Induction of insulin resistance by the adipokines resistin, leptin, plasminogen activator inhibitor-1 and retinol binding protein 4 in human megakaryocytes

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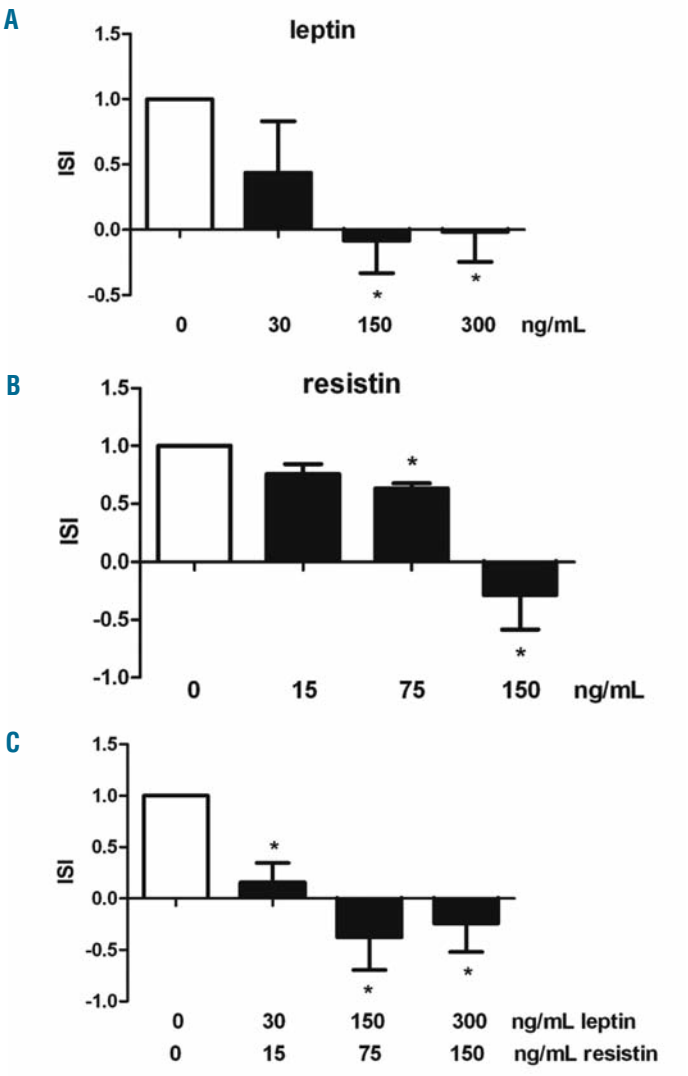
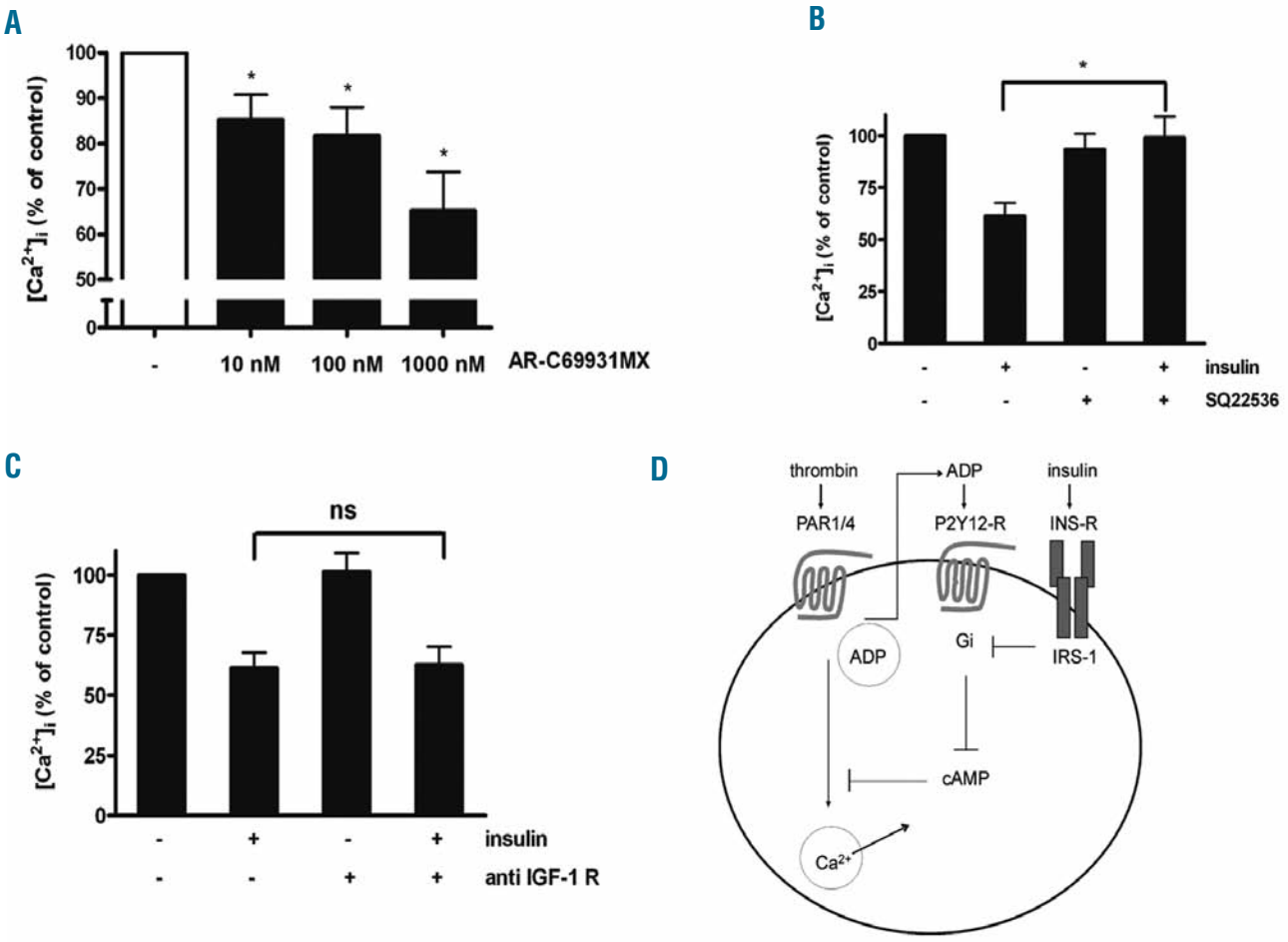
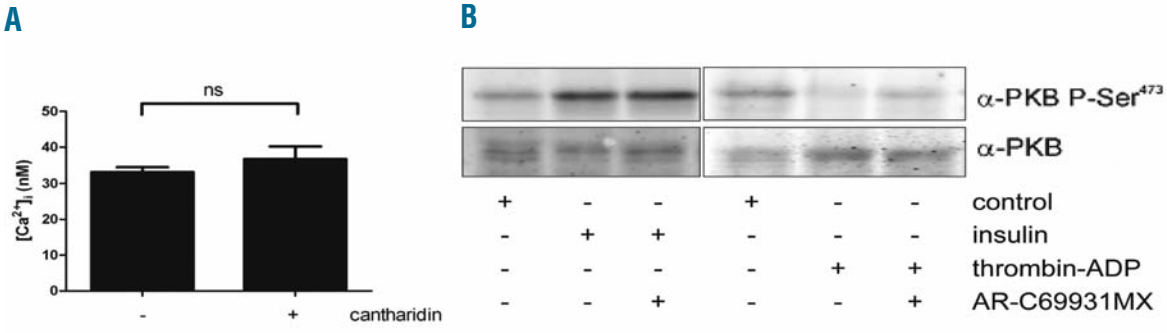


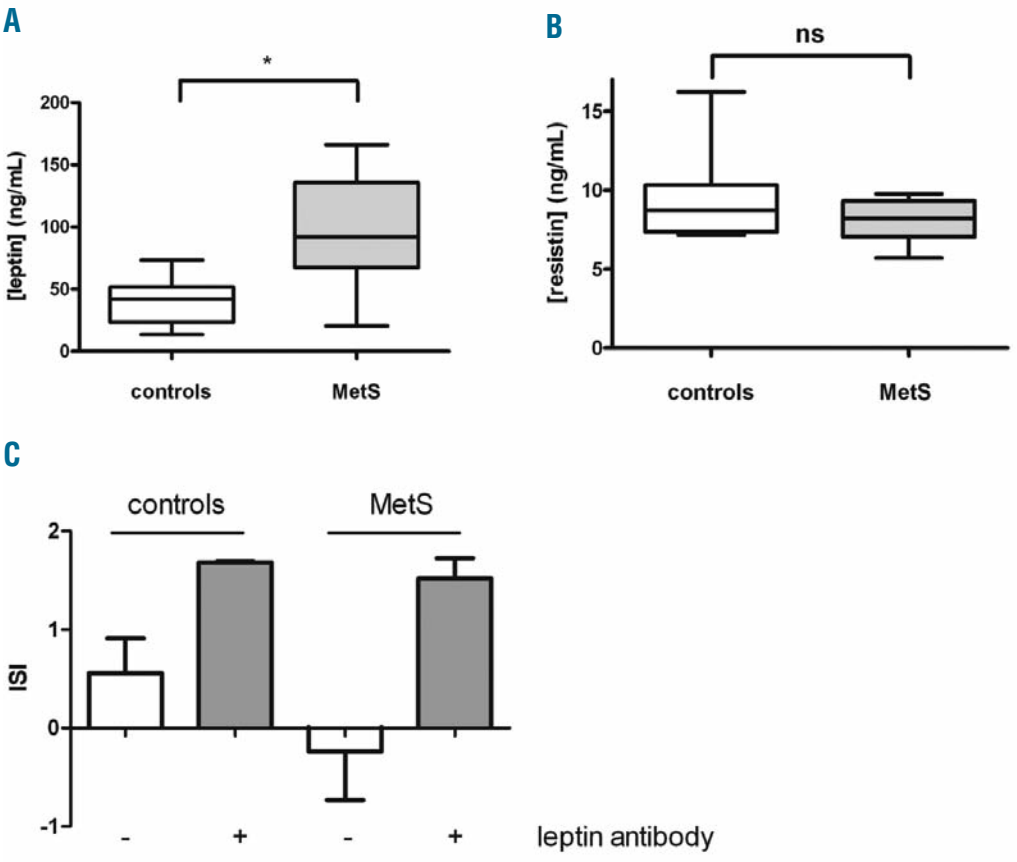
Online Supplementary Figure S1. Dose-dependent interference with insulin resistance by leptin and resistin. CHRF-288-11 cells were incubated with different concentrations of leptin (A), resistin (B) and a combination of leptin and resistin (C) for 2 h (37°C). Insulin inhibition of Ca^{2+} mobilization was measured and expressed as the ISI, as defined in Figure 1E of the main paper.



Online Supplementary Figure S2. Adipokines interfere with insulin suppression of thrombin-induced Ca^{2+} mobilization in CHRF-288-11 cells. (A) Functional P2Y12 signaling in CHRF-288-11 cells. Cells were pre-incubated with different concentrations of AR-C69931MX (5 min, 37°C) and thrombin-induced Ca^{2+} mobilization was measured. (B) Insulin inhibition is mediated by adenylyl cyclase. CHRF-288-11 cells were pre-incubated with the adenylyl cyclase inhibitor SQ22536 (50 μM, 15 min, 37°C) and insulin inhibition of thrombin-induced Ca^{2+} mobilization was measured. (C) No role for the IGF-1 receptor in the inhibition by insulin. CHRF-288-11 cells were pre-incubated with an antibody blocking the insulin-like growth factor-1 receptor (IGF-1 R; 1 μg/mL, 20 min, 37°C) and insulin inhibition of thrombin-induced Ca^{2+} mobilization was measured. (D) Schematic representation of signaling pathways initiated by thrombin and ADP and the interference by insulin in platelets and CHRF-288-11 cells. (For A-C: means±SEM, n=3, data were analyzed with Student's t-test)



Online Supplementary Figure S3. Brief adipokine contact. (A) Cantharidin did not change thrombin-induced Ca^{2+} mobilization. CHRF-288-11 cells were left untreated or incubated with cantharidin (1 μmol/L, 15 min, 37°C) stimulated with thrombin (1 U/mL) and Ca^{2+} mobilization was measured. means±SEM, n=5, data were analyzed with Student's t-test). (B) P2Y12 stimulation does not result in PKB activation. CHRF-288-11 cells were left untreated or preincubated with AR-C69931MX (100 nM, 10 min, 37°C) and subsequently stimulated with different agonists (100 nM insulin or a combination of 20 μM ADP and 1 U/mL thrombin (15 min, 37°C). Total lysates were analyzed for phospho-PKBα Ser^{473} and total PKB levels. The figure shows a representative example of four experiments with similar results.



Online Supplementary Figure S4. Leptin and resistin concentrations in normal and MetS plasma and effect of a leptin neutralizing antibody. (A,B) MetS plasma samples contain elevated concentrations of leptin but resistin levels are normal (n=9). (C) Cells were incubated in control and MetS plasma in the absence and presence of a leptin neutralizing antibody (40 μM, 37 °C). The ISI was determined after 2 h (means±SEM, n=3; data were analyzed with Student's t-test).

Online Supplementary Table S1. Characteristics of male control subjects and male subjects with metabolic syndrome as defined by the Adult Treatment Panel III of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults.[1]

	Control group (n=9)	Metabolic syndrome patients (n=9)	*P*
Age (years)	52±8	54±7	0.62
Height (m)	1.80±0.08	1.83±0.04	0.34
Weight (kg)	80.2±11.3	101.5±10.2	<0.001
Body mass index (kg/m^2)	24.6±2.6	30.1±2.7	<0.001
Body fat (%)	23±2	30.3±4.3	<0.001
Laboratory parameters			
Creatinine (μM)	93±18	95±7	0.77
HbA1c (%)	5.5±0.2	5.8±0.4	<0.05
Hs-CRP (mg/L)	0.96±1.2	3.39±2.3	<0.02
Total cholesterol (mM)	6.05±1.2	6.46±1.2	0.47
LDL-cholesterol (mM)	2.98±0.63	4.33±1.15	<0.01
Plasma insulin (mU/L)	6±4	20±8	<0.001
Homocysteine (μM)	12.6±1.6	9.3±1.7	<0.001
HOMA-IR	1.26±0.27	5.5±2.2	<0.0001
Components of the metabolic syndrome			
Glucose (mM)	5.2±0.6	6.3±0.6	<0.002
Waist circumference (cm)	90±7	111±7	<0.0001
Systolic blood pressure (mmHg)	139±19	131±13	0.30
Diastolic blood pressure (mmHg)	86±10	88±7	0.51
Triglycerides (mM)	1.65±0.83	2.28±0.83	0.13
HDL-c (mM)	1.22±0.20	1.11±0.12	0.19

Online Supplementary Table S2. Characteristics of male control subjects and male subjects with diabetes mellitus type 2 (DM2).

	Control group (n=9)	DM2 patients (n=9)	*P*
Age (years)	55±2	58±3	0.4
Body mass index (kg/m^2)	25±0.7	28±1	0.08
Systolic blood pressure (mmHg)	136±7	132±2	0.5
Diastolic blood pressure (mmHg)	82±4	80±4	0.7
HbA1c (%)	5.4±0.08	7.3±0.4	<0.0006
Cholesterol (mM)	6.3±0.3	4±0.3	<0.0003
Triglycerides (mM)	1.5±0.3	1.9±0.3	0.4
HDL-cholesterol (mM)	1.3±0.07	1.1±0.09	0.05
Creatinine (μM)	100±5.2	100±6	1.0
Management with insulin		9	-
Duration of diabetes (years)	-	14±2	-
Smoking	-	4	-
Medication – aspirin	-	1	-
Medication – statin	-	6	-
Medication – ACE-inhibitor or ARB	-	9	-
Cardiovascular disease	-	2	-

References

1. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001;285:2486-2497.