as mean ±SEM. n=3-4 independent experiments, each independent experiment was done in triplicate. The exact mean and SEM values for data presented in Figure S6 can be found in Tables S41 to S45.

Figure S7. Glycolytic rates in C2C12 myotubes directly exposed to PCB126 in insulin sensitive (IS) or insulin resistant (IR) conditions. Differentiated C2C12 myotubes were exposed for the last 24hrs of differentiation to different PCB126 concentrations in IS (left panel) or IR conditions (right panel). Glycolytic rates were estimated by measuring extracellular acidification rates (ECAR) with a Seahorse analyzer (Agilent). ECAR were first measured in resting conditions, and cells were then treated with 600 ng/mL oligomycin to determine maximal glycolytic capacity (M.G.C.). Data are presented relative to the vehicle as mean ±SEM. n=4 independent experiments, each independent experiment was done in 5 replicates. The exact mean and SEM values for data presented in Figure S7 can be found in Tables S46 and S47.

Table S1. Cyp1a1 mRNA expression in 3T3-L1 adipocytes exposed for 24hrs to different PCB126 concentrations, in IS and IR conditions. Mean and SEM values are the ones presented in Figure 2A.

Table S2. Adiponectin mRNA expression in 3T3-L1 adipocytes exposed for 24hrs to different PCB126 concentrations, in IS and IR conditions. Mean and SEM values are the ones presented in Figure 2B.

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Table S4. Adipokines secreted in the medium of 3T3-L1 adipocytes differentiated in IS conditions and treated for 24hrs to different PCB126 concentrations. Mean and SEM values are the ones presented in Figure 2D.

Table S5. Adipokines secreted in the medium of 3T3-L1 adipocytes differentiated in IR conditions and treated for 24hrs to different PCB126 concentrations. Mean and SEM values are the ones presented in Figure 2E.

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Table S7. Glycerol concentration (μmol/μg of proteins) in the medium of IS and IR 3T3-L1 adipocytes exposed to PCB126 for 24hrs. Mean and SEM values are the ones presented in Figure 3B.

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Table S9. Oxygen consumption rate measured in IR 3T3-L1 adipocytes exposed to PCB126 for 24hrs. Mean and SEM values are the ones presented in Figure 4B.

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Table S21. Extracellular acidification rate (ECAR) in IR 3T3-L1 adipocytes exposed to PCB126 for 24hrs. Mean and SEM values are the ones presented in Figure 6B.

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Table S30. Lipid accumulation in IR 3T3-L1 adipocytes, in C2C12 myotubes exposed to the conditioned medium (CM) of IR adipocytes, and in control myotubes directly treated with PCB126 in IR conditions. Mean and SEM values are the ones presented in Figure S2A, right panel.

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Table S36. IL-6 secretion (pg/ml) in 3T3-L1 adipocytes exposed to PCB126 for 24hrs in IS and IR conditions. Mean and SEM values are the ones presented in Figure S4B.

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Table S46. Glycolytic rates in C2C12 myotubes directly exposed to PCB126 in IS conditions. Mean and SEM values are the ones presented in Figure S7, left panel.

Table S47. Glycolytic rates in C2C12 myotubes directly exposed to PCB126 in IR conditions. Mean and SEM values are the ones presented in Figure S7, right panel.