Kaempferide Improves Glycolipid Metabolism Disorder by Activating PPAR γ in High-Fat-Diet-Fed Mice

Qingfu Zeng

Chongqing Medical University First Affiliated Hospital

Heng Tang

Chongqing Medical University First Affiliated Hospital

Ting Tang

Chongqing Medical University First Affiliated Hospital

Peng Pu (✉ pp841103@sina.com)

Chongqing Medical University First Affiliated Hospital <https://orcid.org/0000-0003-3863-5944>

Research

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Abstract

Background: Kaempferide (Ka, 3,5,7-trihydroxy-4'-methoxyflavone), an active ingredient of *Tagetes erecta* L has been demonstrated to possess many pharmacological effects, including antioxidant, anti-inflammation, anticancer and antihypertension in previous study. However, there is no evidence of Ka on metabolic disorder in former studies. This study investigated the effects of Ka on glycolipid metabolism and explored the underlying mechanisms of action in vivo and vitro.

Methods: High-fat diet (HFD) was used to induce the model of glycolipid metabolism disorder in mice. The hypolipidemic and hypoglycemic effect was detected by several indicators, like blood sample analysis blood glucose, serum insulin, HOMA index and intraperitoneal glucose tolerance tests (IPGTT). The signaling pathways of lipid metabolism (PPAR γ /LXR α /ABCA1) and glucose metabolism (PPAR γ /PI3K/AKT) were evaluated using Real-Time PCR and Western blot. The primary culture of hepatocytes was prepared to confirm the target of Ka by co-culturing with PPAR γ agonist or inhibitor.

Results: Administration of Ka at a dose of 10mg/kg for 16 weeks effectively attenuated obesity, hyperlipidemia, hyperglycemia and insulin resistance in HFD mice. Further studies revealed the hypolipidemic and hypoglycemic effects of Ka depended on the activation of PPAR γ /LXR α /ABCA1 pathway and PPAR γ /PI3K/AKT pathway, respectively. The primary hepatocyte test, co-cultured with PPAR γ agonists or inhibitors, further confirmed the above signaling pathway and key protein.

Conclusion: Ka played an important role in improving glycolipid metabolism disorder, which were causally associated with weight loss. The underlying mechanisms might be associated with the activation of PPAR γ and its downstream signaling pathway. Our study helped to understand the pharmacological actions of Ka, and provides theoretical basis for Ka in the effective treatment of obesity, diabetes and other metabolic diseases.

1. Introduction

As the main source of energy supply, glucose and lipid play a key role in life activities. Long term intake of a large number of calories can induce the glycolipid metabolism disorder which damage the organs of the whole body^[1]. Glycolipid metabolism disorder occurs in several tissues, including liver, muscle and fat. It was always found in various metabolic disorders, such as obesity, diabetes, non-alcoholic liver disease, hypertension and coronary heart disease^[2, 3]. The glycolipid metabolism disorder has become a global disease and is an important cause of death and disability for patients^[1].

Kaempferide (Ka), 3,5,7-trihydroxy-4'-methoxyflavone, one of the main active ingredients from *Tagetes erecta* L, is a natural product which possesses known anti-inflammatory and antioxidant properties^[4, 5]. The previous research suggested that it has anticancer, antihypertension effects and cardiovascular protection^[6, 7]. Recent studies demonstrated that Ka might promote GLUT4 translocation in L6 myotubes of skeletal muscle and exerted a anti-adipogenic activity in 3T3-L1 Cells^[8]. Based on the results, we

believed that Ka may affect the pathogenesis of glycolipid metabolism disorder. However, This hypothesis had not been confirmed by research, whether in *vivo* or *vitro*.

In this present study, we investigated the hypolipidemic and hypoglycemic effects of Ka using a obesity animal model induced by HFD and cell model induced by high glucose. For the underlying mechanisms, we explored the target of Ka and the key pathway of glycolipid metabolism.

2. Material And Methods

2.1 Materials

Kaempferide, purity $\geq 92\%$, was obtained from Hubei ChuShengWei Chemistry Co. Ltd (Hubei, China). Commercial kits for the measurement of triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL) were obtained from Princeton Biotechnology Co., Ltd (Shanghai, China). Insulin was purchased from Novo Nordisk (China) Pharmaceutical Co., Ltd (China). Glucose was bought from Wuhan Fuxing Biological Pharmaceutical Co., Ltd. ELISA kit for insulin was acquired from R&D Systems. Fetal calf serum (FCS) was obtained from Invitrogen. Primers were synthesized by Sangon Biotechnology (Shanghai) Co., Ltd. The antibodies used to recognize PPAR γ and P85 α /AKT signaling pathways were bought from Cell Signaling Technology. Tissue culture grade kaempferide was obtained from Nanjing Aikang Chemical Co., Ltd. Cell culture reagents and all other reagents were purchased from Sigma or China National Medicines Co., Ltd. All chemicals and reagents were analytical grade.

2.2. Animal experiments

2.2.1 Animal model and diet

The study got approval from the Animal Ethics Committee of the Second Affiliated Hospital of Nanchang University. C57BL/6 J male mice, aged 7 weeks, were purchased from the Experimental Animal Center of Nanchang University. The feeding methods and model establishment had been published in our previous studies[9]. The mice were divided into four groups: normal-diet-fed mice (ND group, n = 12), normal-diet-fed mice treated with Ka (10 mg/kg/day, ND + Ka group, n = 12), high fat-diet-fed mice (HFD group, n = 12), high fat-diet-fed mice treated with Ka (10 mg/kg/day, HFD + Ka group, n = 12). The food consumption was measured once a week and the bodyweight twice per week.

2.2.2 Sample collection and preparation

Mice were fed for 16 weeks. At the end of the experiments, animals were fasted overnight, weighed and sacrificed under anesthesia. Blood samples were collected and centrifuged at 4000 rpm, 30 min at 4 °C. Liver was excised for weight and fatty liver index calculation (Index [%] = liver weight [g]*body weight [g]⁻¹*100). A section of each liver was used for molecular experiments. Visceral fat (perirenal, retroperitoneal, epididymal fat pad) was also excised and weighed for adiposity index calculation (adiposity index = white adipose tissue weight[g]/body weight[g] × 100).

2.2.3 Evaluation of insulin resistance

As described previously^[10], intraperitoneal glucose tolerance tests (IPGTT) was completed one week before sacrifice. Mice were fasted for 6 h (8:00–14:00). Blood glucose concentrations were measured at 0, 15, 30, 60, and 120 min after i.p. injection of glucose (2 g/kg). The areas under the curve (AUC) were calculated according to the formula:

$$AUC_{0-120\text{ min}} = (G_0 + G_{15}) \times 15/2 + (G_{15} + G_{30}) \times 15/2 + (G_{30} + G_{60}) \times 30/2 + (G_{60} + G_{120}) \times 60/2.$$

Insulin concentrations were analyzed with ELISA method. Insulin resistance (IR) was evaluated by the homeostasis model assessment-insulin resistance formula (HOMA-IR), homeostasis model assessment of insulin secretion (HOMA-IS), homeostasis model assessment of β cell function (HOMA- β), quantitative insulin sensitivity check index (QUICKI) ^[11].

2.3 Cell culture and treatment

The protocol of hepatocyte culture had been published in our previous researches^[12]. The primary hepatocytes were divided into five groups: normal glucose group (NG, 5 μ M), high glucose group (HG, 25 μ M), high glucose group incubated with PPAR γ agonist (HG + Ag), high glucose group incubated with kaempferide (HG + Ka), high glucose group incubated with kaempferide and PPAR γ inhibitor (HG + Ka + In). The cells were serum-starved for at least 12 h before the experiment and then preincubated with the kaempferide (30 μ M), or PPAR γ agonist (rosiglitazone, 10 μ M) or PPAR γ inhibitor (T0070907, 1 μ M) for 6 h, according to different treatment. Finally, all hepatocytes were stimulated with normal glucose or high glucose for 2 h. Viability was determined by methylthiazolyldiphenyl- tetrazolium bromide (MTT) assay.

2.4 Gene Expression Analysis

For Real-Time PCR, total RNA was extracted from frozen pulverized mouse liver (n = 6) and cultured hepatocytes (n = 4) using TRIzol (Invitrogen), then was transcribed by two-step method with Super script First-Strand Synthesis System. The primers sequences were listed in Table 1. The PCR products were quantified with the SYBR Green PCR Master Mix (Applied Biosystems), and the results were normalized to β -actin gene expression.

Table.1

Sequences of the primers used in the PCR measurements.

Gene	Sense	Sequence (5' to 3')
PPAR γ	PPAR γ -FWD	ATTCTGGCCCACTTTCGG
	PPAR γ -REV	TGGAAGCCTGATGCTTTATCCCCA
LXR- α	LXR- α -FWD	GCTCAGGAGCTGATGATCCA
	LXR- α -REV	GCGCTTGATCCTCGTGTAG
ABCA1	ABCA1-FWD	CAAGGATGGCCATAATGGTCA
	ABCA1-REV	GGCCACATCCACAACTGTCTG
β -actin	β -actin -FWD	CCACTGCCGCATCCTCTTCCTC
	β -actin -REV	TCCTGCTTGCTGATCCACATCT

2.5 Western blotting

For western blot analysis, liver tissue (n = 4) and primary hepatocytes were lysed in radioimmunoprecipitation (RIPA) lysis buffer. Supernatants were gathered, and the protein concentration was determined using a BCA assay kit. In total, 50 μ g of liver tissue lysate or 20 μ g of cell lysate was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and proteins were then transferred to an FL membrane (Millipore). The specific protein expression levels were normalized to GAPDH.

2.6 Statistical analysis

Data were expressed as mean $\pm$ SE. The significance of differences between groups were analyzed statistically using a two-way analysis of variance (ANOVA), followed by a Tukey's multiple-comparison post hoc test. Differences were considered significant at $P < 0.05$.

3. Results

3.1 Glycolipid metabolism disorder was induced by high-fat diet in C57 mice obesity,

In this study, the mice, fed a high-fat diet, developed abdominal obesity (Table 2), hyperlipidemia, hyperglycemia, and insulin resistance (Table 3, Fig. 1), which was proved as a successful establishment of animal model. Not any overall difference exists in food intake for all mice ($P > 0.05$), however, the energy input was more in mice with HFD ($P < 0.05$, Table 2).

Table 2
Kaempferide decreased obesity and organ weights in C57 mice(n = 12).

	ND	ND + Ka	HFD	HFD + Ka
Body weight(g)	30.4 ± 0.96	30.7 ± 1.02	38.2 ± 1.11**	35.1 ± 0.92 [#]
Food intake(g/d)	3.27 ± 0.19	3.24 ± 0.17	3.36 ± 0.21	3.25 ± 0.27
Calorie intake(kcal/d)	12.4 ± 0.65	12.3 ± 0.62	15.4 ± 0.74*	14.9 ± 0.82
Liver weight(g)	1.14 ± 0.07	1.07 ± 0.08	1.78 ± 0.15**	1.30 ± 0.09 [#]
White adipose tissue(g)	1.11 ± 0.13	1.14 ± 0.16	3.26 ± 0.52**	2.12 ± 0.31 [#]
Liver Index(%)	3.75 ± 0.24	3.49 ± 0.26	4.66 ± 0.28*	3.70 ± 0.28 [#]
Adiposity Index(%)	3.65 ± 0.26	3.71 ± 0.33	8.27 ± 0.68**	6.01 ± 0.55 ^{##}
<p>Note: Ka-treated obese mice were compared with obese animals and with the controls. Ka lowered the body weights, organ weight and the index. Visceral fat includes epididymal fat pad, mesentery fat tissue and abdominal adipose tissue. Adiposity Index = white adipose tissue weight(g) / body weight(g) × 100. So was the liver index. All values are the mean ± SEM. *<i>P</i> < 0.05, **<i>P</i> < 0.01 vs ND, [#]<i>P</i> < 0.05, ^{##}<i>P</i> < 0.01 vs HFD.</p>				

Table 3
Effects of kaempferide on glycolipid metabolism in C57 mice(n = 12).

	ND	ND + Ka	HFD	HFD + Ka
Serum TC (mM)	1.98 ± 0.35	2.13 ± 0.41	4.76 ± 0.52**	3.22 ± 0.41 [#]
Serum TG (mM)	0.67 ± 0.08	0.53 ± 0.06	1.22 ± 0.24*	0.77 ± 0.11 [#]
Serum HDL (mM)	1.23 ± 0.15	1.46 ± 0.22	3.25 ± 0.36**	2.17 ± 0.24 ^{##}
Serum LDL (mM)	0.52 ± 0.05	0.39 ± 0.07	1.16 ± 0.11**	0.76 ± 0.12 ^{##}
Blood glucose(mmol/L)	5.58 ± 0.64	5.67 ± 0.63	8.63 ± 1.02*	6.12 ± 0.93 [#]
Serum insulin(mIU/L)	11.56 ± 1.25	12.47 ± 1.37	56.12 ± 6.68**	28.34 ± 3.22 ^{##}
HOMA-IR	2.87 ± 0.21	3.14 ± 0.36	21.53 ± 2.54**	7.71 ± 1.21 ^{##}
HOMA-IS	0.35 ± 0.04	0.32 ± 0.03	0.05 ± 0.01**	0.13 ± 0.02 [#]
HOMA-β	111.15 ± 2.63	114.93 ± 3.76	218.79 ± 4.38**	216.34 ± 4.12
QUICKI	0.55 ± 0.03	0.54 ± 0.03	0.37 ± 0.02**	0.45 ± 0.02 [#]
Note: Ka treatment promoted a significant decrease in serum lipid levels and insulin resistance in comparison to HFD animals. All values are the mean ± SEM. All values are the mean ± SEM. * <i>P</i> < 0.05, ** <i>P</i> < 0.01 vs ND, [#] <i>P</i> < 0.05, ^{##} <i>P</i> < 0.01 vs HFD.				

3.2 Kaempferide alleviated obesity and glycolipid metabolism disorder

3.2.1 Kaempferide reduced obesity state

The mice with HFD developed obesity indicating an increased body weight (Table 2, *P* < 0.05). The average body weight reached 38.2 ± 1.11 g in the HFD group versus 30.4 ± 0.96 g in the ND group before sacrifice(*P* < 0.01). And Ka showed favorable changes in obesity and several other obesity-related parameters, such as liver weight, fatty liver index, visceral fat weight, and visceral fat index, as detailed in Table 2 (*P* < 0.05).

3.2.2 Kaempferide improved abnormal glycolipid metabolism and insulin sensitivity

In the current study, hyperlipidemia was induced in mice fed a high-fat diet. At the end of the experiment, the levels of serum TC were significantly increased by 140.4% in the HFD group (*P* < 0.01) compared with

the ND group. Treatment with Ka significantly reduced TC levels in the HFD mice ($P < 0.01$, Table 3). The levels of serum TG, LDL and HDL presented a trend similar to those of serum TC ($P < 0.05$).

The mice with HFD also presented a notable increase in the blood glucose and serum insulin levels compared to the ND group ($P < 0.01$). Treating with Ka evidently reversed these changes ($P < 0.05$). The changes of other glycometabolism indicators, like HOMA-IR, HOMA-IS, QUICKI, further suggested that Ka could improve insulin sensitivity ($P < 0.05$). The detailed data were shown in Table 3.

Figure 1 showed the IPGTT results in mice. The hyperglycemia was observed at all test points in HFD mice after glucose loading (Fig. 1A). Similarly, the area under AUC_{0-2h} curve of the glucose response was significantly increased compared to that of the ND group ($P < 0.01$, Fig. 1B). After Ka treatment, the hyperglycemia was restrained ($P < 0.05$), and the area under AUC_{0-2h} curve was significantly reduced ($P < 0.01$).

3.3 The mechanism of kaempferide on glycolipid metabolism disorder

3.3.1 Molecular changes of hepatic genes involved in lipometabolism in vivo

PPAR γ is the key transcriptional regulator of adipogenesis, which is expressed in the liver. we evaluated the mRNA levels of PPAR γ , LXR α and ABCA1. Ka supplementation did not affect the mRNA levels in ND group compared with ND + Ka group ($P > 0.05$, Table 4). However, feeding with high-fat diet, the mRNA expressions of PPAR γ , LXR α and ABCA1 decreased by 68%, 57% and 48%, respectively ($P < 0.01$). Treating with Ka, the decreased mRNA levels returned to normal ($P < 0.01$).

Table 4
Changes in hepatic expressions of genes involved in lipid metabolism (n = 6)

	ND	ND + Ka	HFD	HFD + Ka
PPAR γ	1.00 $\pm$ 0.10	1.43 $\pm$ 0.24	0.32 $\pm$ 0.07*	0.78 $\pm$ 0.14 [#]
LXR α	1.00 $\pm$ 0.11	1.13 $\pm$ 0.14	0.43 $\pm$ 0.08*	1.02 $\pm$ 0.21 [#]
ABCA1	1.00 $\pm$ 0.11	1.23 $\pm$ 0.23	0.52 $\pm$ 0.12*	1.12 $\pm$ 0.16 [#]
Note: The expressions of PPAR γ -mediated lipid synthesis gene. Relative mRNA levels are expressed as a ratio relative to β -actin. All values are the mean $\pm$ SEM. * $P < 0.01$ vs ND, [#] $P < 0.01$ vs HFD.				

3.3.2 Changes of key protein expressions of PPAR γ /PI3K/AKT signaling pathway in vivo

PI3K / Akt signaling pathway is closely related to glucose metabolism. In Fig. 2, feeding with HFD could decrease the levels of P-P85α and P-Akt. However, these effects were abolished by Ka treatment. Moreover, Ka activated PPARγ which is an upstream regulator linking the PI3K/AKT signaling pathway (Fig. 2A). The semi-quantitative analysis provided more evidence that Ka could improve glycometabolism by acting on PPARγ/PI3K/AKT signaling pathway (Fig. 2B).

3.3.3 Molecular changes of hepatic genes involved in lipometabolism in vitro

In order to confirm the conclusion that Ka could inhibit lipid synthesis by activating PPARγ, we detected the changes of PPARγ / LXR α / ABCA1 signaling pathway *in vitro*(Table 5). Similar to the results of animal experiments, high glucose stimulation did down-regulate this signaling transduction pathway. Ka was able to reverse these changes, just as the PPAR agonist did. After CO incubation of Ka and T0070907, the PPARγ activation of Ka was neutralized.

Table 5
Effects of kaempferide on PPARγ-mediated lipid synthesis pathway in *vitro* (n = 4)

	NG	HG	HG + Ag	HG + Ka	HG + Ka + In
PPARγ	1.00 ± 0.10	0.35 ± 0.14 [*]	1.34 ± 0.25 ^{##}	0.97 ± 0.18 [#]	0.24 ± 0.08 ^{&}
LXRα	1.00 ± 0.10	0.41 ± 0.06 ^{**}	0.94 ± 0.11 ^{##}	0.85 ± 0.09 ^{##}	0.33 ± 0.06 ^{&&}
ABCA1	1.00 ± 0.10	0.32 ± 0.21 ^{**}	1.06 ± 0.32 ^{##}	0.86 ± 0.26 ^{##}	0.19 ± 0.08 ^{&&}
Note: The expressions of PPARγ-mediated lipid synthesis gene in <i>vitro</i> . Relative mRNA levels are expressed as a ratio relative to β-actin. NG = normal-glucose group (5 μM), HG = high-glucose group (25 μM), HG + Ag = high- glucose + PPARγ agonist (rosiglitazone, 10 μM), HG + Ka = high-glucose + Ka at 30μM HG + Ka + In = high-glucose + Ka(30μM)+PPAR γ inhibitor (T0070907, 1 μM). All values are the mean ± SEM. [*] <i>P</i> < 0.05, ^{**} <i>P</i> < 0.01 vs NG, [#] <i>P</i> < 0.05, ^{##} <i>P</i> < 0.01 vs HG, ^{&} <i>P</i> < 0.05, ^{&&} <i>P</i> < 0.01 vs HG + Ka.					

3.3.4 Changes of key protein expressions of PPARγ mediated glycometabolism signaling molecules in vitro

To confirm the previous results *in vivo*, we carried out the primary hepatocytes cultured test. We examined the expressions of PPARγ and the phosphorylation of P85α and AKT(Fig. 3). In accordance to the results identified in the animal studies, Ka could activate PPARγ / PI3K / Akt signaling pathway, just like PPAR agonist did. And PPAR inhibitor could inhibit the activity of Ka.

4. Discussion

Our research identified for the first time that Ka could be against glycolipid metabolism disorder and reversed existing insulin resistance in *vivo* and in *vitro*. These protective effects depended on PPARγ-mediated activation of the LXRα/ABCA1 and PI3K/AKT signaling pathway. Our research suggested that

the target of Ka was PPAR γ , and Ka was a PPAR γ activator. This pre-clinical study will make the researchers have a better understanding of the pharmacological action of Ka. Meanwhile, it provided clues for developing efficacious natural-based products to achieve glycolipid metabolism disorder.

At a dose of 10 mg/kg/day, we found that Ka owned a novel and promising hypolipidemic and hypoglycemic effects in the model mice, showing a great improvement in a range of glycolipid metabolism disorder. It was consistent with our hypothesis. The key target that Ka ameliorated glycolipid metabolism disorder was to activate PPAR γ , which was consistent with the prediction of Wang JY et al^[13]. In that study, researchers used computer simulation to predict that Ka might act on multiple sites and PPARs may be an important target. Our research confirmed this inference.

It is well known that PPAR γ can regulate all aspects of fatty acid metabolism^[14]. It can increase the expressions of fatty acid transport protein and translocase, then stimulate the fatty acid intake for acyl CoA transformation, which promotes the lipids oxidative metabolism^[15, 16]. PPAR γ is expressed in most tissues and could be activated by ligands to induce LXRA overexpression which regulates the reverse transport of cholesterol^[16]. LXRA can effectively block or delay the occurrence and development of hyperlipidemia by regulating cholesterol outflow transporter ABCA1, promoting cholesterol outflow to apoA-1^[17, 18]. Our study was consistent with the research conclusion of kumkarnjana s et al^[8]. However, the latter was only verified in *vitro*, and its specific signaling transduction pathway was not clear. For the first time, our study confirmed that the hypolipidemic effect of Ka was realized by activating PPAR γ -LXRA-ABCA1 signaling pathway.

PPAR γ regulates glucose metabolism mainly by increasing the sensitivity of peripheral tissues to insulin, stimulating muscle glucose utilization and inhibiting hepatic glycogen output^[19, 20]. PPAR γ may play the role of insulin sensitization through the following ways. (1)PI3K is a key kinase for glucose to enter cells. Activated PPAR γ could promote PI3K/AKT signal pathway for enhancing insulin sensitivity^[21]; (2)Activated PPAR γ can promote the GLUT4 expression, increase the glucose uptake and improve insulin resistance^[22]; (3)PI3K activation could accelerate the TG decomposition in peripheral tissue, increase its synthesis in adipose tissue and inhibit the production of glucagon^[23]. Our study confirmed that the hypoglycemic effect of Ka depended on the activation of PI3K/Akt, which was consistent with the study of Wang D et al. That study showed that Ka attenuated I/R-induced myocardial injury through PI3K/Akt/GSK 3 β pathway^[7]. Our study also showed that Ka reduced TG, which may also be related to the activation of PI3K.

Our work has provided original evidence that Ka as a natural molecule simultaneously possessed weight loss, hypolipidemic and hypoglycemic effects, highlighting the key underlying mechanisms. However, how Ka regulates the PPAR γ in *vivo* and its detailed mechanism, are still not known. Furthermore, it would be interesting to explore the basic pharmacokinetics of Ka as an antiobesity or anti-diabetes agent. Thus, further work is warranted to elucidate the potential of Ka as a new and efficacious natural-based therapy for obesity, diabetes, nonalcoholic hepatitis and other metabolic diseases.

5. Conclusion

The conclusion from the data was that Ka can effectively improve HFD-induced glycolipid metabolism disorder. This property was related to the activation of PPAR γ and its downstream signaling pathway(PPAR γ /LXR α /ABCA, PPAR γ /PI3K/AKT). Based on the above evidence, Ka might be a promising therapeutic agent for obesity, diabetes mellitus, nonalcoholic fatty liver disease and other metabolic diseases. Our clinical research has been prepared and is to carry out soon. Foundational research will also further explore the additional mechanisms and the other therapeutic targets.

6. List Of Abbreviation

Ka (Kaempferide)

HFD (high-fat diet)

ND (normal-diet-fed mice)

NG (normal glucose group)

HG (high glucose group)

IPGTT (intraperitoneal glucose tolerance tests)

TG (triglyceride)

TC (total cholesterol)

HDL (high density lipoprotein)

LDL (low density lipoprotein)

FCS (Fetal calf serum)

HOMA-IR (homeostasis model assessment-insulin resistance formula)

HOMA-IS (homeostasis model assessment of insulin secretion)

HOMA- β (homeostasis model assessment of β cell function)

QUICKI (quantitative insulin sensitivity check index)

7. Declarations

7.1 Ethics approval and consent to participate

The study got approval from the Animal Ethics Committee of the Second Affiliated Hospital of Nanchang University.

7.2 Consent for publication

All the authors have made a significant contribution to this manuscript, have seen and approved the final manuscript, and have agreed to its submission to the "Nutrition & Metabolism"

7.3 Availability of data and material

As part of our basic and clinical research has not been completed, we intend to apply for patent at the right time, and we will not disclose these data temporarily. If researchers want to verify the results of the article, copy the analysis, and conduct a secondary analysis, we can provide some data.

7.4 Competing interests

We declare that we have no conflict of interest.

7.5 Funding Sources

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7.6 Author Contributions

Qingfu Zeng: Substantial contributions to the conception or design of the work and analysis and interpretation of data for the work

Heng Tang: Acquisition analysis and interpretation of data for the work and revising manuscript critically for important intellectual content

Ting Tang: Design of the work and analysis of data for the work

Peng Pu: Drafting the work important intellectual content and final approval of the version to be published. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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Figures

Figure 1

Figure 1

Effects of kaempferide on IPGTT (A) IPGTT performed on mice. (B) The corresponded AUC over 2 hours was shown. Values are expressed as Mean±SEM. *P<0.01 vs ND, #P<0.05, ##P<0.01 vs HFD.

Figure 2

Figure 2

The possible molecular mechanisms of kaempferide in attenuating glycometabolism disorder induced by high-fat diet (A) Representative images of Western blot analysis examining the expressions of PPAR γ /PI3K/AKT signaling pathway in each group; (B) Quantification of the expressions of the key proteins (n=4). GAPDH was used as internal control. ND=normal diet group, ND+Ka=normal diet+Ka at 10mg/kg/day, HFD=high-fat diet, and HFD+Ka=high fat diet+Ka at 10mg/kg/day. Values are expressed as Mean $\pm$ SEM. *P<0.05, **P<0.01 vs ND, #P<0.01, ##P<0.01 vs HFD.

Figure 3

Figure 3

Western blot analysis of the effects of kaempferide on PPAR γ mediated signaling molecules in vitro induced by high-glucose (A) Representative images of Western blot analysis examining the expressions of PPAR γ /PI3K/AKT signaling pathway in each group; (B) Quantification of the expression of the key proteins (n=4). GAPDH was used as internal control. NG = normal-glucose group (5 μ M), HG=high-glucose group (25 μ M), HG+Ag=high- glucose+PPAR γ agonist (rosiglitazone, 10 μ M) HG+Ka=high-glucose+Ka at 30 μ M HG+Ka+In=high-glucose+Ka 30 μ M+PPAR γ inhibitor (T0070907, 1 μ M). Values are expressed as Mean $\pm$ SEM. *P<0.05, **P<0.01 vs NG, #P<0.05, ##P<0.01 vs HG, &P<0.05, &&P<0.01 vs HG+Ka.