Relationships among obesity, Type 2 diabetes and plasma cytokines in African American women

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Supporting Information: Supplementary Online Content

Histology and adipocyte area measurement

Surgically excised breast adipose tissue was fixed, paraffin-embedded and stained with hematoxylin/eosin. Adipocyte size was measured by digital microscopy with the pathologist blinded to sample identity. A subset of the cohort was used for comparisons between T2D and ND groups to ensure number of cells counted.

Immunohistochemistry and measurement of 'crown-like structure' (CLS) frequency

Immunohistochemistry was performed on de-waxed sections of breast adipose tissue using anti-CD68 (clone KP1, Dako) and positive staining was detected using horseradish peroxidase-conjugated secondary antibody and detection kits (Vector Labs, Inc). Selected adjacent negative

control sections received isotype antibody. The number of positively stained immune / CD68+ cell foci per adipocyte section was recorded (# CLS in two sections/subject).

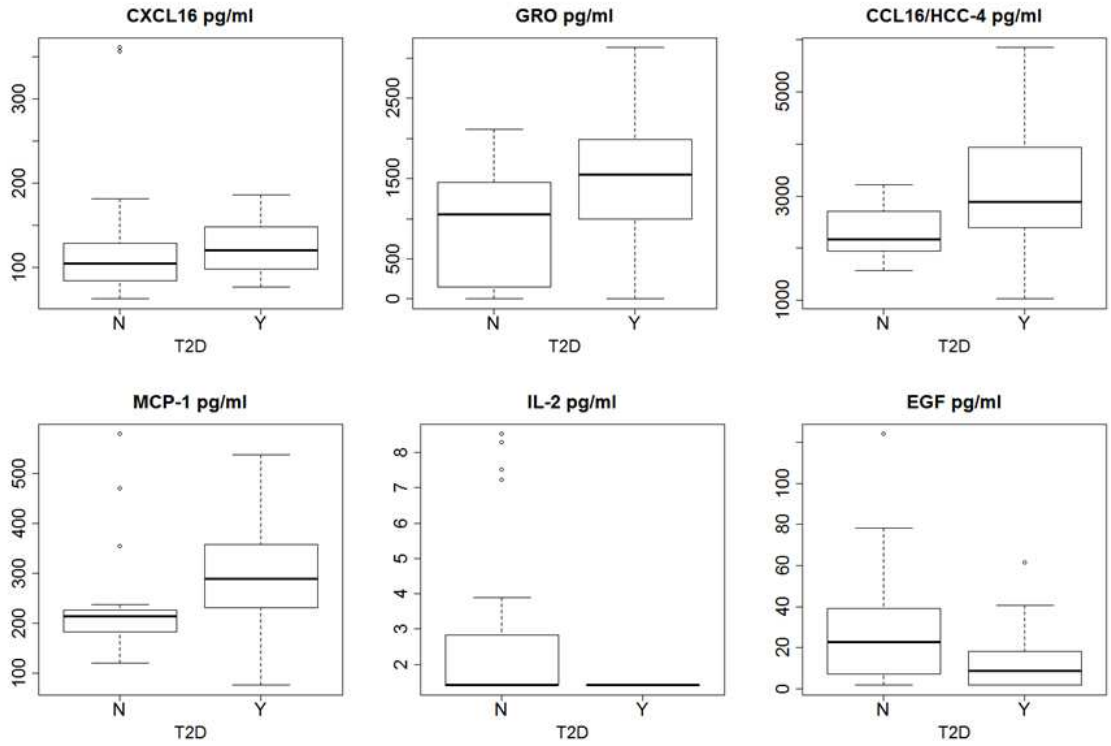
Plasma measures

Blood was drawn by venous puncture and anticoagulated with EDTA. Plasma was prepared by centrifugation, snap-frozen, shipped if required and maintained at -80°C until assay. Aliquots for cytokine measurements were not subjected to more than two freeze thaw cycles.

Protein quantification

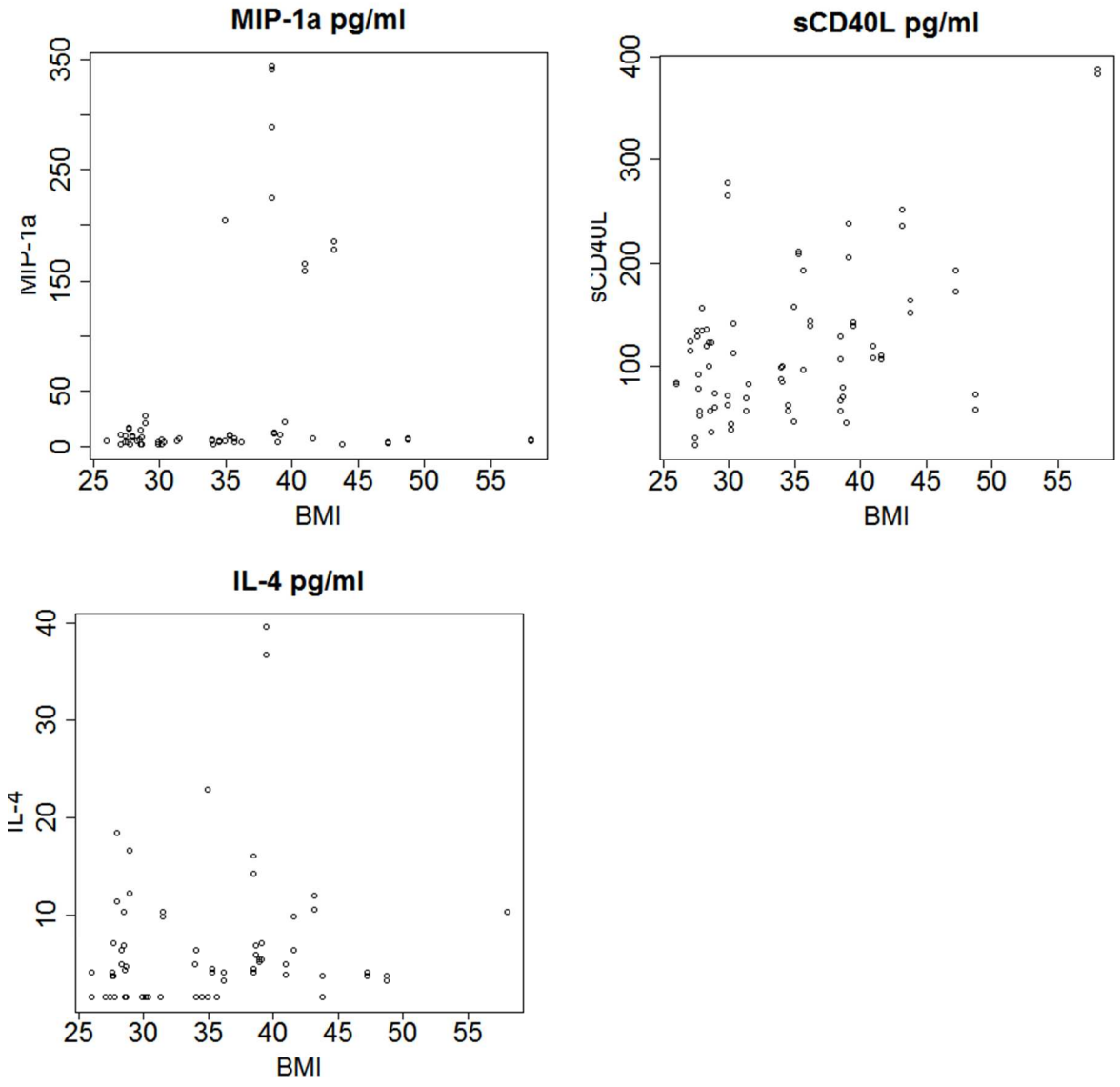
Cytokines and chemokines were measured in plasma and conditioned medium using magnetic bead assays (EMD Millipore, human cytokine/chemokine panels I, II, III and IGF1&2) along with a miniaturization drop array plate and washer system (Curiox Biosystems, Inc), and analyzed on a Magpix instrument with Exponent software for standard curve fitting (Luminex). Median fluorescent intensity data were analyzed after a natural log transformation.

Figure S1. Cytokines significantly associated with ND vs T2D



Cytokine concentration (pg/ml) by T2D status

Figure S2. Cytokines significantly associated with BMI, independently of T2D



Cytokine concentration (pg/ml) by BMI

Table S1. Demographic, clinical and medication information for Komen Tissue Bank subjects.

The Komen Tissue Bank collected as much detailed medical history, including diagnoses and medications, as possible from their non-clinical, volunteer population. This information was obtained for each subject in the analysis, stripped of HIPAA identifiers and is provided in Supplementary Online Content (Table S1) with written permission from the Komen Tissue Bank. The barcode identifiers of each subject have been preserved and link to the annotated data archived in the Virtual Tissue Bank (https://virtualtissuebank.iu.edu/), which is accessible to the general public. Self-reported race of all subjects recruited to this study was African American; all subjects were female gender. Age was reported in years, Body Mass Index (BMI) was reported in kg/m^2 and rounded to two significant figures. T2D, high blood pressure (HBP), heart disease (HeartD), immune complications, nonfasted state (food prior to donation), metformin as well as metformin combinatory drugs (Metformin), diabetes drug prescription (Diabetic), lipid medication (Lipid), hypertension medication (Hypertensive), cardiovascular disease medication (Heart.Disease), hypertension and cardiovascular disease medication (HTN.and.CVD), anti-inflammatory medication, topical anti-inflammatory medication or non-steroidal anti-inflammatory drug or steroidal medication; or other anti-inflammatory prescription, are also reported.

Table S2. Differential expression of each plasma cytokine and chemokine in the analysis and adjustments for medications

Cytokine	log (fold change)	SE	Model	p-value[a]
α-Taxilin/IL-14	-0.012916301	0.05521592	crude	0.815044369
APRIL/TNFSF13	-0.038415578	0.18888793	crude	0.838839891
BAFF/BLys	-0.027372694	0.073047148	HBP	0.707863961
BCA-1/CXCL13	0.107763214	0.111744486	crude	0.334859628
CCL16/HCC-4	0.314523382	0.135513075	crude	0.020287825
CCL18/PARC	0.032725122	0.072484988	crude	0.651647621
CCL23/MPIF-1	-0.074960677	0.222755341	crude	0.736481989
CCL28	-0.013324271	0.054340174	crude	0.806300716
CHI3L1	0.242756187	0.222776457	Metf-Lipid	0.275851881
CTACK/CCL27	0.083651765	0.15705785	crude	0.594298363
CXCL14/BRAK	0.169250589	0.139108731	crude	0.223726598
CXCL16	0.178713333	0.087486975	crude	0.041077967
EGF	-0.546213116	0.248476434	crude	0.027931354
ENA-78/CXCL5	0.046165804	0.285147972	crude	0.871383644
Eotaxin-1/CCL11	0.026256032	0.291863056	Lipid-HBP	0.928318915
Eotaxin-2/CCL24	-0.28081047	0.221693482	crude	0.205276558
Eotaxin-3/CCL26	-0.169373391	0.118996682	crude	0.154636023
FGF-2	-0.159302674	0.114979216	BMI-Infl	0.165902366
FLT-3L	0.066082894	0.192633807	crude	0.731561366
Fractalkine/CX3CL1	-0.185239698	0.134388615	BMI	0.168083426
G-CSF	-0.151599613	0.127341677	crude	0.233851944
GM-CSF	-0.075063703	0.126380201	crude	0.552544591
GRO-1/CXCL1	0.789525587	0.356001874	HBP	0.026571417
HMGB1/HMG-1	0.047311411	0.043435028	HBP	0.276045613
I-309/CCL1	-0.052651683	0.104498834	crude	0.614367154
IFN-α2	-0.105807923	0.122109373	crude	0.386215351
IFN-β	-0.021994858	0.051797312	HBP	0.671104094
IFN-γ	-0.036662261	0.23877507	HBP	0.877970015
IFN-ω	-0.079746242	0.068135702	crude	0.241838761
IGF-I	0.007465366	0.081726509	crude	0.927217896
IGF-II	0.030972599	0.028205861	Metf-Lipid	0.272164805
IL-10	-0.153236049	0.140909869	crude	0.276826664
IL-12p70	-0.215928946	0.128433145	BMI	0.092713276
IL-13	-0.318783685	0.38098239	HBP	0.402738022
IL-15	-0.138074088	0.124473561	crude	0.267316145
IL-16	-0.219758312	0.180437846	crude	0.223255533
IL-17α	-0.165689435	0.16121281	BMI	0.304058775

IL-19	-0.059060357	0.06117475	HBP-Infl	0.334326063
IL-1α	0.007734377	0.148645893	HBP	0.958503017
IL-1β	0.049319925	0.145766339	HBP	0.735100048
IL-1rα	-0.196737236	0.286886234	crude	0.492859778
IL-2	-0.205316083	0.088465647	Infl	0.020294584
IL-20	-0.059696525	0.088165901	crude	0.498346871
IL-21	-0.053873547	0.121017747	crude	0.656196446
IL-23	-0.006337372	0.189266642	HBP	0.97328876
IL-24	-0.003053021	0.053900096	crude	0.95483021
IL-28A	-0.217008122	0.122209642	crude	0.075781756
IL-28B/IFN-L3	-0.014346288	0.056487287	crude	0.799515834
IL-3	-0.177139975	0.116118585	BMI	0.127132129
IL-32α	-0.019175743	0.050957596	crude	0.706688051
IL-33	0.004093124	0.210469637	HBP	0.984484059
IL-34	-0.05530245	0.058970597	HBP	0.348348735
IL-35	-0.015964385	0.051791692	HBP-Infl	0.757898048
IL-36β/IL-1F8	0.001934517	0.063630713	crude	0.975746252
IL-37/IL-1F7	-0.017061849	0.076615259	crude	0.823772752
IL-38/IL-1F10	-0.052010576	0.040077861	crude	0.194377265
IL-4	-0.206792094	0.111134331	BMI	0.062780903
IL-5	-0.309826831	0.331222527	HBP	0.349580208
IL-6	0.018762917	0.146744594	crude	0.898258938
IL-7	-0.006886965	0.114034611	crude	0.951842123
IL-8	0.135903398	0.252128974	HBP	0.589870761
IL-9	-0.126993901	0.138032525	HBP	0.357557697
IL-12p40	-0.21988319	0.196082841	crude	0.262126551
IP-10/CXCL10	0.100653125	0.229473024	crude	0.660931639
LIF	0.115352054	0.136527027	Metf	0.398165167
MCP-1/CCL2	0.311045757	0.155819145	crude	0.04591245
MCP-2/CCL8	-0.041653717	0.110922651	crude	0.707273483
MCP-4/CCL13	-0.118766689	0.151694915	HBP	0.433667485
MCP-3/CCL7	-0.373902687	0.479765296	HBP	0.43577651
MDC/CCL22	-0.308542586	0.278605945	BMI-lipid	0.268098749
MIP-1α/CCL3	0.143676717	0.669886885	BMI-Metf-Lipid	0.830173486
MIP-1β/CCL4	-0.052068655	0.195790691	crude	0.790285107
MIP-1δ/CCL15	0.134082981	0.162492137	crude	0.409277374
PDGF-AA	0.18815646	0.159696841	crude	0.238712785
PDGF-AB/BB	-0.047498129	0.164435045	crude	0.772691204
RANTES/CCL5	-0.011320346	0.110520757	crude	0.918417493
sCD40L	0.295183565	0.184103826	crude	0.108856569
SCF	-0.127767308	0.191320363	crude	0.504249357
SDF-1α+β/CXCL12	-0.072244537	0.200217581	Metf-Lipid-HBP	0.718226435
TARC/CCL17	0.353751619	0.323201925	Metf	0.273726102

TGF-α/TFGA	-0.275997141	0.298707213	BMI-HBP	0.355500825
TNF-α	0.115826485	0.118080753	crude	0.326637567
TNF-β	-0.472057497	0.411403543	HBP	0.251203216
TPO	-0.047409144	0.117727737	crude	0.68716778
TRAIL	0.209814633	0.145475618	Lipid-HBP	0.149227163
TSLP	-0.241682885	0.192233017	crude	0.208667032
VEGF	-0.336845324	0.222804346	BMI	0.130573655
6CKine/CCL21	0.148690589	0.121117586	crude	0.219576572

[a] In comparison of T2D/ND status, Table 2A.

Adjustment for medications is essential when human leukocyte or plasma cytokines and chemokines are assayed in the context of metabolic disease. Column 4 describes the confounders used to adjust the association between cytokine and T2D/ND status. The selection of confounders was conducted using a backward model selection to remove non-significant variables in the regression model. See the methods section for details. Crude: no adjustment needed; HPB: anti hypertensive medication; Lipid: lipid lowering medication; Infl: anti-inflammatory medication; Metf: metformin.

Table S1. Demographic, clinical and medication information for Komen Tissue Bank subjects

Barcode	AGE	BMI	T2D	HBP	HeartD	immune	nonfasted	Metformin	Diabetic	Lipid
K102484	62	39.45	N	N	N	N	NA	NA	NA	NA
K102495	62	27.56	N	Y	N	N	NA	NA	NA	NA
K102524	61	28.29	N	N	Y	N	NA	NA	NA	NA
K102540	57	27.98	N	N	N	N	NA	NA	NA	NA
K104421	47	28.53	Y	N	Y	Y	NA	NA	NA	NA
K104606	52	34.45	Y	N	N	N	NA	NA	NA	NA
K104616	51	33.94	Y	Y	N	N	NA	Metformin HCl	NA	Simvastatin
K104662	64	29.91	Y	Y	N	Y	NA	NA	Glipizide ER	Simvastatin
K105323	51	27.73	N	Y	N	N	Yes	NA	NA	NA
K105326	47	27.72	N	N	Y	N	Yes	NA	NA	NA
K105339	70	27.37	N	Y	N	N	Yes	NA	NA	Atorvastatin
K105369	62	28.49	Y	Y	N	Y	Yes	Avandamet	NA	Rosuvastatin
K105412	66	35.6	Y	Y	N	N	Yes	Kombiglyze	NA	Rosuvastatin
K105464	60	38.47	Y	Y	N	N	Yes	NA	NA	Atorvastatin, Simvastatin
K105494	63	37.6738	Y	N	N	N	Yes	Metformin HCl	NA	Rosuvastatin
K105500	69	36.18	Y	Y	Y	Y	Yes	NA	Insulin glargine, Insulin regular	NA
K105613	61	41.53	Y	Y	N	N	Yes	NA	Insulin glargine, insulin aspart	Atorvastatin
K105679	65	35.3	Y	Y	N	N	Yes	Metformin HCl	NA	Ezetimibe, Pravastatin
K105684	69	43.12	Y	Y	N	N	Yes	NA	Sitagliptin, Insulin glargine,	NA
K105685	61	28.62	Y	N	N	N	Yes	NA	NA	NA
K105693	51	34.01	N	N	N	N	Yes	NA	NA	NA
K105744	64	30.18	N	Y	N	N	Yes	NA	NA	NA

K105758	44	39.06	Y	Y	N	N	Yes	NA	Sitagliptin, Insulin glargine	NA
K105911	70	27.07	Y	N	N	N	Yes	Metformin HCl	Sitagliptin	Simvastatin
K105947	67	48.71	Y	Y	N	N	Yes	Metformin HCl	NA	Rosuvastatin
K105952	57	26	Y	Y	N	N	Yes	Metformin ER	Sitagliptin	Atorvastatin
K105963	52	34.95	N	Y	N	N	No	NA	NA	Simvastatin, Ezetimibe
K106195	51	40.92	Y	Y	N	N	Yes	NA	NA	NA
K106231	71	30.36	Y	Y	N	N	Yes	NA	Glimepiride	Simvastatin
K106240	53	38.46	Y	Y	Y	N	Yes	NA	Human Insulin 70/30	Atorvastatin
K106265	51	28.95	N	Y	Y	N	Yes	NA	NA	NA
K106362	61	38.67	Y	Y	Y	N	NA	NA	NA	NA
K106512	53	43.74	Y	Y	N	N	Yes	Metformin	Insulin glargine, Insulin lispro, sitagliptin	NA
K106513	49	57.9975	Y	Y	N	N	Yes	Metformin	Insulin glargine, glimepiride, insulin aspart	NA
K106731	57	31.44	Y	N	N	N	Yes	NA	Insulin glargine, humalog kwikpen	Rosuvastatin
K106802	50	31.32	Y	Y	N	N	Yes	NA	Sitagliptin	NA
K106809	68	29.85	Y	Y	N	N	Yes	Metformin	Acarbose	Atorvastatin
K107223	54	38.94	Y	N	Y	Y	No	Metformin HCl	NA	Atorvastatin
K107430	54	47.25	Y	Y	N	N	Yes	NA	Insulin lispro	NA

Hypertensive	Heart.Disease	HTN+CVD	Anti.inflammatory	Topical.Anti.inflamm.NSAID.steroid	Other.Anti.inflamm.NSAID.steroid
NA	NA	NA	NA	NA	NA
Metoprolol-XL, valsartan	Metoprolol-XL	Metoprolol-XL	NA	NA	NA
NA	NA	NA	Ibuprofen	NA	NA
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
Amlodipine, Hydrochlorothiazide	NA	NA	NA	NA	NA
Lisinopril, Triamterene, Hydrochlorothiazide, Timolol	NA	NA	NA	NA	NA
Metoprolol-XL, valsartan, Hydrochlorothiazide	Metoprolol-XL	Metoprolol-XL	NA	NA	NA
NA	NA	NA	Meloxicam	NA	NA
NA	NA	NA	Aspirin	NA	NA
Lisinopril, Hydrochlorothiazide	NA	NA	NA	NA	NA
Amlodipine, Carvedilol, Losartan	Carvedilol	Carvedilol	NA	NA	NA
Benazepril, Amlodipine	NA	NA	NA	NA	NA
Losartan	NA	NA	NA	NA	NA
Benazepril, Amlodipine, Clonidine HCl	Furosemide	NA	NA	NA	NA
Lisinopril, Hydrochlorothiazide	NA	NA	Aspirin	NA	NA
Valsartan	NA	NA	Meloxicam	NA	NA
Metoprolol-XL, Amlodipine, Quinapril	NA	NA	NA	NA	NA
NA	NA	NA	Budesonide	NA	NA
NA	NA	NA	NA	NA	NA
Hydrochlorothiazide	NA	NA	Naproxen	NA	NA

Metoprolol Tartrate, Clonidine HCl, Nifedical XL, Hydralazine	Furosemide, Metoprolol tartrate	Metoprolol Tartrate	NA	NA	NA
NA	NA	NA	NA	NA	NA
Triamterene, Hydrochlorothiazide	NA	NA	Aspirin, Meloxicam	NA	NA
Lisinopril	NA	NA	NA	NA	NA
Atenolol, Triamterene, Hydrochlorothiazide	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
Losartan	NA	NA	Aspirin	NA	NA
Lisinopril, Carvedilol, Diltiazem SR, Isosorbide mononitrate	Furosemide, Carvedilol, Isosorbide mononitrate	Carvedilol, Isosorbide mononitrate	Aspirin	NA	Fluticasone
NA	NA	NA	Montelukast	NA	NA
NA	NA	NA	NA	NA	NA
Ramipril	Ramipril	Ramipril	NA	NA	NA
Lisinopril	Furosemide	NA	NA	NA	NA
Losartan	NA	NA	Etodolac	NA	NA
Carvedilol, Lisinopril, Hydrochlorothiazide	Carvedilol	Carvedilol	NA	NA	NA
Lisinopril	NA	NA	NA	NA	NA
Metoprolol-XL, Lisinopril, Hydrochlorothiazide, Isosorbide mononitrate	Metoprolol-XL, Isosorbide mononitrate	Metoprolo-XL, Isosorbide mononitrate	Montelukast, Aspirin	NA	NA
Amlodipine, valsartan	NA	NA	NA	NA	NA