Palmitate-Induced S1P Signaling Pathway Attenuates by Chicoric Acid in Human Peripheral Blood Mononuclear Cells

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Research Article

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Abstract

Objective: Sphingosine 1-phosphate (S1P) signaling pathway is involved in the pathogenesis of type 2 diabetes (T2D). So, targeting S1P signaling pathway could be considered as potential therapeutic target for T2D. The aim of this study was to investigate the effects of palmitate and chicoric acid (CA) on S1P signaling pathway in peripheral blood mononuclear cells (PBMCs) from newly diagnosed patients with T2D and healthy subjects.

Materials and Methods: 20 newly diagnosed patients with T2D and 20 healthy subjects, aged 40-60 years, were enrolled in the study. PBMCs were isolated and treated with palmitate and CA. Then, Sphingosine kinase 1 (SPHK1) and Sphingosine 1-phosphate receptor 1 (S1PR1) genes expression were evaluated by real-time PCR and S1PR1 protein levels were quantified using ELISA.

Results: Palmitate significantly increased SPHK1 and S1PR1 genes expression and S1PR1 protein levels in PBMCs of both patients and healthy subjects. However, CA ameliorates palmitate-increased SPHK1 and S1PR1 genes expression and S1PR1 levels in these cells. Furthermore, a significant positive correlation between SPHK1 and S1PR1 genes expression with the S1PR1 protein levels was observed.

Conclusions: These data indicate that CA could be considered as a novel S1P signaling pathway inhibitor through down regulation of SPHK1 and S1PR1.

Key Points

1. Palmitate increases sphingosine kinase 1 (SPHK1) and sphingosine 1-phosphate receptor 1 (S1PR1) genes expression, as well as S1PR1 protein levels.
2. Chicoric acid ameliorates palmitate-induced sphingosine 1-phosphate signaling pathway through decreasing SPHK1 and S1PR1.

1. Introduction

Type 2 diabetes (T2D) is an endocrine disorder worldwide. The prevalence of T2D has increased markedly around the world and constitutes one of the major public healthcare problem [1]. Peripheral blood mononuclear cells (PBMCs) consist of lymphocytes and monocytes. These cells are helpful in revealing the complex nature of T2D [2]. Increasing evidence has implicated that inflammation is essential contributors to T2D. The signaling pathways involved in the production of inflammatory mediators play crucial role in the induction of inflammation in tissues, followed by progression of diseases [3]. Now, understanding the molecular mechanism involved in the inflammation has attracted interest of researchers. Undoubtedly, knowing the molecular details of the inflammatory pathways lead to identify and develop appropriate treatment.

Sphingosine signaling pathway is one of the newly pathways involved in the tissue inflammation and plays an important role in the activation of tissue macrophages and induction of inflammation.

Sphingosine 1 phosphate (S1P) is a bioactive lipid with structural role in plasma membranes. In addition to its structural function, it has been demonstrated that S1P has a critical role in many cellular processes such as inflammation and immunity, cell survival and proliferation, cell migration, metastases and etc [4]. The role of S1P in modulating the relationship between monocytes and endothelium in diabetic and healthy mice has been evaluated and it has been shown that S1P in diabetic mice increases the adhesion of monocytes to the endothelial wall [5]. Today, S1P recognized as a vital regulator of many pathological processes including diabetes, cancer, atherosclerosis and osteoporosis [6].

S1P is generated from sphingosine by two kind of sphingosine kinase (SPHK) including SPHK1 and SPHK2. SPHK1 present mainly in the cytosol. However, SPHK2 localized in the several intracellular compartments [6]. It has been found that SPHK1 gene expression increases in high-fat diet [7]. Various studies clearly showed that inhibition of SPHK1 is a therapeutic target for inflammatory diseases [6]. S1P is secreted out of cells and regulates cellular processes through binding to its receptors. Five specific cell surface G protein-coupled receptors, named S1PR1–5, have been recognized for S1P. Binding of S1P to S1PR1 activates S1PR1. Activated S1PR1 plays an important role in the PBMCs trafficking and induces inflammation. Therefore, the regulation of S1P signaling pathway could be a good therapeutic strategy for T2D [6].

Palmitate has been proposed to induce activation of inflammatory cascade in PBMCs [2]. Palmitate increases CCL4 levels in monocytes and macrophages by activating NF- κ B and PI3K signaling pathway [8]. CCL4 increases the production of inflammatory cytokines such as IL-6. Increased CCL4 and inflammatory cytokines production leads to metabolic inflammation. Metabolic inflammation plays a vital role in many diseases such as diabetes, atherosclerosis, cancer and etc. There are several reports that the circulating level of free fatty acids (FFAs), especially palmitate increases during T2D [8, 9]. Indeed, palmitate is a major risk factor for the development of T2D. Recently, it has been reported that palmitate increases S1P through up regulation of SPHK1 [10].

Chicoric acid (CA) is a phenolic compound with anti-inflammatory, anti-oxidants and anti-hyperglycemic effects [11]. CA found in several widely used herbal medicinal plants including *Cichorium intybus* L., *Echinacea purpurea* and *Orthosiphon stamineus* B. Recently, the beneficial effects of CA on T2D has been demonstrated [12, 13]. However, the exact molecular mechanism underlying the effect of CA on T2D remains unexplored.

In this study, we aimed to evaluate the effects of palmitate and CA on SPHK1 and S1PR1 genes expression, as well as protein levels of S1PR1 in PBMCs from newly diagnosed patients with T2D and healthy subjects.

2. Materials And Methods

2.1. Participants and study design

A total of 40 subjects (20 newly diagnosed patients with T2D and 20 healthy subjects) with an age between 40 and 60 years old were recruited in this study. Diagnosis of T2D was performed based on American Diabetes Association (ADA) criteria [14]. Subjects with fasting blood sugar (FBS) ≥ 126 mg/dl were considered to be diabetic and subjects with FBS < 100 mg/dl were grouped as healthy subjects. It should be noted that all patients with T2D had a first-time diagnosis of T2D at time of sampling and do not took any anti-diabetic medication. Patients with acute and chronic inflammatory diseases, cardiovascular disease, uncontrolled hypertension, T1DM and gestational diabetes were excluded from the study. Also, none of the studied subjects have smoking and alcohol consumption. All participants gave written informed consent before the study. The protocol of present study approved by Ethics Committee of Shahrekord University of Medical Sciences (code: IR.SKUMS.REC.1399.115). After an overnight fast, anthropometric measurements including age, height, weight, BMI (weight/height²), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded. Then, venous blood samples were collected for biochemical parameters analysis and PBMCs isolation. FBS, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine were measured by colorimetric methods (Pars Azmoon, Tehran, Iran) on a BIOLIS24i Premium autoanalyzer (Tokyo Boeki Machinery Ltd., Japan). Insulin was detected by enzyme-linked immunosorbent assay (ELISA) kit (Monobind Inc., CA, USA). Homeostasis model assessment of insulin resistance (HOMA-IR) was measured using the formula: fasting insulin (μ U/ml) $\times$ FBS (mg/dl) / 405. Hemoglobin A1C (HbA1C) was measured with HPLC method using Tosoh G8 instrument (South San Francisco, CA).

2.2. PBMCs isolation and cell culture

PBMCs were purified from the blood sample immediately by the use of Ficoll–Hypaque density gradient centrifugation (Lympholyte-H; Cedarlane Laboratories, Ontario, Canada) as previously described. Briefly, blood was diluted (1:1) with phosphate buffered saline (PBS), and placed on ficoll medium and then centrifuged. After collecting PBMCs, the cells were washed twice with sterile PBS and suspended in RPMI 1640 medium (GIBCO; Invitrogen Laboratories, UK). The cell viability test was assessed with trypan blue staining. Isolated PBMCs plated in 12-well flat-bottom plate containing RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) (GIBCO; Invitrogen Laboratories, UK) and 1% penicillinstreptomycin (GIBCO; Invitrogen Laboratories, UK) for genes expression and ELISA analysis. After 2 h incubation under 37°C with 5% CO₂, cells were treated as follows:

1. Control groups (untreated, treated with BSA 1% for 12 h).
2. CA groups (treated with 50 μ M CA for 6 h).
3. Palmitate groups (treated with 500 μ M palmitate for 12 h).
4. Palmitate + CA groups (treated with 500 μ M palmitate for 12 h and then treated with 50 μ M CA for 6 h).

Finally, PBMCs were harvested and stored at -80°C for evaluation of SPHK1 and SIPR1 genes expression and measuring SIPR1 protein levels. It is important to note that optimum concentration and exposure

time for palmitate and CA treatment were used according to the results of our previous study [2, 15].

2.3. Preparation of treatments

Sodium palmitate (Sigma Aldrich, St. Louis, MO, USA) was dissolved in 50 % (v/v) ethanol by brief heating at 55°C. Then, palmitate-BSA complexes were prepared by slowly adding palmitate to pre-warmed RPMI 1640 medium containing 1 % (w/v) fatty acid free-bovine serum albumin (BSA), followed by 2 h shaking in incubator under 37°C. CA (Sigma Aldrich, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO). It should be noted that a 500 μ M palmitate and a 50 μ M CA solution were prepared and filtrated prior to treatment.

2.4. Quantitative real-time PCR

Total RNA was extracted from treated PBMCs using the Hybrid-R RNA purification kit (GeneAll Biotechnology, Seoul, Korea). The quantity and integrity of RNA was determined by a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and 1% agarose gel electrophoresis, respectively. Then, cDNA was synthesized using Revert Aid RT Kit according to the manufacturer's instructions (Thermo Fisher scientific, CA, USA). Real-time PCR was conducted by a Corbett Rotor Gene 6000 Light Cycler (Qiagen, Hilden, Germany) using SYBR Green detection kit (Ampliqon, Denmark) and gene-specific primers. Primers sequences of the reaction were as follows: ACTB: 5'-ACAGAGCCTCGCCTTTGC-3' (forward) and 5'-ATCACGCCCTGGTGCCT-3' (reverse), SPHK1: 5'-CCTTCCTCCTTCCCTAGGG-3' (forward) and 5'-TAGAAGGCCTTACATAGGCAG-3' (reverse), S1PR1: 5'-CAGCAAATCGGACAATTCCT-3' (forward) and 5'-GCCAGCGACCAAGTAAAGAG-3' (reverse). The data were normalized against β -actin transcript level. Fold change in SPHK1 and S1PR1 genes expression were calculated using the $2^{-\Delta\Delta C_t}$ method.

2.5. Determination of S1PR1 levels

Concentrations of S1PR1 in the PBMCs were measured by an ELISA kit with a sensitivity of 0.057 ng/ml, according to the protocol provided by the manufacturer (catalog number: MBS2021994).

2.6. Statistical analysis

Data are reported as mean $\pm$ standard deviations (SD). All data were analyzed using SPSS software (version 16.0, Chicago, IL, USA) and GraphPad Prism software (version 7, San Diego, CA, USA). Comparisons between data were made by Two-way analysis of variance (Two-way ANOVA). Pearson's correlation test was used to examine the relationship between the variables. A value of $p < 0.05$ was considered significant.

3. Results

3.1. Anthropometric and Biochemical findings

Anthropometric characteristics of the studied subjects are illustrated in Table 1. Age, height, weight, BMI, SBP and DBP of the subjects were not significantly differences between two groups. As shown in Table 2

the patients with T2D had significantly higher FBS, insulin, HOMA-IR, HbA1C and TG as compared to healthy subjects ($p < 0.001$). However, other biochemical parameters including TC, LDL-C, HDL-C, urea, creatinine, AST and ALT were not significantly differences between the two studied groups.

Table 1
Anthropometric indices of the study population

Variable	Healthy subjects (n=20)	Patients with T2D (n=20)	<i>p</i> value
Female/male	10/10	10/10	n.s.
Age (year)	51.24±6.54	50.33±7.78	n.s.
Height (cm)	174.42±8.21	171.43±9.18	n.s.
Weight (kg)	75.55±7.28	76.54±6.44	n.s.
BMI (kg/m ²)	22.89±3.19	22.40±2.37	n.s.
SBP (mmHg)	123.40±4.18	128.14±2.38	n.s.
DBP (mmHg)	79.19±3.49	82.39±2.60	n.s.
Data are expressed as Means ± SD. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.			

Table 2
Biochemical parameters of the study population

Variable	Healthy subjects (n=20)	Patients with T2D (n=20)	p value
FBS (mg/dl)	93.21±5.14	195.41±52.11	<0.001
Insulin (μIU/ml)	5.25±1.04	10.34±5.31	<0.001
HOMA-IR	0.94±0.22	6.61±3.21	<0.001
HbA1C (%)	4.36±0.71	8.38±3.18	<0.001
TG (mg/dl)	155.53±39.26	288.19±85.93	<0.001
TC (mg/dl)	179.33±31.84	201.32±52.02	n.s.
LDL-C (mg/dl)	84.42±17.54	93.11±16.5	n.s.
HDL-C (mg/dl)	48.25±11.26	44.18±6.42	n.s.
Urea (mg/dl)	27.35±5.98	32.10±5.35	n.s.
Creatinine (mg/dl)	1.03±0.11	1.04±0.18	n.s.
AST (U/L)	25.64±7.22	28.44±7.16	n.s.
ALT (U/L)	27.33±6.10	25.11±6.49	n.s.
Data are expressed as Means ± SD. FBS: fasting blood sugar; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance, TG: triglycerides; TC: total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.			

Table 3

Correlation coefficients of SPHK1 and S1PR1 genes expression with S1PR1 protein levels in each group

		S1PR1 protein levels			
		Untreated PBMCs	Chicoric acid treated PBMCs	Palmitate treated PBMCs	Palmitate + Chicoric acid treated PBMCs
		r	r	r	r
SPHK1 gene expression	Healthy subjects	0.453	0.865	0.108	0.277
	Patients with T2D	0.456	0.287	0.498	0.439
S1PR1 gene expression	Healthy subjects	0.064	0.753	0.613	-0.017
	Patients with T2D	0.497	0.290	0.299	0.526
Bold texts are statistically significant. *p < 0.05.					

3.2. Effects of palmitate and CA on SPHK1 and S1PR1 genes expression

To elucidate the effects of palmitate and CA on S1P signaling pathway, we investigated SPHK1 and S1PR1 genes expression in response to palmitate and CA. As shown in Fig. 1, palmitate significantly increased SPHK1 gene expression by 2.23 and 2.78 fold in healthy volunteers and patients with T2D, respectively ($p < 0.001$). Inversely, CA decreased SPHK1 gene expression in PBMCs of healthy subjects and patients with T2D approximately 0.25 and 0.24 fold, respectively ($p < 0.001$). Also, palmitate-increased in SPHK1 gene expression remarkably has been reversed by CA in healthy subjects (from 2.23 to 1.24 fold) ($p < 0.001$) and patients with T2D (from 2.78 to 1.54 fold) ($p < 0.001$). The between group evaluation showed that SPHK1 gene expression in untreated cells from patients with T2D is significantly decreased as compared with untreated cells from healthy subjects ($p < 0.001$).

Our findings revealed that treatment of PBMCs with palmitate increases S1PR1 gene expression in non-diabetic subjects and patients with T2D approximately 1.93 and 1.80 fold, respectively ($p < 0.001$). Unlike palmitate, treatment of PBMCs with CA caused a decrease in S1PR1 gene expression in healthy volunteers (0.72 fold) and patients with T2D (0.68 fold) ($p < 0.001$). Moreover, we found that in healthy subjects increased S1PR1 gene expression induced by palmitate significantly has been restored via CA (from 1.93 fold to 1.46) ($p < 0.05$). In the current study, we did not detect significant difference in S1PR1 gene expression in untreated PBMCs from healthy and diabetic groups (Fig. 2).

3.3. Effects of palmitate and CA on S1PR1 protein levels

We also evaluated the effects of palmitate and CA on S1PR1 protein levels. Fig. 3 showed that S1PR1 protein levels is dramatically increased in PBMCs exposed to palmitate as compared to untreated cells in healthy volunteers and patients with T2D ($p < 0.001$). However, CA significantly decreased S1PR1 protein levels in PBMCs of healthy subjects ($p < 0.001$). Furthermore, our study revealed that CA significantly ameliorates palmitate-increased S1PR1 protein levels in PBMCs from healthy subjects ($p < 0.05$).

3.4. Association between SPHK1 and S1PR1 genes expression with S1PR1 protein levels

Analysis of the relationships of SPHK1 and S1PR1 genes expression with S1PR1 protein levels showed that S1PR1 protein levels had a significant positive association with SPHK1 gene expression in palmitate-treated PBMCs from patients with T2D ($r = 0.498$, $p = 0.004$) as well as S1PR1 gene expression in chicoric acid-treated PBMCs from healthy subjects ($r = 0.753$, $p = 0.028$).

4. Discussion

It is well known that inflammation impairs cellular function and leads to several disease such as T2D, IR, atherosclerosis, cancer and etc [16]. Increased FFAs stimulate inflammation-related pathways such as S1P signaling pathway [10]. Based on the data from previous studies, CA exerts the anti-inflammatory

effects. However, the exact molecular mechanism in which CA regulates anti-inflammatory pathways has not been recognized [2, 11]. Here, we investigated the effects of CA on S1P signaling pathway.

We first showed that SPHK1 gene expression in untreated cells of T2D was significantly lower as compared to healthy subjects. However, the gene expression and protein levels of S1PR1 in untreated PBMCs of patients with T2D is not significantly difference from healthy subjects. Consistent with our results, it was showed that HFD-induced muscle IR is attenuated in SPHK1 transgenic mice. SPHK1 prevents ceramide accumulation through promoting its metabolism into S1P and, therefore, protects against the development of IR. Accumulation of ceramide impairs the insulin signaling pathway through activating JNK [17]. In another study, it was demonstrated that injection of an adenoviral vector containing the human SphK1 cDNA in diabetic mice improves glucose tolerance and decreases total cholesterol, triglycerides, and LDL [18]. Also, it was showed that administration of the S1P analogue FTY720 in HFD animals attenuates IR [19]. However, another studies reported the opposite results. It was demonstrated that SPHK1 gene expression in subcutaneous adipose tissue from ob/ob mice was elevated [20]. Also, Blachnio-Zabielska and et al, found that S1P levels was increased in subcutaneous adipose tissue of obese subjects as compared to lean subjects [21].

We examined the effects of palmitate and CA on S1P signaling pathway in PBMCs. Our study indicates for the first time that SPHK1 and S1PR1 genes expression and the protein levels of S1PR1 are significantly increased in response to palmitate. In fact, palmitate, which has a crucial role in the development of IR stimulates S1P signaling via increasing SPHK1 and S1PR1. This is in accordance with the fact that palmitate is necessary for the de novo synthesis of sphingosine. Also, palmitate increases the S1P formation by inducing SPHK1 gene expression [22]. In this regard, it was shown that palmitate increases SPHK1 expression and activity in C2C12 myotubes [4]. This may suggest that SPHK1 and S1PR1 mediate pathological effects of palmitate. Elevated S1P in response to upregulation of SPHK1 not only plays a role in the impairment of insulin pathway but also increases inflammation [7]. In a study by Wang et al. on adipocytes of HFD-mice it was demonstrated that SPHK1 deficiency, which leads to reduced S1P, increases anti-inflammatory cytokines and decreases proinflammatory cytokines [23]. On the other hand, based on our previous results that palmitate induces inflammatory cytokine production, we can suggest that palmitate increases inflammation through up regulation of S1P signaling. In accordance with our result, Jin et al. reported that palmitate and LPS synergistically increases S1P through stimulation of SPHK1 expression. Also, their result reveal increased proinflammatory cytokine expression in response to palmitate and LPS [10].

To evaluate the molecular mechanism underlying the effect of CA on diabetes, we investigated S1P pathway proteins including SPHK1 and S1PR1. Our study provides the first evidence that CA decreases the SPHK1 and S1PR1 genes expression and S1PR1 protein levels. Binding of S1P to its receptors induces inflammatory cytokine production. Increased inflammation is contribute to development of IR [23]. These results were accompanied by previous studies that implicated resveratrol, as a poly phenol, inhibits SPHK1 activity in HEK 293 cells [24]. Here, we provide novel evidence that CA could be as a new SPHK1 inhibitor. Down regulation of SPHK1 gene expression maybe due to changes in SPHK1 protein

turnover. Like other SK1 inhibitors, CA probably stimulates the SPHK1 ubiquitin-proteasomal degradation or lysosomal-cathepsin B catalysed proteolysis or changes in activity of gene promoter [25, 26]. Together, anti-inflammatory effects of CA might be a consequence of the inhibition of S1P signaling pathway. Therefore, inhibition of S1P signaling pathway by CA could be considered as a novel therapeutic target for the improvement of diabetes [6].

Another finding in our study was positive correlation between SPHK1 and S1PR1 genes expression with S1PR1 protein levels. These data suggest that SPHK1 and S1PR1 are essential in S1P signaling pathway [6].

Conclusion

In conclusion, our data identify CA as an important regulator of S1P signaling pathway by decreasing SPHK1 and S1PR1 expression. Since it is known that activated S1P signaling pathway exacerbates T2D, thereby targeting S1P signaling pathway by CA may improve T2D. However, more works are needed to evaluate the efficacy of CA in the management of T2D.

Abbreviations

T2D

Type 2 diabetes

IR

Insulin resistance

PBMCs

Peripheral blood mononuclear cells

CA

Chicoric acid

FFAs

Free fatty acids

SPHK1

Sphingosine kinase 1

S1PR1

Sphingosine 1-phosphate receptor 1.

Declarations

Compliance with Ethical Standards

Acknowledgment

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Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

Authors' contributions

RM designed the research and wrote the manuscript. ZAS performed the experiments. KGS analyzed the results. FY drafted the manuscript. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission. The authors declare that all data were generated in-house and that no paper mill was used.

Conflicts of interest

The authors declare no conflict of interest.

Ethical approval

Written informed consent was obtained from all individual participants included in the study. This study followed the ethical rules for medical research involving human subjects of the Declaration of Helsinki (1964), and the protocol of present study approved by Ethics Committee of Shahrekord University of Medical Sciences (code: IR.SKUMS.REC.1399.115).

References

1. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H and Al Kaabi J (2020) Epidemiology of type 2 diabetes–global burden of disease and forecasted trends. *Journal of epidemiology and global health* 10:107.
2. Sadeghabadi ZA, Ziamajidi N, Abbasalipourkabir R, Mohseni R and Borzouei S (2019) Palmitate-induced IL6 expression ameliorated by chicoric acid through AMPK and SIRT1-mediated pathway in the PBMCs of newly diagnosed type 2 diabetes patients and healthy subjects. *Cytokine* 116:106-114.
3. TEMELKOVA KT, Henkel E, Koehler C, Karrei K and Hanefeld M (2002) Subclinical inflammation in newly detected type II diabetes and impaired glucose tolerance.
4. Hu W, Bielawski J, Samad F, Merrill AH and Cowart LA (2009) Palmitate increases sphingosine-1-phosphate in C2C12 myotubes via upregulation of sphingosine kinase message and activity. *Journal of lipid research* 50:1852-1862.
5. Keul P, Lucke S, von Wnuck Lipinski K, Bode C, Gräler M, Heusch G and Levkau B (2011) Sphingosine-1-phosphate receptor 3 promotes recruitment of monocyte/macrophages in inflammation and atherosclerosis. *Circulation research* 108:314-323.

6. Maceyka M, Harikumar KB, Milstien S and Spiegel S (2012) Sphingosine-1-phosphate signaling and its role in disease. *Trends in cell biology* 22:50-60.
7. Nagahashi M, Yamada A, Katsuta E, Aoyagi T, Huang W-C, Terracina KP, Hait NC, Allegood JC, Tsuchida J and Yuza K (2018) Targeting the SphK1/S1P/S1PR1 axis that links obesity, chronic inflammation, and breast cancer metastasis. *Cancer research* 78:1713-1725.
8. Kennedy A, Martinez K, Chuang C-C, LaPoint K and McIntosh M (2009) Saturated fatty acid-mediated inflammation and insulin resistance in adipose tissue: mechanisms of action and implications. *The Journal of nutrition* 139:1-4.
9. Ahmad R, Al-Roub A, Kochumon S, Akther N, Thomas R, Kumari M, Koshy MS, Tiss A, Hannun YA and Tuomilehto J (2018) The synergy between palmitate and TNF- α for CCL2 production is dependent on the TRIF/IRF3 pathway: implications for metabolic inflammation. *The Journal of Immunology* 200:3599-3611.
10. Jin J, Lu Z, Li Y, Ru JH, Lopes-Virella MF and Huang Y (2018) LPS and palmitate synergistically stimulate sphingosine kinase 1 and increase sphingosine 1 phosphate in RAW264. 7 macrophages. *Journal of leukocyte biology* 104:843-853.
11. Xiao H, Xie G, Wang J, Hou X, Wang X, Wu W and Liu X (2013) Chicoric acid prevents obesity by attenuating hepatic steatosis, inflammation and oxidative stress in high-fat diet-fed mice. *Food research international* 54:345-353.
12. Ghamarian A, Abdollahi M, Su X, Amiri A, Ahadi A and Nowrouzi A (2012) Effect of chicory seed extract on glucose tolerance test (GTT) and metabolic profile in early and late stage diabetic rats. *DARU Journal of Pharmaceutical Sciences* 20:56.
13. Shim D-W, Han J-W, Ji Y-E, Shin W-Y, Koppula S, Kim M-K, Kim T-K, Park P-J, Kang T-B and Lee K-H (2016) *Cichorium intybus* Linn. extract prevents type 2 diabetes through inhibition of NLRP3 inflammasome activation. *Journal of medicinal food* 19:310-317.
14. Alberti KGMM and Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic medicine* 15:539-553.
15. Arab Sadeghabadi Z, Abbasalipourkabir R, Mohseni R and Ziamajidi N (2019) Chicoric acid does not restore palmitate-induced decrease in irisin levels in PBMCs of newly diagnosed patients with T2D and healthy subjects. *Archives of Physiology and Biochemistry*:1-7.
16. Stump CS, Henriksen EJ, Wei Y and Sowers JR (2006) The metabolic syndrome: role of skeletal muscle metabolism. *Annals of medicine* 38:389-402.
17. Bruce CR, Risis S, Babb JR, Yang C, Kowalski GM, Selathurai A, Lee-Young RS, Weir JM, Yoshioka K and Takuwa Y (2012) Overexpression of sphingosine kinase 1 prevents ceramide accumulation and ameliorates muscle insulin resistance in high-fat diet-fed mice. *Diabetes* 61:3148-3155.
18. Ma M, Chen J, Wang G, Wang H, Lu Y, Li J, Yi J, Yuan Y, Zhang Q and Mi J (2007) Sphingosine kinase 1 participates in insulin signalling and regulates glucose metabolism and homeostasis in KK/Ay diabetic mice. *Diabetologia* 50:891-900.

19. Guitton J, Bandet CL, Mariko ML, Tan-Chen S, Bourron O, Benomar Y, Hajduch E and Le Stunff H (2020) Sphingosine-1-Phosphate Metabolism in the Regulation of Obesity/Type 2 Diabetes. *Cells* 9:1682.
20. Hashimoto T, Igarashi J and Kosaka H (2009) Sphingosine kinase is induced in mouse 3T3-L1 cells and promotes adipogenesis. *Journal of lipid research* 50:602-610.
21. Blachnio-Zabielska AU, Koutsari C, Tchkonja T and Jensen MD (2012) Sphingolipid content of human adipose tissue: relationship to adiponectin and insulin resistance. *Obesity* 20:2341-2347.
22. Fayyaz S, Henkel J, Japtok L, Krämer S, Damm G, Seehofer D, Püschel GP and Kleuser B (2014) Involvement of sphingosine 1-phosphate in palmitate-induced insulin resistance of hepatocytes via the S1P 2 receptor subtype. *Diabetologia* 57:373-382.
23. Wang J, Badeanlou L, Bielawski J, Ciaraldi TP and Samad F (2014) Sphingosine kinase 1 regulates adipose proinflammatory responses and insulin resistance. *American Journal of Physiology-Endocrinology and Metabolism* 306:E756-E768.
24. Lim KG, Gray AI, Pyne S and Pyne NJ (2012) Resveratrol dimers are novel sphingosine kinase 1 inhibitors and affect sphingosine kinase 1 expression and cancer cell growth and survival. *British journal of pharmacology* 166:1605-1616.
25. Tonelli F, Lim KG, Loveridge C, Long J, Pitson SM, Tigyi G, Bittman R, Pyne S and Pyne NJ (2010) FTY720 and (S)-FTY720 vinylphosphonate inhibit sphingosine kinase 1 and promote its proteasomal degradation in human pulmonary artery smooth muscle, breast cancer and androgen-independent prostate cancer cells. *Cellular signalling* 22:1536-1542.
26. Ren S, Xin C, Pfeilschifter J and Huwiler A (2010) A novel mode of action of the putative sphingosine kinase inhibitor 2-(p-hydroxyanilino)-4-(p-chlorophenyl) thiazole (SKI II): induction of lysosomal sphingosine kinase 1 degradation. *Cellular physiology and biochemistry* 26:97-104.

Figures

Figure 1

Effects of palmitate and CA on SPHK1 gene expression in PBMCs of healthy subjects and patients with T2D. Left side of the graph shows mRNA expression ($2^{-\Delta Ct}$) and right side of the graph shows mRNA fold change ($2^{-\Delta\Delta Ct}$). The non-dotted and dotted lines show fold change for healthy subjects and patients with T2D, respectively. Data are shown as mean $\pm$ SD. * $p < 0.05$ (between group), + $p < 0.05$ compare to untreated cells (within group), # $p < 0.05$ compare to palmitate treated cells (within group).

Figure 2

Effects of palmitate and CA on S1PR1 gene expression in PBMCs of healthy subjects and patients with T2D. Left side of the graph shows mRNA expression ($2^{-\Delta Ct}$) and right side of the graph shows mRNA fold change ($2^{-\Delta\Delta Ct}$). The non-dotted and dotted lines show fold change for healthy subjects and patients with T2D, respectively. Data are shown as mean $\pm$ SD. +p < 0.05 compare to untreated cells (within group), #p < 0.05 compare to palmitate treated cells (within group).

Figure 3

Effects of palmitate and CA on S1PR1 concentration in PBMCs of healthy subjects and patients with T2D. Data are shown as mean $\pm$ SD. +p < 0.05 compare to untreated cells (within group), #p < 0.05 compare to palmitate treated cells (within group).