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Supplemental Material

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

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Table of Contents

Figure S1. Effect of insulin sensitive (IS) and insulin resistant (IR) conditions on 3T3-L1 adipocyte glucose uptake and lipid content. A. Glucose uptake after treatment with insulin normalized to basal conditions (i.e. fold increase in response to insulin). Glucose uptake was measured in 3T3-L1 adipocytes differentiated in IS and IR conditions, under basal condition (no insulin) and after 20 min insulin treatment (100 nM). n=3 independent experiment, each independent experiment was done in triplicate. Mean \pm SEM. #: $P < 0.05$ compared to IS (Student's t-test). B. Representative image of lipid droplet visualization by Oil Red O staining in 3T3-L1 adipocytes differentiated in IS or IR conditions. Lipid droplets were visualized using a light microscope with a 40X objective. For this experiment, 3T3-L1 adipocytes were in basal conditions (not acutely treated with insulin). These images were representative of 4 independent experiments. The exact mean and SEM values for data presented in Figure S1 can be found in Table S28.

Figure S2. Effect of PCB126 or conditioned medium (CM) treatments on lipid accumulation and cell viability in 3T3-L1 adipocytes and C2C12 myotubes. A-B. Insulin sensitive (IS, left panel) and insulin resistant (IR, right panel) 3T3-L1 adipocytes and C2C12 myotubes (control C2C12) were directly exposed to different concentrations of PCB126 for 24hrs. The conditioned media from adipocytes were subsequently transferred to C2C12 myotubes for 24hrs (CM C2C12). A. After treatments, cells were fixed, and lipid droplets stained with Oil Red O. Stained lipid droplets were extracted with isopropanol and a triplicate for every well was read at 492 nm. Mean \pm SEM. n=4 independent experiments, each independent experiment was done at least in triplicate. B. After treatment, cells were incubated with PrestoBlue for 30 min. Plates were read at 570 nm and 600 nm (reference wavelength). n=3 independent experiments, each independent experiment was done at least in triplicate. A. Data are presented relative to the vehicle (0 nM PCB126) in 3T3-L1. B. Data are presented relative to the vehicle (0 nM PCB126) for each cell line independently. Mean \pm SEM. The exact mean and SEM values for data presented in Figure S2 can be found in Tables S29 to S32.

Figure S3. Effect of direct or indirect PCB126 exposure on *Cyp11a1* 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. *Cyp11a1* mRNA levels were normalized to β -actin mRNA levels and analyzed using the $\Delta\Delta$ CT method. Average of normalized $\Delta\Delta$ CT is presented relative to the vehicle (IS, no PCB) \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 100 nM insulin for 20 min and exposed to 10 μ M 2-deoxyglucose and 0.5 μ Ci/mL [3 H]2-deoxyglucose for 10 min. Data are presented relative to the vehicle as mean \pm 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 \pm 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.

Table S3. IL-6 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 2C.

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.

Table S6. Free fatty acid (FFA) concentration ($\mu\text{mol}/\mu\text{g}$ 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 3A.

Table S7. Glycerol concentration ($\mu\text{mol}/\mu\text{g}$ 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.

Table S8. Oxygen consumption rate measured in IS 3T3-L1 adipocytes exposed to PCB126 for 24hrs. Mean and SEM values are the ones presented in Figure 4A.

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.

Table S10. Mitochondrial complex levels in 3T3-L1 adipocytes exposed for 24hrs to PCB126-treated 3T3-L1 adipocytes in IR conditions. Mean and SEM values are the ones presented in Figure 4C.

Table S11. Oxygen consumption rate measured in C2C12 myotubes exposed to the conditioned medium (CM) of PCB126-treated IS adipocytes. Mean and SEM values are the ones presented in Figure 4E.

Table S12. Oxygen consumption rate measured in C2C12 myotubes exposed to the conditioned medium (CM) of PCB126-treated IR adipocytes. Mean and SEM values are the ones presented in Figure 4F.

Table S13. Oxygen consumption rate measured in C2C12 myotubes exposed directly to PCB126 in IS conditions. Mean and SEM values are the ones presented in Figure 4G.

Table S14. Oxygen consumption rate measured in C2C12 myotubes exposed directly to PCB126 in IR conditions. Mean and SEM values are the ones presented in Figure 4H.

Table S15. Glucose uptake in IS 3T3-L1 adipocytes exposed to PCB126 for 24hrs. Mean and SEM values are the ones presented in Figure 5A and B.

Table S16. Glucose uptake in IR 3T3-L1 adipocytes exposed to PCB126 for 24hrs. Mean and SEM values are the ones presented in Figure 5C and D.

Table S17. Glucose uptake in C2C12 myotubes exposed to the conditioned medium (CM) of PCB126-treated IS adipocytes. Mean and SEM values are the ones presented in Figure 5E.

Table S18. Glucose uptake in C2C12 myotubes exposed to the conditioned medium (CM) of PCB126-treated IR adipocytes. Mean and SEM values are the ones presented in Figure 5F.

Table S19. Glucose uptake in mouse primary myotubes exposed to the conditioned medium (CM) of PCB126-treated IS adipocytes treated with PCB126. Mean and SEM values are the ones presented in Figure 5 G and H.

Table S20. Extracellular acidification rate (ECAR) in IS 3T3-L1 adipocytes exposed to PCB126 for 24hrs. Mean and SEM values are the ones presented in Figure 6A.

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.

Table S22. Extracellular acidification rate (ECAR) in C2C12 myotubes exposed to the conditioned medium (CM) of PCB126-treated IS adipocytes for 24hrs. Mean and SEM values are the ones presented in Figure 6C.

Table S23. Extracellular acidification rate (ECAR) in C2C12 myotubes exposed to the conditioned medium (CM) of PCB126-treated IR adipocytes for 24hrs. Mean and SEM values are the ones presented in Figure 6D.

Table S24. Oxidative stress markers in 3T3-L1 adipocytes exposed to PCB126 in insulin resistant (IR) conditions. Mean and SEM values are the ones presented in Figure 7A to E.

Table S25. Oxidative stress markers in C2C12 myotubes exposed to the conditioned medium (CM) of PCB126-treated IR adipocytes. Mean and SEM values are the ones presented in Figure 7G to K.

Table S26. AMP-activated protein kinase (AMPK) levels in 3T3-L1 adipocytes exposed to PCB126 in insulin resistant (IR) conditions. Mean and SEM values are the ones presented in Figure 8A.

Table S27. AMP-activated protein kinase (AMPK) levels in C2C12 myotubes exposed to the conditioned medium (CM) of PCB126-treated IR adipocytes. Mean and SEM values are the ones presented in Figure 8C.

Table S28. Effect of insulin sensitive (IS) and insulin resistant (IR) conditions on 3T3-L1 adipocyte glucose uptake. Mean and SEM values are the ones presented in Figure S1A.

Table S29. Lipid accumulation in IS 3T3-L1 adipocytes, in C2C12 myotubes exposed to the conditioned medium (CM) of IS adipocytes, and in control myotubes directly treated with PCB126 in IS conditions. Mean and SEM values are the ones presented in Figure S2A, left panel.

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.

Table S31. Cell viability measured in IS 3T3-L1 adipocytes, in C2C12 myotubes exposed to the conditioned medium (CM) of IS adipocytes, and in control myotubes directly treated with PCB126 in IS conditions. Mean and SEM values are the ones presented in Figure S2B, left panel.

Table S32. Cell viability measured 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 S2B, right panel.

Table S33. Cyp1a1 mRNA expression in control C2C12 myotubes exposed for 24hrs to different PCB126 concentrations in IS and IR conditions. Mean and SEM values are the ones presented in Figure S3A.

Table S34. Cyp1a1 mRNA expression in C2C12 myotubes exposed for 24hrs to PCB126-treated 3T3-L1 adipocytes in IS and IR conditions. Mean and SEM values are the ones presented in Figure S3B.

Table S35. Leptin secretion in IR 3T3-L1 adipocytes exposed to PCB126 for 24hrs. Mean and SEM values are the ones presented in Figure S4A.

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.

Table S37. Adiponectin secretion ($\mu\text{g/ml}$) in 3T3-L1 adipocytes exposed to PCB126 for 24hrs in IS and IR conditions. Mean and SEM values are the ones presented in Figure S4C.

Table S38. Leptin 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 S4D.

Table S39. Insulin signaling in 3T3-L1 adipocytes exposed to PCB126 in insulin resistant (IR) conditions. Mean and SEM values are the ones presented in Figure S5A.

Table S40. Insulin signaling in C2C12 exposed to the conditioned medium (CM) of PCB126-treated IR adipocytes. Mean and SEM values are the ones presented in Figure S5B.

Table S41. Glucose uptake in control C2C12 myotubes directly exposed to PCB126 in IS conditions. Mean and SEM values are the ones presented in Figure S6A, left panel.

Table S42. Glucose uptake in control C2C12 myotubes directly exposed to PCB126 in IR conditions. Mean and SEM values are the ones presented in Figure S6A, right panel.

Table S43. Glucose uptake in control mouse primary myotubes directly exposed to PCB126 in IS conditions. Mean and SEM values are the ones presented in Figure S6B, left panel.

Table S44. Glucose uptake in control mouse primary myotubes directly exposed to PCB126 in IR conditions. Mean and SEM values are the ones presented in Figure S6B, right panel.

Table S45. Glucose uptake in mouse primary myotubes exposed to the conditioned medium (CM) of PCB126-treated 3T3-L1 adipocytes in IR conditions. Mean and SEM values are the ones presented in Figure S6C.

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.