Resolvin D1 reduces inflammation in co-cultures of primary human macrophages and adipocytes by triggering macrophages

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Abstract: Obesity leads to chronic inflammation of the adipose tissue which is tightly associated with the metabolic syndrome, type 2 diabetes and cardiovascular disease. Inflammation of the adipose tissue is mainly characterized by the presence of crown-like structures composed of inflammatory macrophages in the neighborhood of adipocytes. Resolvin D1 (RvD1), a potent anti-inflammatory and pro-resolving lipid mediator derived from the omega-3 fatty acid docosahexaenoic acid, has been shown to reduce the inflammatory tone of adipose tissue in animal models but the underlying mechanism is not clear. We investigated the effect of RvD1 on the inflammatory state of a human co-culture system of adipocytes and macrophages. For this, human mesenchymal stem cells were differentiated into mature adipocytes and overlaid with human primary macrophages. In this co-culture, 10-500 nM RvD1 dose-dependently reduced the secretion of the pro-inflammatory cytokine IL-6 (-21%) and its soluble receptor IL-6R (22%), of the chemokine MCP-1 (-13%), and of the adipokine leptin (-22%). Similarly, we observed a reduction in secretion of the soluble receptor IL-6R (-20%), and TNF- (-11%) when macrophages alone were treated with RvD1, while no change of cytokine secretion was observed when adipocytes were treated with RvD1. We conclude that RvD1 polarizes macrophages to an anti-inflammatory phenotype, which in turn modulates inflammation in adipocytes.

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ABSTRACT

Obesity leads to chronic inflammation of the adipose tissue which is tightly associated with the metabolic syndrome, type 2 diabetes and cardiovascular disease. Inflammation of the adipose tissue is mainly characterized by the presence of crown-like structures composed of inflammatory macrophages in the neighborhood of adipocytes. Resolvin D1 (RvD1), a potent anti-inflammatory and pro-resolving lipid mediator derived from the omega-3 fatty acid docosahexaenoic acid, has been shown to reduce the inflammatory tone of adipose tissue in animal models but the underlying mechanism is not clear. We investigated the effect of RvD1 on the inflammatory state of a human co-culture system of adipocytes and macrophages. For this, human mesenchymal stem cells were differentiated into mature adipocytes and overlaid with human primary macrophages. In this co-culture, 10–500 nM RvD1 dose-dependently reduced the secretion of the pro-inflammatory cytokine IL-6 (-21%) and its soluble receptor IL-6Rα (-22%), of the chemokine MCP-1 (-13%), and of the adipokine leptin (-22%). Similarly, we observed a reduction in secretion of the soluble receptor IL-6Rα (-20%), and TNF-α (-11%) when macrophages alone were treated with RvD1, while no change of cytokine secretion was observed when adipocytes were treated with RvD1. We conclude that RvD1 polarizes macrophages to an anti-inflammatory phenotype, which in turn modulates inflammation in adipocytes.

Abbreviations  
RvD1, Resolvin D1.

1. Introduction

Obesity is associated with chronic, low-grade inflammation, which is recognized as a critical factor for the development of the metabolic syndrome, diabetes and chronic inflammatory diseases like cardiovascular disease [1, 2, 3]. In particular, the white visceral adipose tissue is recognized as an important player in the low-grade inflammation [4, 5]. The white adipose tissue is an active endocrine organ secretting a variety of cytokines, chemokines and hormone-like factors, commonly termed adipokines [6, 7, 8, 9], which include the inflammation associated TNFα, IL-1β, IL-6, MCP-1, PAI-1, leptin, chemeerin, visfatin, secretin, and adiponectin [2, 6, 10]. There is evidence that the white adipose tissue can expand several times in obese individuals and that this enlarged adipose tissue secretes higher amounts of pro-inflammatory cytokines, including TNFα, IL-1β, and IL-6 [11, 12].

This increased secretion of adipokines is associated with infiltration of immune cells in the obese adipose tissue [5]. In particular, macrophages accumulate in the adipose tissue acting as scavengers of dead adipocytes [3, 13, 14]. These macrophages also contribute to the cytokine release of the adipose tissue and therefore to the local and systemic low-grade inflammation. Several mouse models manipulating macrophage inflammatory pathways established the causal relationship of macrophages in adipose tissue inflammation, suggesting that macrophages are the major contributors to cytokine secretion of adipose tissue [5]. While in non-obese mouse models, non-phlogistic M2 macrophages are ubiquitously present in adipose tissues between adipocytes, dietary obesity induces M1 macrophage recruitment into fat clustering around dead adipocytes (crown-like structures), where they induce inflammation and insulin resistance [15].

Specialized pro-resolving lipid mediators (SPMs) have recently been
shown to have anti-inflammatory effects and to orchestrate the resolution of inflammation [16]. A set of recent studies showed that there is a physiological role for the resolvins and especially resolvin D1 (RvD1) in mouse adipose tissue homeostasis [17]. Comparing adipose tissue between lean and obese mice, two mouse studies found that obesity was associated with reduced levels of RvD1 and its precursor 17-HDHA and that supplementation of these lipid mediators dampened adipose tissue inflammation and improved plasma glucose levels and insulin sensitivity in obese mice [18, 19, 20]. In addition, application of RvD1 to mice and to adipose tissue explants led to an increase in the secretion of adipokines TNF-α, IL-6 and IL-1β [19, 21]. These beneficial changes on inflammation and insulin sensitivity following RvD1 treatment were associated in the adipose tissue with a polarization of macrophages towards an anti-inflammatory, non-phenotypic, M2 like phenotype with reduced reactive oxygen species production [21, 22].

In addition, there are human studies showing that omega-3 fatty acid supplementation will increase plasma and adipose tissue levels of SPMs and that such supplementation will reduce subcutaneous adipose tissue inflammation. Using targeted lipidomic approaches, it was shown that RvD1 and its precursor 17-HDHA are detectable in human plasma and in human visceral adipose tissue following omega-3 fatty acid supplementation [23, 24, 25]. The increase in RvD1 in human visceral and subcutaneous adipose tissue was accompanied by a reduction in staining for a M1-macrophage marker, indicating that omega-3 fatty acid supplementation may lead to reduced adipose tissue inflammation [25]. The combined findings of the mouse and human studies imply that omega-3 fatty acid supplementation will increase plasma and adipose tissue levels of SPMs.

2. Material and methods

2.1. Reagents and chemicals

RvD1 (7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid) produced by stereospecific total synthesis [26] was purchased from Cayman Chemicals (Ann Arbor, MI) and was verified by liquid chromatography–tandem mass spectrometry analysis (LC-MS/MS) in the laboratory [27, 28].

2.2. Differentiation of human adipocytes from mesenchymal stem cells

Human mesenchymal stem cells (MSC) were isolated from visceral adipose tissue that was obtained during surgeries [29]. Each patient gave written informed consent and the study was approved by the local ethics committee. Adipocytes were differentiated from human adipose tissue-derived mesenchymal stem cells by a modified approach based on the protocols from Pittenger et al. [30] and Scott et al. [31]. Therefore, human mesenchymal stem cells were seeded in 6-well plates (TPP, Trasadingen, Switzerland) containing Dulbecco’s modified eagle’s medium (DMEM; Sigma-Aldrich, St. Louis, USA) with 4500 mg/L glucose, 10% fetal calf serum (Sigma-Aldrich), and 1% penicillin/streptomycin (Sigma-Aldrich) and cultured until they were confluent. Subsequently, differentiation was induced by 60 μg/mL insulin (Sigma-Aldrich), 1 μM dexamethasone (Sigma-Aldrich), 80 μg/mL indomethacin (Sigma-Aldrich) and 115 μg/mL 1-methyl-3-isobutylxanthine (Sigma-Aldrich) in DMEM with 4500 mg/L glucose, 10% fetal calf serum, and 1% penicillin/streptomycin. After 28 days of differentiation, mature adipocytes were observed and used for experiments.

To visualize lipid accumulation in mature adipocytes, the cells were washed with PBS (Sigma-Aldrich), fixed with 2% paraformaldehyde (Merck Millipore, Burlington, USA), rinsed with 60% isopropanol (Sigma-Aldrich), and stained with Oil red O solution (Sigma- Aldrich) diluted with H2O (1:1). Images were taken using a Zeiss Axioskop (Carl Zeiss Vision GmbH, Jena, Germany).

2.3. Preparation of primary human macrophages

Peripheral blood mononuclear cells were isolated by Histopaque-1077 (Sigma Aldrich) density gradient centrifugation for 20 min at 600 g from buffy coats of healthy human blood donors (Blutspendezentrum Schlieren, Zurich, Switzerland). Each blood donation was from a different donor and was used for one experiment (n = 8 donors in total). The PBMCs were washed thrice with PBS and monocytes were purified by positive selection using anti-CD14 antibodies coupled to magnetic beads (MACS) according to the manufacturer’s instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of monocytes was determined by flow cytometry on a TX-1000 hematology analyzer (Sysmex, Lincolnshire, IL, USA) and reached >98% for all isolations. The total number of isolated monocytes was estimated using a Neubauer Chamber (Sigma-Aldrich). Isolated monocytes were differentiated into macrophages for 6 days in RPMI 1640 (Sigma-Aldrich) cell culture medium supplemented with 10% fetal calf serum (Sigma-Aldrich), 1% penicillin/streptomycin (Sigma-Aldrich) and 20 ng/mL GM-CSF (Miltenyi Biotec) [32-34]. After 6 days the macrophages were washed with PBS and stimulated with RvD1 in macrophage medium for 48 h.

2.4. Co-culture of human adipocytes and primary human macrophages

Macrophages were washed with PBS and were detached from the plastic surfaces using dissociation buffer containing PBS and 1% EDTA (Thermo Fisher Scientific, Waltham, USA) at 37 °C for 30 min. The cell pellet was resuspended in 1 mL DMEM with 4500 mg/L glucose (Sigma-Aldrich) supplemented with 10% fetal calf serum (Sigma-Aldrich) and 1% penicillin/streptomycin (Sigma-Aldrich). One million suspended macrophages each were then added to 200’000 confluent adipocytes in culture, corresponding to a 5:1 ratio. The co-cultures were incubated for 48 h in adipocyte medium with supplementation of RvD1 at times 0 and 24 h.

2.5. Trans-well co-culture of human adipocytes and primary human macrophages

For the trans-well co-culture system, monocytes were differentiated into macrophages for 6 days in RPMI 1640 (Sigma-Aldrich) cell culture medium supplemented with 10% fetal calf serum (Sigma-Aldrich), 1% penicillin/streptomycin (Sigma-Aldrich) and 20 ng/mL GM-CSF (Miltenyi Biotec) in special trans-well plates (Thermo Fisher Scientific, Waltham, MA, USA). After 6 days the macrophages were washed with PBS and stimulated with RvD1 in macrophage medium for 48 h. These trans-well plates were inserted into 6-well plates containing 28-day old mature adipocytes and the co-cultures were cultivated in DMEM with 4500 mg/L glucose, supplemented with 10% FCS and 1% penicillin/ streptomycin for 48 h with supplementation of RvD1 at times 0 and 24 h.

2.6. RT Q-PCR analysis

RNA was isolated with the RNeasy® Mini Kit (Qiagen AG, Hilden, Germany). 500 ng mRNA, 0,5 mM dNTP mix (Solis Biodyne, Tartu, Estonia) and 250 ng random primers (Thermo Fisher Scientific) were mixed and incubated at 65 °C for 5 min to denature secondary structures. Reverse transcription was performed to produce cDNA using the Superscript III Reverse Transcriptase kit (Thermo Fisher Scientific) with the addition of 40 U RNase inhibitor (Thermo Fisher Scientific) on the T3000 Thermocycler (Biometra GmbH, Goettingen, Germany). Q-PCR was performed on a LightCycler® 480 II (Roche, Rotkreuz, Switzerland) using the LightCycler FastStart Master SYBR Green 1 K (Roche). For this 2 μl cDNA, 0.5 μM forward primer and 0.5 μM reverse primer (see Table 1) were mixed with the SYBR Green Master Mix. The following cycling conditions were used: 10 min at 95 °C; 45 cycles of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 10 s; followed by a melting curve procedure.
of 5 s at 95 °C; 1 min at 65 °C and a gradual increase to 95 °C with an increment of 0.1 °C/sec.

2.7. Multiplex ELISA

The secretory profile of macrophages, adipocytes and their co-cultures was determined by a magnetic multiplex immunoassay with a customized human premixed multi-analyte kit (Bio-Techne, Minneapolis, USA) to quantify the peptide concentrations of IL-6, IL-6 Rα, IL-8, MIP-1α, MIP-1β, TNF-α, MCP-1, leptin and adiponectin on a Bio-Plex® 200 System (Bio Rad, Hercules, CA, USA).

2.8. Lipid mediator measurement

The concentrations of resolvin D1 was measured by LC-MS/MS analysis as previously described \[27,\] \[28\].

2.9. Statistical analysis

The data were analyzed with GraphPad Prism Version 9 software (GraphPad Software, La Jolla, California, USA). Differences between independent variables were evaluated using the conservative non-parametric One-way ANOVA (Kruskal-Wallis test followed by Dunn’s test) and a p-value of < 0.05 was considered significant. Graphs were created using Microsoft Excel Professional Plus 2013 (Microsoft Corporation, Redmond, WA, USA) presenting data as mean ± SD.

3. Results

The macrophage-adipocyte interaction was shown to play a major role in obesity associated adipocyte inflammation, with pro-inflammatory M1 macrophages infiltrating the adipose tissue and aggravating adipocyte inflammation and vice versa [35]. To characterize the individual contribution of human macrophages and adipocytes to the elevated adipokine secretion observed in inflamed adipose tissue, we initially measured the secretion of a set of adipokines in individual cell cultures of primary human macrophages and human MSC derived adipocytes. Primary human monocytes were isolated from buffy coat of healthy blood donors and differentiated into macrophages as we have previously done [32, 33, 34]. In parallel, differentiation of human MSC to adipocytes was induced in adipocyte differentiation medium [30, 31] and tracked by measuring the mRNA expression of PPAR-γ, leptin and FABP4 over time [4, 5, 8, 9]. The differentiated adipocytes were then characterized by the presence of intracellular lipid droplets (Fig. 1A).

Analyzing the adipokine secretion pattern of such primary human macrophages and human MSC derived adipocytes, showed that human macrophages secreted more IL-6 Rα, MCP-1, and TNF-α into the supernatant under basic cell culture conditions (Figs. 2A, 2B, and 2C), while

### Table 1

| Primer  | Sequence from 5’ to 3’ | Length in bp |
|---------|------------------------|--------------|
| HPRT1 Forward | CCT GGC GTG ATT AGT GA | 17 |
| HPRT1 Reverse | GCA CAC AGA GGG CTA CAA TG | 20 |
| PPARγ Forward | AGG GGA GGG GTA TCA CAG | 21 |
| PPARγ Reverse | GAT GCG GAT GGC CAC TCT TTT | 21 |
| FABP4 Forward | GCC AAG ATG AGG AGC ACA C | 22 |
| FABP4 Reverse | TTC TGC ACA TGT ACC AGG ACA C | 22 |
| Leptin Forward | GCC CTA TCT TTT CTA TGT CC | 20 |
| Leptin Reverse | TTC GTG GAG TAG CCT GAA G | 19 |

Fig. 1. Differentiation of human adipocytes from MSC. Differentiation of human MSCs to adipocytes was induced in adipocyte differentiation medium and was verified at day 28 (d28) by the presence of intracellular lipid droplets stained with Oil red O at 25 x magnification (A). The differentiation was followed by measuring the mRNA expression of PPAR-γ, leptin and FABP4 (Figs. 1B, 1C, and 1D), and showing the characteristic presence of lipid droplets [30].

Analyzing the adipokine secretion pattern of such primary human macrophages and human MSC derived adipocytes, showed that human macrophages secreted more IL-6 Rα, MCP-1, and TNF-α into the supernatant under basic cell culture conditions (Figs. 2A, 2B, and 2C), while...
human adipocytes were responsible for IL-6, leptin and adiponectin secretion (Figs. 2D, 2E, and 2F).

To investigate whether a direct contact of human macrophages and adipocytes alters the secretion pattern of these two major cell types of adipose tissue, we developed a co-incubation system. At day 20 of adipocyte differentiation, primary human monocytes were isolated from buffy coat of healthy blood donors and differentiated into macrophages in separate plates. Such differentiated 8-day old macrophages were then overlaid on top of the adipocytes to establish the direct co-culture system at day 28 of adipocyte differentiation. Co-culturing of human macrophages and adipocytes for 48 h increased the secretion of IL-6 into the supernatant (Fig. 2D, CC) compared to the secretion of the same number of individually cultured macrophages and adipocytes added together (Fig. 2D, stacked bar of MØ and Adip), while the release of IL-6 Rα into the supernatant was reduced (Fig. 2A). These results suggest that direct co-incubation of these two major cell types of adipose tissue leads to enhanced IL-6 release and reduced IL-6 Rα secretion.

To answer the question whether a direct cell contact is necessary or whether paracrine factors mediate the observed IL-6 modulation in the co-cultures, we repeated the experiment in a trans-well co-culture system (TW) lacking direct cell contact. In contrast to the co-cultures with cell contact (CC), trans-well co-cultures (TW) only showed a reduction in MCP-1 secretion (Fig. 2B), arguing that the stimulation of IL-6 depends on direct contact of human macrophages and adipocytes. These results agree with previous studies in men and mice, which showed a similar dependency on cell contact for IL-6 and MCP-1 stimulation, when splenocytes or macrophages and differentiated adipocytes were co-incubated [36, 37].

To investigate whether RvD1 elicits an anti-inflammatory and pro-resolution effect in the presented co-culture system comparable to its effect observed in mouse adipose tissue in vivo [18, 19, 21, 22], we performed the co-culture experiments in the presence of 10, 100, and 500 nM RvD1, covering the concentration range previously shown to affect human cells expressing the RvD1 receptor ALX/FPR2 and/or GPR32 [32, 38, 39]. Therefore, 6 day old macrophages were incubated with RvD1 for two days and then transferred to 28 day old adipocytes. These co-cultures were subsequently incubated in the presence of 10, 100, and 500 nM RvD1 for 48 h and the adipokines were measured in the supernatant. Since some SPMs have a short half-live in cell culture media [40], we analyzed the RvD1 levels in these co-cultures over time and observed that 18–37% of the original RvD1 dose was still present after 48 h (data not shown). Such RvD1 treatment led to a dose-dependent reduction of the secretion of IL-6, IL-6Rα, MCP-1 and leptin (Fig. 3A, 3B, 3C, and 3D), showing a RvD1 mediated reduction of inflammatory adipokines in the supernatant of these co-cultures (Fig. 3).

To dissect the cell type triggered by RvD1 in the co-culture system, we repeated the experiments with the 10, 100, and 500 nM RvD1 stimulation in separate cultures of primary human macrophages or human adipocytes. Incubation of primary human macrophages with RvD1 led to a trend in the reduction of MCP-1 secretion at the 10 nM

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**Fig. 2. Different adipokine secretion profiles from human macrophages, adipocytes, and their co-cultures.** The secretion of IL-6 Rα (A), MCP-1 (B), TNFα (C), IL-6 (D), leptin (E), and adiponectin (F) into the supernatant was measured for 48 h from 10⁶ primary human macrophages (MØ), confluent human adipocytes (Adip), co-cultures of 10⁶ macrophages and confluent adipocytes with direct contact (CC), and co-cultures of 10⁶ macrophages and confluent adipocytes in trans-wells with no direct contact (TW). The cell number in the co-culture systems corresponds to the sum of the cell number in the individual adipocyte and macrophage cultures. For the purpose of comparison and statistics, the cytokine concentrations for the macrophage (MØ, light gray) and adipocyte (Adip, dark gray) cultures are stacked above each other and were added together. Data is represented as mean ± SD (n = 3–5). * = p < 0.05. ** = p < 0.01.
Fig. 3. RvD1 reduces IL-6, IL-6Rα, MCP-1 and leptin in the supernatant of human co-cultures of macrophages and adipocytes. The secretion of IL-6 (A), IL-6 Rα (B), MCP-1 (C), leptin (D) and TNFα (below the quantification limit, data not shown) into the supernatant was measured following 10 nM, 100 nM, and 500 nM RvD1 treatment of co-cultures of 10^6 macrophages and confluent adipocytes with direct contact for 48 h. The cell number in the co-culture systems corresponds to the sum of the cell number in the individual adipocyte and macrophage cultures. Data is represented as mean ± SD (n = 5). * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

In contrast, when adipocytes were incubated with RvD1, we observed no change in protein secretion for any of these adipokines, arguing for a lack of a direct effect of RvD1 on human adipocytes (Fig. 5). To corroborate these finding, we analyzed the expression of the two RvD1 receptors, FPR2/ALX and GPR32, in human adipocytes [41]. Only very low mRNA levels were detected for the two RvD1 receptors, FPR2/ALX and GPR32, in human adipocytes that RvD1 attenuates the inflammation stimulated by glucose levels and insulin sensitivity [42]. We corroborate the evidence for the necessity of cell contact for the observed IL-6 and MCP-1 stimulation in the co-cultures in vitro because we did not observe such stimulation in the trans-well system lacking the direct contact (Fig. 2D and 2B).

Treatment of these co-cultures with RvD1 dose-dependently decreased the secretion of IL-6, IL-6 Rα, MCP-1, and leptin into the supernatant, arguing for altered inflammation in the co-culture system upon RvD1 treatment. These results are comparable to the recently observed in vivo effect of RvD1 in obese mice, where RvD1 supplementation dampened adipose tissue inflammation and improved plasma glucose levels and insulin sensitivity [18, 19]. In particular, the application of RvD1 to mice and to adipose tissue explants led to an increase in the secretion of adiponectin and to a reduction in the secretion of leptin and the pro-inflammatory cytokines TNF-α, IL-6 and IL-1β [19, 21, 44]. These beneficial changes on insulin sensitivity and inflammation following RvD1 treatment were associated with a polarization of macrophages towards an anti-inflammatory non-phlogistic M2-like phenotype with reduced reactive oxygen species production [21, 22]. This mechanism may be similar in humans because RvD1 was previously shown to polarize and even re-polarize primary human macrophages towards an anti-inflammatory pro-resolution type macrophage with

4. Discussion

RvD1 has been shown to promote the resolution of inflammation and to have anti-inflammatory effects in several mouse models of inflammation [43]. In particular, the administration of RvD1 to mice and to adipose tissue explants led to an increase in the secretion of adiponectin and to a reduction in the secretion of leptin and several pro-inflammatory adipokines from adipose tissue [19, 21]. We show in a co-culture system of primary human macrophages and MSC derived human adipocytes that RvD1 attenuates the inflammation stimulated by direct contact between these two cell types in vitro, leading to decreased secretion of IL-6, IL-6 Rα, MCP-1 and leptin into the supernatant of primary human macrophages. The secretion of IL-6 (A), IL-6 Rα (B), TNFα (C), MCP-1 (D), and leptin (below the quantification limit, data not shown) into the supernatant was measured following 10 nM, 100 nM, and 500 nM RvD1 treatment of 10^6 macrophages for 48 h. Data is represented as mean ± SD (n = 5). * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

(Fig. 3A, 3B, 3C, and 3D). Investigating the effect of RvD1 on the individual cell types, we show that RvD1 reduces the secretion of TNF-α and IL-6 Rα from primary human macrophages (Fig. 4C and 4B) but does not directly affect the secretion of adipokines from human adipocytes (Fig. 5).

Co-culturing of primary human macrophages and MSC derived human adipocytes in vitro has previously been shown to result in increased IL-6 and MCP-1 secretion [37]. This process involves consumption of adipokines by macrophages motivating the macrophages to increase the secretion of IL-6 and MCP-1 but not of TNF-α or IL-1β [37]. We corroborate the evidence for the necessity of cell contact for the observed IL-6 and MCP-1 stimulation in the co-cultures in vitro because we did not observe such stimulation in the trans-well system lacking the direct contact (Fig. 2D and 2B).

We observed no change in protein secretion for any of these adipokines, arguing for a lack of a direct effect of RvD1 on human adipocytes (Fig. 5). To corroborate these finding, we analyzed the expression of the two RvD1 receptors, FPR2/ALX and GPR32, in human adipocytes [41]. Only very low mRNA levels were detected for the two RvD1 receptors in human adipocytes with Ct values of 30.8 ± 0.8 for FPR2/ALX and 30.7 ± 0.6 for GPR32 (Supplementary figure 1). These levels are comparable to the levels of GPR32 in human macrophages not expressing GPR32 (Ct values 29.4 ± 0.4 and 30.2 ± 0.5), and for FPR2/ALX in resting human macrophages not expressing FPR2/ALX (Ct values 28.9 ± 0.7) [32, 42].
Fig. 5. RvD1 has no effect on the secretion of inflammatory cytokines in human adipocytes. The secretion of IL-6 (A), IL-6 Rα (B), MCP-1 (C), leptin (D), and TNFα (below the quantification limit, data not shown) into the supernatant was measured following 10 nM, 100 nM, and 500 nM RvD1 treatment of confluent MSC derived adipocytes for 48 h. Data is represented as mean ± SD (n = 5). * = p < 0.05.

some M2-like features [32, 45]. Such RvD1 treated macrophages secreted less IL-6, TNF-α [45], and MCP-1 [32], similar to the results presented here. Although the M1 and M2 polarization concept of macrophage polarization is an oversimplification for adipose tissue inflammation and homeostasis [46], a RvD1 mediated reduction in secretion of TNF-α and MCP-1 is usually considered beneficial for adipose inflammation [47], while IL-6 and IL-6 Rα have pleiotropic effects depending on the level and tissue context [48]. In light of the recent findings that macrophages from obese mouse adipose tissue display a broad range of macrophage characteristics with M0-, M1-, and M2-like features, the observed RvD1 mediated reduction in secretion of pro-inflammatory cytokines without induction for a formal repolarization of the adipose tissue macrophages [32, 45] may well reduce chronic low-grade inflammation in obesity [47].

Enhanced phagocytosis and macrophagocytosis could be another beneficial effect of RvD1 in the context of obese adipose tissue [32, 45]. While phagocytosis is a receptor mediated uptake and mediates the uptake of adipocyte debris, macrophagocytosis is receptor-independent and non-saturable [49], and was shown to mediate the uptake of lipid-rich adipocyte derived exosomes [13, 50]. Such lipid-rich exosomes contribute to adipose tissue lipid homeostasis [50]. However, it is currently unknown whether RvD1 increases macrophagocytosis of adipocyte exosomes and further studies are necessary to investigate such a mechanism. Our studies only show that RvD1 reduces the secretion of inflammatory cytokines in a human macrophage and adipocyte co-culture system by polarizing primary human macrophages towards an anti-inflammatory pro-resolution type macrophage [32, 45].

In contrast, we show that human MSC derived adipocytes from several donors do not alter the secretion of adipokines when treated with RvD1 (Fig. 5). This seems to depend on the lack of expression of the two RvD1 receptors, ALXR and GPR32, which were detected in adipocytes only at very low mRNA levels. These mRNA levels are 2–60 times lower than the mRNA levels observed in monocytes and macrophages expressing the ALXR and GPR32 receptors (Supplementary figure 1A and 1B), and are similar to the levels previously measured in macrophages not expressing the two receptors [32, 42]. We did not succeed in corroborating the mRNA data on the protein level because several currently available antibodies against FPR2/ALX and GPR32 did neither work for western blot nor for immunohistochemistry, and because FACS analysis is not feasible with differentiated adipocytes in cell culture (data not shown). Nevertheless, our results argue that RvD1 reduces adipose tissue inflammation through a direct effect on macrophages which then modify the secretion of adipokines by adipocytes.

There are several limitations of this study. First, it is an in vitro study involving only two primary human cell types of the adipose tissue, missing the contribution of stromal and other inflammatory cells like dendritic cells and T-cells, which have been shown to influence inflammation in adipose tissue [35, 51]. However, we observed no effect of RvD1 on human adipocytes, arguing that the RvD1 suppresses adipose tissue inflammation through macrophages and eventually other immune cells [52]. Second, the concentrations of RvD1 applied in the study ranged from 10 – 500 nM and some effects have only been observed with the highest concentrations, which are about 30 - 100 times higher than the 3 pmol/g RvD1 measured in whole extracts of mouse adipose tissue [19]. This may question the validity of our in vitro study but in light of the recent indication that there is large heterogeneity in the adipose tissue [53], the RvD1 concentrations may be much higher in certain areas of the adipose tissue, eventually reaching some of the concentrations used in our in vitro experiments.

In summary, we show that RvD1 stimulates human primary macrophages leading to an anti-inflammatory and pro-resolution macrophage, which in turn will reduce its stimulation of human adipocytes to secrete pro-inflammatory cytokines. Such a mechanism may well occur in vivo in human adipose tissue if a sufficient local production of RvD1 is available due to an adequate supplementation of DHA.

CRediT authorship contribution statement

Claudio Gemperle: Methodology, Investigation, Formal analysis, Writing – review & editing. Syndi Tran: Methodology, Investigation, Writing – review & editing. Mattia Schmid: Conceptualization, Methodology, Investigation, Writing – review & editing. Nicole Rimann: Methodology, Investigation, Writing – review & editing. Jacqueline Marti-Jaun: Methodology, Investigation, Writing – review & editing. Ivan Hartling: Methodology, Investigation, Writing – review & editing. Paulina Wawrzyniak: Formal analysis, Writing – review & editing. Martin Hersberger: Conceptualization, Resources, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in
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References

[1] G. Engstrom, L. Stavenov, B. Hedblad, et al., Inflammation-sensitive plasma proteins, diabetes, and mortality and incidence of myocardial infarction and stroke: a population-based study. Diabetes 52 (2003) 442–447.

[2] J.S. Yudkin, M. Kumari, S.E. Humphries, V. Mohamed-Ali, Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 148 (2004) 209–214.

[3] S.P. Weinsberg, D. McCann, M. Desai, M. Rosenbaum, R.L. Leibel, A.W. Ferrante Jr., Obesity is associated with macrophage accumulation in adipose tissue, J. Clin. Invest. 112 (2003) 1796–1806.

[4] Y. Wang, T. Nakayama, Inflammation, a link between obesity and cardiovascular disease, Mediators Inflamm. 2010 (2010), 535916.

[5] G.R. Romeo, J. Lee, S.E. Shoelson, Metabolic syndrome, insulin resistance, and roles of inflammation–mechanisms and therapeutic targets, Arterioscler Thromb Vasc Biol. 32 (8) (2012) 1771–1776.

[6] T. Skurk, C. Alberti-Huber, C. Herder, H. Hauner, Relationship between adipocyte size and adipokine expression and secretion, J. Clin. Endocrinol. Metab. 92 (3) (2007) 1023–1033.

[7] G. Fruehbeck, J. Gomez-Ambrosi, F.J. Muruzabal, M.A. Burrell, The adipocyte: a regulator of inflammation and other physiological processes, Am. J. Physiol. Endocrinol. Metab. 299 (6) (2010) 1590–1612.

[8] S.P. Ahima, J.S. Flier, Adipose tissue as an endocrine organ, Trends Endocrinol. Metab. 11 (6) (2000) 327–332.

[9] A. Festa, R. D’Agostino Jr., K. Williams, et al., The relation of body fat mass and distribution to markers of chronic inflammation, Int J Obes Relat Metab Disord 25 (10) (2001) 1487–1495.

[10] H.S. Mattu, H.S. Rendava, Role of adipokines in cardiovascular disease, J. Endocrinol. 216 (1) (2011) 717–736.

[11] M. Bullo, P. Garcia-Lorada, I. Megias, J. Salan-Salvado, Systemic inflammation, adipose tissue macrophage, and atherosclerotic lesion progression, Arterioscler Thromb Vasc Biol. 28 (6) (2008) 1225–1232.

[12] P.A. Kern, S. Ranganathan, C. Li, L. Wood, G. Ranganathan, Adipose tissue macrophage infiltration is required for obesity-induced insulin resistance, J. Clin. Invest. 128 (10) (2018) 4267–4281.

[13] S. Cinti, G. Mitchell, G. Barbatelli, et al., Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans, J. Lipid Res. 46 (11) (2005) 2347–2355.

[14] H. Xu, G.T. Barnes, Q. Yang, et al., Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance, J. Clin. Invest. 112 (1) (2003) 1821–1830.

[15] C.N. Lumeng, J.B. DelPorto, D.J. Westcott, A.R. Saltiel, Adipose tissue macrophages: a source of cytokines that govern local inflammatory tone in obese mice and humans, J. Lipid Res. 51 (7) (2010) 1497–1502.

[16] J. Hellmann, Y. Tang, M. Kosuri, A. Bhatnagar, M. Spite, Resolvin D1 decreases chronic inflammation in fat, J. Clin. Invest. 123 (6) (2013) 2741–2752.

[17] J.S. Yudkin, M. Kumari, S.E. Humphries, V. Mohamed-Ali, Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 148 (2004) 209–214.

[18] M. Wonnita, K. Hemmrich, A. Groger, S. Graber, N. Pallia, Plasticity of human adipose stem cells to perform adipogenic and endothelial differentiation, Differentiation 75 (1) (2007) 12–23.

[19] M.F. Pittenger, A.M. Mackay, S.C. Beck, et al., Multilineage potential of adult human mesenchymal stem cells, Science 284 (5411) (1999) 143–147.

[20] M.A. Scott, V.T. Nguyen, B. Levi, A.W. James, Current methods of adipogenic differentiation of mesenchymal stem cells, Stem Cells Dev. 20 (10) (2011) 1793–1804.

[21] M. Schmid, C. Gemperle, N. Rimann, M. Hersberger, Resolvin D1 Polarizes Primary Human Macrophages toward a Proresolution Phenotype through GPR32, J. Immunol. 196 (8) (2016) 3429–3437.

[22] M. Herova, M. Schmid, C. Gemperle, M. Hersberger, ChemR23, the receptor for chemerin and resolvin E1, is expressed and functional on M1 but not on M2 macrophages, J. Immunol. 194 (5) (2015) 2330–2337.

[23] C. Gemperle, M. Schmid, M. Herova, et al., Regulation of the formyl peptide receptor 1 (FPR1) gene in primary human macrophages, PLoS ONE 7 (11) (2012) e95195.

[24] N. Ouchi, J.L. Parker, J.J. Lugus, K. Walsh, Adipokines in inflammation and metabolic disease, Nat. Rev. Immunol. 11 (2) (2011) 85–97.

[25] C.F. Nitta, R.A. Orlando, Crossstalk between immune cells and adipocytes requires both paracrine factors and cell contact to modify cytokine secretion, PLoS ONE 8 (10) (2013) e77306.

[26] A.K. Sarvari, Q.M. Doan-Xuan, Z. Bacso, I. Csomos, Z. Balajthy, L. Fesus, J.F. Maddox, C.N. Serhan, Lipoxin A4 and B4 are potent stimuli for human T cells, J. Immunol. 193 (20) (2014) 9480–9487.

[27] J.F. Maddox, C.N. Serhan, Lipoxin A4 and B4 are potent stimuli for human phagocytes and adipocytes to perform adipogenic and endothelial differentiation by dehydrogenation and reduction, J. Exp. Med. 183 (1) (1996) 137–146.

[28] S. Krishnamoorthy, A. Rocchiuti, N. Chiang, et al., Resolvin D1 binds human phagocytes with evidence for proresolving receptors, Proc. Natl. Acad. Sci. U S A, 107 (4) (2010) 1660–1665.

[29] V. Waechter, M. Schmid, M. Herova, et al., Characterization of the promoter and the transcriptional regulation of the lipoxin A4 receptor (FPAR2/ALX) gene in human monocytes and macrophages, J. Immunol. 188 (4) (2012) 1856–1867.

[30] C.N. Serhan, N. Chiang, J. Dalli, B.D. Levy, Lipid mediators in the resolution of inflammation, Cold Spring Harb Perspect Biol. 7 (2) (2014) a016311.

[31] E. Titos, B. Rius, C. Lopez-Vicario, et al., Signaling and immunoresolving actions of Resolvin D1 in inhibited human viscerale adipose tissue, J. Immunol. 197 (8) (2016) 3366–3370.

[32] A. Crossland, T.H. Thatcher, R.M. Kottmann, et al., Resolvin D1 attenuates polynonsopic-polycyctidal acid-induced inflammatory signaling in human airway epithelial cells via TAK1, J. Immunol. 193 (10) (2014) 4980–4987.

[33] J.S. Yudkin, M. Kumari, S.E. Humphries, V. Mohamed-Ali, Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 148 (2004) 209–214.

[34] J.S. Yudkin, M. Kumari, S.E. Humphries, V. Mohamed-Ali, Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 148 (2004) 209–214.

[35] J.S. Yudkin, M. Kumari, S.E. Humphries, V. Mohamed-Ali, Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 148 (2004) 209–214.

[36] J.S. Yudkin, M. Kumari, S.E. Humphries, V. Mohamed-Ali, Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 148 (2004) 209–214.

[37] J.S. Yudkin, M. Kumari, S.E. Humphries, V. Mohamed-Ali, Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 148 (2004) 209–214.

[38] J.S. Yudkin, M. Kumari, S.E. Humphries, V. Mohamed-Ali, Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 148 (2004) 209–214.