SUPPLEMENTARY DATA

Drugs and solutions

Kits for glucose, insulin, total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL) and low density lipoproteins (LDL) were purchased from Abbott Laboratories (Abbott Park, IL, USA). Lpa and (hs-CRP) kits were supplied by Beckman Corp (Brea, A, USA). The HbA1c kit was purchased from Menarini Diagnostics (Florence, Italy).

Sodium cyanide, NADH, glucose, trypan blue, arginine, glutathione reductase, NEM, BPDS, H_2O_2, haemoglobin, RPMI1640 supplemented with 20 mM HEPES, HBSS and fibronectin were obtained from Sigma-Aldricht (Sigma Chem. Co., St. Louis, MO, USA). Dextran was acquired from Fluka (St. Louis, MO, USA). HBSS was supplied by Cambrex (Verviers, Belgium). DCFH-DA was provided by Calbiochem (San Diego, CA, USA). TMRM was supplied by Molecular Probes (Eugene, OR, USA). Dulbecco's PBS with (DPBS$^+$) or without (DPBS-) Ca^{2+} and Mg^{2+}, endothelial cell growth medium culture media and foetal bovine serum were obtained from LONZA (Verviers, Belgium). Recombinant TNF−α, human serum albumin (HSA, Albuminate 25%) and Ficoll-Paque TM Plus were purchased from GE Healthcare (Little Chalfont, Buckinghamshire,UK). Plastic coverslips with a diameter of 25 mm were obtained from Nunc (supplied by Thermo Fisher Scientific). PBS, collagenase, and trypsin-EDTA were obtained from Invitrogen (Eugene, OR, USA).

Determination of glutathione content

An HPLC method was used to evaluate whole blood (collected in heparinised tubes) for GSH and GSSG. For determination of GSSG, blood samples (0.5 mL) were treated at 4ºC with 0.5 mL ice-cold perchloric acid (12%) containing $40x10^{-3}$ mol/L N-ethylmaleimide (NEM) - in order to prevent GSH oxidation - and with 2 mM bathophenanthroline disulfonic acid (BPDS), as described by Asensi et al.(1994) (1). To measure total glutathione, blood (0.5 mL) was treated at 4ºC with 0.5 mL trichloroacetic acid (30%). Samples were then centrifuged at 15,000g for 5 min at 4ºC and the acidic supernatants were used for measurement of GSH, GSSG and total glutathione.

For GSSG determinations, 0.5 mL of the acidic supernatants were derivatized by adding 50 μL of 1 mM γ-glutamyl-glutamate prepared in 0.3% perchloric acid. Afterwards, pH was adjusted to 8.0 with KOH (2 mol/L). Samples were then centrifuged and a 25 μL aliquot of the supernatant was mixed with 50 μL of 1% 1-fluoro-2,4-dinitrobenzene. Derivatization was subsequently completed in 45 min and desiccated samples were maintained stable at -20ºC until injection. This procedure reduces GSH oxidation in blood to about 1%.

Adhesion assay. Levels of cytokines and adhesion molecules

The parallel plate flow chamber *in-vitro* model has been described in detail previously (2-3). For adhesion assays, coverslips containing confluent HUVEC monolayers were inserted into a circular recess in the bottom plate of the flow chamber (maintained at 37^{o}C), to which a portion (5x25mm) of the endothelial cells were exposed. The entire chamber was mounted on an inverted microscope (Nikon Eclipse TE 2000-S, Amstleveen, The Netherlands) connected to a video camera. Experiments were performed using a 40x objective lens. PMNs were resuspended in Dulbecco's Phosphate-Buffered Saline (DPBS) containing $20X10^{-3}$ mol/L HEPES and 0.1% human serum albumin at $1x10^6$ cells/mL and were then drawn across the HUVEC monolayer at a controlled flow rate of 0.36 mL/min (estimated shear stress of 0.7 dyne/cm^2). A circular glass window in the top plate of the chamber allowed microscopic examination of the monolayer exposed to the flow in real time. Images in a single field of view were recorded over a 5-min period during which leukocyte parameters were determined. Leukocyte rolling was calculated by counting the number of leukocytes rolling over 100 μm^2 of the endothelial monolayer during a 1-min period. The velocity of 20 consecutive leukocytes in the field of focus was determined by measuring the time required to travel a distance of 100 μm. Adhesion was evaluated by counting the number of PMN that maintained stable contact with the HUVEC for 30 s. Tumoral necrosis factor

(TNF-α, 10 ng/mL, 4h) and platelet activating factor (PAF, 1 μmol/L, 1h) were used as positive controls for HUVEC and leukocytes, respectively. A Luminex 100 flow analyser system was employed to analyse VCAM-1, E-selectin, ICAM-1, IL-6 and TNF-α in serum from controls and diabetic patients (Austin, TX, USA).

1. Asensi M, Sastre J, Pallardo FV, Estrela JM, Viña J. Determination of oxidized glutathione in blood: high-performance liquid chromatography. *Methods Enzymol* 1994;234:367-371.
2. De Pablo C, Orden S, Apostolova N, Blanquer A, Esplugues JV, Alvarez A. Abacavir and didanosine induce the interaction between human leukocytes and endothelial cells through Mac-1 upregulation. *AIDS* 2010; 24:1259-1266.
3. Victor VM, Rocha M, Bañuls C, Alvarez A, de Pablo C, Sanchez-Serrano M, Gomez M, Hernandez-Mijares A. Induction of oxidative stress and human leukocyte/endothelial cell interactions in polycystic ovary syndrome patients with insulin resistance. *J Clin Endocrinol Metab*.2011;96:3115-22.

SUPPLEMENTARY DATA

Supplementary Table 1. Correlation coefficient of leukocyte mitochondrial function, ROS (total and mitochondrial) and leukocyte-endothelial interactions in diabetic patients with and without SMI.

	Rolling velocity		Rolling flux		Adhesion		O_2 Consumption		$\Delta\Psi_m$	
	r	P value	r	P value	r	P value	r	P value	r	P value
Without SMI										
O_2 Consumption	0.628	0.095	-0.770	0.025	-0.979	<0.001	--------	--------	--------	--------
$\Delta\Psi_m$	0.294	0.479	-0.706	0.050	-0.865	0.005	--------	--------	--------	--------
ROS	-0.387	0.344	0.820	0.013	0.965	<0.001	-0.407	0.011	0.128	0.358
Mitocondrial ROS	-0.132	0.777	0.548	0.203	0.744	0.055	-0.312	0.060	-0.115	0.491
With SMI										
O_2 Consumption	0.954	<0.001	-0.683	0.091	-0.873	0.010	--------	--------	--------	--------
$\Delta\Psi_m$	0.622	0.135	-0.765	0.045	-0.929	0.002	--------	--------	--------	--------
ROS	-0.616	0.141	0.777	0.040	0.838	0.018	-0.647	0.006	-0.156	0.563
Mitocondrial ROS	-0.814	0.026	0.848	0.016	0.989	<0.001	-0.412	0.071	-0.468	0.037

Correlation coefficients were estimated by Pearson's correlation for parametric parameters or Spearman's correlation when data were not normally distributed.

SUPPLEMENTARY DATA

Supplementary Figure 1. Effects of tumoral necrosis factor (TNF-α, 10 ng/mL, 4h) and platelet activating factor (PAF, 1 μmol/L, 1h) (used as positive controls for HUVEC and leukocytes, respectively) on PMN from control subjects. PMN rolling velocity (microsecond-1) (A), rolling flux (PMN per minute) (B) and PMN adhesion (PMN per square millimetre) (C). Data are expressed as percentage or mean + SEM. *P<0.05 and ***P<0.001 when compared by an independent T-test.