

SUPPLEMENTARY TEXT 1

MEASUREMENT OF INFLAMMATORY AND ADIPOSITY RELATED HORMONES

Briefly, fresh blood from each participant was collected after fasting in each recruiting centre. Blood was immediately centrifuged at 2000 x g for 10 min at 4°C and separated into plasma and serum according to a standardized operating procedure. All the specimens were stored at -80 until the time of analysis and sent to the project partners responsible for the analyses. A magnetic bead-based multiplex immunoassay (Bio-Plex) (BIO-RAD laboratories, Milan, Italy) was used to measure the inflammatory and adiposity related markers according to the manufacturer's instructions. In particular, Interleukin (IL) 1beta, 1Ra, 2, 4, 5, 6, 7, 8, 10, 12p70, 13, 17, 17A, 18, Tumor Necrosis Factor alpha (TNF α), Interferon gamma (INF γ), Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF), Granulocyte Colony-Stimulating Factor (G-CSF), Macrophage inflammatory protein-1beta (MIP1 β) and Monocyte Chemotactic Protein-1 (MCP-1), were measured in multiplex with Bio-Plex Pro Cytokine, Chemokine, and Growth Factor Assays (intra-assay coefficient of variation (CV) was lower than 4.55% for all the molecules); Transforming Growth Factor beta1 (TGF- β 1 intra-assay CV, 3.83%) with Bioplex Pro TGF- beta assay; Ghrelin (inter-assay CV, 2%) and Resistin (inter-assay CV, 4%) in multiplex with Bio-Plex Pro human diabetes assay. Plates were read and analyzed by Bio-Plex Manager Software. The level of Interleukin 6 receptor alpha (IL6 α , inter-assay CV, 3.1%), Glycoprotein 130 (gp130, inter-assay CV, 5.9%), Pentraxin-3 (inter-assay CV, 6.8%) and soluble TNFalpha receptors R1 (TNF-R1, inter-assay CV, 6.1%) and R2 (TNF-R2, inter-assay CV, 7.7%) were assessed in multiplex in a subgroup of 360 samples with Bioplex Pro human inflammation assay (gp-130, inter-assay %CV 5.9). The quantitative determination of hsCRP, leptin, adiponectin has been performed by ProcartaPlexTM Immunoassay (eBioscience, Hatfield, UK) according to the manufacturer's instructions. Analysis was performed using Luminex 200 instrumentation (Luminex Corporation, The Netherlands). Assay sensitivities were 19.31 pg/mL for Leptin, 4.39 pg/mL for hsCRP, and 47.46 pg/mL for adiponectin.