Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 99 (2022) 108837

Journal of Nutritional Biochemistry

RESEARCH PAPER

Changing the dietary composition improves inflammation but not adipocyte thermogenesis in diet-induced obese mice

Sabrina Folie[a,b], Bernhard Radlinger[a,b], Georg Goebel[c], Karin Salzmann[a,b], Gabriele Staudacher[a,b], Claudia Ress[a,b], Herbert Tilg[a], Susanne Kaser[a,b,*]

[a] Department of Internal Medicine I, Medical University Innsbruck, Innsbruck, Austria
[b] Christian Doppler Laboratory for Metabolic Crosstalk, Medical University Innsbruck, Innsbruck, Austria
[c] Department of Medical Statistics, Informatics and Health Economics, Medical University Innsbruck, Innsbruck, Austria

Received 27 October 2020; received in revised form 11 July 2021; accepted 14 July 2021

Abstract

Pronounced weight loss was shown to improve adipocyte dysfunction and insulin sensitivity in obese subjects. While bariatric surgery is frequently accompanied by adverse side effects, weight loss due to caloric restriction is often followed by weight regain. Here we aimed to determine whether switching the diet from a metabolically harmful Western type diet to a balanced standard diet is sufficient to reverse adipocyte dysfunction in diet-induced obese mice.

Male C57BL/6 mice were fed a Western diet for 10 weeks and afterwards switched to a standard diet for eight more weeks (WD/SD mice) or continued to be fed a Western diet (WD/WD mice) *ad libitum*. Mice fed SD for 18 weeks served as control group (SD/SD). Insulin sensitivity was similar in WD/SD and SD/SD mice despite increased body weight in WD/SD mice. Beiging markers *Ucp-1, Cidea* and *Cox8b* were drastically reduced in subcutaneous adipose tissue of WD/SD mice when compared with SD/SD mice. Also, in brown adipose tissue morphologic features and markers of thermogenesis were still altered in both WD/SD and WD/WD mice. However, adipocyte size, *Hif1α* and macrophage infiltration were significantly lower in both, brown and white adipose tissues of WD/SD compared to WD/WD mice and additionally, a shift toward anti–inflammatory M2 phenotype was found in WD/SD mice only.

In conclusion our data suggest that switching the diet is sufficient to improve adipose tissue inflammation, while western diet negatively affects thermogenic capacity of brown adipose tissue, and inhibits beiging of white adipose tissue in the long-term.

Keywords: Adipose tissue; Inflammation; Type of diet; Obesity; Caloric restriction.

1. Introduction

Overweight and obesity are one of the major health problems of modern civilization as they carry a high risk to develop chronic diseases such as metabolic syndrome, type II diabetes (T2D), and non–alcoholic fatty liver disease [1]. T2D, affecting more than 450 million patients worldwide [2], does not only lead to hyperglycemia but is highly associated with micro- and macrovascular disease such as chronic kidney disease, neuropathy or cardiovascular disease, and is even associated with increased mortality [3–5]. Adipose tissue is not a passive reservoir for energy but a complex endocrine, paracrine and autocrine organ which orchestrates a vast number of cytokines [6] referred to as adipokines [7]. In response to changes in nutritional status adipose tissue undergoes dynamic changes, often described as adipose tissue remodelling. Adipose tissue remodelling includes alterations in adipocyte number, size, cytokine expression, cell death, hypoxia, fatty acid homeostasis, and inflammation [8]. Diminished adipogenesis and inflammation are thought to be key drivers of adipose tissue dysfunction. Brown and beige adipocytes which are located within white adipose tissue are responsible for the generation of non–shivering thermogenesis and thus protect against metabolic disorders [9–15]. Obesity and insulin resistance are characterized by impaired beige adipogenesis and an altered brown-versus-white plasticity and a hypertrophic adipocyte phenotype. [16,17]. Low-grade chronic inflammation in adipose tissue has been described as a mechanism for obesity-related complications. While predominately anti–inflammatory cytokines are secreted from adipose tissue of healthy, lean subjects, higher amounts of proinflammatory adipokines are typically seen in obese individuals. [18].

Excessive weight loss by reduced caloric intake or bariatric surgery has extensively been demonstrated to improve glucose

* Corresponding author at: Susanne Kaser, Department of Internal Medicine I & Christian Doppler Laboratory for Metabolic Crosstalk, Medical University Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria
E-mail address: susanne.kaser@i-med.ac.at (S. Kaser).

https://doi.org/10.1016/j.jnutbio.2021.108837

metabolism and reverse adipocyte dysfunction [19–25]. However, in clinical practice non–surgical weight reduction is very challenging. For some patients it might be more practicable to change the type of diet than restrict caloric intake in the long-term. Thus, we investigated whether switching the diet to a more beneficial one is sufficient to reverse Western diet- induced alterations in adipose tissue, and systemic glucose metabolism.

2. Material and methods

2.1. Animals and diets

C57BL/6J mice were purchased from Charles River Laboratories (Germany). After one additional week of acclimatisation the experiments were started. Mice were housed in a group of five to avoid additional stress of from individual housing. Twenty 7-weeks-old mice were fed a mixed high sucrose, high fat diet (WD) (referred as Western diet, TD.88137, 42.7%kcal carbohydrate, 15.2%kcal protein, 42%kcal fat) (Table S1a) for 10 weeks. After 10 weeks mice were randomly split into two equal groups and either switched to a standard diet (SD) (TD.05075, 66.9%kcal carbohydrate, 20.2%kcal protein, 12.9%kcal fat) (Table S1b) or continued to fed a Western diet (WD) for eight more weeks. Additionally, 10 mice were fed a SD for 18 weeks. This leads to three groups which will be referred as: SD/SD mice (mice fed a standard diet for 18 weeks), WD/WD mice (mice fed a western diet for 18 weeks) and WD/SD mice (mice where after 10 weeks diet was changed from a western to standard diet for eight more weeks). Diets were purchased from Envigo (UK). Mice were fed ad libitum and kept in a 12-h light/dark cycle at a temperature range between 20 and 24°C. Food was changed and weighed twice a week, body weight once a week. After 18 weeks, oral gavage glucose tolerance test (oGTT, 1 mg glucose/g bodyweight) and intraperitoneal insulin tolerance test (ipITT, 0.75 mU insulin/g bodyweight) were performed. Areas under the curve (AUC) were calculated by the linear trapezoidal method. Finally, mice were anesthetized and sacrificed via removal of cardiac blood, and adipose tissue was harvested. Perigonadal visceral adipose tissue (VAT), posterior subcutaneous adipose tissue (SAT) and intrascapular brown adipose tissue samples were taken. All animal experiments were conducted according to the regulations of Medical University Innsbruck and according to the regulation of the Austrian Ministry for Science and Education based on the Animal Testing Act of 2012. Approval was given under the reference number BMWFW-66.011/0027-WF/V/3b2017.

2.2. Blood analysis and plasma leptin levels

Hematological parameters were measured from whole blood samples with heparin used as anticoagulant and analysed with scil VET abc 2003 (scil animal care company GmbH, Viernheim, Germany). Plasma leptin was measured by a commercially available ELISA kit (*Invitrogen*) according to the manufacturer's protocol.

2.3. RNA Extraction and quantitative RT-PCR analysis

RNA extraction was performed for subcutaneous (SAT), visceral (VAT), and brown (BAT) adipose tissue. 30–60 mg of frozen adipose tissue was homogenized and sonicated for 30 s in 500µl QIAzol Lysis Reagent (Qiagen). For white adipose tissue RNeasy Lipid Tissue Mini Kit (Qiagen) according to the manufacturer's instruction was used and for brown adipose tissue chloroform (Scharlau) and an overnight step in 1:2 Isopropanol (Merck) was used to extract RNA. 0.5µg RNA was translated in cDNA by using High-Capacity cDNA Reverse Transcription Kit. Subsequently, TaqMan probe (Luna Universal Probe qPCR Master Mix, NE BioLabs) or SyberGreen (Luna Universal Master Mix, NE BioLabs) based relative quantitative PCR was performed. For primer sequences see supplements (Table S2). TATA-binding protein was used as housekeeping gene and qPCR was performed in duplicates.

2.4. Protein Extraction and Western blot analysis

Protein extraction was performed for SAT, VAT and BAT. Frozen tissue was homogenized and sonicated in lysis buffer (25 mM Tris-HCl, pH 7.4, 40 mM KCl, 1% Triton X-100) (Roche). Protein was quantified by Pierce BCA Protein Assay Kit (Thermo Fisher). Mini-Protean TGX stain free precast gels (Biorad) and nitrocellulose membrane (Amersham Protran 0.45 µm NC) were used for separation and blotting. Visualisation was performed by chemiluminescence (Amersham ECL Prime Western Blotting Detection Reagent). Results were normalized to total amount of protein. Used antibodies are listed in supplements (Table S2).

2.5. Histology

For immunohistochemistry (IHC), samples from subcutaneous visceral and brown adipose tissue depots were dissected, fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned and stained with haematoxylin and eosin (H&E). Adipocyte size was calculated in ImageJ manually by using the ROI manager. Lipid droplets were manually counted by using cell counter plugin for a 37,000 μm^2 area.

2.6. Statistical analysis

All statistical analyses were performed with the statistical analysis software package (Prism GraphPad). Descriptive data are expressed as means ± SEM. Kolmogorov-Smirnov test was performed to determine normality. Subsequently, one-Way Anova or Kruskal-Wallis-Test with Bonferroni correction was used to compare data from different groups. Statistical significance was set at P-value $\leq .05$.

3. Results

3.1. Animal characteristic, food intake, and measures of glucose homeostasis

As expected, mice fed a WD gained more weight than mice fed a SD (Fig. 1A, Table S3). Initially, switching diet from a WD to a SD caused stress leading to reduced food intake which was associated with short-term weight loss. But after two weeks food consumption increased again and after additional two weeks mice started to regain weight and plateaued after six weeks (Fig. 1A-C). After 18 weeks caloric intake/g body weight was similar in all groups and caloric intake/individual was higher in WD/SD and WD/WD mice when compared to SD/SD mice (Fig. 1B-C, Table S4a-b). Cumulative food intake per mouse was highest in the WD/WD group, while WD/SD mice had the highest food intake per body weight (Fig. 1D-E, statistics in Table S5a-b). Total plasma leptin levels and leptin concentration/g body weight were elevated in both, WD/WD and SD/WD mice (Fig. 1F-G). Leptin expression in SAT and VAT was highest in WD/WD and lowest in SD/SD with WD/SD lying in-between (Fig. S1 A-B). White blood cells were only upregulated in WD/WD but not in WD/SD mice (Fig. 1H). Adiponectin was only downregulated in VAT in WD/WD mice (Figure S1 C-D). Insulin sensitivity estimated by ipITT (AUC) was significantly decreased in WD/WD but not in WD/SD mice when compared to SD/SD mice (Fig. 1-J). Glucose tolerance determined by oGTT tended to be diminished in WD/WD and WD/SD mice without reaching statistical significance (Fig. 1K-L).

3.2. Switching diet from a harmful to a more beneficial one is sufficient to reverse adipose tissue inflammation

Adipose tissue inflammation is a hallmark of insulin resistance [26]. Accordingly, decreased inflammation is thought to be a major contributor to metabolic benefits of weight loss. In previous studies [27] we found that the type of diet is a critical determinant of adipose tissue inflammation. Here we investigated whether these detrimental alterations are reversible by changing dietary composition.

3.2.1. Subcutaneous adipose tissue

Macrophage infiltration in SAT estimated by EGF-like module-containing mucin-like hormone receptor-like 1 (*F4/80*) expression analysis was highest in WD/WD and lowest in SD/SD mice with WD/SD lying in-between suggesting partial reversibility of adipose tissue inflammation by switching the type of diet (Fig. 2A). Activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Fig. 2B) and tumour necrosis factor alpha (*Tnf-α*) expression was significantly higher in WD/WD than in WD/SD and SD/SD mice (Fig. 2A). Similarly, pro-inflammatory M1 phonotype markers *Cd11c* (integrin), *Ccl2* (chemokine [C-C motif] ligand 2) and *Ccr2* (chemokine [C-C motif] receptor 2) were significantly increased in WD/WD mice only. For *Il1β* (interleukin 1 beta) no

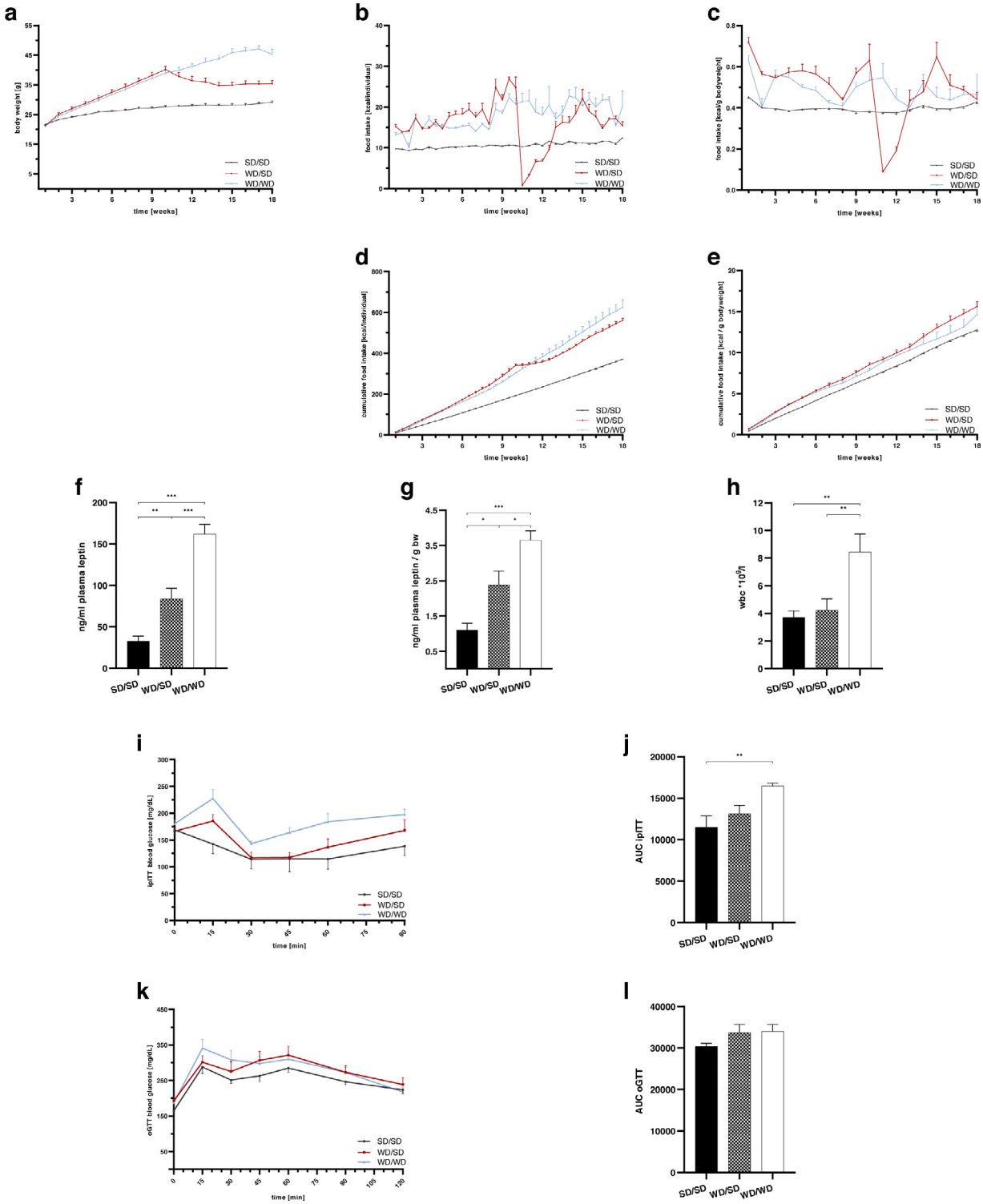

Fig. 1. Animal characteristics, food intake and measures of glucose homeostasis (A) mean body weight (g) of SD/SD, WD/SD and WD/WD mice during treatment (18 weeks); (B) mean food intake (kcal) of SD/SD, WD/SD and WD/WD mice per individual during treatment; (C) mean food intake (kcal) of SD/SD, WD/SD and WD/WD mice per g body weight during treatment; (D) cumulative food intake (kcal) of SD/SD, WD/SD and WD/WD mice per individual during treatment; (E) cumulative food intake (kcal) of SD/SD, WD/SD and WD/WD mice per g body weight during treatment; (F) absolute plasma leptin levels (ng/mL); (G) plasma leptin levels (ng/mL) relative to body weight (g); (H) blood count of white blood cells (10^9/l) (I) blood glucose levels (mg/dL) during intraperitoneal insulin tolerance test (0.75mU insulin/g bodyweight) (J) calculated area under the curve (AUC) of glucose levels during intraperitoneal insulin tolerance test; (K) blood glucose levels (mg/dL) during oral glucose tolerance test (1mg glucose/g bodyweight); (L) calculated area under the curve (AUC) of glucose levels during oral glucose tolerance test are shown; SD/SD = mice fed a standard diet for 18 weeks; WD/SD mice which were fed a Western diet for 10 weeks followed by a standard diet for 8 more weeks; WD/WD=mice fed a western diet for 18 weeks; $n = 10$; means ± SD; $^{*}P < .05$; $^{**}P < .005$; $^{***}P < .0005$

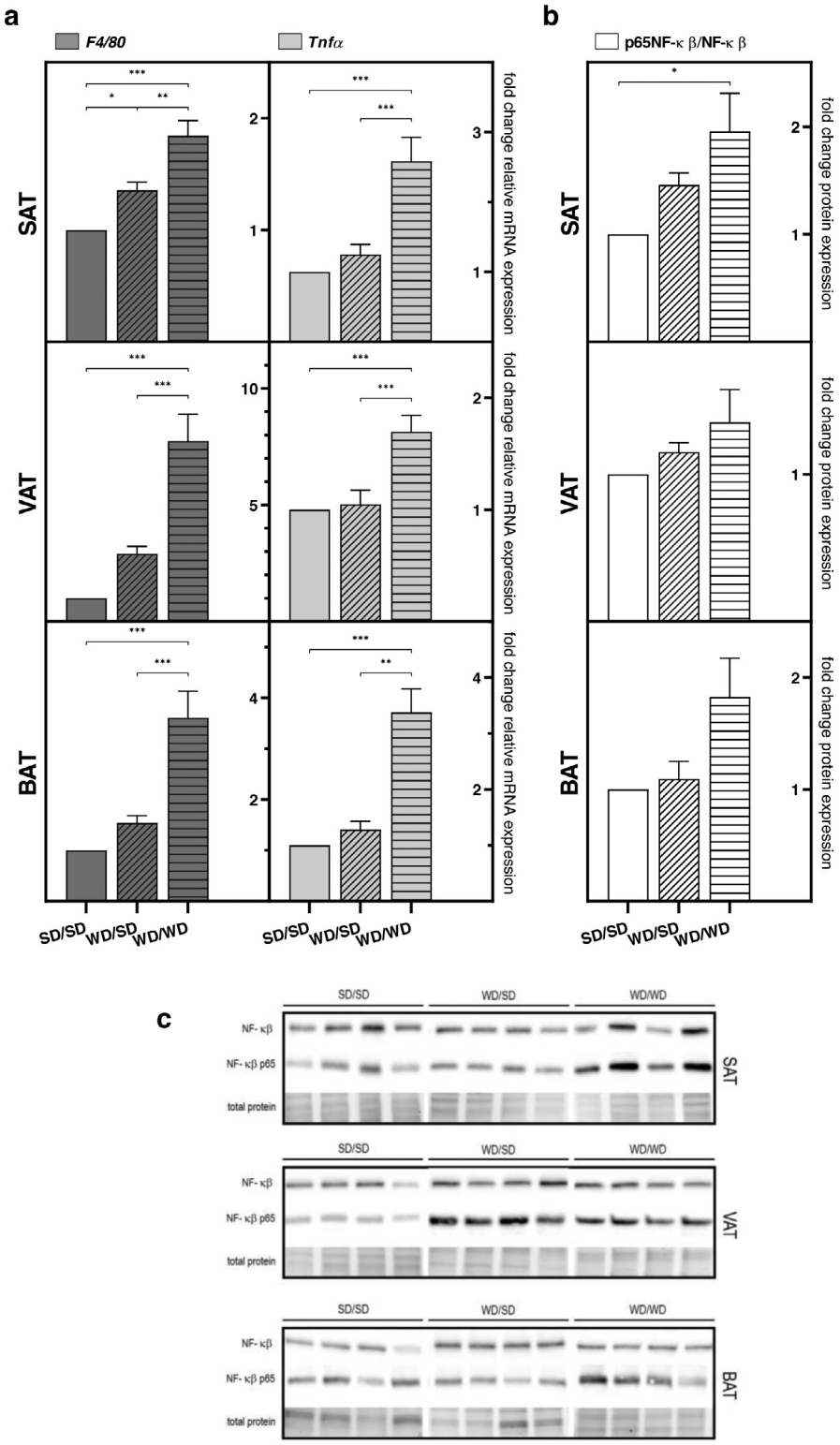

Fig. 2. Effects of switching the diet from a harmful to a more beneficial one on adipose tissue inflammation; mean fold change of (A) relative mRNA expression of *F4/80* (left column) and *Tnfα* (right column) and (B) activation of NF-$\kappa\beta$ estimated as protein levels of phosphorylated NF-$\kappa\beta$ / total amount of NF-$\kappa\beta$ in subcutaneous (top) visceral (middle) and brown (bottom) fat depot from SD/SD, WD/SD and WD/WD mice; (C) representative western blots of NF-$\kappa\beta$ and pNF-$\kappa\beta$; SD/SD = mice fed a standard diet for 18 weeks; WD/SD mice which were fed a western diet for 10 weeks followed by a standard diet for 8 more weeks; WD/WD = mice fed a western diet for 18 weeks; mRNA expression levels corrected for TATA-binding protein and fold change relatively to SD/SD; $n = 10$; means ± SEM; $^{*}P < .05$; $^{**}P < .005$; $^{***}P < .0005$

Table 1
Expression of M1 and M2 markers; arrows show gene expression of M1 and M2 macrophage markers of WD/SD mice in comparison to SD/SD and WD/WD mice respectively in subcutaneous (SAT) visceral (VAT) and brown (BAT) adipose tissue; ↓ = downregulated; ↑ = upregulated; ↔ = no significant difference; mRNA expression levels relative to TATA-binding protein $n = 10$; $P < .05$

Marker		SAT		VAT		BAT	
		WD/SD vs. SD/SD	WD/SD vs. WD/WD	WD/SD vs. SD/SD	WD/SD vs. WD/WD	WD/SD vs. SD/SD	WD/SD vs. WD/WD
M1/M2	***F4/80***	↑	↓	↔	↓	↔	↓
M1	***Tnfa***	↔	↓	↔	↓	↔	↓
Macrophage	***Cd11c***	↔	↓	↔	↓	↔	↓
marker	***Ccl2***	↔	↓	↔	↓	↔	↓
	Ccr2	↔	↔	↔	↓	↑	↔
	Il1b	↔	↔	↔	↔	↑	↔
M2	***Il10***	↓	↔	↔	↑	↑	↔
Macrophage	***Mrc2***	↔	↓	↑	↓	↔	↔
marker	***Mgl1***	↔	↔	↑	↔	↔	↔

differences of expression between groups were detected (Table 1 and Fig. S2A). Anti–inflammatory M2 marker *Il10* was downregulated in WD/WD and WD/SD mice when compared to SD/SD fed mice. M-RNA expression of M2 markers mannose receptor (*Mrc2*) was increased in WD/WD mice only. *Mgl1* (macrophage galactosidase-type lectin-1) was similarly expressed in all groups (Table 1 and Fig. S2B).

3.2.2. Visceral adipose tissue

In accordance with SAT, *F4/80* mRNA expression, activated NF-κB, *Tnf-α* (Fig. 2A-B) and M1 markers *Cd11c Ccl2* and *Ccr2* were increased in WD/WD but not in WD/SD mice when compared to SD/SD mice (Table 1 and Fig. S2A). *Il1β* and *Il10* expression levels were comparable between all groups. *Mrc2* was highest in WD/WD mice and lowest in SD/SD mice, while *Mgl1* was upregulated in both WD/SD, and WD/WD mice (Table 1 and Fig. S2b).

3.2.3. Brown adipose tissue

Similar results were found in brown adipose tissue showing increased *F4/80* (Fig. 2A) and M1 markers in WD/WD but not in WD/SD fed mice. Surprisingly, *Il1β* expression was only increased in WD/SD mice (Table 1 Fig. S2A). While M2 marker *Il10* showed upregulation in both WD/SD and WD/WD mice, *Mrc2,* and *Mgl1* expression levels did not differ between the groups (Table 1 and Fig. S2b).

In summary, we found that increased adipose tissue inflammation in WD/WD mice is reversed in all types of adipose tissue by switching the diet.

3.3. Switching diet from a Western to a standard diet reduces adipocyte size and Hif1α expression in white and brown adipose tissue

Dietary intake of saturated fatty acids and sucrose is accompanied by a shift toward larger adipocytes with diminished insulin sensitivity [27]. Until now it is not clear whether switching the diet is sufficient to reverse altered adipocyte morphology.

In subcutaneous and visceral adipose tissue adipocyte size was significantly smaller in WD/SD than in WD/WD mice and comparable with SD/SD mice (Fig. 3A). In accordance with adipocyte size, hypoxia-inducible factor 1-alpha (*Hif1α*) was only significantly increased in WD/WD but not in WD/SD mice (Fig. 3B).

Also, in brown adipose tissue adipocytes were largest in WD/WD mice. Although in comparison to WD/WD mice cell size was reduced in WD/SD mice, adipocytes of WD/SD mice were still larger than those of SD/SD mice (Fig. 3A). *Hif1α* expression was highest in WD/WD and lowest in SD/SD mice (Fig. 3B). Additionally, quantity of lipid droplets in brown adipocytes was highest in SD/SD mice, and lowest in WD/WD Mice (Fig. 3C).

To summarize switching the diet normalizes adipocyte size in white adipose tissue while in brown adipose tissue adipocyte morphology is only partly reversed.

3.4. Effects of switching the diet on thermogenic capacity are dependent on the type of adipose tissue

Adipocyte differentiation and their capacity to store lipids is controlled by a cascade of transcription factors and adipokines such as *Pparγ*, adiponectin, and leptin [28,29]. Similar to adipocyte size intake of diets enriched with fat, especially saturated fatty acids, inhibits adipogenesis, and reduces markers of thermogenic capacity [27] why we aimed to investigate whether changes in dietary composition is able to reverse beiging capacity of adipose tissue.

3.4.1. Effects on differentiation in white adipose tissue

Adipocyte differentiation was irreversibly affected by WD as shown by decreased *Pparγ* expression in VAT (Fig. S1G) and increased leptin expression in SAT and VAT of both, WD/WD and WD/SD mice (Fig. S1A-B). In VAT, expression of *Ucp1*, which is a key marker of thermogenesis, *Cidea,* and mitochondrial marker cytochrome c oxidase subunit 8b (*Cox8b*) were comparable between all groups (Fig. 4B). In rodents predominantly subcutaneous adipocytes are capable to generate thermogenesis by differentiation into beige adipocytes [30]. In our study, expression of *Ucp1* was drastically downregulated in SAT of both WD/WD and WD/SD mice. Other markers of beiging such as *Cidea, Cox8b* and beta 3 adrenergic receptor (*Adrb3)* were also significantly downregulated in both, WD/WD and WD/SD suggesting irreversibility or long-term inhibition of whitening of adipose tissue by WD (Fig. 4A). In both, SAT (Fig. 5A) and VAT (Fig. 5B) fibroblast growth factor 21 (*Fgf-21*) expression was significantly higher in WD/WD than in WD/SD and SD/SD mice. Fibroblast growth factor receptor 1 (*Fgfr1*) and Fgf-21 cofactor beta klotho (*βKl*) were downregulated in all adipose tissue depots of WD/WD mice and in SAT of WD/SD (Fig. 5A-B)

3.4.2. Effects on differentiation in brown adipose tissue

Surprisingly, in BAT expression of *Pparγ* was increased in WD/WD mice as well as in WD/SD mice when compared to SD/SD

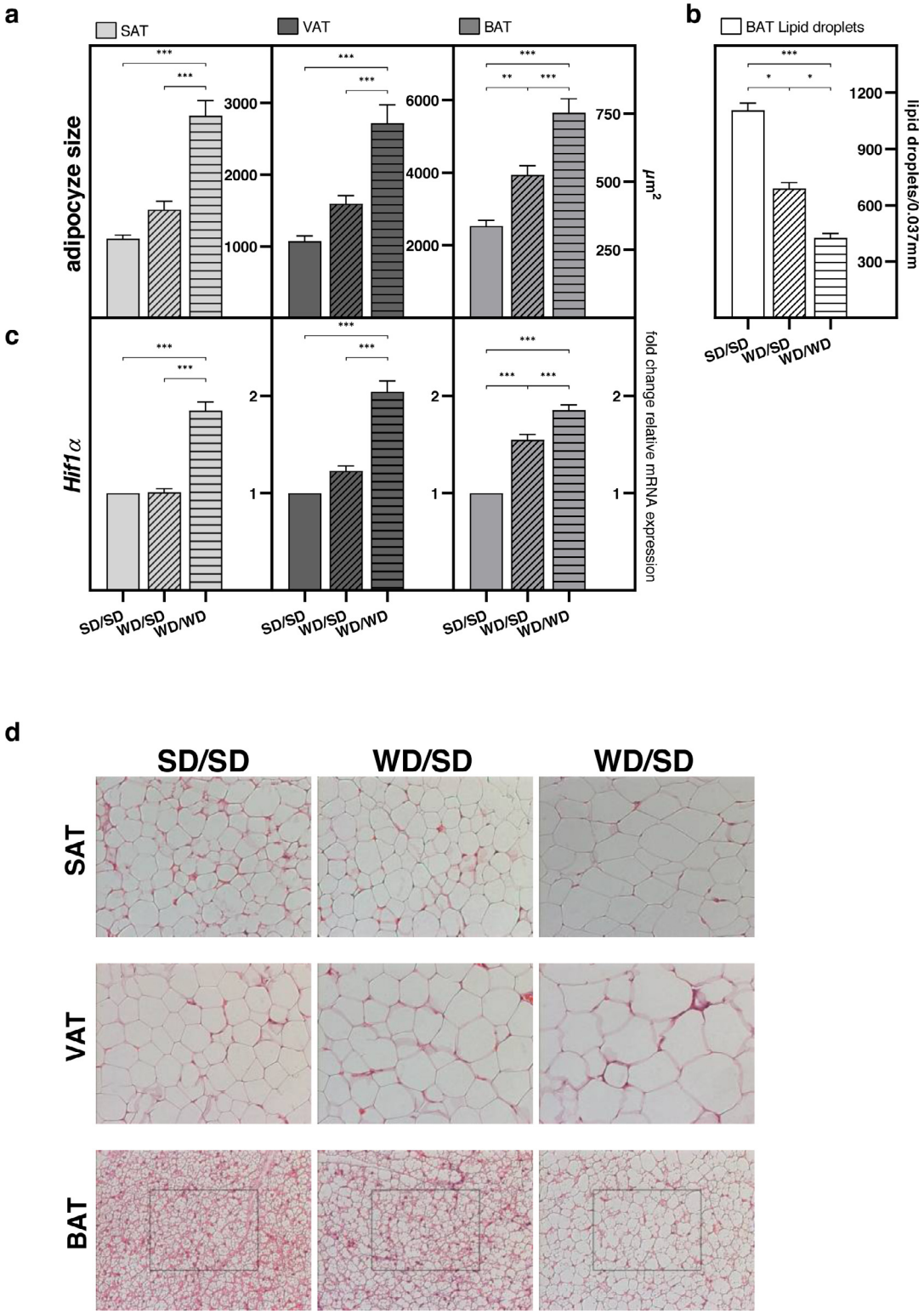

Fig. 3. Adipocyte size and *Hif1α* expression in white and brown adipose tissue: (A) mean adipocyte size (μm^2) of subcutaneous (SAT), visceral (VAT) and brown (BAT) adipose tissue and (B) mean number of lipid droplets (LD) / $0.37mm^2$ in brown adipose tissue ($n = 6$) (C) *Hif1a* / TATA-binding protein mRNA expression of in SAT, VAT and BAT; (C) representative images of hematoxylin and eosin staining of formalin-fixed paraffin-embedded tissue specimens of SAT, VAT and BAT of SD/SD, WD/SD and WD/WD mice; ($n = 10$); SD/SD = mice fed a standard diet for 18 weeks; WD/SD mice which were fed a western diet for 10 weeks followed by a standard diet for 8 more weeks; WD/WD = mice fed a western diet for 18 weeks; fold changes relatively to SD/SD; means ± SEM; $^{*}P < .05$; $^{**}P < .005$; $^{***}P < .0005$ (For interpretation of the references to colour in their figure legend, the reader is referred to the web version of this article.)

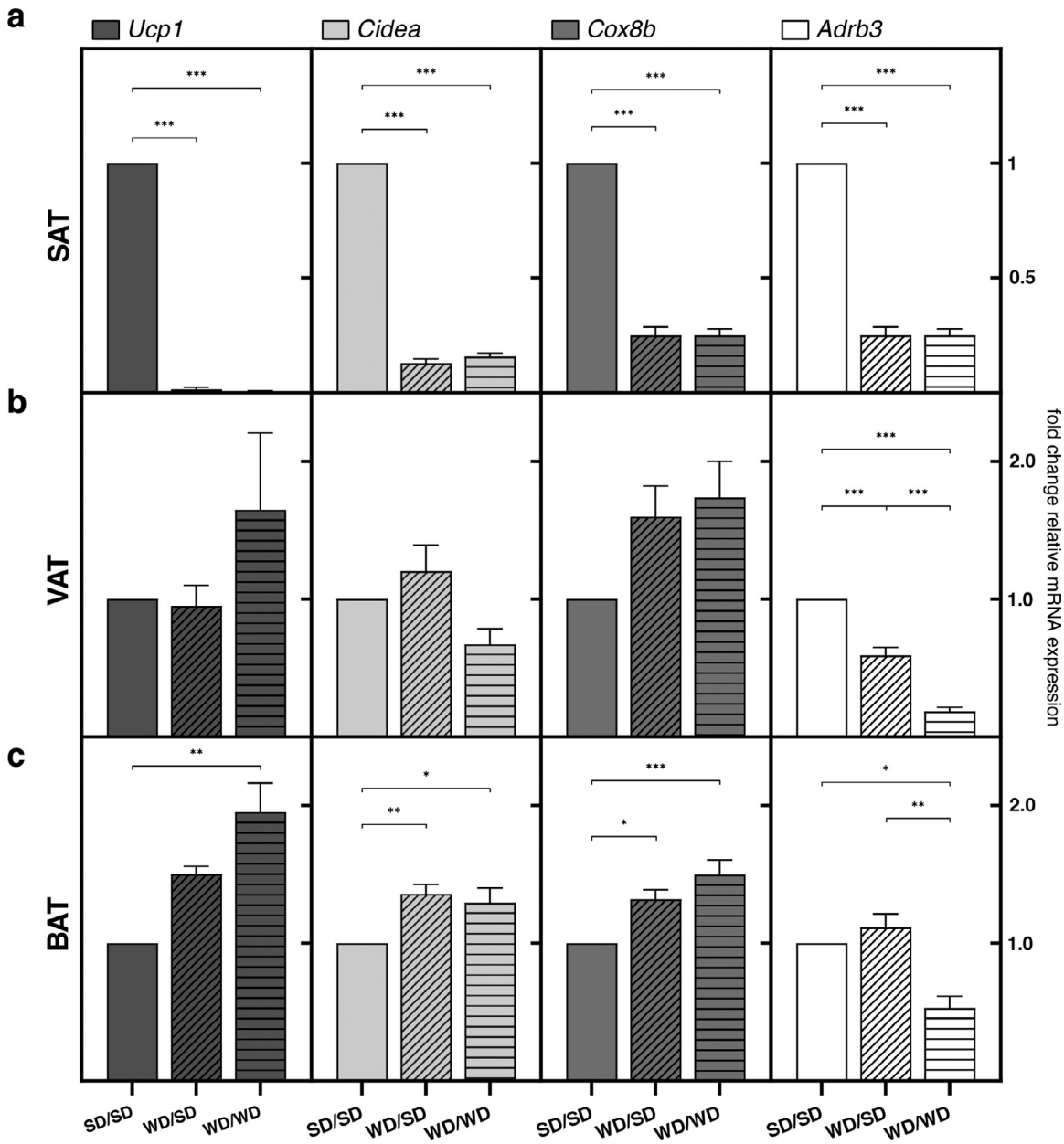

Fig. 4. Effects of switching the diet on the ability of beiging of SAT and markers of thermogenic capacity of various adipose tissue depots: relative mRNA expression levels of *Ucp1, Cidea, Cox8b* and *Adrb3* in (A) subcutaneous (B) visceral and (C) brown adipose tissue of SD/SD, WD/SD and WD/WD mice; SD/SD=mice fed a standard diet for 18 weeks; WD/SD mice which were fed a western diet for 10 weeks followed by a standard diet for 8 more weeks; WD/WD = mice fed a western diet for 18 weeks; mRNA expression levels were corrected for TATA-binding protein and fold changes relatively to SD/SD are shown; $n = 10$; means ± SEM; $^{*}P < .05$; $^{**}P < .005$; $^{***}P < .0005$

mice (Fig. S1H). Furthermore, *Ucp-1* was increased in WD/WD but not in WD/SD mice and expression of *Cidea,* which is a transcription factor of UCP1 [31] and *Cox8b* were increased in both WD/WD and WD/SD mice (Fig. 4C). Importantly, *Adrb3* which is critically involved in activation of thermogenesis was significantly reduced in WD/WD but not in WD/SD mice (Fig. 4C). *Fgf21* expression was highest in WD/WD and lowest in SD/SD mice with WD/SD lying in-between (Fig. 5C) and mRNA levels of *Fgfr1* and *βKl* were only reduced in WD/WD mice, expression was similar between SD/SD, and WD/SD mice (Fig. 5C).

To summarize beiging of subcutaneous adipose tissue is irreversibly inhibited by Western diet while thermogenic capacity of brown adipose tissue is partially restored by switching the type of diet.

4. Discussion

Adipocyte dysfunction is a hallmark of obesity related diseases such as insulin resistance and non–alcoholic fatty liver disease (NAFLD). While the concept of metabolically healthy obesity underlines its pivotal role in obesity related disorders, therapies that specifically address adipocyte dysfunction are very limited. Pronounced weight loss resulting from bariatric surgery has extensively shown to improve systemic glucose metabolism and adipocyte function [32–35], however, it is frequently associated with significant risk of long-term complications such as malabsorption or bone disease [36]. Weight loss by caloric restriction has also been shown to improve whole body energy metabolism and adipose tissue function, however, maintenance of weight loss

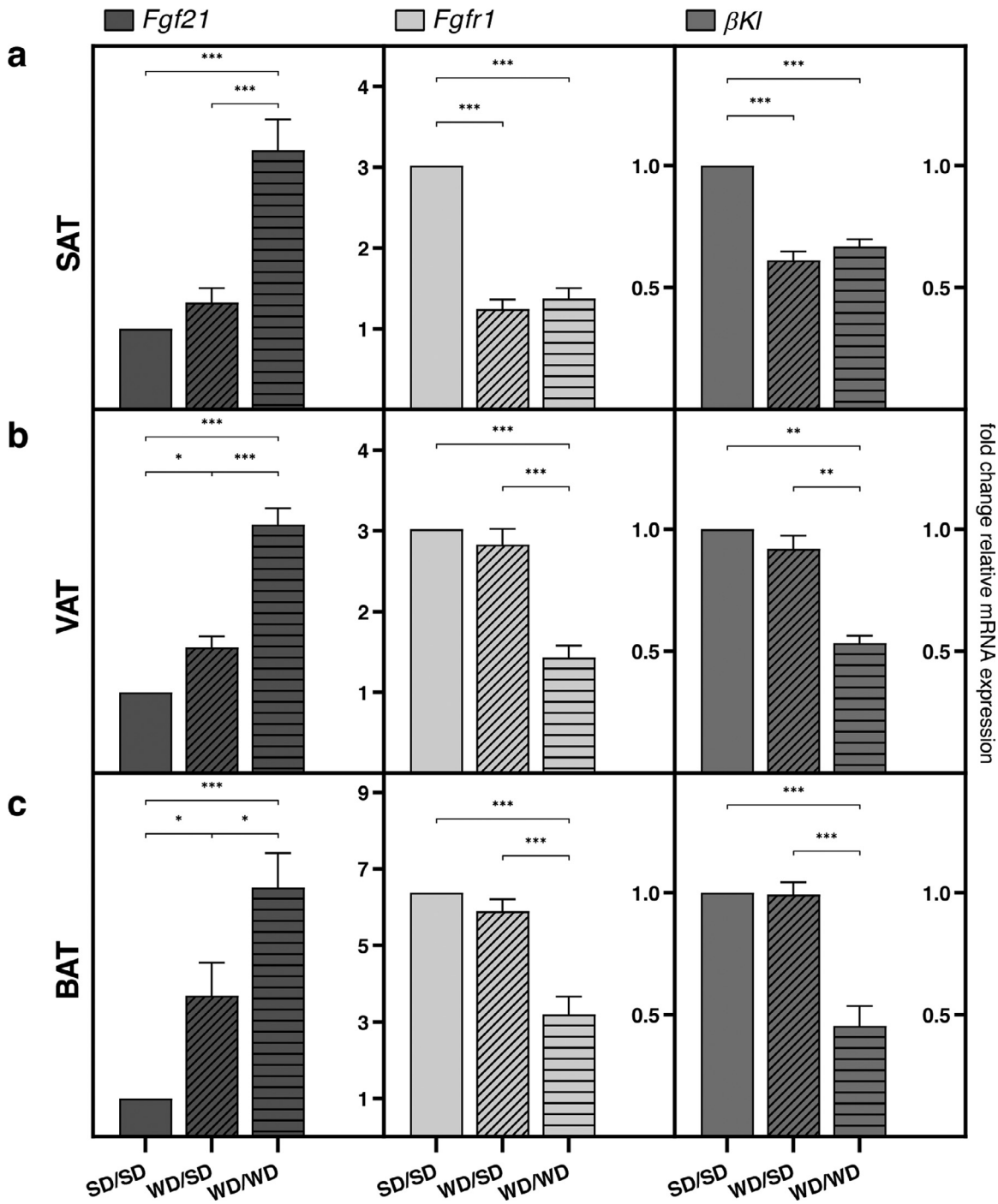

Fig. 5. Influence of changing dietary composition on fibroblast growth factor (*Fgf21*) and related genes in various adipose tissue depots: mean fold change of relative mRNA expression of *Fgf21*, FGF receptor 1 (*Fgfr1*) and *βKlotho* in (A) subcutaneous (B) visceral and (C) brown adipose tissue of SD/SD, WD/SD and WD/WD mice; SD/SD = mice fed a standard diet for 18 weeks; WD/SD mice which were fed a western diet for 10 weeks followed by a standard diet for 8 more weeks; WD/WD = mice fed a western diet for 18 weeks; mRNA expression levels corrected for TATA-binding protein and fold change relatively SD/SD; $n = 10$; means ± SEM; $^{*}P < .05$; $^{**}P < .005$; $^{***}P < .0005$

is commonly very challenging and often frustrating. The aim of this study was to test whether switching the diet is sufficient to reverse Western diet induced alterations in adipogenesis and adipose tissue inflammation. In this study, experiments were only performed in male C57Bl/6 mice as previous studies from our group revealed that harmful effects of Western diets on adipose tissue and systemic glucose metabolism are more pronounced in male than in female C57BL/6 mice [27]. Accordingly, results from our studies are applicable to male mice only.

In states of overnutrition adipose tissue expansion leads to adipocyte hypertrophy which is often associated with hypoxia and increased expression of *Hif1α*. The latter is associated with decreased β-oxidation, dysregulation of adipokines, and induction of necrosis [37,38].

In our study we were able to show that switching the diet is sufficient to restore adipocyte size in SAT and VAT as shown by comparable diameters in WD/SD and SD/S mice (Fig. 3). Also, in BAT feeding a WD caused an increase in size of adipocytes, and

a decrease in amount of lipid droplets resembling typical white adipocytes (Fig. 3). This whitening of BAT can be induced by multiple factors such as impaired leptin action or reduced β-adrenergic signaling both of which are seen in WD fed mice suggesting that WD irreversibly alters morphology of BAT. Whitening of BAT is associated with cell death and consequently macrophage infiltration and tissue inflammation [39,40]. Interestingly, even if brown adipocyte size was increased in WD/WD mice, at the same time expression of markers associated with thermogenesis were upregulated. *Ucp1* is a BAT-specific transporter protein of the inner mitochondrial membrane responsible for the non–shivering thermogenetic ability of BAT [41–43]. Although mechanisms are poorly understood, it was reported before that high fat diets lead to an upregulation of Ucp1 mRNA expression and protein [44–46]. In our study *Ucp1* was significantly upregulated in WD/WD mice only, while *Cox8b,* and *Cidea* which are also considered as beiging markers were also increased in WD/SD mice (Fig. 4). Albeit *Cidea* is highly expressed in brown adipocytes its function is not fully clarified. It was positively associated with lipid droplet fusion [47]. Mice overexpressing *Cidea* show reduced UCP1 activity [48]. The latter is controlled by beta adrenergic receptors [44]. In our experiments the β3-adrenoceptor (*Adrb3),* which upon stimulation triggers a beiging response [49] was downregulated in WD/WD mice but not in WD/SD mice. Therefore, we hypothesize that despite increased *Ucp1* expression in WD/WD mice thermogenetic capability is reduced in WD/WD mice and not fully restored by switching the diet as shown by altered *Cox8b* and *Cidea* expressions.

Thermogenesis capable cells are not restricted to brown adipose tissue. Brown-like adipocytes exist in several adipose tissue depots such as white adipose tissue. Despite deriving from different precursor cells, the so-called beige/bright adipocytes resemble typical brown adipocyte morphologic features such as multivesicular intracellular lipid droplets and a high mitochondrial content [50]. White adipocytes are able to differentiate into beige adipocytes upon cold exposure or direct β-adrenergic exposure [51]. This feature of "browning" gained a lot of interest as it opens up possibilities for new medical treatments against obesity. In mice subcutaneous adipose tissue was found to be especially susceptible to beiging [52,53]. In SAT beiging marker *Ucp1, Cidea* and *Cox8b* [54] were drastically downregulated in both WD/WD and WD/SD mice (Fig. 4). Furthermore, *Adrb3* was also reduced in both WD/WD and WD/SD mice suggesting that browning of subcutaneous adipocytes is irreversibly inhibited by Western diet. Our hypothesis is supported by FGF-21 data from our study showing increased expression levels of FGF-21 in SAT and reduced FGFR1 and βklotho levels in both WD/WD and WD/SD mice indicating reduced FGF-21 activity (Fig. 5). FGF-21 was found to be not only a critical determinant of energy balance, glucose, and lipid metabolism [55] but also to regulate browning of white adipose tissue [56].

Importantly, despite higher body weight, switching the diet from WD to SD protected from macrophage infiltration in VAT, and SAT estimated via F4/80 mRNA expression (Fig. 2).

These data suggest that mononuclear infiltration in adipose tissue is rather influenced by the type of diet than by body weight. Not only quantity but also characteristics of infiltrated immune cells were altered by switching the diet. Adipose tissue macrophages (ATM) are classified as pro-inflammatory classically activated M1 macrophages or alternatively activated M2 macrophages [57,58]. The anti–inflammatory M2 phenotype is typically dominant in lean individuals while the predominately proinflammatory M1 macrophages are associated with insulin resistant adipose tissue [59]. In M1 macrophages activation of the NF-$\kappa\beta$ pathway leads to the release of pro-inflammatory cytokines such as IL-1β, IL-6, CCL2, and TNF-α [58,59] [60–62]. In our study, NF-κB-activity and expression of proinflammatory M1 cytokines in SAT, VAT and BAT of WD/SD mice were comparable with those in SD/SD mice (Fig. 2, S2 and Table 1) suggesting that switching the diet alone without weight reduction is sufficient to reduce proinflammatory predominance in adipose tissue. However, our data based on mRNA expression levels do not allow to provide exact information on the grade of mononuclear infiltration of adipose tissue.

In our study switching the diet from a WD to a SD was associated with weight loss for the first days, however, WD/SD mice regained weight during the experiment, and stabilized for the last three weeks (Fig. 1). Despite the initial weight loss WD/SD mice were still significantly heavier than SD mice. These scenario – especially when comparing WD/SD and SD/SD mice allows to clearly distinguish beneficial effects of switching the diet from those of weight loss. While it will be interesting to investigate the effects of changing the diets on body fat distribution in future studies, lacking data are a limitation of this work.

Our data showing comparable insulin sensitivity in WD/SD and SD/SD mice suggest that switching the diet might positively affect insulin resistance in states of obesity.

In summary, our data suggest that switching the diet from a harmful WD to a beneficial SD is sufficient to improve adipose tissue inflammation in *ad libitum* fed mice. In contrast, feeding a Western diet induces whitening of brown adipose tissue and inhibits beiging of white adipose tissue in the long-term.

Future studies investigating detailed effects of switching the diet from a metabolically harmful Western to a standard diet on energy expenditure are necessary to fully understand the kinetics of the observed weight loss in our study.

Author contributions

FS performed the experiments, analysed the data and wrote the paper, RB, SK, SG, RC performed experiments, GG performed statistical analysis, TH reviewed the manuscript, SK conducted the study, reviewed the manuscript, supervised the work, and acquired funding for the study.

Funding

The financial support by the Federal Ministry for Digital and Economic Affairs and the National Foundation for Research, Technology, and Development is gratefully acknowledged (to SK). We would like to thank Merck Sharp & Dohme Austria for financial support of these studies (Christian Doppler Laboratory for Metabolic Crosstalk).

Declaration of competing interests

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2021.108837.

References

[1] Kopelman P. Health risks associated with overweight and obesity. Obes Rev 2007;8(Suppl 1):13–17.
[2] Federation. ID. IDF Diabetes Atlas, 9th edn. Brussels. Accessed from: https://www.diabetesatlas.org2019, access date 1st June 2019.
[3] Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: distinct or continuum? Indian J Endocrinol Metab 2016;20:546–51.

[4] Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. Phys Ther 2008;88:1322–35.
[5] Fowler MJ. Microvascular and macrovascular complications of diabetes. Clinical Diabetes 2008;26:77–82.
[6] Conde J, Scotece M, Gómez R, López V, Gómez-Reino JJ, Lago F, et al. Adipokines: Biofactors from white adipose tissue. A complex hub among inflammation, metabolism, and immunity. BioFactors 2011;37:413–20.
[7] Rondinone CM. Adipocyte-derived hormones, cytokines, and mediators. Endocrine 2006;29:81–90.
[8] Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose tissue remodeling: its role in energy metabolism and metabolic disorders. Front Endocrinol 2016;7:30. doi:10.3389/fendo.2016.00030.
[9] Ricquier D, Kader J-C. Mitochondrial protein alteration in active brown fat: a sodium dodecyl sulfate-polyacrylamide gel electrophoretic study. Biochem Biophys Res Commun 1976;73:577–83.
[10] Nicholls DG, Locke RM. Thermogenic mechanisms in brown fat. Physiol Rev 1984;64:1–64.
[11] Ricquier D. UCP1, the mitochondrial uncoupling protein of brown adipocyte: a personal contribution and a historical perspective. Biochimie 2017;134:3–8.
[12] Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J. Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. J Biol Chem 2010;285:7153–64.
[13] Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 2012;150:366–76.
[14] Carobbio S, Guénantin AC, Samuelson I, Bahri M, Vidal-Puig A. Brown and beige fat: From molecules to physiology and pathophysiology. Biochim Biophys Acta Mol Cell Biol Lipids 2019;1864:37–50.
[15] Villarroya F, Cereijo R, Gavaldà-Navarro A, Villarroya J, Giralt M. Inflammation of brown/beige adipose tissues in obesity and metabolic disease. J Intern Med 2018;284:492–504.
[16] Villarroya F, Cereijo R, Gavaldà-Navarro A, Villarroya J, Giralt M. Inflammation of brown/beige adipose tissues in obesity and metabolic disease. J Intern Med 2018;284:492–504.
[17] Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, et al. Adipose tissue dysfunction as determinant of obesity-associated metabolic complications. Int J Mol Sci 2019;20:2358.
[18] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011;11:85–97.
[19] Buse JB, Caprio S, Cefalu WT, Ceriello A, Del Prato S, Inzucchi SE, et al. How do we define cure of diabetes? Diabetes Care 2009;32:2133–5.
[20] Ang GY. Reversibility of diabetes mellitus: narrative review of the evidence. World J Diabetes 2018;9:127–31.
[21] Lean ME, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, et al. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. Lancet 2018;391:541–51.
[22] Lean MEJ, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, et al. Durability of a primary care-led weight-management intervention for remission of type 2 diabetes: 2-year results of the DiRECT open-label, cluster-randomised trial. Lancet Diabetes Endocrinol 2019;7:344–55.
[23] Buchwald H, Avidor Y, Braunwald E, Jensen MD, Pories W, Fahrbach K, et al. Bariatric surgery: a systematic review and meta-analysis. Jama 2004;292:1724–37.
[24] Chang SH, Stoll CR, Song J, Varela JE, Eagon CJ, Colditz GA. The effectiveness and risks of bariatric surgery: an updated systematic review and meta-analysis, 2003-2012. JAMA Surg 2014;149:275–87.
[25] Pi-Sunyer X. The look AHEAD trial: a review and discussion of its outcomes. Curr Nutr Rep 2014;3:387–91.
[26] Burhans MS, Hagman DK, Kuzma JN, Schmidt KA, Kratz M. Contribution of adipose tissue inflammation to the development of type 2 diabetes mellitus. Compr Physiol 2018;9:1–58.
[27] Dobner J, Ress C, Rufinatscha K, Salzmann K, Salvenmoser W, Folie S, et al. Fat-enriched rather than high-fructose diets promote whitening of adipose tissue in a sex-dependent manner. J Nutr Biochem 2017;49:22–9.
[28] Medina-Gomez G, Gray S, Vidal-Puig A. Adipogenesis and lipotoxicity: role of peroxisome proliferator-activated receptor gamma (PPARgamma) and PPARgammacoactivator-1 (PGC1). Public Health Nutr 2007;10:1132–7.
[29] Stern JH, Rutkowski JM, Scherer PE. Adiponectin, leptin, and fatty acids in the maintenance of metabolic homeostasis through adipose tissue crosstalk. Cell Metab 2016;23:770–84.
[30] Zuriaga MA, Fuster JJ, Gokce N, Walsh K. Humans and mice display opposing patterns of "browning" gene expression in visceral and subcutaneous white adipose tissue depots. Front Cardiovasc Med 2017;4:27.
[31] Jash S, Banerjee S, Lee MJ, Farmer SR, Puri V. CIDEA transcriptionally regulates UCP1 for britening and thermogenesis in human fat cells. iScience 2019;20:73–89.
[32] Frikke-Schmidt H, O'Rourke RW, Lumeng CN, Sandoval DA, Seeley RJ. Does bariatric surgery improve adipose tissue function? Obesity Rev 2016;17:795–809.
[33] Frikke-Schmidt H, Zamarron BF, O'Rourke RW, Sandoval DA, Lumeng CN, Seeley RJ. Weight loss independent changes in adipose tissue macrophage and T cell populations after sleeve gastrectomy in mice. Mol Metab 2017;6:317–26.
[34] Madsbad S, Dirksen C, Holst JJ. Mechanisms of changes in glucose metabolism and bodyweight after bariatric surgery. Lancet Diabetes Endocrinol 2014;2:152–64.
[35] Jørgensen NB, Bojsen-Møller KN, Dirksen C, Martinussen C, Svane MS, Kristiansen VB, et al. Sustained improvements in glucose metabolism late after roux-en-y gastric bypass surgery in patients with and without preoperative diabetes. Sci Rep 2019;9:15154.
[36] Stein EM, Silverberg SJ. Bone loss after bariatric surgery: causes, consequences, and management. Lancet Diabetes Endocrinol 2014;2:165–74.
[37] Am A, Berbecaru-Iovan A, Anghel FRID, Sn Ce, Berbecaru-Iovan S, Banjd I, et al. Interplay between Hypoxia, Inflammation and Adipocyte Remodeling in the Metabolic Syndrome; 2017.
[38] Krishnan J, Danzer C, Simka T, Ukropec J, Walter KM, Kumpf S, et al. Dietary obesity-associated Hif1α activation in adipocytes restricts fatty acid oxidation and energy expenditure via suppression of the Sirt2-NAD+ system. Genes Dev 2012;26:259–70.
[39] Kotzbeck P, Giordano A, Mondini E, Murano I, Severi I, Venema W, et al. Brown adipose tissue whitening leads to brown adipocyte death and adipose tissue inflammation. J Lipid Res 2018;59:784–94.
[40] Shimizu I, Aprahamian T, Kikuchi R, Shimizu A, Papanicolaou KN, MacLauchlan S, et al. Vascular rarefaction mediates whitening of brown fat in obesity. J Clin Invest 2014;124:2099–112.
[41] Aquila H, Link TA, Klingenberg M. The uncoupling protein from brown fat mitochondria is related to the mitochondrial ADP/ATP carrier. Analysis of sequence homologies and of folding of the protein in the membrane. Embo J 1985;4:2369–76.
[42] Bouillaud F, Weissenbach J, Ricquier D. Complete cDNA-derived amino acid sequence of rat brown fat uncoupling protein. J Biol Chem 1986;261:1487–90.
[43] Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev 2004;84:277–359.
[44] Fromme T, Klingenspor M. Uncoupling protein 1 expression and high-fat diets. Am J Physiol Regul Integr Comp Physiol 2011;300:R1–8.
[45] Ohtomo T, Ino K, Miyashita R, Chigira M, Nakamura M, Someya K, et al. Chronic high-fat feeding impairs adaptive induction of mitochondrial fatty acid combustion-associated proteins in brown adipose tissue of mice. Biochem Biophys Rep 2017;10:32–8.
[46] Oliver P, Sánchez J, Caimari A, Miralles O, Picó C, Palou A. The intake of a high-fat diet triggers higher brown adipose tissue UCP1 levels in male rats but not in females. Genes Nutr 2007;2:125–6.
[47] Barneda D, Planas-Iglesias J, Gaspar ML, Mohammadyani D, Prasannan S, Dormann D, et al. The brown adipocyte protein CIDEA promotes lipid droplet fusion via a phosphatidic acid-binding amphipathic helix. Elife 2015;4:e07485.
[48] Fischer AW, Shabalina IG, Mattsson CL, Abreu-Vieira G, Cannon B, Nedergaard J, et al. UCP1 inhibition in Cidea-overexpressing mice is physiologically counteracted by brown adipose tissue hyperrecruitment. Am J Physiol Endocrinol Metab 2017;312:E72–87.
[49] Jiang Y, Berry DC, Graff JM. Distinct cellular and molecular mechanisms for β3 adrenergic receptor-induced beige adipocyte formation. Elife 2017;6:e30329.
[50] Hepler C, Vishvanath L, Gupta RK. Sorting out adipocyte precursors and their role in physiology and disease. Genes Dev 2017;31:127–40.
[51] Barneda D, Frontini A, Cinti S, Christian M. Dynamic changes in lipid droplet-associated proteins in the "browning" of white adipose tissues. Biochim Biophys Acta 2013;1831:924–33.
[52] Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, et al. Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J Clin Invest 2011;121:96–105.
[53] van der Stelt I, Hoevenaars F, Široká J, de Ronde L, Friedecký D, Keijer J, et al. Metabolic response of visceral white adipose tissue of obese mice exposed for 5 days to human room temperature compared to mouse thermoneutrality. Front Physiol 2017;8:179.
[54] Garcia RA, Roemmich JN, Claycombe KJ. Evaluation of markers of beige adipocytes in white adipose tissue of the mouse. Nutr Metab (Lond) 2016;13:24.
[55] Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, et al. FGF-21 as a novel metabolic regulator. J Clin Invest 2005;115:1627–35.
[56] Tretter EM, Berger JM. Mechanisms for defining supercoiling set point of DNA gyrase orthologs: II. The shape of the GyrA subunit C-terminal domain (CTD) is not a sole determinant for controlling supercoiling efficiency. J Biol Chem 2012;287:18645–54.
[57] Chylikova J, Dvorackova J, Tauber Z, Kamarad V. M1/M2 macrophage polarization in human obese adipose tissue. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2018;162:79–82.
[58] Chawla A, Nguyen KD, Goh YP. Macrophage-mediated inflammation in metabolic disease. Nat Rev Immunol 2011;11:738–49.
[59] Pahlavani M, Ramalho T, Koboziev I, LeMieux MJ, Jayarathne S, Ramalingam L, et al. Adipose tissue inflammation in insulin resistance: review of mechanisms mediating anti-inflammatory effects of omega-3 polyunsaturated fatty acids. J Investig Med 2017;65:1021–7.
[60] Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res 2005;46:2347–55.
[61] Lee YS, Kim JW, Osborne O, Oh DY, Sasik R, Schenk S, et al. Increased adipocyte O2 consumption triggers HIF-1alpha, causing inflammation and insulin resistance in obesity. Cell 2014;157:1339–52.
[62] Liu R, Nikolajczyk BS. Tissue immune cells fuel obesity-associated inflammation in adipose tissue and beyond. Front Immunol 2019;10:1587.