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Human macrophages stimulate expression of inflammatory mediators in adipocytes; effects of second-generation antipsychotics and glucocorticoids on cellular cross-talk

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ABSTRACT

Objective: Adipose tissue inflammation and distorted macrophage-adipocyte communication are positively associated with metabolic disturbances. Some pharmacological agents, such as second-generation antipsychotics (SGAs) and synthetic glucocorticoid (GC) dexamethasone, tend to induce adverse metabolic side effects and the underlying mechanisms are not fully understood. Our work aimed to study whether SGAs and dexamethasone affect macrophage phenotype and macrophage-adipocyte communication on gene expression level. We selected the model involving THP-1-derived macrophages, polarized into M0, M1, and M2 phenotypes, and primary human mature subcutaneous adipocytes.

Methods: Abdominal subcutaneous adipose tissue needle biopsies were obtained from 6 healthy subjects (4F/2M; age: 22–64 yr; BMI: 21.7–27.6 kg/m2) followed by isolation of mature adipocytes. THP-1-human monocytic cell line was used for the study. THP-1 monocytes were differentiated and polarized into M0 (naïve), M1 (classically activated), and M2 (alternatively activated) macrophages. During and after polarization the macrophages were treated for 24 h without (control) or with therapeutic and supra-therapeutic concentrations of olanzapine (0.2 μM and 2.0 μM), aripiprazole (1.0 μM and 10 μM) and its active metabolite dehydroaripiprazole (0.4 μM and 4.0 μM). Isolated mature human adipocytes were co-incubated with THP-1-derived polarized macrophages pre-treated with SGAs after their polarization. Adipocytes and macrophages were collected before and after co-culture for mRNA expression analysis of genes involved in inflammation.

Results: Co-incubation of mature human adipocytes with human macrophages, regardless of polarization, resulted in a marked induction of pro-inflammatory cytokines in adipocytes, including *IL1B*, *IL6*, *TNFA*, and *IL10*. Remarkably, it did not affect the expression of adipokines and genes involved in the regulation of energy, lipid, and glucose metabolism in adipocytes. Dexamethasone markedly reduced gene expression of pro-inflammatory cytokines in macrophages and prevented macrophage-induced inflammatory response in adipocytes. In contrast, SGAs did not affect macrophage-adipocyte communication and had a minute anti-inflammatory effect in macrophages at supra-therapeutic concentrations. Interestingly, the adipocytes co-incubated with M1 macrophages pre-treated with dexamethasone and SGAs particularly the supra-therapeutic concentration of olanzapine, reduced expression of *LPL*, *LIPE*, *AKT1*, and *SLC2A4*, suggesting that the expression of metabolic genes in adipocytes was dependent on the presence of pro-inflammatory M1 macrophages.

Conclusion: Together, these data suggest that macrophages induce expression of pro-inflammatory genes in human subcutaneous adipocytes without affecting the expression of adipokines or genes involved in energy regulation. Furthermore, our findings demonstrated that SGAs and dexamethasone had a mild effect on macrophage-adipocyte communication in M1 macrophage phenotype.

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Nomenclature

ADIPOQ	adiponectin
AKT	Protein kinase B
ARI	aripiprazole
BMI	body mass index
BSA	bovine serum albumin
CPT1A	Carnitine Palmitoyltransferase 1A,
dARI	dehydroaripiprazole
Dex	dexamethasone
DMEM	Dulbecco's modified Eagle medium
FABP4	Fatty Acid Binding Protein 4
FASN	Fatty Acid Synthase
HOMA IR	homeostatic model assessment of insulin resistance
IL	interleukin
LEP	leptin
LIPE	lipase E
LPL	Lipoprotein lipase
MEM	minimum essential medium
OLA	olanzapine
PBMC	peripheral blood mononuclear cells
PEST	penicillin-streptomycin
PPARGC1A	PPARG Coactivator 1 α,
RPMI 1640	Roswell Park Memorial Institute 1640
SGA	second-generation antipsychotic
SLC2A4	glucose transporter 4
TNF	tumor necrosis factor

1. Introduction

Being an essential endocrine organ, adipose tissue releases several hormones and cytokines necessary for modulation of whole-body metabolism as well as the function of the adipose tissue itself. Adipose tissue consists of several different cells, including adipocytes, pre-adipocytes, and immune cells (Blüher, 2016). Among these, macrophages attract a considerable amount of interest, as they are implicated in the induction of chronic adipose tissue inflammation and insulin resistance via secretion of different pro-inflammatory mediators (Boutens and Stienstra, 2016; Makki et al., 2013).

Macrophages are innate immune cells, which play a vital role in host defense (Sica and Mantovani, 2012). Traditionally they are identified as classically activated, or M1, and alternatively activated M2 macrophages. However, this nomenclature is dichotomous, as macrophages can polarize and develop different phenotypes within the M1/M2 spectrum (Sica and Mantovani, 2012). M1 macrophages are characterised by secretion of nitric oxide and pro-inflammatory cytokines, such as tumor necrosis factor (TNF)α, interleukin (IL)12, IL6, IL1β and others. In contrast, M2 macrophages are involved in subsiding the inflammation and maintaining tissue homeostasis. M2 macrophages are characterised by high expression of anti-inflammatory cytokines, such as IL10, and non-opsonic receptors, for instance, mannose receptor – cluster of differentiation (CD)206 (Mantovani et al., 2004; Morris et al., 2011). Macrophages are known to have high functional plasticity that allows them to adapt and accommodate to the surrounding environment in order to create an appropriate immune response to invading pathogens (Biswas and Mantovani, 2010). They can switch their established phenotype when exposed to a different cytokine milieu, in other words, get re-programmed (Shapouri-Moghaddam et al., 2018).

Macrophage infiltration in adipose tissue has been shown both in obese humans and mice (Weisberg et al., 2003; Xu et al., 2003). In obese mice, there is an increased number of M1-like adipose tissue macrophages (Boutens and Stienstra, 2016). On the contrary, in humans with obesity, M2-like macrophages, which were able to secrete high amounts of pro-inflammatory cytokines, were more prevalent (Boutens and Stienstra, 2016). To investigate the mechanisms underlying dysfunctional macrophage-adipocyte communication and its contribution to adipose tissue inflammation, co-culturing murine and human adipocytes with macrophages has been attempted (Keuper et al., 2011; Sárvári et al., 2015; Suganami et al., 2005). However, most of these studies were based on using rodent cells or human adipocytes differentiated *in vitro*, due to the limited survival time of isolated mature adipocytes. Additionally, there is a lack of studies involving M1 and M2 macrophage effects on adipocytes.

Several reports have indicated that second-generation antipsychotics (SGAs) and glucocorticoids lead to an increased incidence of metabolic side effects, such as weight gain, development of insulin resistance, aggravated plasma lipid profile, increased adiposity, and type 2 diabetes mellitus (Mitchell et al., 2013; Wang, 2005). Individual agents have been reported to induce metabolic adverse effects to a different extent, with olanzapine (OLA) and clozapine being considered high-risk drugs (Mitchell et al., 2013) and aripiprazole (ARI) a low-risk agent (Rummel-Kluge et al., 2010).

Very little is known about the ability of SGAs to directly disrupt human adipose tissue metabolism, particularly via interfering with macrophage-adipocyte cross-talk. Therefore, our work aimed to study the effects of macrophages on isolated mature adipocytes gene expression. Additionally, we investigated whether SGAs and the synthetic glucocorticoid dexamethasone (Dex), affect human macrophage phenotype and macrophage-adipocyte communication. Based on the individual tendency of the drugs to induce metabolic adverse effects, OLA, ARI and its active metabolite dehydroaripiprazole (dARI) were chosen for this study. dARI was included to test whether the effects exerted by the drug are due to the parent compound or the active metabolite.

To the best of our knowledge, this is the first study with SGAs and Dex conducted on a human macrophage cell line and isolated mature human adipocyte co-culture, including all three M0-, M1-, and M2-polarized macrophage subtypes.

2. Methods

2.1. Adipose tissue biopsies

Adipose tissue needle biopsies were collected from abdominal subcutaneous adipose tissue from 6 healthy individuals (4F/2M; age: 22–64 yr; BMI: 21.8–27.7 kg/m^2) after administration of local anaesthetic lidocaine. Fasting blood samples from study participants were obtained for biochemical profiling (Table 1) at the Department of Clinical Chemistry, Uppsala University Hospital. Individuals with type 2 diabetes, endocrine disorders, cancer or other major illnesses, as well as patients receiving antipsychotic, antidepressant, or neuroleptic medications, were excluded from the study. The study was approved by the Swedish Ethical Review Authority in Uppsala (DNR 2018/385), and all participants gave their written informed consent. The study was conducted in accordance with the relevant regulations and guidelines.

2.2. Human mature adipocytes isolation

Mature adipocytes were isolated by digestion of the adipose tissue for 60 min in 37 °C water bath shaking at 105 rpm with 1 mg/ml collagenase A (from *Clostridium histolyticum*, Roche, Manheim, Germany) in Medium 199 (Gibco, Life Technologies, Paisley, UK) (6 mM glucose, 4% bovine serum albumin (BSA, Sigma, MO, USA), 150 nM adenosine (Sigma, MO, USA), pH = 7.4). Digested adipose tissue was further

Table 1
Anthropometric and biochemical characteristics of the subjects involved in the study.

Variables	
Sex (male/female, n)	2/4
Age (years)	42 ± 17
Body mass index (kg/m^2)	25.1 ± 2.6
Waist-hip ratio (WHR)	0.85 ± 0.09
Systolic blood pressure (mmHg)	117 ± 22
Diastolic blood pressure (mmHg)	64 ± 9
HbA_{1c} (mmol/mol)	34 ± 2
HbA1c (%)	5.3 ± 0.2
Plasma glucose (mmol/L)	5.4 ± 0.4
Serum insulin (mU/L)	8.0 ± 3.9
HOMA IR (mmol x mU/L^2)	1.7 ± 0.6
Body fat mass (%)	29.3 ± 8.2
P-triglycerides (mmol/L)	1.2 ± 0.9
P-total cholesterol (mmol/L)	5.3 ± 1.3
P-LDL-cholesterol (mmol/L)	3.5 ± 1.1
P-HDL-cholesterol (mmol/L)	1.5 ± 0.6
Cell size, μm	100.3 ± 5.7

Data are shown as mean ± SD; HbA1c, glycosylated haemoglobin; HOMA IR, homeostatic model assessment of insulin resistance index (fasting blood glucose x fasting insulin/22.5); LDL, low-density lipoprotein; HDL high-density lipoprotein.

filtered through 250 μm nylon mesh. The floating adipocyte fraction was collected and washed four times with Dulbecco's modified Eagle's medium, DMEM, supplemented with 6 mM glucose (Gibco, Life Technologies, Paisley, UK), 10% fetal bovine serum (FBS, Gibco, Life Technologies, Paisley, UK) and 1% penicillin-streptomycin (PEST, Gibco, Life Technologies, Paisley, UK). The cells were then diluted with DMEM to 6–7% lipocrit, and 1 ml of the cell lipocrit was co-cultured with 80,000 THP-1-derived macrophages pre-treated without or with different concentrations of SGAs (see 2.4).

2.3. THP-1 monocytes differentiation and polarization

THP-1 cell line differentiation and polarization has been performed in accordance with previously reported protocols (Chanput et al., 2014; Genin et al., 2015). In brief, the human monocytic cell line THP-1 (ATCC, TIB202, Manassas, Virginia) was maintained and expanded in culture medium RPMI 1640 GlutaMAX (Gibco, Life Technologies, Paisley, UK) supplemented with 10% FBS (Gibco, Life Technologies, Paisley, UK), 1% penicillin-streptomycin (PEST, Invitrogen), 1X L-glutamine (Sigma, MO, USA), 1X MEM Non-essential amino acids (MEM NEAA) (Gibco, Life Technologies, Paisley, UK), 1 mM sodium pyruvate to the density of 1–8 × 10^5 cells/ml. The cells were then plated at the density of 80,000 cells in 12 well plates (Sarstedt, Nümbrecht, Germany) and differentiated with the addition of 150 nM phorbol 12-myristate 13-acetate (PMA) (Sigma, MO, USA) for 48 h, followed by 24 h rest in RPMI 1640 culture medium (described previously). Monocytes were further polarized for 24 h to:

- M1 (classically activated macrophages) in the presence of human recombinant interferon (IFN) γ (20 ng/ml) (PeproTech, NJ, USA) and lipopolysaccharide from *E.Coli* (10 pg/ml) (Sigma, MO, USA).
- M2 (alternatively activated macrophages) in the presence of human recombinant IL4 (20 ng/ml) and IL13 (20 ng/ml) (PoproTech, NJ, USA).
- M0 (non-polarized naïve macrophages) were treated with the vehicle.

2.4. THP-1 macrophage treatment with SGAs

To facilitate the understanding of the experimental design, a visual representation is shown in Fig. 1. The results obtained during the study will be reported according to the indicated labelling of the samples. The treatment of THP-1-derived macrophages was carried as follows:

1. In order to understand whether SGAs and Dex affected the macrophage polarization and altered their phenotype, THP-1 macrophages (described in 2.3) were polarized in the absence (Control) or in the presence of therapeutic and supra-therapeutic concentrations of OLA (0.2 and 2.0 μM), ARI (1.0 μM and 10 μM), dARI (0.4 μM and 4 μM, dARI) for 24 h. The cells were then snap-frozen and used for gene expression analysis. These cells are labelled as P-M0, P-M1, and P-M2 (see Fig. 1).
2. To study whether the SGAs and Dex are able to affect the established phenotype of polarized macrophages and alter their cytokine profile, the differentiated macrophages were further polarized into M0, M1, and M2 macrophages. This was followed by the treatment of the cells without or with SGAs for 24 h. SGA- and Dex-containing medium was removed entirely, and macrophages were either snap-frozen and used for gene expression analysis (labelled as M0, M1, and M2), or co-incubated with isolated mature adipocytes (see 2.5).

The concentrations of the SGAs were chosen according to the reported plasma concentrations (Citrome et al., 2009; Kirschbaum et al., 2008). We have also tested the concentrations ten times higher in order to ensure that we cover all the relevant concentrations in case of drug lipophilicity and accumulation in the adipose tissue. Very little is known about the concentration of the drugs in adipose tissue in humans, however, animal studies reported that the concentration of OLA in adipose tissue is comparable to its plasma concentration (Aravagiri et al., 1999). The concentration of Dex 0.3 μM has been chosen as the standard concentration previously used for *in vitro* and *ex vivo* experiments with adipocytes (Carter-Su and Okamoto, 1985; Lundgren et al., 2004; Sarsenbayeva et al., 2019).

2.5. THP-1 macrophage and isolated mature adipocytes co-culture

Following treatment of THP-1-differentiated macrophages without (control) or with SGAs (see 2.4), the medium containing SGAs was removed entirely, cells were washed with phosphate buffer saline, and 1 ml of 6–7% lipocrit of isolated mature adipocytes was added to THP-1-differentiated macrophages (80,000 cells) and co-cultured for 24 h. The culture allowed clear separation between macrophages and adipocytes since mature adipocytes are floating cells, while macrophages are adherent cell culture. After the co-incubation the adipocytes were collected and snap-frozen for further gene expression analysis. The co-culture of differentiated macrophages with mature adipocytes was adapted from previous studies using membrane inserts and demonstrating the cross-talk between two cell types (Harms et al., 2019; Suganami et al., 2005). The study also indicated that floating adipocytes have a similar gene profile and can be used for short culture times (Harms et al., 2019).

Adipocytes incubated without the macrophages are labelled as no MΦ and adipocytes co-incubated with macrophages as +M0, +M1, and +M2 (depending on the macrophages subtypes used for the co-incubation). Adipocyte cell size was measured in 100 consecutively selected adipocytes from the same individual.

2.6. RNA extraction and cDNA transcription

Macrophages and adipocytes were lysed with Qiazol lysis buffer (Qiagen, Hilden, Germany). RNA from adipocytes was isolated using RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). RNA from macrophages was extracted via the addition of chloroform to the tissue lysate and precipitated with the addition of 2-propanol. RNA was eluted, and its concentration and quality were checked with NanoDrop 2000 Spectrophotometer (Thermo Scientific, DE, USA). RNA was converted to cDNA using high-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific, CA, USA) according to the

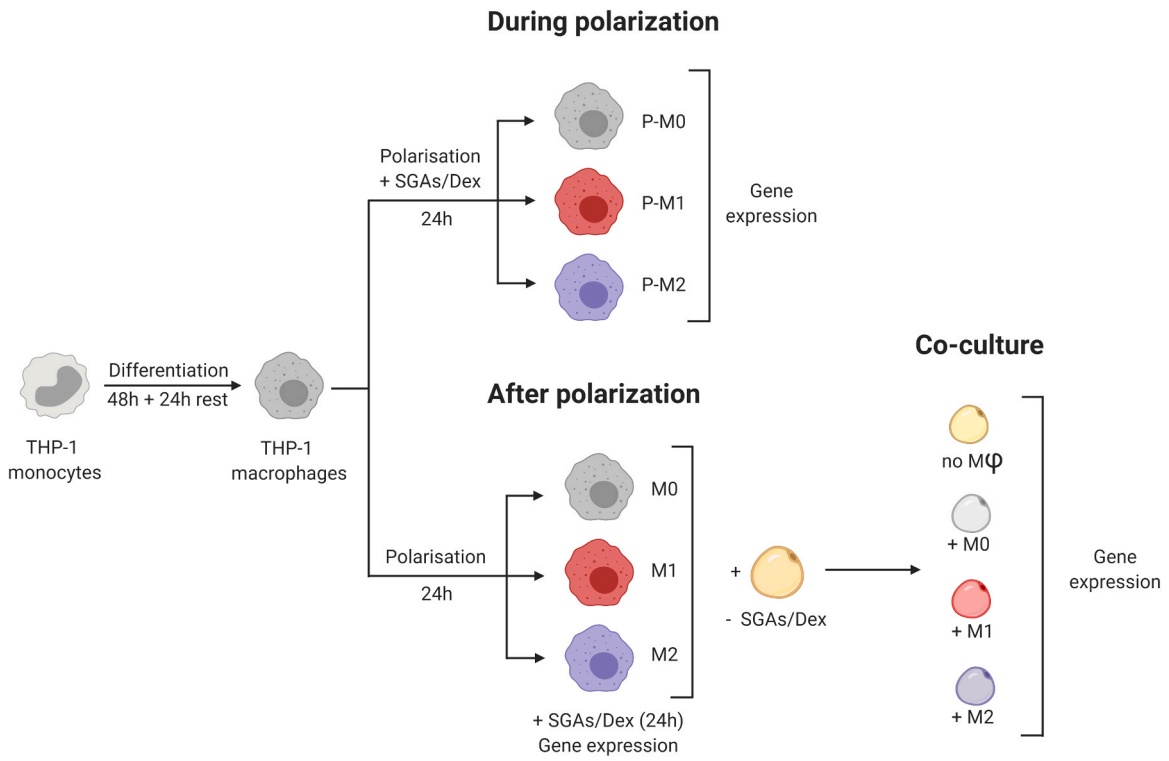

Fig. 1. Schematic representation of the experimental setup for THP-1 monocyte differentiation and polarization. Annotation: macrophages treated with SGAs after their polarization – M0 (naïve), M1 (classically-activated), and M2 (alternatively activated) macrophages. Macrophages treated with SGAs during their polarization – P-M0, P-M1, and P-M2. Adipocytes co-incubated without (no MΦ) and with: +M0 (co-incubated with M0), +M1 (co-incubated with M1), and +M2 (co-incubated with M2) treated without (Control) or with SGAs and Dex. Created with BioRender.com

manufacturer's protocol.

2.7. Gene expression analysis

Adipocytes and macrophages were used for measuring mRNA expression of several macrophage differentiation and polarization markers: cluster of differentiation 68, ***CD68*** (Hs02836816_g1), mannose receptor C-type 1/CD206, ***MRC1*** (Hs00267207_m1), pro- and anti-inflammatory genes: interleukin 6, ***IL6*** (Hs00985639_m1), interleukin 1β, ***IL1B*** (Hs01555410_m1), tumor necrosis factor α, ***TNFA*** (Hs00174128_m1), interleukin 10, ***IL10*** (Hs00961622_m1); adipokines: leptin, ***LEP*** (Hs00174877_m1), adiponectin, ***ADIPOQ*** (Hs00605917_m1); genes involved in glucose, lipid and energy metabolism regulation: Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1 α, ***PPARGC1A*** (Hs00173304_m1) fatty acid-binding protein 4, ***FABP4*** (Hs01086177_m1), carnitine palmitoyltransferase 1A, ***CPT1A*** (Hs00912671_m1), lipase E/hormone-sensitive lipase, ***LIPE*** (Hs00193510_m1), fatty acid synthase, ***FASN*** (Hs00163653_m1), protein kinase B, ***AKT1*** (Hs00178289_m1), glucose transporter 4, ***SLC2A4*** (Hs00168966_m1). The gene expression measurement was performed with the QuantStudio 3 System (Thermo Fisher Scientific, MA, USA). TaqMan assay probes were purchased from Thermo Fisher Scientific, MA, USA. Gene expression was normalised to the expression of *18S* ribosomal RNA or Glyceraldehyde 3-phosphate dehydrogenase, ***GAPDH*** (Hs02786624_g1) and calculated using $2^{-\Delta\Delta Ct}$ method relative to the respective control condition, which is indicated in figure/table legends.

2.8. Cell toxicity assay

After the treatment of THP-1 monocyte-derived macrophages without or with different concentrations of SGAs and Dex (see 2.4) viability assay was performed using Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells (Biotium, CA, USA) according to the manufacturer's protocol. Fluorescence intensity was measured at 530 and 635 nm with Spectramax ID3 (Molecular Devices, CA, USA). Cell viability was calculated relative to the control condition.

2.9. Statistical analysis

GraphPad Prism 7 and IBM SPSS Statistics 25 software were used to create figures and to analyze the data. Data distribution was tested with the Shapiro–Wilk test, and the data were analyzed either with Friedman's test or repeated measure One-way ANOVA test comparing each drug treatment group with control. For multiple comparisons, the p-value was adjusted with Dunn's or Dunnett's correction, respectively. Gene expression for Dex-treated groups was analyzed with paired *t*-test in comparison to control. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Macrophage differentiation and phenotype confirmation

The gene expression of the macrophage surface marker *CD68* was used to verify the differentiation of THP-1 monocytes into macrophage-like cells. The expression of the marker was 2–3-fold significantly higher ($p < 0.05$) in all three macrophage subtypes (M0, M1 and M2) compared to the monocytes from the corresponding cultures (Supplementary Fig. S1a).

To confirm the phenotype of the macrophages after polarization, specific classically activated, pro-inflammatory M1 and alternatively activated, anti-inflammatory M2 macrophage markers expression was measured. M1 polarized macrophages presented increased gene expression of the M1 pro-inflammatory cytokines genes *TNFA* and *IL6* ($p < 0.05$, Supplementary Fig. S1b), but low expression of *IL10* ($p < 0.001$, Supplementary Fig. S1c), compared to M0 and M2

macrophages. Meanwhile, M2 macrophages had higher expression of the M2 markers *MRC1* and *IL10* ($p < 0.001$, Supplementary Fig. S1c) compared to M0 and M1 macrophages, respectively. Therefore, the macrophage subtypes expressed the expected specific markers.

3.2. Effects of SGA and dexamethasone treatment on macrophage during polarization

To study whether treatment with SGAs during polarization would affect THP-1 macrophage phenotype and cytokine profile, THP-1-derived macrophages were polarized into all three subtypes (P-M0, P-M1, and P-M2) for 24 h in the absence or presence of different concentrations of SGAs (see Methods) (Fig. 2A, C, E, G, and I). The effects of SGAs on the macrophage phenotype was assessed via measuring changes in the expression of M1- and M2-specific markers, *IL1B*, *IL6*, *TNFA*, *IL10*, and *MRC1* (i.e. *CD206*) after treatment with SGAs.

In P-M0 macrophages, therapeutic concentrations of ARI and OLA did not have a significant effect on cytokine gene expression. However, dARI 0.4 μM induced the expression of *IL1B* by 20% ($p < 0.01$) (Fig. 2A). Supra-therapeutic concentrations of ARI and OLA reduced the gene expression of *IL6* and *TNFA* by almost 50% ($p < 0.05$) and ca 25% (ns), respectively (Fig. 2C, E). Both ARI 10 μM and OLA 2.0 μM also reduced the expression of the M2-specific marker *MRC1* by 45% (ns) and by 60% ($p < 0.05$), respectively (Fig. 2I).

Interestingly, in P-M1 macrophages, all SGAs at both concentrations led to a marked or nominal increase in *IL1B* expression by up to 50% (Fig. 2A). At therapeutic concentrations, SGAs had no significant effect on *MRC1* or other cytokine gene expression, except upregulation of *IL6* expression by ca 30% ($p < 0.05$) by OLA 0.2 μM.

Similarly, cytokine and *MRC1* gene expression in P-M2 macrophages was not significantly affected by the therapeutic concentrations of SGAs. However, dARI μM 4.0 induced *IL1B* expression ($p < 0.05$) and ARI 10 μM suppressed *IL10* expression by 50% ($p < 0.01$) in P-M2 cells (Fig. 2A, G).

Supra-therapeutic concentrations of ARI and OLA seem to cause a mild reduction in the expression of the pro-inflammatory cytokines *IL6* and *TNFA* in P-M0 and P-M2 macrophages, suggesting an anti-inflammatory role in these cells. However, SGAs seem to enhance pro-inflammatory phenotype via inducing *IL1B* and *IL6* expression, when added during M1 polarization.

Dex 0.3 μM, demonstrated a sustained significant or nominal downregulation of 60–70% ($p < 0.05$) of pro-inflammatory genes in all three macrophage subtypes and consistent induction of *MRC1* expression up to 100-fold ($p < 0.05$) (Fig. 2I). Interestingly, Dex 0.3 μM did not reduce the expression of pro-inflammatory cytokines to the same level in all three macrophage subtypes. In other words, the expression of pro-inflammatory cytokines in Dex-treated P-M1 macrophages was higher than in Dex-treated P-M0 and P-M2 macrophages.

3.3. Effects of SGAs and dexamethasone on macrophage plasticity and phenotype switch after polarization

In order to understand whether SGAs are able to change the established phenotype in already polarized macrophages, M0, M1, and M2 macrophages were treated without or with different concentrations of SGAs for 24 h (Fig. 2B, D, F, H, and J). Similarly, the effects of the drugs on macrophage phenotype was assessed via measuring the expression of the M1- and M2-specific macrophage markers, as the ones indicated in Sections 3.1 and 3.2.

We observed that in M0 macrophages, OLA at both concentrations significantly or nominally reduced the expression of *IL6* and *TNFA* by 25–40% ($p < 0.05$) (Fig. 2D and F). By contrast, dARI 4.0 induced *IL1B* expression by 65% ($p < 0.05$) (Fig. 2B).

Neither of the SGAs affected the cytokine gene expression in M1 macrophages, apart from ARI 10 μM reducing *IL10* expression by 45% ($p = 0.054$) (Fig. 2H). ARI 10 μM also reduced the expression of *IL6* by 50% in M2 macrophages ($p = 0.054$), while dARI 0.4 μM numerically upregulated *IL6* ($p = 0.054$) (Fig. 2D, F).

Our data show that SGAs, apart from dARI, tend to keep their mild anti-inflammatory effect in M0 macrophages, while they do not seem to significantly affect the established M1 and M2 macrophage phenotypes when added after their polarization.

Dex 0.3 μM demonstrated a significant and nominal reduction in the gene expression of pro-inflammatory cytokines in macrophages of up to 60–70% ($p < 0.05$) and induced the expression on M2-marker *MRC1* by ca 60–100 fold. Similar to P-M1 macrophages, Dex 0.3 μM did not suppress pro-inflammatory cytokine gene expression in all three macrophage phenotypes to the same level, as it remained higher in Dex-treated M1 macrophages compared to Dex-treated M0 and M2 macrophages.

3.4. Cell toxicity assay

In order to understand whether the changes in the gene expression are due to changes in the viability of macrophages, cell toxicity testing was performed following 24 h incubation without or with the SGAs. Incubation of differentiated and polarized THP-1 macrophages with ARI, OLA and dARI, did not affect the cell viability of all three macrophage subtypes, M0, M1, and M2 (Supplementary Fig. S2), compared to control (set as 1.0). Therefore, SGAs were not cytotoxic to cells after 24 h incubation. However, Dex 0.3 μM significantly reduced the cell viability of M1 ($p < 0.05$) macrophages by ca 25%.

3.5. SGAs and Dex effects on macrophage-induced expression of pro-inflammatory genes in adipocytes

Co-culture of adipocytes with all three macrophages subtypes (M0, M1 and M2) led to a consistent significant increase in the gene expression of pro-inflammatory cytokines in adipocytes up to 100-fold in *IL1B*, 50-fold in *IL6*, and 10-fold in *TNFA*, $p < 0.05$ (Fig. 3). Interestingly, adipocytes incubated with macrophages pre-treated with Dex 0.3 μM demonstrated reduced expression of *IL1B and IL6* ($p < 0.01$) compared to the adipocytes co-incubated with non-treated macrophages (Control) (Fig. 3, Supplementary Table S1). However, SGA pre-treatment of all three macrophage phenotypes led to a nominal, but non-significant, increase in the expression of pro-inflammatory cytokine genes (*TNFA, IL6* and *IL1B*) in adipocytes following their co-incubation (Fig. 3). This indicates that SGAs do not have a significant effect on macrophage-induced pro-inflammatory gene expression in adipocytes, while Dex partially prevented it.

We also compared the effects of ARI and OLA on the pro-inflammatory gene expression on differentiated human adipocytes alone ($n = 3$) and observed no effects on expression of pro-inflammatory genes following 72 h of treatment (data not shown).

3.6. Effects of SGAs and Dex pre-treatment of macrophages on metabolic gene expression in adipocytes

Co-culturing mature adipocytes with M0, M1-, and M2-polarized macrophages did not change the gene expression of the adipokines *LEP* and *ADIPOQ* (Fig. 4) Similarly, other genes involved in the regulation of adipocyte lipid and glucose metabolism, including *LPL*, *LIPE*, *FASN*, *FABP4*, *SLC2A4* (Fig. 5), and *CPT1A* and *PPARGC1A* (Supplementary Fig. S3 and Supplementary Table S1) were not affected by the co-incubation of adipocytes with all three macrophage subtypes. Interestingly, co-incubation of adipocytes with M1 macrophages slightly increased the expression of *AKT1* in adipocytes by 1.5-fold ($p < 0.05$, Fig. 5F).

SGA- and Dex-treatment of M0 and M2 macrophages did not affect the expression of most metabolic genes in co-cultured adipocytes (Fig. 5), apart from a significant reduction in the expression of *AKT1*, and *SLC2A4* in adipocytes after co-incubation with M0 macrophages

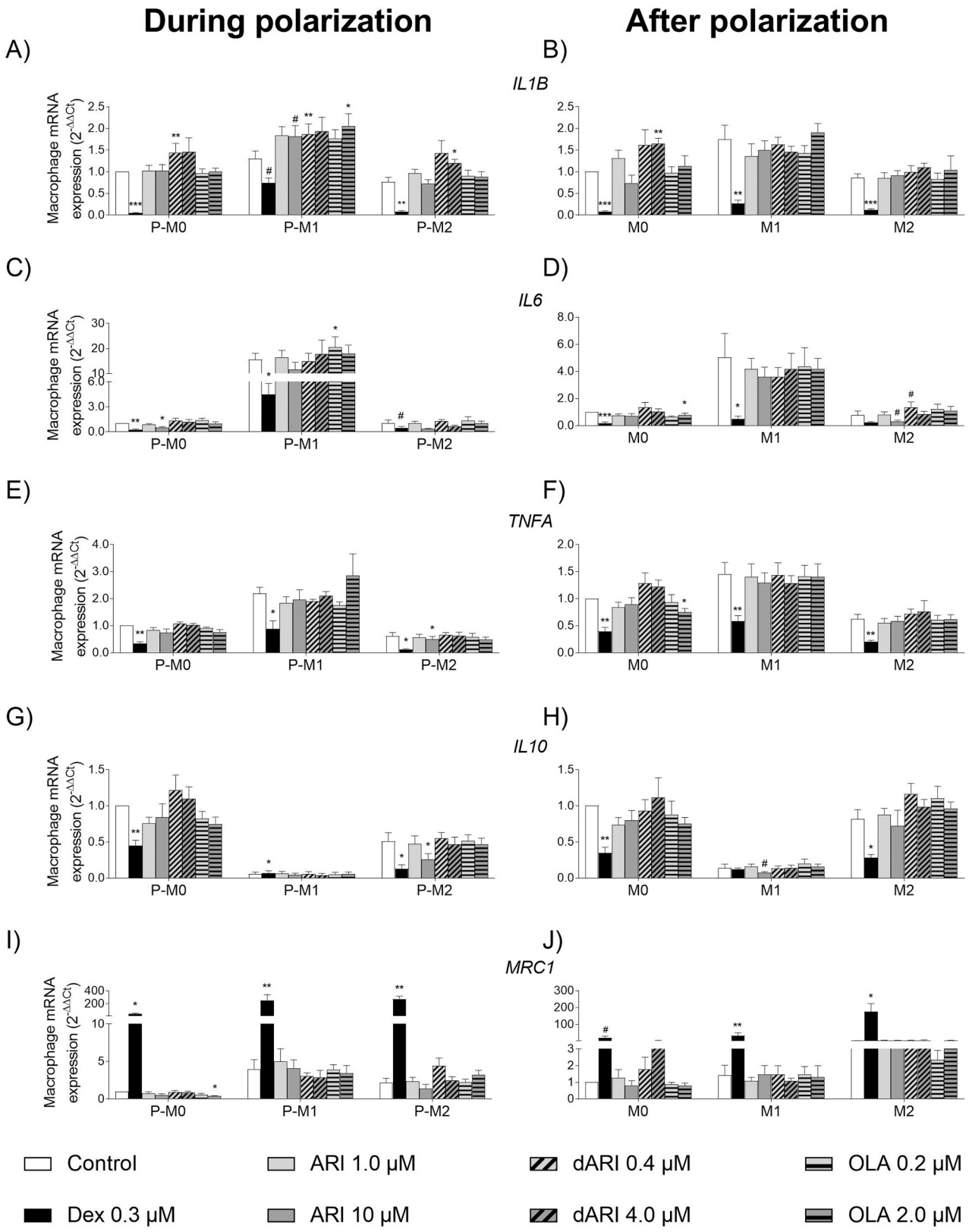

Fig. 2. Effects of SGAs and Dex treatment during polarization (P-M0, P-M1, and P-M2, left column) and after polarization (M0, M1, and M2, right column) on macrophage gene expression. The figure illustrates the expression of A) and B) *IL1B*, C) and D) *IL6*, E) and F) *TNFA*, H) and G) *IL10*, and I) and J) *MRC1*. Data show mean ± SEM from n = 5 independent experiments. Gene expression was calculated as $2^{-\Delta\Delta Ct}$ relative to the naïve (P-M0 or M0) Control gene expression. Data were analyzed with paired *t*-test (Dex) and Friedman's test with multiple comparisons of each drug groups vs control (no SGAs), p-values were adjusted with Dunn's correction.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, # $p < 0.09$, relative to the respective Control.

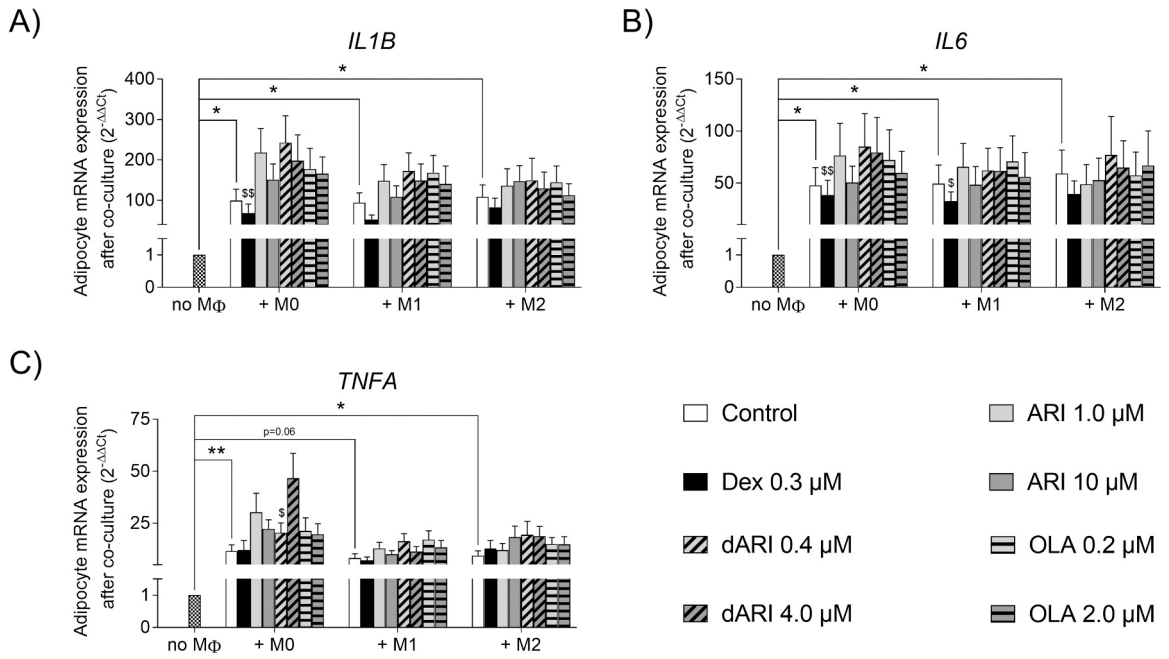

Fig. 3. Expression of pro-inflammatory genes in adipocytes following co-culture without (no MΦ) or with M0, M1, and M2 macrophages pre-treated with SGAs and Dex. The figure illustrates the expression of A) *IL1B*, B) *IL6*, and C) *TNFA* of adipocytes co-incubated without (no MΦ) or with THP-1-derived M0, M1, and M2 macrophages. Data show mean ± SEM of n = 6 independent experiments. Gene expression was calculated as $2^{-\Delta\Delta Ct}$ relative to the Adipocytes (no MΦ). *p < 0.05, * *p < 0.01, relative to no MΦ. $ p < 0.05, $$ p < 0.01, relative to the respective Control. Data were analyzed with Friedman test, p-values were adjusted with Dunn's correction.

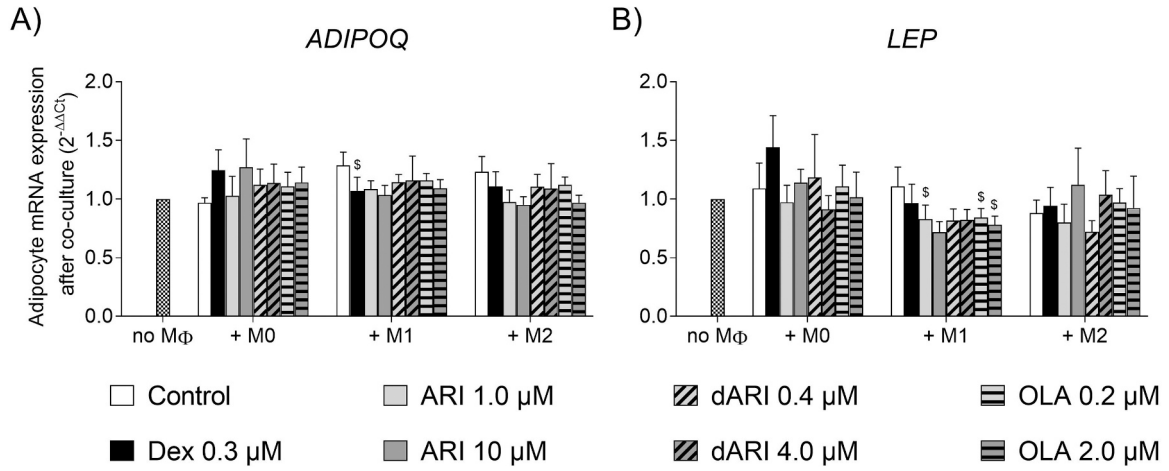

Fig. 4. Adipokines gene expression in adipocytes following co-culture without (no MΦ) or with M0, M1, and M2 macrophages pre-treated with SGAs and Dex. The figure illustrates the expression of A) *ADIPOQ* and B) *LEP*. Data show mean ± SEM of n = 6 independent experiments. Gene expression was calculated as $2^{-\Delta\Delta Ct}$ relative to the Adipocytes (no MΦ). *p < 0.05, **p < 0.01, relative to no MΦ. $ p < 0.05, $$ p < 0.01, relative to the respective Control. Data were analyzed with Friedman test, p-values were adjusted with Dunn's correction.

treated with ARI and OLA (Fig. 5). Interestingly, most of the effects of SGAs on macrophage-adipocyte communication were observed with M1 macrophages. Treatment of M1 macrophages with OLA and ARI nominally or significantly reduced the expression of *LEP*, *FABP4*, *LPL*, *LIPE*, *AKT1*, and *FASN* in co-cultured adipocytes in a dose-dependent manner, with a more prominent reduction of 20–30% with OLA 2.0 μM treatment (Figs. 4 and 5). We also observed that treatment of M1 macrophages with Dex resulted in a significant reduction in the expression of *LPL*, *AKT1*, and *SLC2A4* in adipocytes. Having measured basal lipolysis in adipocytes via glycerol release in the co-incubation medium, we observed that treatment of macrophages with SGAs and Dex did not affect adipocyte lipolysis (data not shown).

We did not detect any changes in adipocyte cell size after co-incubation with macrophages treated without or with the drugs (Supplementary Fig. S4). Additionally, we observed large individual variance in the effects of the SGAs on adipocyte gene expression between the subjects. However, there were no significant correlations between the effects of SGAs on the expression of the explored genes and individual subject characteristics such as age, sex, BMI, waist/hip ratio, body fat%, HOMA-IR, and adipocyte cell size (data not shown).

4. Discussion

To our best knowledge, there have been no previous studies conducted on the effects of Dex and SGAs on human macrophage-adipocyte communication involving isolated mature human adipocytes and all

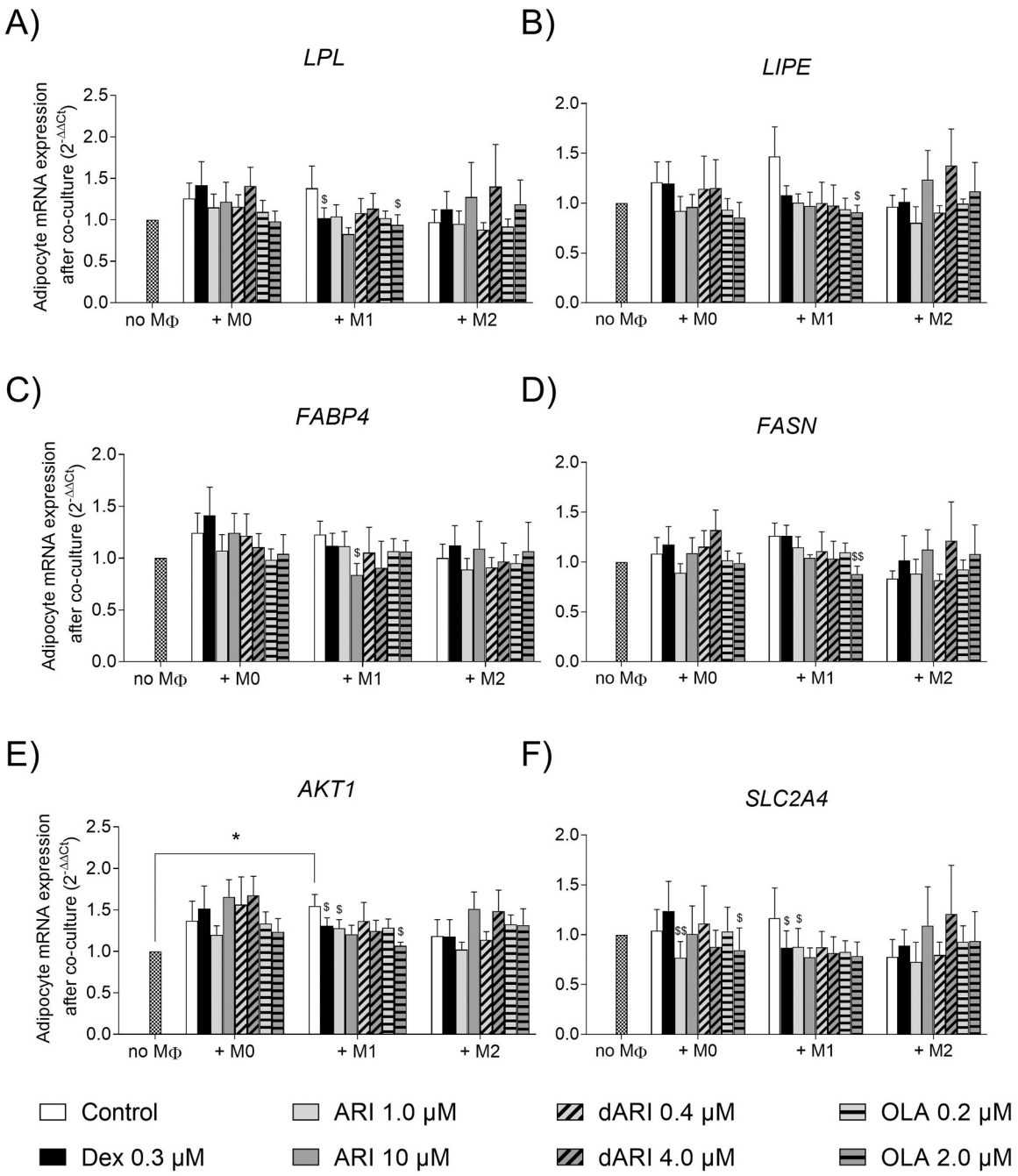

Fig. 5. Expression of metabolic genes in adipocytes following co-culture without (no MΦ) or with M0, M1, and M2 macrophages pre-treated with SGAs and Dex. The figure illustrates the expression of: A) *LPL*, B) *LIPE*, C) *FABP4*, D) *FASN*, E) *AKT1*, F) *SLC2A4*. Data show mean ± SEM of n = 6 independent experiments. Gene expression was calculated as $2^{-\Delta\Delta Ct}$ relative to the Adipocytes (no MΦ). *p < 0.05, * *p < 0.01, relative to no MΦ. $ p < 0.05, $$ p < 0.01, relative to the respective Control. Data were analyzed with Friedman test, p-values were adjusted with Dunn's correction.

three M0, M1, and M2 macrophage subtypes.

The phenotype of the monocyte-derived macrophages was verified by the increased expression of the surface marker *CD68* as well as the adherence of cells to the plastic, as reported previously (Genin et al., 2015). Furthermore, the correct polarization of the macrophages was confirmed via measuring the expression of several markers specific to M1 or M2 macrophages, which indicated that the obtained cells were differentiated and polarized according to the attempted phenotype.

Treatment of macrophages with supra-therapeutic concentrations of SGAs during their polarization seems to enhance M1 phenotype in P-M1 macrophages. However, in P-M0 and P-M2 macrophages, supra-therapeutic concentrations of ARI and OLA acted as anti-inflammatory agents, reducing the expression of *IL6* and *TNFA*.

Twenty four hour treatment of macrophages after their polarization demonstrated a similar effect in M0 macrophages as during polarization (P-M0), albeit to a lesser extent. This would be expected, as M0 macrophages were not treated with any polarising agents and should be phenotypically similar. Administration of the drugs to M1 and M2-polarized macrophages did not have a significant effect on macrophage phenotype apart from ARI 10 μM, reducing *IL6* and *IL10* expression both during and after polarization.

Therefore, our data indicate a mild anti-inflammatory effect of SGAs at supra-therapeutic concentration on macrophage phenotype in polarized macrophages. At the same time, they seem to be able to enhance M1

phenotype in the cells undergoing polarization process. Since macrophage polarization is a highly dynamic process, the time of exposure to the stimuli can also modulate their polarization (Murray, 2017). Dex 0.3 μM, had a potent anti-inflammatory effect on the cells and significantly upregulated the expression of *MRC1* in all macrophage subtypes. Although it had some toxicity in M1 macrophages, which would affect the gene expression in the cells, the reduction in pro-inflammatory cytokine gene expression was twice or three times higher than the cytotoxic effect of the drug. Our results are in accordance with reported anti-inflammatory effects of glucocorticoids in macrophages characterised by reduced pro-inflammatory cytokine expression and increased *MRC1* expression (Desgeorges et al., 2019; Shepherd et al., 1985). The anti-inflammatory effect of glucocorticoids on gene regulation in macrophages was also reported to be dependent on the inflammatory status of macrophages (van de Garde et al., 2014).

Anti-inflammatory effects of SGAs have been demonstrated both *in vivo* and *in vitro*. Elevated pro-inflammatory cytokine plasma levels, seen in schizophrenia patients (compared to healthy controls (Lesh et al., 2018; Miller et al., 2011)), are reduced after administration of antipsychotic treatment despite weight gain induction (Müller et al., 1997; Tourjman et al., 2013). Treatment of THP-1 monocytes and primary peripheral blood mononuclear cells (PBMCs) with 10 μM ARI and 100 nM OLA also showed a significant reduction in the expression of pro-inflammatory cytokines such as IL6, IL1B, and TNFα (Stapel et al., 2018). In our study on *ex vivo* treated human subcutaneous adipose tissue with supra-therapeutic concentrations of OLA and ARI, the drugs also had an anti-inflammatory effect (Sarsenbayeva et al., 2019). Adipose tissue-derived pro-inflammatory cytokines substantially contribute to the systemic levels of these agents, for instance, up to one-third of the total circulating IL6 levels is secreted by adipose tissue (Makki et al., 2013). Therefore, the reduction in the peripheral concentration of pro-inflammatory cytokines observed during antipsychotic treatment in schizophrenia patients could partially be due to the anti-inflammatory effects of these drugs on adipose tissue. To our knowledge, the inflammatory capabilities of dARI have not been investigated, and more studies on the direct effects of dARI on inflammation are warranted. Additionally, our results need to be interpreted with caution since we mostly observed significant effects with supra-therapeutic concentrations of the drugs and the clinical relevance of our findings need further investigation.

Immune-modulatory functions of SGAs and Dex could contribute to their adverse metabolic effects, as different cytokines are involved in regulation of appetite and energy homeostasis (Fonseka et al., 2016; Pollmächer et al., 2000; Tourjman et al., 2012; Wang, 2005). Such properties of SGAs could be linked to their receptor-binding profile, as native ligands, such as dopamine, serotonin, and histamine have been reported to affect macrophage phenotypes (Branco et al., 2018; de las Casas-Engel et al., 2013; Gaskill et al., 2014). In the clinical setting, ARI and dARI are reported to have a similar affinity to dopamine D2 receptors, and the main effect is thought to be via the parent compound (Otsuka Pharmaceutical, 2005). However, in our *in vitro* model, we did not find significant differences in the dARI and ARI effects on the expression of the studied genes. Both drugs have a long half-life: 75 and 94 h for ARI and dARI, respectively (Otsuka Pharmaceutical, 2005). Our study was conducted with 24 h incubation, and perhaps additional time points could have rendered different results.

We observed a remarkable induction of pro-inflammatory genes in adipocytes upon their co-incubation with macrophages and our results are in accordance with previous studies in both murine and human pre-adipocytes differentiated into adipocytes (Bassols et al., 2009; Harms et al., 2019; Nitta and Orlando, 2013; Sárvári et al., 2015; Suganami et al., 2005). In our setting, different macrophage phenotypes M0, M1, and M2 had similar regulatory effects in the gene expression of pro-inflammatory cytokines in adipocytes. This suggests that inflammatory genes are regulated in adipocytes independently of the macrophage phenotype. However, we only tested one type of immune cells in this *in vitro* setting, while adipose tissue contains a vast number of different immune cells, such as B and T cells, NK cells, which contribute to adipose tissue inflammation (Blüher, 2016). Additionally, the macrophage-adipocyte ratio is highly important, as adipose tissue inflammation is characterised by an increased number of pro-inflammatory macrophages in adipose tissue (Morris et al., 2011), and hence, it needs to be taken into consideration.

Pre-treatment of macrophages with SGAs does not seem to interfere with macrophage-mediated induction of pro-inflammatory genes in adipocytes directly. We observed that pre-treatment of macrophages with dexamethasone partially reverses the induction of pro-inflammatory genes in adipocytes. However, the clinical impact of this effect was not addressed in this work and warrants additional investigations. It may be speculated that the anti-inflammatory effects of glucocorticoids in adipose tissue (Pickering et al., 2016; Sarsenbayeva et al., 2019) are partly mediated via macrophage to adipocyte communication. Anti-inflammatory effects of Dex could potentially affect adipose tissue expansion and remodelling via facilitating hypertrophic adipocyte expansion and reduced adipogenesis (Wernstedt Asterholm et al., 2014).

Interestingly, the expression of adipokines, *LEP* and *ADIPOQ*, in adipocytes was not altered after co-incubation with macrophages in our setting, while Suganami et al. reported that co-incubation of 3T3-L1 adipocytes with RAW264 macrophages significantly reduced the expression of adiponectin (Suganami et al., 2005). The discrepancy could be due to species-specific differences as well as the difference in the experimental setup. However, there is a lack of studies on human macrophage-adipocyte cross-talk, and these studies are warranted. Additionally, we observed that co-incubation with SGAs- and Dex-treated M1 macrophages led to a reduction in the gene expression of leptin in adipocytes. Aberrant leptin expression can lead to disturbances in appetite regulation and adipocyte metabolism, including lipolysis and glucose-uptake (Harris, 2014). Although in a clinical setting, patients receiving SGAs treatment present elevated serum leptin concentration, this effect could also be secondary to increased adiposity observed in these individuals (Jin et al., 2008).

We observed that co-incubation of adipocytes with M0 and polarized macrophages did not affect the expression of genes involved in glucose and lipid metabolism in adipocytes, apart from increased *AKT1* expression in adipocytes co-cultured with M1 macrophages. This could be a compensatory mechanism, as macrophage-conditioned media have been previously reported to maintain the survival of 3T3-L1 adipocytes via induction of Akt and ERK1/2 (Molgat et al., 2011). Mostly adipocytes co-cultured with M1 (but not M0 and M2) macrophages pre-treated with the SGAs, particularly OLA 2.0 μM, and Dex, demonstrated a significant downregulation of genes involved in lipid and glucose metabolism, such as *LIPE*, *LPL*, *AKT1*, and *SLC2A4*. This suggests that these drugs could impact lipid turnover and glucose uptake in adipocytes (Wang et al., 2006; Zhang et al., 2017). We did not detect any difference in adipocyte basal lipolysis after co-incubation with macrophages treated with the drugs (data not shown). This is consistent with our previous study, where we only detect a reduction in lipolysis in OLA-treated adipocytes following short-term incubation, but not after longer incubation of adipose tissue (Sarsenbayeva et al., 2019). Dex and ARI 10 μM have been shown to reduce glucose uptake in adipocytes (Lundgren et al., 2004; Sarsenbayeva et al., 2019). Studies measuring lipid storage and glucose uptake in adipocytes after their co-incubation with macrophages treated with SGAs and Dex are warranted.

Our study has several limitations. Primarily, we did not explore the mechanisms involved in the macrophage-adipocyte communication, therefore, studies directed to the analysis of the potential mechanisms are needed. Another limitation is a small sample size. We also used relatively short-term treatment with the drugs, while patient exposure in the clinical setting is long term. Additionally, we have used THP-1 macrophages, while it would be more clinically relevant to use PBMCs for the co-culture with adipocytes. Furthermore, Th17 response seems to

be highly important in schizophrenia, as the expression of pro-inflammatory cytokine IL17 is downregulated in first-episode schizophrenia patients and is modulated by the SGA-treatment (Borovcanin et al., 2012; Himmerich et al., 2011). However, IL17 is a signature cytokine of CD4+T helper 17 cells and its expression in the cells of myeloid lineage (neutrophils, microglia), although reported, remains controversial (McGeachy et al., 2019). We have not measured the effects of SGAs on cross-talk between adipocytes and other immune cells, such as T helper cells. Therefore, studies involving other immune cells are needed.

5. Conclusion

To conclude, this is the first study on human cells exploring the direct effects of SGAs and Dex on paracrine macrophage to mature adipocyte communication involving all three macrophage subtypes. Our data show that macrophages, regardless of polarization phenotype, induce expression of pro-inflammatory cytokines in adipocytes without altering the expression of adipokines or metabolic genes. SGAs at supra-therapeutic concentrations had mild anti-inflammatory effects on macrophages but did not affect the macrophage-adipocyte communication, while Dex prevented macrophage-induced upregulation of pro-inflammatory genes in adipocytes. Based on our data and previous work results, SGAs at pharmacological concentrations seem to have a minute direct effect on macrophage-mediated adipose tissue inflammation. It is likely that the observed metabolic adverse effects in patients treated with OLA and ARI result mainly from the drug effects on the central nervous system or other insulin-sensitive tissues in the body. However, Dex seems to have a direct effect on macrophage-adipocyte communication, which requires further examination.

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Conflict of interest

All authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2020.105071.

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