Expression of inflammation-related genes in gluteal and abdominal subcutaneous adipose tissue during weight-reducing dietary intervention in obese women

Running title: Gene expression in gluteal and abdominal fat during diet

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Summary

Accumulation of adipose tissue in lower body lowers risk of cardiovascular and metabolic disorders. The molecular basis of this protective effect of gluteofemoral depot is not clear. The aim of this study was to compare the profile of expression of inflammation-related genes in subcutaneous gluteal (sGAT) and abdominal (sAAT) adipose tissue at baseline and in response to multiphase weight-reducing dietary intervention (DI). 14 premenopausal healthy obese women underwent a 6 months´ DI consisting of 1 month very-low-calorie-diet (VLCD), subsequent 2 months´ low-calorie-diet and 3 months´ weight maintenance diet (WM). Paired samples of sGAT and sAAT were obtained before and at the end of VLCD and WM periods. mRNA expression of 17 genes (macrophage markers, cytokines) was measured using RT-qPCR on chip-platform.

At baseline, there were no differences in gene expression of macrophage markers and cytokines between sGAT and sAAT. The dynamic changes induced by DI were similar in both depots for all genes except for three cytokines (IL6, IL10, CCL2) that differed in their response during weight maintenance phase. The results show that, in obese women, there are no major differences between sGAT and sAAT in expression of inflammation-related genes at baseline conditions and in response to the weight-reducing DI.

Keywords: body fat distribution, hypocaloric diet, macrophages, cytokines

Introduction

Obesity is associated with higher risk of metabolic and cardiovascular diseases. In addition to body mass index (BMI), body fat distribution plays a major role in the development of the above mentioned diseases. While upper body fat accumulation is associated with increased obesity-related health risk, the lower body fat accumulation was shown to be linked with the reduction of metabolic (Snijder*, et al.* 2004), cardiovascular risk (Canoy*, et al.* 2007, Faloia*, et al.* 2009, Seidell*, et al.* 2001) and with lower morbidity and mortality (Folsom Ar and Et Al. 1993, Pischon*, et al.* 2008). The increased amount of lower body fat - expressed as hip circumference - was associated with lower triacylglycerol and higher HDL cholesterol levels (Ruige and Van Gaal 2009).

Possible mechanisms that may contribute to the subcutaneous gluteal adipose tissue (sGAT) protective role have not been fully elucidated. Different uptake and release of fatty acids in the subcutaneous abdominal (sAAT) when compared to sGAT have been suggested as a candidate underlying cause (Berman*, et al.* 1998, Berman*, et al.* 2004). In several studies, the activity of lipoprotein lipase (LPL) was found to be higher (Arner*, et al.* 1991, Ferrara*, et al.* 2002) in sGAT in obese women. This might suggest a higher capacity of sGAT for lipid accumulation.

In addition to alteration of adipose tissue (AT) fatty acid handling, the obesity-related metabolic disturbances are linked with pro-inflammatory state of AT characterized by enhanced recruitment of macrophages in AT and modified AT secretion of cytokines (Klimcakova*, et al.* 2011, O'hara*, et al.* 2009, Suganami and Ogawa 2010, Trayhurn and Wood 2004). Thus, it may be hypothesized that differences in the inflammation-related characteristics underlie the differences in metabolic role of sGAT when compared with sAAT. Few studies paid attention to this topic. Recently, Evans et al.

63 showed that sGAT had, contrary to the expectation, greater mRNA expression of a set of pro-
64 inflammatory genes than sAAT (Evans*, et al.* 2011).
65 Thus the first aim of our study was to compare, in obese women, expression of wider range of
66 cytokines and macrophage markers in sGAT *vs.* sAAT (selected according to our previous studies of
67 Capel*, et al.* 2009, Klimcakova*, et al.* 2011, Siklova-Vitkova *et al*. 2012) and their association with
68 indices of metabolic syndrome. Our second aim was to explore the regulation of expression of the
69 above mentioned genes in a dynamic condition that was realized by a dietary intervention (DI) using
70 hypocaloric diet. It was shown previously that hypocaloric diet-induced changes in adipocyte
71 lipolysis (Mauriege*, et al.* 1999) or in adipocyte size (Bjorntorp*, et al.* 1975) were less pronounced in
72 sGAT when compared with sAAT. We hypothesized that this impaired responsiveness, or
73 "inflexibility", of sGAT might also appear in respect to the diet-induced modulation of gene
74 expression of immunity-related genes. Thus, we measured mRNA expression of the respective genes
75 in the paired samples of sGAT and sAAT obtained in obese women before and during multiple
76 phases of a 6 months´ hypocaloric DI.
77

Materials and Methods

STUDY DESIGN

Subjects

14 premenopausal obese women (BMI 34.2 ± 2.6 kg/m^2, range 27 – 49 yr) without medication and diseases except for obesity participated in this study. Their body weight had been stable for 3 months prior to the examination. The informed consent was obtained from each patient before the study. The study was performed according to the Declaration of Helsinki protocols and was approved by Ethical Committee of the Third Faculty of Medicine, Charles University in Prague.

Dietary intervention and clinical investigation

The entire DI lasted 6 months. During the first dietary period, obese subjects received a very low calorie diet (VLCD) of 800 kcal/day (liquid formula diet, Redita, Promil, Czech Republic) for 1 month. The subsequent period consisted of a 2 months` low-calorie diet (LCD) followed by 3 months` weight maintenance (WM) diet. LCD was designed to provide 600 kcal/day less than the individually estimated energy requirement based on an initial resting metabolic rate multiplied by 1.3, the coefficient of correction for physical activity level. Patients consulted a dietician once a week during the first 3 months of the program and once a month during subsequent phase. They provided a written 3 days´ dietary record at each dietary consultation.

Complete clinical investigation including anthropometric measurements, blood sampling and AT biopsies was performed in the morning in the fasting state before the beginning of the diet and at the end of VLCD and WM periods.

The whole body composition was evaluated by multi-frequency bioimpedance (Bodystat, Quad scan 4000, Isle of Man, UK). The blood was collected and centrifuged at 1300 RPM, 4^{o}C, separated plasma was stored at -80^{o}C until analysis. The paired samples of subcutaneous AT were obtained from the subcutaneous abdominal (10 cm lateral to the umbilicus) and gluteal (right upper quadrant) region using needle biopsy under local anesthesia (1% Xylocaine). AT samples were obtained from superficial sAAT, as we verified on several occasions using ultrasonography. AT was washed in physiological saline, aliquoted, snap-frozen in liquid nitrogen and stored at -80°C until processing.

Laboratory measurements

Plasma glucose was determined using the glucose-oxidase technique (Beckman Instruments, Fullerton, CA). Plasma insulin was measured using an Immunotech Insulin Irma kit (Immunotech, Prague, Czech Republic). Homeostasis model assessment of the insulin resistance index (HOMA-IR) was calculated as follows: ((fasting insulin in mU/l) x (fasting glucose in mmol/l) / 22.5). Plasma levels of other relevant substances were determined using standard clinical biochemistry methods. Plasma levels of cytokines were determined using multiplex human cytokines Milliplex panels (Millipore-Merck, Bedford, MA, USA).

Gene expression analysis

Total RNA was isolated from 100-300 mg aliquots of AT using RNeasy Lipid Tissue RNA Mini kit (Qiagen, Hilden, Germany). RNA concentration was measured by Nanodrop1000 (Thermo Fisher Scientific, Wilmington, Delaware, USA). Genomic DNA was removed by DNAse I treatment (Invitrogen, Carlsbad CA, USA). cDNA was obtained by reverse transcription (High Capacity cDNA Reverse Transcription Kit, Applied Biosystem, Carlsbad, CA, USA) using 200 ng of total RNA. 1 ng

of cDNA was then preamplified to improve detection of target genes during subsequent Real Time qPCR (16 cycles, TaqMan Pre Amp Master Mix Kit, Applied Biosystem). For the preamplification, 20 x TaqMan gene expression assays of all target genes were pooled together and diluted with 1x TE buffer to the final concentration 0.2x (each probe). The RT-qPCR was performed on Biomark Real Time qPCR system and 96x96 chip (Fluidigm, USA) in triplicates. This part of analysis was carried out as a paid service by Biotechnology Institute, AS CR.

Expression of 17 genes grouped according to their origin or function was measured: cytokines (IL6, TNF, CCL2, CXCL1, IL10, TGFβ1, IL10RA) and cytokine receptor (CCR2), macrophage markers (SPP1, CD68, MSR1, PLA2G7, ACP5, FCGBP, CD14, TLR4, TLR2). Macrophage markers were selected according to the work of Capel (Capel*, et al.* 2009) and Klimcakova (Klimcakova*, et al.* 2011) and cytokines produced predominantly by cells of stromavascular fraction were chosen according to study of Siklova (Siklova-Vitkova*, et al.* 2012) so that comparisons of outcomes between this and our previous studies were enabled. Expression data were normalized to expression of reference gene, PPIA, and delta Ct was log-transformed for statistical analysis.

Statistical analysis

The data from RT-qPCR were analyzed with Genex software (MultiD Analysis AB, Sweden) and SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). For all the analyses the data were log-transformed. One way ANOVA was used for comparison of gene expression separately in each depot, the effect of sAAT *vs.* sGAT on the diet-induced changes was estimated by two way ANOVA with repetitive measures and Tukey´s posthoc analysis. The level of significance was set at $p<0.05$. Clinical and anthropometrical data were analyzed with GraphPad Prism 5.0. (La Jolla, CA, USA). Correlations were performed using Pearson's parametric test.

Results

Effect of dietary intervention on anthropometrical and plasma variables

The clinical data of the entire group of subjects at baseline and during the DI are presented in Table 1. When compared to baseline, the subjects' body weight decreased by 8.0 % after the VLCD and decreased further during subsequent period so that the weight loss at the end of WM represented 11.1% of the original weight. BMI, fat mass, waist and hip circumference showed a similar pattern. The relative decrease of waist circumference was greater than that of hip circumference at the end of VLCD as well as at the end of the entire DI (VLCD: waist: - 6.8 ± 0.3%, hip: - 4.2. ± 0.3%, $P< 0.05$, the end of DI: waist: -8.6 ± 0.2%, hip: -5.9 ± 0.3%, $P <0.01$). Plasma levels of insulin were lower at the end of VLCD when compared to baseline condition and remained lower at the end of WM. Glycaemia and total cholesterol decreased after VLCD and returned to the baseline levels at WM. Free fatty acid levels were increased after VLCD and decreased below the baseline values at the end of the WM. Insulin resistance assessed by HOMA-IR decreased during VLCD and remained reduced at the end of WM phase. The changes of plasma levels of cytokines IL10, IL6, TNF, CCL2 were in line with our previous study (Siklova-Vitkova*, et al.* 2012).

Comparison of gene expression in gluteal and abdominal subcutaneous AT in obese women at pre-diet condition (Figure1)

To compare gene expression profile in sAAT and sGAT, we measured mRNA expression of 17 genes divided into 2 functional groups: macrophage markers (9 genes), cytokines (8 genes), and reference gene PPIA. The expression of macrophage markers was similar in both depots except for two genes, ACP5 and MSR1 that had higher expression in sGAT compared to sAAT (Figure 1A). There were no

163 depot-related differences in the expression of all measured cytokines (TNF, IL6, CCR2, CXCL1,
164 IL10, TGFβ1) with exception of IL10RA (higher in sGAT) and CCL2 (lower in sGAT) (Figure 1B).
165
166 *Effect of dietary intervention on gene expression in gluteal and abdominal subcutaneous AT*
167 *(Figure2, Table 2)*
168 The evolution of the mRNA expression for individual genes during DI is shown in Figure 2.
169 Schematic representation of the direction and significance of the diet-induced changes of gene
170 expression during VLCD and during the entire DI in each depot is presented in Table 2.
171 *Macrophage markers (Figure 2A-2B)*
172 The expression of 6 macrophage markers (CD68, ACP5, FCGBP, MSR1, PLA2G7, SPP1) increased
173 during VLCD in both depots while expression of 3 remaining markers (CD14, TLR4, TLR2) was not
174 changed in both depots. At the end of WM, the mRNA levels of all macrophage markers were not
175 different from the baseline values in both depots *(Figure 2A-2B)*.
176 *Cytokines (Figure 2C-2D)*
177 During VLCD, the mRNA levels of 5 cytokines (TNF, IL6, IL10RA, TGFβ1, CXCL1) did not
178 change in either depot, CCL2 increased and CCR2 decreased in both depots and IL10 showed a
179 significant increase selectively in sAAT.
180 At the end of DI, IL10 and CCL2 mRNA levels were higher than baseline values in sGAT while, in
181 sAAT, the mRNA levels of these two cytokines as well as those of IL6, IL10RA and TNF were lower
182 when compared with the baseline.
183 The diet-induced changes of mRNA levels were similar in the two depots for all of the cytokine genes
184 with 3 exceptions: during the entire DI, expression of IL10, IL6, and CCL2 decreased in sAAT while
185 it increased or was unaltered in sGAT.

Summary of the comparison of the diet-induced responses of the gene expression in sGAT and sAAT

For majority of measured genes no differences in the diet-induced changes between sGAT and sAAT were found. Different responses were found only for the 3 cytokines (IL10, IL6, CCL2): their decrease during the entire DI was pronounced in sAAT but not in sGAT.

Correlations

No correlations between the diet-induced changes of mRNA expression of examined genes in sGAT *vs*. sAAT were found. No correlations were found between the diet-induced changes of plasma levels of IL10, IL6, TNF, CCL2 and the changes in the expression of these genes in either sGAT or sAAT. In addition, no correlations between the diet-induced changes of mRNA expression of examined genes and those of BMI or HOMA-IR were found.

Discussion

It was hypothesized that the protective role of AT accumulation in the lower body, in respect to cardiovascular risk and metabolic disturbances, might be based on the lower pro-inflammatory profile of sGAT. However, recent work of Evans et al. (Evans*, et al.* 2011) reported that the expression of several pro-inflammatory markers was higher in sGAT compared to sAAT in a mixed group of lean and obese black and white South African women. Our work extended the range of explored genes and showed that, in a wide group of cytokines and macrophage markers, there were, with exception of 4 genes, no differences between sAAT and sGAT at baseline. Taken together, our and Evans' results do not support the hypothesis of the lower pro-inflammatory profile of sGAT. This is in line with the finding reported by Tchoukalova et al. (Tchoukalova*, et al.* 2010) that subcutaneous abdominal and femoral fat depot did not differ in number of macrophages in lean men and women.

The main interest of this study lies in the comparison of gene regulation in sAAT *vs*. sGAT in dynamic condition represented by two phases of a 6 months´ dietary intervention. The pattern of the expression of macrophage markers observed in this study, including the increase during initial VLCD phase is in agreement with our previous work carried out in sAAT in another cohort of subjects (Capel*, et al.* 2009). The pattern is bi-phasic, characterized by an increased expression during VLCD and a decrease towards baseline values during subsequent weight maintenance phase of the diet. Increased expression of macrophage markers during VLCD might be associated with enhanced fatty acids release from adipocytes as a possible trigger of macrophage activation and infiltration mediated by TLR4 signaling as shown before (Kosteli*, et al.* 2010, Suganami*, et al.* 2005). Importantly, this bi-phasic response of macrophage markers expression was similar in sGAT when compared with sAAT (*Figure 2, Table 2*) and the magnitudes of the diet-induced changes were not different in the two fat depots (*Table 2*). This finding suggests the same regulation of macrophage infiltration in sGAT and sAAT during weight-reducing dietary intervention.

The bi-phasic pattern of the diet-induced regulation was found also in the expression of cytokines derived predominantly from the cells of stromavascular fraction. This pattern was in accordance with previous results obtained in sAAT in a different cohort of subjects (Siklova-Vitkova*, et al.* 2012). The magnitudes of the diet-induced changes were not different between sGAT and sAAT - except for three cytokines - IL6, IL10 and CCL2. The observed variability in respect to the individual cytokine genes is, again, in agreement with the above mentioned study of Siklova et al.: in that study the same three cytokine genes were the only ones that showed a significant decrease in sAAT at the end of dietary intervention. This partial differential depot-related response - limited to the expression of the three cytokines - might be linked to differential response of endocannabinoid system as observed in the study of Bennetzen (Bennetzen*, et al.* 2011). In fact, during the weight-reducing diet the authors

found different change of expression of cannabinoid receptor type 1 in sGAT when compared with sAAT. And endocannabinoids were shown to inhibit production of several proinflammatory cytokines in primary human Muller cells (Krishnan and Chatterjee 2012). This mechanism could be taken into account, although no such regulation has been reported in adipose tissue. Moreover, in resting condition, Rantalainen et al. found differential expression of 12% of measured microRNA (e.g. miR146-5b, miR-21, miR155) in sGAT when compared with sAAT which could imply a differential expression of targets of these microRNA such as IL10 (Quinn and O'neill 2011) in the two fat depots (Rantalainen*, et al.* 2011).

The lack of correlations in gene expression changes between sAAT and sGAT suggests that, in spite of the similar pattern of the diet-induced response of the gene expression in the two fat depots, there is no direct quantitative association of the magnitude of the change between these two depots. In light of our and others results demonstrating the absence of major differences between sGAT and sAAT it has been suggested that the deleterious effect of upper body obesity could be mediated by the excess of visceral adipose tissue (VAT) and not excess of sAAT. Nevertheless, several studies showed that both, VAT and sAAT, are associated with the increased risk of metabolic profile and pro-inflammatory status (although the association was stronger with VAT) (Fox *et al.* 2007, Oka *et al.* 2010, Pou *et al.* 2007).

Furthemore, it should be noted that the present study compared sGAT and sAAT on transcriptional level. Due to the lack of sufficient amount of adipose tissue we did not explore the protein levels of cytokines in adipose tissues or their levels of secretion. It is not excluded that the underlying causes of different physiological impact of sGAT *vs*. sAAT may be at the level of translation or post-translational regulations of cytokine production or release.

It is to be noted that the results of this study are limited to women. Female adipose tissue shows different metabolic and endocrine characteristics (Kern*, et al.* 2003, Montague*, et al.* 1997) when compared with men. Moreover, the initial fat distribution in our set of women (mean WHR= 0.861±0.0) might play a role in the diet-induced response of the two fat depots although the reports on the effect of initial fat distribution on the body fat reduction are not unequivocal (Svendsen*, et al.* 1995, Jones and Edwards, 1999).

In conclusion, we did not find major differences in mRNA levels of macrophage markers and cytokines between sAAT and sGAT at baseline condition or in the pattern of their regulation in response to two phases of hypocaloric weight-reducing dietary intervention (with exception of 3 cytokines that were differentially regulated during weight maintenance phase). Therefore, our results do not bring evidence of an altered pro-inflammatory status or an altered "responsiveness" of immune cells in sGAT when compared with sAAT. Further research elucidating the molecular base of the protective role of sGAT fat depot is therefore warranted.

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Conflicts of interests

The authors declare no conflict of interest.

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Table1. Clinical characteristics of subjects and plasma levels of cytokines before dietary intervention (basal) and at the end of VLCD and weight maintenance (WM) phase of the dietary intervention.

	Basal	**VLCD**	**WM**
Age (year)	27-49		
BMI (kg/m^2)	34.2±0.2	31.5±0.2***	30.4±0.2 †††
Weight (kg)	93.5 ± 0.6	86.0 ±0.6***	83.1±0.7 †††
Waist circumference (cm)	102.3±0.4	95.4±0.6***	93.6±0.6 †††
Hip circumference (cm)	119.1±0.5	114.1±0.5***	112.1±0.5 †††
Waist to hip ratio (cm)	0.861±0.0	0.838±0.0*	0.836±0.0††
Fat mass (%)	41.9±0.3	39.4±0.4***	37.2±0.4†††
FFM (%)	58.1±0.3	60.1±0.4*	63.0±0.5†††
Glucose (mmol/l)	4.9 ±0.4	4.6 ±0.0*	4.7±0.0
Insulin (mIU/l)	11.7±0.8	8.0±0.3*	9.0±0.4†
FFA (µmol/l)	842±60.1	1190±28.2*	676±13.5†
Triglycerides (mmol/l)	1.6±0.0	1.2±0.0	1.5±0.0
HDLcholesterol (mmol/l)	2.0±0.0	1.2±0.0	1.6±0.0

Total cholesterol (mmol/l)	5.2±0.0	4.3±0.0***	5.2±0.0
HOMA–IR	2.6±0.1	1.7±0.1*	1.9±0.1††
hs-CRP (mg/l)	5.8±0.4	4.2±0.3	4.0±0.3†
IL10 (pg/ml)	1.4±0.4	1.5±0.6	1.5±0.6
IL6 (pg/ml)	3.7±0.1	3.1±0.1	2.6±0.1
TNF (pg/ml)	2.0±0.1	2.3±0.1**	2.0±0.1
CCL2 (pg/ml)	104.3±4.7	103.1±3.2	86.4±2.7†

Values are means ± SEM, n = 14. Significance was set as follows: VLCD *vs.* basal: * $p<0.05$, ** $p< 0.01$, *** $p< 0.001$, WM phase *vs.* basal: † $p<0.05$, †† $p< 0.01$, ††† $p< 0.001$

BMI, body mass index; CCL2, chemokine (C-C motif) ligand 2; FFM, fat-free mass; HOMA-IR, homeostasis model assessment of the insulin resistance index; hs-CRP, high-sensitivity C-reactive protein; IL6, interleukin 6; IL10, interleukin 10; FFA, free fatty acids; TNF, tumor necrosis factor

Table 2. Changes of gene expression in subcutaneous abdominal (sAAT) and subcutaneous gluteal (sGAT) adipose tissue during VLCD and weight maintenance (WM) phases of dietary intervention in relation to the pre-diet (basal) level.

Name of gene	sAAT		sGAT	
	VLCD	WM	VLCD	WM
1. Macrophage markers				
CD68	**↑	↔	*↑	↔
ACP5	***↑	↔	**↑	↔
CD14	↔	↔	↔	↔
FCGBP	***↑	↔	**↑	↔
MSR1	***↑	↔	**↑	↔
PLA2G7	**↑	↔	*↑	↔
SPP1	*↑	↔	*↑	↔
TLR4	↔	↔	↔	↔
TLR2	↔	↔	↔	↔
2. Cytokines				
TNF	↔	*↓	↔	↔
IL10	*↑	*↓	↔	*↑ (# #)
IL6	↔	*↓	↔	↔ (# #)
IL10RA	↔	**↓	↔	↔
CCL2	*↑	*↓	*↑	*↑ (# #)
CCR2	**↓	↔	**↓	↔
TGFβ	↔	↔	↔	↔
CXCL1	↔	↔	↔	↔

↑ denotes a higher level of gene expression at the end of respective dietary phase (VLCD or WM) in relation to the pre-diet (basal) level
↓ denotes a lower level of gene expression in relation to the pre-diet (basal) level
↔ denotes no change in gene expression in relation to the pre-diet (basal) level
*,**,*** denotes the level of significance ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively) of the difference between the value at the end of respective dietary phase *vs*. pre-diet (basal) level

denotes the level of significance ($p<0.01$) of the difference between sGAT and sAAT in respect to the change of gene expression during respective dietary phase (as assessed by two-way ANOVA)

Legend of figures

Figures 1 : Adipose tissue gene expression in basal state, before the diet. A: macrophage markers, B: cytokines. Subcutaneous abdominal adipose tissue □, subcutaneous gluteal adipose tissue ▩. Each column represents mean of gene expression levels calculated as delta Ct (normalized to PPIA). *p<0.05, ** p<0.01, *** p<0.001.

Figures 2 : Profile of gene expression in subcutaneous abdominal (sAAT) and gluteal (sGAT) adipose tissue during the two phases (VLCD and WM) of dietary intervention. A and B: Expression of macrophage markers in sAAT (A) and sGAT (B). C and D: Expression of cytokines in sAAT (C) and sGAT (D). The data are presented as fold change in respect to the pre-diet (basal) levels. Data are presented as means ± SEM.

Figure 1.

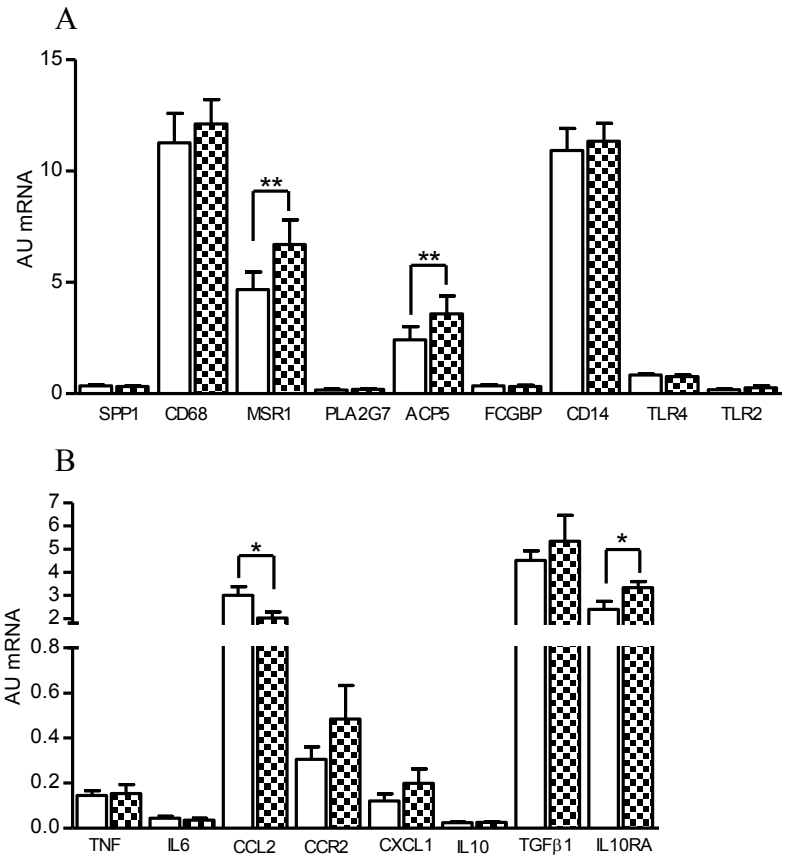
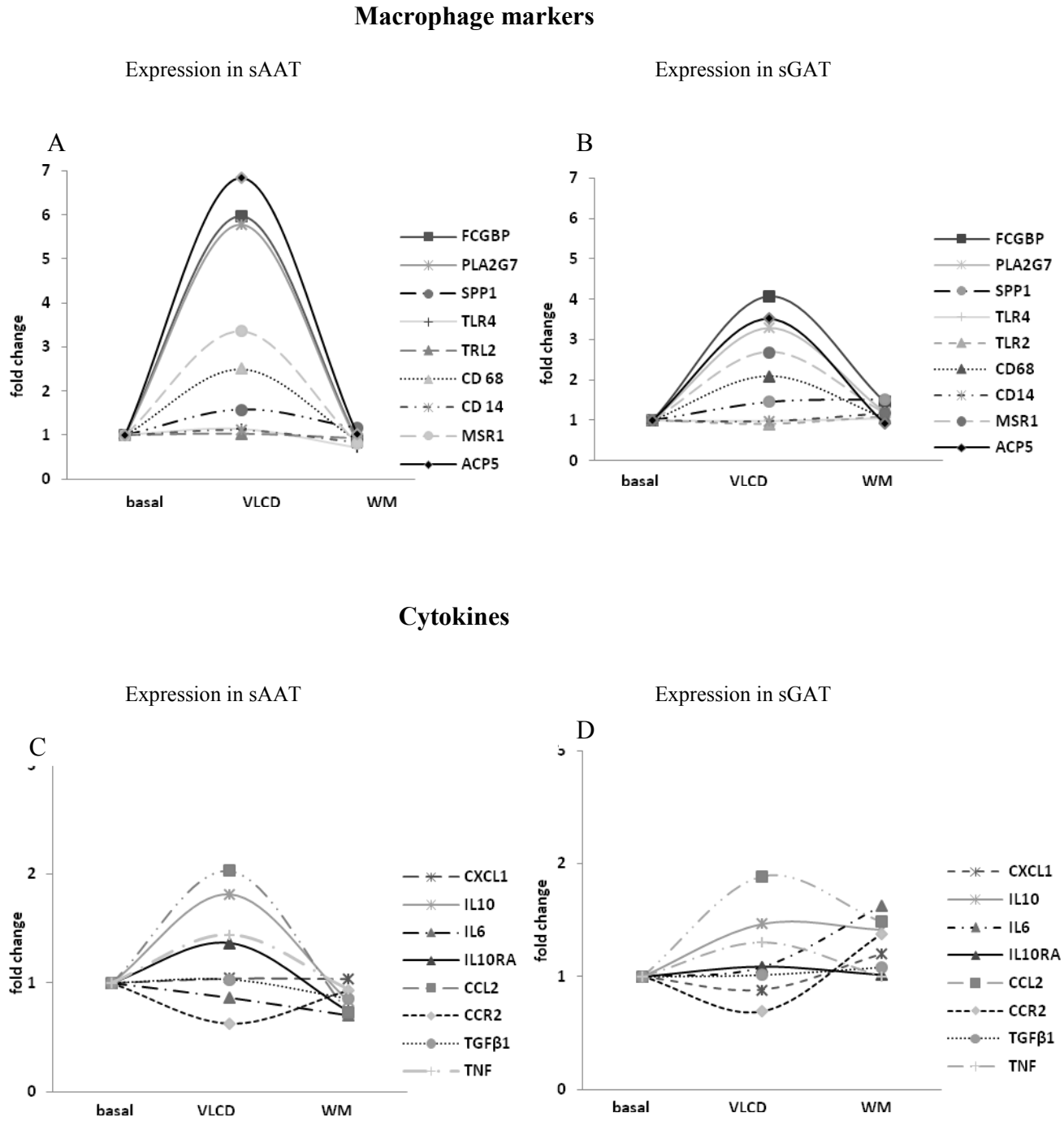

A
AU mRNA
15
10
5
0
**
**
SPP1
CD68
MSR1
PLA2G7
ACP5
FCGBP
CD14
TLR4
TLR2
B
AU mRNA
7
6
5
4
3
2
0.8
0.6
0.4
0.2
0.0
*
*
TNF
IL6
CCL2
CCR2
CXCL1
IL10
TGFβ1
IL10RA

Figure 2


Macrophage markers
Expression in sAAT
Expression in sGAT
A
B
fold change
basal
VLCD
WM
FCGBP
PLA2G7
SPP1
TLR4
TRL2
CD 68
CD 14
MSR1
ACP5
TLR2
CD68
CD14
Cytokines
C
D
CXCL1
IL10
IL6
IL10RA
CCL2
CCR2
TGFβ1
TNF