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Fucoidan from the sea cucumber *Acaudina molpadioides* exhibits anti-adipogenic activity by modulating the Wnt/β-catenin pathway and down-regulating the SREBP-1c expression

Hui Xu, Jingfeng Wang,* Yaoguang Chang, Jie Xu, Yuming Wang, Tengteng Long and Changhu Xue *

A novel fucoidan, which consists of a 1→3-linked tetrafucose repeating unit that is distinctive in its sulphation pattern, was isolated from the sea cucumber *Acaudina molpadioides*. In the present study, we examined the anti-adipogenic effect of the fucoidan from *Acaudina molpadioides* (*Am*-FUC) in vitro and in vivo. Results showed that *Am*-FUC exhibited an inhibitory effect on the proliferation and differentiation of 3T3-L1 cells. *Am*-FUC suppressed the differentiation of 3T3-L1 cells, decreasing the content of intracellular triglyceride by 34.07% at the concentration of 200µg/ml. In vivo experiments showed that the subcutaneous, perirenal and epididymal fat content of *Am*-FUC-treated mice were significantly reduced compared to the HFFD-fed mice. A reverse transcriptase-polymerase chain reaction assay revealed that *Am*-FUC significantly increased the mRNA expressions of Wnt/β-catenin pathway related factors, namely, Wnt10b, β-catenin, Fz and LRP5 and decreased that of the key transcriptional factors, such as SREBP-1c, PPARγ and C/EBPα. β-catenin acts as an anti-adipogenic factor to inhibit the expression of PPARγ and C/EBPα, while SREBP-1c can promote the adipocyte differentiation by enhancing the activity of PPARγ. Western blotting results showed that *Am*-FUC significantly increased the protein level of the total β-catenin and nuclear β-catenin and suppressed that of the SREBP-1c. *Am*-FUC also significantly inhibited the mRNA expressions of the lipid synthesis related genes such as FAS and GPAT, while had no effect on that of the lipolysis related genes such as HSL and ATGL. These findings suggest that *Am*-FUC possesses marked anti-adipogenic activity by modulating the Wnt/β-catenin pathway and down-regulating the expression of SREBP-1c.

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Introduction

Adipocytes have long been regarded as passive vessels for the storage of excess energy. However, recent studies show that adipocytes can also act as endocrine cells by actively secreting various adipocytokines to regulate glucose and lipid metabolism. The exhausted capacity of white adipose tissue to store lipid or the deregulated adipocytokine secretion from the expanded adipocytes caused by obesity contribute to the development of systemic insulin resistance and metabolic diseases, such as type 2 diabetes, hypertension and hyperlipidaemia. Adipocyte differentiation, namely adipogenesis, is the process of changes in cell morphology, hormone sensitivity and genes expression. Studies show that adipogenesis involves many stages related to obesity and disordered adipocyte differentiation may lead to the pathogenesis of obesity-associated conditions.

Many signaling pathways participate in regulating the adipogenesis and one of them is Wnt/β-catenin signaling pathway. Wnt proteins can regulate various cellular activities, including cell proliferation and cell differentiation, through activating the cell surface receptor-mediated signal transduction pathways. There are several Wnt isoforms in the Wnt proteins family and the one involved in the adipogenesis is Wnt10b. Once secreted, Wnt10b combines to the cell surface Lipoprotein receptor-related protein 5 (LRP5)/Frizzled (Fz) receptor complex and activates dishevelled to initiate the Wnt/β-catenin signaling pathway. Dishevelled helps to prevent the formation of the destruction complex and release β-catenin, which then translocates to the nucleus and binds to the T cell factor/lymphoid enhancer factor (TCF/LEF) family to mediate the Wnt response genes. CCAAT/enhancer binding protein-α (C/EBPα) and peroxisome proliferator-activated receptor-γ (PPARγ) are key transcriptional factors in the adipogenesis. Researches show that they can be regulated by various upstream transcriptional factors, such as β-catenin and sterol regulatory element-binding protein-1c (SREBP-1c), an activator of the PPARγ ligand.

The sea cucumber *Acaudina molpadioides*, with little edible value, is widely distributed in bay of Bengal, Indonesia, Philippines, Australian, Japan and China. It contains high content of fucoidan, possessing up to 3.8% of the body wall dried matter. Fucoids from the sea cucumber are mainly composed of L-fucose and sulphate ester groups. They are linear polysaccharides consisted of regular tandem
repeat, e.g. di-, tri- or tetrasaccharide repeating unit, with diverse glycosidic linkages and sulfation patterns.\textsuperscript{16} Am-FUC consists of a 1→3-linked tetrafucose repeating unit and its sulfation pattern is different from other investigated fucoidans.\textsuperscript{17} The chemical structure of Am-FUC was deduced to be \[-3-\alpha-L\text{-fuc}2,4(\text{OSO}_3)\text{-fuc}-1\rightarrow3-\alpha-L\text{-fuc}-1\rightarrow3-\alpha-L\text{-fuc}-1\rightarrow\ldots\text{]}_n\) (Figure 1).\textsuperscript{17} There are limited reports on the biological activities of the fucoidan from the sea cucumber. Wang et al. proved that Am-FUC protected the ethanol-induced gastric damage.\textsuperscript{15} Kariya et al. found that the fucoidan extracted from the sea cucumber \textit{Stichopus japonicus} inhibited osteoclastogenesis.\textsuperscript{18} To the best of our knowledge, no information is available about the anti-adipogenic activity of the fucoidan from the sea cucumber. Here, for the first time, we investigated the anti-adipogenic activities of the fucoidan from the sea cucumber \textit{Acaudina molpadioides} which possesses novel sulphation pattern,\textsuperscript{17} and the underlying mechanism was further researched by studying the Wnt/\(\beta\)-catenin pathway related factors.

\textbf{Materials and Methods}

\textbf{Materials}

Dried \textit{Acaudina molpadioides} was purchased from a seafood market in Qingdao, China and was identified by Professor Yulin Liao of the Chinese Academy of Sciences Institute of Oceanography (Qingdao, China). The Dulbecco’s modified eagle medium (DMEM) was purchased from Gibco (Gaithersburg, MD, USA). The Fetal bovine serum (FBS) and trypsin was Hyclone product (Logan, UT, USA). Isobutylmethylxanthine (IBMX), dexamethasone (DEX) and insulin were all provided by Sigma (St. Louis, MO, USA). TRIzol reagent was Invitrogen products (California, USA). Moloney murine leukemia virus reverse transcriptase (M-MLV) was from TaKaRa Bio Inc (Otsu, Shiga, Japan). Rabbit anti-rat \(\beta\)-catenin, SREBP-1c and \(\beta\)-actin were Abcam products (Burlingame, CA, USA). Western blot IP lysis buffer, BCA protein concentration kit, and super-enhanced chemiluminescence (ECL) detection kit were provided by Applygen Technologies Inc (Beijing, China). The primers of genes examined and \(\beta\)-actin were synthesized by ShanGon Ltd. Co. (Shanghai, China).

\textbf{Preparation of Am-FUC}
Am-FUC was isolated and purified using the methods described by Chang and Yu et al. Its molecular weight was 1614.1 kDa and the sulfate content is 26.3 ± 2.7%.

Cell lines and cell culture

3T3-L1 cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, America) and cultured in Dulbecco’s modified eagle medium (DMEM) with 10% (v/v) Fetal bovine serum (FBS), 100µg/ml streptomycin, and 100U/ml penicillin at 37°C in a humidified atmosphere of 5% CO₂. All the experiments were repeated at least three times to ensure the accuracy.

Animals

Male C57BL/6J mice (4 - 5 weeks) were purchased from Vital River Laboratory Animal Center (Beijing, China; Licensed ID: SCXK2009-0007). The animals were housed in a 12-12 h light-dark condition at a temperature of 23 ± 1 °C daily. The use of animals in this study was approved by the ethical committee of experimental animal care at Ocean University of China.

Adipocyte differentiation

3T3-L1 cells were induced into mature adipocytes using the standard hormonal cocktail method. Briefly, 3T3-L1 cells were seeded into culture plates and cultured to be confluent. Having been contact inhibited for 2 days, cells were cultured with the differentiation medium containing 0.5 mM IBMX, 1 µM DEX, 10 µg/ml insulin and 10% FBS (marked as day 0). After 2 days, the culture medium was replaced by DMEM medium containing 10% FBS and 10µg/ml insulin (day 2). Then the medium was changed with the complete medium at day 4 and day 6. Different concentrations of Am-FUC were added at each medium change during the differentiation from day 0. The adipocytes were collected at day 8.

3T3-L1 cells viability assay

Preadipocytes or mature adipocytes of 3T3-L1 cells (2×10⁴/ml) were seeded in 96-well plates. After 24 hours, cells were treated with different concentrations of Am-FUC (0, 100, 200, 400µg/ml) for 96 h. Cell viability was determined by MTT method and the cell culture supernatant was collected. The activity of the lactic
dehydrogenase (LDH) in the supernatant was examined using a LDH kit (Beyotime, Jiangsu, China).

To measure the viability of cells during the differentiation process, 3T3-L1 cells (2×10⁴/ml) were seeded into 96-well plates and induced to mature adipocytes. Cells were treated with Am-FUC (0, 100, 200, 400µg/ml) from day 0. Cell viability was measured at day 8 by MTT method.

**Oil-red O staining**

3T3-L1 cells (3×10⁴/ml) were seeded into 24-well plates and induced to mature adipocytes with the method described above. Cells were fixed with 10% fresh formaldehyde for 1 h, then washed with PBS and stained with filtered Oil-red O solution (60% isopropanol and 40% water) for half an hour at room temperature. Then, cells were washed with 60% isopropanol for three times. Images of the stained lipid droplets were collected using an inverted microscope (IX51, Olympus, Tokyo, Japan).

**Intracellular triglyceride assay**

3T3-L1 cells (3×10⁴/ml) were seeded into 24-well plates and induced to mature adipocytes with the method described above. Cells were then lysed with lysis solution for half an hour at 4°C. The cell lysate was repeatedly thawed and freezeed for three times and then centrifuged to get the supernatant. At last, the total triglyceride (TG) level was determined using the triglyceride assay kit (Aibio, Beijing, China) and the protein content was measures by bicinchoninic acid (BCA) kit (Beyotime, Jiangsu, China) to revise the result. TG content is expressed as the concentration of TG/ the concentration of Protein (mg/mg).

**Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis**

The expressions of genes that regulate the adipogenesis, such as Wnt10b, β-catenin, Fz, LRP5, SREBP-1c, PPARγ, C/EBPα and the lipid metabolism related genes, such as FAS, GPAT, HSL, ATGL were examined by RT-PCR. β-actin was used as a control. The total RNA of the adipocytes was extracted using TRIzol reagent. RNA (1µg) of each sample was reverse transcribed to cDNA by MMLV reverse transcriptase. Two microliters of the reverse transcription product was used for PCR.
The amplification cycling conditions were as follows: denatured at 94°C for 45s, annealing for 30s, extended at 72°C for 45s, followed by a 10-min extension at 72°C. The Primer sequences, annealing temperature and cycles performed of different genes are listed in Table 1. The PCR products were separated by electrophoresis on 1% agarose and stained with ethidium bromide (EB). Images were captured and the relative expression of mRNA was achieved by quantifying the densities of the bands with Image J software (Version 1.41o, NIH, USA).

**Western blotting analysis**

The protein expression levels of the key transcriptional regulator β-catenin and SREBP-1c were examined by western blotting. β-actin was used as the internal control for total protein and TATA box binding protein (TBP) was used as the endogenous control of nuclear protein. The adipocytes were lysed, centrifuged and proteins are collected. The proteins were then fractionated by SDS-PAGE, transferred to nitrocellulose, and incubated sequentially with antibodies against β-catenin and SREBP-1c and then with horseradish peroxidase-conjugated secondary antibodies. Protein bands were visualised using a Tiangen KC-420 Chemiluminescent Substrate Kit and quantified with Image J program (Version 1.41o).

**Analysis of nuclear β-catenin level**

3T3-L1 cells (3×10⁴/ml) were seeded into 24-well plates and induced to mature adipocytes. Cells were digested by trypsin and then nuclear extracts were obtained using a nuclear extract kit (Solarbio, Beijing, China). Protein content of the nuclear extracts was determined by the BCA protein kit. The protein level of nuclear β-catenin was examined by the western blotting analysis.

**In vivo anti-adipogeneic activity**

The obesity model mice were established by fed a high-fat-high-fructose diet (HFFD). The composition of the diet was listed in Table 2. The mice were randomly divided into four groups (10 animals each): control, HFFD-fed, 20mg/kg/d Am-FUC-treated and 80mg/kg/d Am-FUC-treated. The control group mice were fed with a low-fat-low-fructose diet and the others were all fed with HFFD. After 13 weeks, the subcutaneous, perirenal and epididymal fat of each mouse were measured. Then the
subcutaneous fat were fixed with 10% fresh formaldehyde, sectioned using paraffin sections method and stained with hematoxylin-eosin staining method.

Statistical analysis

All data were presented as mean ± standard deviation (SD) of at least three independent experiments. Differences between the means of the individual groups were assessed by one-way analysis of variance (ANOVA) followed by the Tukey's test. Differences were considered significant at \( P < 0.05 \).

Results

Am-FUC inhibited the proliferation activity of 3T3-L1 cells

The inhibitory effects of Am-FUC on the proliferation activity of 3T3-L1 preadipocytes and adipocytes were determined by MTT assay. As shown in Fig. 2, Am-FUC significantly inhibited the viability of the preadipocytes and adipocytes in a dose-dependent manner, with 15.93% \( (P < 0.05) \) and 29.52% \( (P < 0.01) \) reduction, respectively, when the cells were treated with 200 µg/ml Am-FUC for 96 h. To test whether the reduction is result of the cytotoxicity of Am-FUC, the LDH levels of the cell culture supernatant were measured. Figure 2 showed that the LDH levels of preadipocytes and adipocytes culture supernatant had no significant change, which means that Am-FUC had no cytotoxicity to preadipocytes and adipocytes.

Am-FUC exhibited anti-adipogenic activity both in vitro and in vivo

The lipid accumulation in 3T3-L1 cells were examined by Oil-red O staining and triglyceride assay. Figure 3 indicated that Am-FUC had a significant inhibitory effect on the differentiation of 3T3-L1 preadipocytes. In comparison with the control level, the TG content was decreased by 29.36% averagely after Am-FUC treatment. To know whether the reduction was associated with the inhibition on the proliferation, cells were induced to mature adipocyte and the viability of cells treated with Am-FUC from day 0 was measured by MTT assay at day 8. Figure 3 showed that Am-FUC has no significant effect on the viability of adipocytes during the differentiation process. To further verify the anti-adipogenic activity of Am-FUC, in vivo experiments were performed. As shown in Fig. 3, the droplets in the adipocytes of Am-FUC-treated mice are much smaller than that of the HFFD-fed group. Table 3 showed that Am-
FUC significantly reduced the mass of the subcutaneous, perirenal and epididymal fat at the dose of 80mg/kg/d.

**Am-FUC modulated the Wnt/β-catenin pathway**

Wnt/β-catenin pathway acts as a negative regulation factor in adipocyte differentiation. The expression levels of its key factors, such as Wnt10b, β-catenin, Fz and LRP5 were measured. As shown in Fig.4, the expressions of Fz and LRP5, the receptors of Wnt10b, were significantly enhanced by *Am*-FUC, producing 173.96% and 40.96% increase after the cells were treated with 200µg/ml *Am*-FUC. Wnt10b, an anti-adipogenic factor, was drastically reduced during the adipocyte differentiation. However, *Am*-FUC had no marked effect on the mRNA expression of Wnt10b compared to the control level. β-catenin can transfer into the nucleus to regulate the late-acting regulators of adipogenesis after the Wnt/β-catenin pathway is activated. Figure 4 indicated that the expression level of β-catenin was significantly enhanced by *Am*-FUC. Compared to the control level, *Am*-FUC (200µg/ml) increased the mRNA and protein expressions of β-catenin by 329.20% and 803.56%, respectively. The level of nuclear β-catenin was further examined. As shown in Fig. 4, *Am*-FUC increased the nuclear β-catenin level by 236.99% averagely.

**Am-FUC suppressed the mRNA expressions of SREBP-1c, C/EBPα and PPARγ**

SREBP-1c is proved to promote the adipocyte differentiation by enhancing the adipogenic activity of PPARγ. As shown in Fig. 5, the mRNA and protein expression of SREBP-1c was significantly suppressed by *Am*-FUC. After treated with 200µg/ml *Am*-FUC, its mRNA and protein expression were decreased by 72.69% and 70.23%. PPARγ and C/EBPα cooperates to induce the expression of genes leading to the mature adipocyte phenotype. Figure 5 indicated that the mRNA expressions of C/EBPα and PPARγ were significantly down-regulated by *Am*-FUC. In comparison with the control level, *Am*-FUC (200µg/ml) reduced the expression of C/EBPα and PPARγ by 65.81% and 81.61%, respectively.

**Am-FUC down-regulated the mRNA expressions of FAS and GPAT**

Fatty acid synthase (FAS) and glycerol 3 phosphate acyltransferase (GPAT) have been suggested to be the rate-limiting enzyme in TG biosynthesis. As shown in Fig. 6, the mRNA expressions of GPAT and FAS had a significant decrease (*P*<0.01)
after treated with *Am*-FUC. At the concentration of 200µg/ml, *Am*-FUC reduced the expression of GPAT and FAS by 69.01% and 53.62%, respectively.

*Am*-FUC **had no effect on the HSL and ATGL mRNA expression**

Adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) catalyze the first two steps of the intracellular TG hydrolysis. Figure 7 indicated that *Am*-FUC cannot significantly affect the mRNA expression of the lipolysis related genes, which means that *Am*-FUC may not be able to increase the rate of lipolysis.

**Discussion**

Adipocytes play critical roles in regulating the whole-body energy metabolism and maintaining the energy homeostasis. A massive adipocyte differentiation process leads to the increased adipocyte number and adipocyte mass, which causes the obesity and other metabolism diseases. Marine-derived sulfated polysaccharides have been shown to inhibit the adipocyte differentiation and improve the insulin resistance and type 2 diabetic. Several studies had reported that the fucoidan derived from brown algae inhibited the adipocyte differentiation. Hu *et al.* proved that fucosylated chondroitin sulfate from sea cucumber improved hyperglycemia and glucose metabolism. In the present study, we obtained a novel fucoidan from the *Acaudina molpadioides* and found that it significantly inhibited the proliferation and differentiation of the 3T3-L1 cells *in vitro*. Our findings indicated that *Am*-FUC may have potent anti-obesity activity. However, because of its high molecular weight, *Am*-FUC seems to be restricted to the gastrointestinal tract and it is unknown whether the degradation products of *Am*-FUC in the gastrointestinal tract still have the anti-adipogenic activity. In *vivo* experiments showed that *Am*-FUC can reduce the fat content of HFFD-fed mice by oral ingestion, which means that *Am*-FUC may be absorbed by oral route. The mechanism of how *Am*-FUC is absorbed needs to be further researched yet.

Adipogenesis is controlled by a regulated transcriptional cascade, in which C/EBPα and PPARγ play important roles and are considered to be the most important transcription factors in the mediation of the adipogenesis. Overwhelming evidences have shown that C/EBPα is significant for adipocyte differentiation. The activation of C/EBPα genes expression is sufficient to induce the differentiation of
3T3-L1 preadipocytes without the exogenous hormonal stimuli.\textsuperscript{33} PPARγ is the only transcription factor that has been proved to be indispensable in the adipogenesis.\textsuperscript{34} Several loss-of-function studies in adipogenesis prove that PPARγ is necessary and sufficient to promote adipocyte differentiation.\textsuperscript{35} Our data showed that \textit{Am}-FUC down-regulated the mRNA expression of C/EBPα and PPARγ, which further proves the anti-adipogenic activity of \textit{Am}-FUC.

C/EBPα and PPARγ are regulated by a series of upstream transcriptional factors and β-catenin is one of them. Nuclear β-catenin binds to the TCF/LEF family and enhances the expression of its target genes, such as cyclin D, c-Myc and c-jun.\textsuperscript{36} Those factors then inhibit the adipogenesis by inhibiting the expression of C/EBPα and PPARγ.\textsuperscript{36} However, cytoplasmic β-catenin is unstable and degraded without the Wnt stimulation.\textsuperscript{37} Studies show that high levels of β-catenin could be important in preventing the adipogenesis.\textsuperscript{38} In the present study, the expression level of β-catenin was enhanced, which indicates that \textit{Am}-FUC may inhibit the adipogenesis through up-regulating the β-catenin.

The translocation of β-catenin from the cytoplasm to the nuclear is controlled by the Wnt/β-catenin pathway.\textsuperscript{39} It is proved that Wnt/β-catenin acts as a molecular switch that represses adipogenesis when activated and promotes adipogenesis when turned off.\textsuperscript{2} Wnt10b, a Wnt ligand that inhibits the adipogenesis, stabilizes free cytoplasmic β-catenin and inhibits the expression of C/EBPα and PPARγ.\textsuperscript{40} Wnt10b is just largely expressed in preadipocytes and declines rapidly upon induction of differentiation,\textsuperscript{41} which is in keeping with our results. However, not all the activation of Wnt/β-catenin pathway is accompanied by the regulating of Wnt10b in 3T3-L1 cells. It is proved that TNFα activates the pathway through up-regulating Wnt10b, while IL-6 activates the pathway through modulate the dishevelled with no change in the expression of Wnt10b.\textsuperscript{9} In the present study, \textit{Am}-FUC had no significant effect on the expression of Wnt10b, which is similar to the IL-6. To activate the Wnt/β-catenin signaling pathway, Wnt10b combines to the Fz receptors and LRP coreceptors and initiate a series signal transduction in adipocyte to help the β-catenin transfer to the nucleus.\textsuperscript{42} Our research showed that \textit{Am}-FUC significantly up-regulated the mRNA expression of Fz and LRP5. These results indicate that \textit{Am}-FUC may modulate the Wnt/β-catenin signaling pathway by up-regulating the expression of Fz and LRP5.
The other upstream transcriptional factor that regulates the C/EBPα and PPARγ is SREBP-1c. It is under transcriptional controlled by insulin. Overexpression of SREBP-1c increases the expression of PPARγ ligand and further enhances the adipocyte differentiation. Our results showed that the expression of SREBP-1c was down-regulated suggesting that Am-FUC may inhibit the adipogenesis via suppressing the SREBP-1c. In addition, SREBP-1c also has crucial role in regulating genes related to fatty acid synthesis. The inhibition of SREBP-1c mRNA expression was accompanied by a sharp reduction in the expression of FAS and Acetyl-CoA carboxylase. In the present study, the mRNA expression of FAS was decreased, which is in keeping with the declining SREBP-1c expression. This result further proves our conclusion.

**Conclusion**

The present study provides evidences that Am-FUC possesses marked anti-adipogenic activity, which is exhibited by modulating the Wnt/β-catenin pathway and down-regulating the expression of SREBP-1c. Further in-depth investigations are necessary to better understand this complex mechanism. To our knowledge, this is the first report that provides a scientific basis for the application of the fucoidan from the sea cucumber in therapeutic intervention against obesity.

**Acknowledgement**

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**References**


Figure legends:

Fig. 1 The chemical structure of Am-FUC

Fig. 2 Effects of Am-FUC on the proliferation of 3T3-L1 preadipocytes (A) and adipocytes (B). 3T3-L1 cells were treated with various concentrations of Am-FUC for 96h. Cell viability was estimated by the MTT assay. The LDH level was measured by a LDH kit. Data are represented as mean ± S.D. of three independent experiments. Multiple comparisons were done using one-way ANOVA analysis. *P < 0.05 versus control; **P < 0.01 versus control.

Fig. 3 The anti-adipogenic activity of Am-FUC. 3T3-L1 cells were induced to mature adipocytes and stained with oil-red O at day 8. TG content was measured by TG assay kit. Cell viability at day 8 was estimated by the MTT assay. The adipose tissue is stained with HE staining method. Data are represented as mean ± S.D. of three independent experiments. Multiple comparisons were done using one-way ANOVA analysis. **P < 0.01 versus control.

Fig. 4 Effects of Am-FUC on the Wnt/β-catenin pathway. The mRNA expressions of Wnt10b, Fz, LRP5 and β-catenin were measured by RT-PCR. The protein level of total β-catenin and nuclear β-catenin was examined by western blotting. β-actin was used as an internal control for total protein and TBP was used as the endogenous control of nuclear protein. Data are represented as mean ± S.D. of three independent experiments. Multiple comparisons were done using one-way ANOVA analysis. *P < 0.05 versus control; **P < 0.01 versus control; ***P < 0.01 versus preadipocyte (Pre.).

Fig. 5 Effects of Am-FUC on the key transcriptional factors of adipocyte. The mRNA expressions of SREBP-1c, C/EBPα and PPARγ were measured by RT-PCR. The protein expression of SREBP-1c was examined by western blotting. β-actin was used as an internal control. Data are represented as mean ± S.D. of three independent experiments. Multiple comparisons were done using one-way ANOVA analysis. *P < 0.05 versus control; **P < 0.01 versus control.

Fig. 6 Effects of Am-FUC on the lipid synthesis related genes. The mRNA expressions of GPAT and FAS were measured by RT-PCR. β-actin was used as an internal control. Data are represented as mean ± S.D. of three independent experiments. Multiple comparisons were done using one-way ANOVA analysis. *P < 0.05 versus control; **P < 0.01 versus control.

Fig. 7 Effects of Am-FUC on the lipolysis related genes. The mRNA expressions of HSL and ATGL were measured by RT-PCR. β-actin was used as an internal control. Data are represented as mean ± S.D. of three independent experiments. Multiple comparisons were done using one-way ANOVA analysis.
Fig. 1 The chemical structure of Am-FUC
Table 1  Primer sequences, annealing temperature and cycles of different genes

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Table 2 The compositions of diets

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<tr>
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<td>150</td>
<td>450</td>
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<tr>
<td>20 Am-FUC</td>
<td>200</td>
<td>150</td>
<td>450</td>
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<td>3</td>
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<tr>
<td>80 Am-FUC</td>
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<td>10</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1.07</td>
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Fig. 2 Effects of Am-FUC on the proliferation of 3T3-L1 preadipocytes (A) and adipocytes (B). 3T3-L1 cells were treated with various concentrations of Am-FUC for 96h. Cell viability was estimated by the MTT assay. The LDH level was measured by a LDH kit. Data are represented as mean ± S.D. of three independent experiments. Multiple comparisons were done using one-way ANOVA analysis. *P < 0.05 versus control; **P < 0.01 versus control.
Fig. 3 The anti-adipogenic activity of Am-FUC. 3T3-L1 cells were induced to mature adipocytes and stained with oil-red O at day 8. TG content was measured by TG assay kit. Cell viability at day 8 was estimated by the MTT assay. The adipose tissue is stained with HE staining method. Data are represented as mean ± S.D. of three independent experiments. Multiple comparisons were done using one-way ANOVA analysis. **P < 0.01 versus control.
Fig. 4 Effects of Am-FUC on the Wnt/β-catenin pathway. The mRNA expressions of Wnt10b, Fz, LRP5 and β-catenin were measured by RT-PCR. The protein level of total β-catenin and nuclear β-catenin was examined by western blotting. β-actin was used as an internal control for total protein and TBP was used as the endogenous control of nuclear protein. Data are represented as mean ± S.D. of three independent experiments. Multiple comparisons were done using one-way ANOVA analysis. *P < 0.05 versus control; **P < 0.01 versus control; ***P < 0.01 versus preadipocyte (Pre.).
Table 3  Effect of *Am*-FUC on the content of subcutaneous, perirenal and epididymal fat in obesity mice fed with HFFD

<table>
<thead>
<tr>
<th>Groups</th>
<th>subcutaneous fat (g/kg.bw)</th>
<th>perirenal fat (g/kg.bw)</th>
<th>epididymal fat (g/kg.bw)</th>
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<tr>
<td>Control</td>
<td>11.65±1.58</td>
<td>4.55±0.47</td>
<td>9.52±0.79</td>
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<tr>
<td>HFFD</td>
<td>19.89±2.49&quot;&quot;</td>
<td>11.04±1.62&quot;&quot;</td>
<td>21.20±1.95&quot;&quot;</td>
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<tr>
<td>20 <em>Am</em>-FUC</td>
<td>18.11±2.53</td>
<td>8.97±1.00</td>
<td>18.90±2.23</td>
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<tr>
<td>80 <em>Am</em>-FUC</td>
<td>12.71±1.95&quot;</td>
<td>5.36±0.91&quot;&quot;</td>
<td>15.07±2.30&quot;</td>
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</tbody>
</table>

The obesity model mice were established by fed HFFD. Data are represented as mean ± S.D. (n=10 per group). Multiple comparisons were done using one-way ANOVA analysis. *P < 0.05 versus HFFD-fed group; **P < 0.01 versus HFFD-fed group; ""P < 0.01 versus control. bw: body weight.
Fig. 5 Effects of Am-FUC on the key transcriptional factors of adipocyte. The mRNA expressions of SREBP-1c, C/EBPα and PPARγ were measured by RT-PCR. The protein expression of SREBP-1c was examined by western blotting. β-actin was used as an internal control. Data are represented as mean ± S.D. of three independent experiments. Multiple comparisons were done using one-way ANOVA analysis. *P < 0.05 versus control; **P < 0.01 versus control.
Fig. 6 Effects of Am-FUC on the lipid synthesis related genes. The mRNA expressions of GPAT and FAS were measured by RT-PCR. β-actin was used as an internal control. Data are represented as mean ± S.D. of three independent experiments. Multiple comparisons were done using one-way ANOVA analysis. *P < 0.05 versus control; **P < 0.01 versus control.
Fig. 7 Effects of Am-FUC on the lipolysis related genes. The mRNA expressions of HSL and ATGL were measured by RT-PCR. β-actin was used as an internal control. Data are represented as mean ± S.D. of three independent experiments. Multiple comparisons were done using one-way ANOVA analysis.