SUPPLEMENTARY INFORMATION

**Material**

Antibodies to ADRP were obtained from Research Diagnostics (Flanders, NJ), and horseradish peroxidase (HRP)-conjugated anti-guinea pig immunoglobulin (Ig) was purchased from Dako (Dakopatts A/S, Denmark). The BCA Protein assay kit was purchased from Pierce (Rockford, IL). Fatty acid-free bovine serum albumin (BSA), palmitic acid and Oil Red O were obtained from Sigma Aldrich Chemical Corp. Hematoxylin was from Histolab Products AB (Sweden) and [3H]-palmitate was purchased from Amersham (UK). Reagents for reverse transcription and real-time PCR including pre-designed Assay-On-Demand for all genes were obtained from Applied Biosystems.

**Macrophage Experiments**

Human mononuclear cells were isolated from buffy coats obtained from the blood bank at Sahlgrenska University Hospital, and isolated using Ficoll-Paque discontinuous gradient centrifugation (Amersham Biosciences AB, Sweden). The cells were seeded at a density of 2 × 10^6 cells/mL and cultured as previously described. Macrophages in 6-well plates (2 mL/well) were used to study lipid content and protein expression. Macrophages were incubated with or without 50 μg/mL oxLDL under normal cell culture conditions (21% O_2) or hypoxic (1% O_2) conditions, as previously described. Total cell protein extracts were collected in 0.2 M NaOH, and protein concentrations were determined using the Bradford assay. Potentially cytotoxic or apoptotic effects of the different culture conditions were measured by Trypan blue exclusion test and caspase 3 protease activity detection assay (Upstate, Lake Placid, NY).

**DNA Microarray and RT-PCR Analysis**
Total RNA was isolated with the RNeasy kit (Qiagen) from macrophages incubated under normoxia or hypoxia for 24 hours. Two micrograms of RNA from each of 4 donors incubated under normoxia, and from each of 4 donors under hypoxia, were pooled separately, and these 2 pools were used for the target preparation and analyzed on duplicate DNA microarrays (Hu95A; Affymetrix, Santa Clara, CA). Target preparation, DNA microarray hybridization, and scanning were performed as described previously. Scanned output files were analyzed with MAS5 software (Affymetrix) and globally scaled to an average intensity of 500. Comparisons were made between the results from the duplicate DNA microarrays used for analysis of the macrophages incubated under hypoxia, and from the duplicate DNA microarrays used for analysis of the control macrophages (i.e. normoxia), generating a total of four comparisons. The DNA microarray data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE4630.

Regulated genes were identified by the “change call” algorithm (Affymetrix). With the change call, the expression of a gene is classified as increased (I), marginally increased (MI), no change (NC), marginally decreased (MD), or decreased (D). Genes having a change call of I, MI or MD, D in three out of the four comparisons were classified as regulated. Regulated genes with putative function in lipid metabolism were identified using the Netaffix database (www.affymetrix.com). Fold change was also calculated from average signal values between normoxic and hypoxic groups.

Reverse transcription of RNA was performed using random hexamer primers. cDNA was analyzed in a ABI PRISM 7700, and re-calculated to pg cDNA by using a standard curve for each gene. Values were normalized to beta-actin, which was not changed by treatment.
Quantification of Oil Red O-Stained Lipid Droplets

Quantification of the total Oil Red O surface area was performed as described in 4. Briefly, cells were grown on chamber slides (Lab-Tek Systems) and stained with Oil Red O and hematoxylin prior to being viewed with a Zeiss epifluorescence microscope. Twenty images were obtained, digitized (TIFF format; with a resolution of 8 pixels/µm), and analyzed for red pixels (lipid droplets) using BioPix software (see www.biopix.se for further information).

Beta oxidation and triglyceride synthesis assay

The β-oxidation assay was performed as described previously 5. Briefly, cells were incubated with medium containing 0.5 µCi/mL [3H]-palmitate with 1% fatty acid-free BSA and 110 µmol/L palmitic acid for 105 min. The medium was then collected, and fatty acids were precipitated with perchloric acid and a high concentration of BSA. The precipitation procedure was repeated three times. The unprecipitated medium (containing metabolites of β-oxidation) was then analyzed for radioactivity. The triglyceride synthesis assay was performed as described 6. Briefly, cells was incubated indicated times with medium containing 1 µCi/mL [3H]-palmitate. Cells were then washed twice, scraped, lipid was extracted and triglycerides were separated with thin layer chromatography. Finally, triglycerides were analyzed for radioactivity.

Phosphatidic Acid Analysis

The isolation and quantification of radiolabeled phosphatidic acid was carried out as described previously 7, with the exception that cells were labeled with [3H]-palmitic acid (1 µCi/mL culture medium) for 24 hours.

Other Methods
Cholesterol, cholesterol esters and triglycerides were extracted as described previously \(^8\) and quantified using straight phase HPLC with ELS detection \(^9\), and the isolation of triglycerides by high-performance thin-layer chromatography has been described elsewhere \(^10\). Western blot was performed as described before \(^11\). Immunostaining of ADRP was performed as recommended in \(^12\). Low-density lipoprotein (LDL) were isolated by the method described earlier \(^13\). Oxidation of LDL was carried out as described previously \(^14\). Lactate was analyzed in cell culture medium by an enzymatic method \(^15\). \textit{In vitro} lipase assay was carried out as described \(^16\).

**Statistical Analysis**

Results are shown as mean ± standard deviation (SD). Differences between groups were assessed with Student’s two-tailed paired t-test or one-way ANOVA. A p-value of less than 0.05 was considered to be statistically significant.
Supplementary Figures

Figure I. Exposure of human macrophages to hypoxia does not affect cell viability. Cell viability and activity of caspase 3 in macrophages incubated at hypoxia (black bar) or normoxia (open bar). A. Trypan blue exclusion test was performed after different time points. B. Caspase 3 activity in macrophage cell lysates. Results are mean ± SD, from three different macrophage donors analysed in triplicates.

Figure II. Exposure of human macrophages to hypoxia does not give rise to an increase in the uptake of glucose but increases the production of lactate. Triglyceride turnover was not affected. A. The uptake of 2-deoxy-D-[2,6-3H]-glucose in human macrophages controls (open bar) or exposed to hypoxia (black bar) for 24 hours was investigated. Results are mean ± SD, from three different macrophage donors analysed in triplicates. B. The accumulation of lactate in the culture medium of human macrophages control (open bar) or exposed to hypoxia (black bar) for 24 and 48 hours was determined. Results are mean ± SD, from 4 different donors (P < 0.05 for control vs. hypoxia-treated macrophages). C. The cells, control (circles) and hypoxia treated (squares), were incubated with [3H]- palmitic acid (0,5 µCi/ml culture medium) for 24 hours to obtain a steady-state labelling of the triglycerides. The incubation was followed by chase in the presence of triacsin C for the indicated period. The amount of radioactive triglycerides was determined after each chase period. D. Lipase activity was determined in vitro as the release of radio labelled fatty acids from an exogenous triglyceride substrate. Results are mean ± SD, n= 3. There was no statistical significant effect between hypoxia treated cells and control cells.

REFERENCES


Supplementary Figure I
Supplementary Figure II