Supplementary Material

Methods and Results

Cell viability test
Cell viability tests were done as described in Sertié et al. (1). Antihypertensive-induced cytotoxicity during in vitro treatment was measured by colorimetric XTT viability assay (Cell Proliferation Kit II-XTT, Roche, USA) in accordance with the instructions of the supplier. Briefly, adipocytes from periepididymal fat pads were isolated and cultured at a density of 0.35×10^6 in 12-well culture plates and treated with aliskiren hemifumarate (Novartis, Italy), captopril (Sigma, USA) and losartan potassium (Sigma-Fluka, USA) and then incubated for 24 h at 37°C. After the reaction, resulting formazan dye was quantified with an ELISA plate reader at 450–690 nM. The absorbance correlated directly with the number of viable cells. Results are reported in Supplementary Figure S1.

![Figure S1](image)

**Figure S1.** Cell viability assay. Percentage of formazan production was estimated in isolated fat cells incubated with 1 µM or 1 mM of aliskiren (A), captopril (B) and losartan (C) for 24 h. XTT: tetrazolium salt 3′-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro) benzene-sulfonic acid hydrate. Data are reported as means±SE (n=6–9). ***P<0.001, *P<0.05 vs control (one-way ANOVA, Bonferroni’s post hoc test).

UCP-1 gene expression
Gene expression was assessed by real-time quantitative reverse transcription PCR (qRT-PCR) in fat cells after 24 h of treatment. Briefly, cells were homogenated with TRIzol® Reagent right after the end of incubation, washed and stored at −80°C. PureLink® RNA Mini Kit (Cat. No. 12183-018A, Ambion by Life Technologies, USA) was used for RNA extraction. RNA determination was measured with Qubit® Fluorometer 2.0 (Invitrogen, USA). RNA (2 µg) was treated with DNase [0.05U+MgCl₂ (3.75 mM)] for 30 min at 37°C followed by 10 min at 75°C for enzyme inactivation. Complementary DNA was synthesized by reverse transcription with a specific mix containing
Superscript® III enzyme and buffer (1:20); DTT (0.1 M); dNTP mix (2.5 mM of each base); Random Primer (65 ng) and incubated for 10 min at 25°C, 50 min at 50°C, and 15 min at 70°C (Thermocycler, Eppendorf®, Germany).

Real-time polymerase chain reaction (qPCR) was performed using 40 ng of cDNA, TaqMan® Gene Expression Master Mix (Applied Biosystems, UK) and a StepOnePlus™ Real-Time PCR System (Applied Biosystems). β-Actin was defined as an internal control gene. Relative quantification of mRNA was calculated by 2−ΔΔCT. Results are reported in Supplementary Figure S2.

Figure S2. UCP-1 gene expression. qRT-PCR (real-time quantitative reverse transcription PCR) estimated in isolated fat cells incubated with 1 µM aliskiren (Alisk), captopril (Cap) or losartan (Los) for 24 h. Data are reported as means±SE (n=6–9). P>0.05 (one-way ANOVA, Bonferroni’s post hoc test).

Reference