Indian Gooseberry and Barley Sprout Mixture Inhibits Adipogenesis and Lipogenesis Activity in 3T3-L1 Adipocytes

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Abstract: This study aimed to confirm the synergistic effect of an Indian gooseberry (IG) and barley sprout (BP) mixture in differentiated adipocytes. To this end, 3T3-L1 adipocytes were treated with IG, BP, and IGBP mixtures during the differentiation period. On the last day of differentiation, we measured intracellular cAMP, triglyceride (TG), and fatty acid (FA) levels, as well as performed Oil Red O staining, glycerol release, and Western blot assays. During adipogenesis, IGBP (200 µg/mL) increased the cAMP levels by more than 2-fold and decreased the protein expression levels of p-CREB (66.3%), C/EBPα (79.4%), C/EBPβ (85.9%), and PPARγ (74.1%) compared to those in the C group. Furthermore, the expression levels of the adipogenesis-related genes and GLUT4 (more than 3-fold) were regulated. During lipogenesis, the IGBP (200 µg/mL) activated AMPK and ACC levels and reduced the protein expression levels of SREBP1c, FAS, and LPL. This reduced the FA and TG contents in the cells by 47.6% and 76.3%, respectively, compared to those in the differentiated control (C) group, resulting in a more than 5-fold increase in glycerol release. In conclusion, we found that IGBP inhibited TG synthesis during adipogenesis and lipogenesis, and thus, displayed potential as a functional health food for preventing obesity.

Keywords: Indian gooseberry; barley sprout; 3T3-L1; adipogenesis; lipogenesis

1. Introduction

Obesity is a serious civilization-related health problem in the 21st century. This disease affects a variety of other conditions, including cardiovascular diseases, diabetes, osteoporosis, and alimentary tract diseases [1]. An increase in body mass is a typical feature of obesity, which is dependent on an increase in the size of a single adipocyte, termed as hypertrophy, or an increase in the number of adipocytes, termed as hyperplasia [2].

Adipogenesis, or the generation of mature adipocytes from pre-adipocytes, accelerates lipid accumulation, and the research basis for adipogenesis has been established using the 3T3-L1 cell line of murine pre-adipocytes in vitro [3–5]. Pre-adipocytes exposed to differentiation–induction substances demonstrated the full maturation of adipocytes [6]. When the differentiation of pre-adipocytes begins, the specific transcriptional factors CCAAT/enhancer-binding protein delta (C/EBP δ) and C/EBPβ are combined to induce the expression of peroxisome proliferation-activated receptor gamma (PPARγ)
and C/EBPα [7,8]. PPARγ and C/EBPα regulate various adipogenic genes, such as fatty acid-binding protein 4 (FABP4), adiponectin, leptin, and phosphoenolpyruvate carboxy kinase 1 (PCK1) [9,10]. The action of high-carbohydrate absorption and insulin signaling activates lipogenesis in adipocytes. Acetyl coenzyme A (acetyl-CoA), which migrates from the intracellular mitochondrial membrane into the cytoplasm, is involved in fatty acid (FA) biosynthesis and is converted to malonyl-CoA by the multifunctional enzyme acetyl-CoA carboxylase (ACC) [11].

Indian gooseberry (IG), or Emblica officinalis, has been widely used for thousands of years as a therapeutic substance in Southeast Asia. IG is a rich source of vitamin C and is known to contain phenolic compounds, tannins, and alkaloids. Other known active materials include gallic acid, ellagic acid, quercetin, and kaempferol [12,13]. IG has been used in several studies modeling various diseases, including hyperlipidemia, diabetes, and cancer [14–17]. Barley sprout (BP), or Hordeum vulgare L., is a young plant (10–20 cm after sowing) that is cultivated around the world [18]. BP is rich in dietary fiber and contains strong antioxidants, as well as other active compounds, including vitamin C, vitamin E, carotenoids, saponarin, and lutonarin [19,20]. In addition, BP is known to be effective for anti-inflammatory, lipid metabolism, and blood sugar control [21–23]. We already confirmed the lipolytic effects of IG, BP, and an IGBP mixture in a previous in vitro screening experiment. Therefore, in the present work, we studied whether the IGBP mixture has an inhibitory effect on adipogenesis and lipogenesis in vitro.

2. Materials and Methods

2.1. Preparation of Materials

The IG and BP used in the experiment were provided by HL Science Co., Ltd. (Uiwang, Korea) in a water extract and juice powder, respectively. The IG extract was obtained by extracting the IG fruit with water, concentrating, and drying, and the yield was 10–15%. The BP juice powder was obtained by juicing BP, filtering, and drying, and the yield was 4–6%. Each sample was sealed and stored at −20 °C until use. High-glucose Dulbecco’s Modified Eagle Medium (DMEM), newborn calf serum (NCS), non-essential amino acids (NEAA), sodium pyruvate, penicillin/streptomycin, and L-glutamine were purchased from Hyclone (Logan, UT, USA). 3-Isobutyl-1-methylxanthine (IBMX), dexamethasone, and insulin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Metformin (Met) was purchased from Nelson (Seoul, Korea).

2.2. Cell Culture and Treatments

We used the 3T3-L1 cell line, which was purchased from the American Type Culture Collection (Rockville, MD, USA). The cells were incubated in a 95% air and 5% CO2 environment at 37 °C. For the cell culture, DMEM containing 10% NCS, 1% NEAA, 1% sodium pyruvate, 1% penicillin/streptomycin, and 1% L-glutamine was used. In the adipocyte differentiation experiments, we set up the experiment according to our existing methods [24], and the cells were seeded at a density of 3 × 10^5 cells/well into a six-well plate. After the cells reached nearly 100% confluence, the medium was changed to a differentiation medium containing 10% fetal bovine serum instead of NCS with an adipogenic cocktail (0.5 mM IBMX, 1 μM dexamethasone, and 10 μg/mL insulin). After 3 days of differentiation, the medium was replaced with a medium containing only insulin (10 μg/mL), and after another 3 days, it was replaced with a medium excluding the adipogenic cocktail. The processing information of each group in this cell experiment was as follows: normal control (NC, no treatment), differentiation control (C), Met 1 mM, IG 200 μg/mL, BP 200 μg/mL, and IGBP (50, 100, or 200 μg/mL). The samples were treated daily during the differentiation period (9 days).

2.3. Water Soluble Tetrazolium Salt (WST) Assay

Cell viability was measured by using the WST assay method with an EZ-Cytox kit (Daeil Lab Service, Seoul, Korea). The cells were seeded at a density of 1 × 10^4 cells/well into a 96-well plate.
After the cells were attached to the bottom of the plate, IG, BP, and IGBP were added to each well at a concentration of 0–1000 µg/mL for 1 day. After 1 day of incubation, 10 µL/100 µL of EZ-Cytox reagent was added to each well and incubated for 2 h. After gently shaking the plate, the optical density (OD) was measured at 450 nm (ELISA reader; Bio-Rad Laboratories, Hercules, CA, USA).

2.4. Western Blotting

After completion of the adipocyte differentiation experiments, the cells were harvested and homogenized with CellLytic™ MT Cell Lysis Reagent (Sigma-Aldrich) containing the Halt™ Protease & Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific, Rockford, IL, USA). The cell lysate was obtained using a refrigerated microcentrifuge (Micro 17R; Hanil Science Industrial Co., Ltd., Gimpo, Korea) at 14,000×g and 4 °C for 20 min. All Western blotting processes, such as protein quantification, sample loading, and transfer, were performed according to our existing methods [25]. The primary antibody information was as follows: phosphorylated cAMP response element-binding protein (p-CREB/CREB; 1:500; Cell Signaling Technology, Beverly, MA, USA), mitogen-activated protein kinase (MAPK; 1:1000; Cell Signaling Technology), C/EBPβ (1:500; Cell Signaling Technology), C/EBPα (1:500; Cell Signaling Technology), PPAR-γ (1:500; Cell Signaling Technology), FABP4 (1:200; Abcam, Cambridge, MA, USA), adiponectin (1:1000; Abcam), leptin (1:1000; Abcam), glucose 6-phosphatase (G6Pase; 1:1000; Abcam), phospho-insulin receptor substrate 1 (p-IRS1/IRS1; 1:1000; Cell Signaling Technology), phospho-phosphoinositide 3-kinase (p-P13K/P13K; 1:500; Cell Signaling Technology), phospho-protein kinase B (p-AKT/AKT; 1:500; Cell Signaling Technology), glucose transporter type 4 (GLUT4; 1:400; Cell Signaling Technology), phospho-AMP-activated protein kinase (p-AMPK/AMPK; 1:500; Cell Signaling Technology), sterol regulatory element binding protein-1c (SREBP1c; 1:500; Abcam), p-ACC/ACC (1:400; Cell Signaling Technology), fatty acid synthase (FAS; 1:1000; Cell Signaling Technology), lipoprotein lipase (LPL; 1:1000; Abcam), and actin (1:1000; Bethyl, Montgomery, TX, USA). As the secondary antibody, anti-rabbit or anti-goat IgG HRP-conjugated secondary antibodies (1:2000; Bethyl) were used. The membrane was exposed using EzWestLumi plus (ATTO, Tokyo, Japan) and Ez-Capture II equipment (ATTO), and analyzed using CS Analyzer 3.0 software (ATTO).

2.5. Enzyme-Linked Immunosorbent Assay (ELISA)

The cyclic-adenosine monophosphate (cAMP) level in 3T3-L1 adipocytes was measured using a cAMP ELISA kit (Cell Biolabs Inc., San Diego, CA, USA). The FA level was measured using a Free Fatty Acid Quantification Colorimetric Kit (Biovision Inc., Milpitas, CA, USA). All experiments were performed according to the respective manufacturer’s manual and our existing methods [24].

2.6. Oil Red O Staining

The staining method related to lipid accumulation followed our existing methods [24]. After completion of the adipocyte differentiation experiments, the cells on the plate were washed with Dulbecco’s phosphate-buffered saline (DPBS) and fixed with 10% formalin. After drying the cells with 60% isopropanol, the lipids were stained with Oil Red O working solution for 2 h, and then the cells were washed with distilled water four times and photographed. Lipid accumulation was measured by eluting the Oil Red O dye that had penetrated the lipids using 100% isopropanol and measuring the OD (520 nm) of the eluted solution.

2.7. Glycerol Release Assay

For the measurement of the release of free glycerol, a triglyceride (TG) decomposition product, the glycerol phosphate oxidase (GPO)-TRINDER enzyme reaction method was used [24]. After completion of the adipocyte differentiation experiments, the cultured medium and free glycerol reagent (Sigma-Aldrich) were mixed. After incubation, the OD (540 nm) was measured. Glycerol standard solution (Sigma-Aldrich) was used to calculate the glycerol content by substituting the OD value into the standard curve.
2.8. Statistical Analysis

All results are presented as mean ± standard deviation (SD). The significance of each group was determined using a one-way analysis of variance and Duncan’s multiple range test using SPSS Statistics 22.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance was considered to be at the p < 0.05 level.

3. Results

3.1. The IGBP Mixture Activated the cAMP Level Related to the Adipogenesis Mechanism in Adipocytes

Upon measuring the cAMP level acting in the process of suppressing adipogenesis in cells, we found that in the C group (716.7 ± 35.1 pmol/mL), it significantly decreased compared to that of the NC group (1097.0 ± 35.8 pmol/mL). Furthermore, the Met (1014.0 ± 162.8 pmol/mL), IG200 (1362.7 ± 26.1 pmol/mL), and BP200 groups (1033.3 ± 45.1 pmol/mL) showed significant increases relative to the C group. In the case of the IGBP 2:1 group, the values of the 50, 100, and 200 μg/mL doses (1556.3 ± 65.0, 1761.3 ± 39.0, and 1975.0 ± 60.5 pmol/mL, respectively) increased in a dose-dependent manner compared to those of the C group. Finally, a synergistic effect of IG and BP was observed in the IGBP 2:1 group (Figure 1A).

3.2. The IGBP Mixture Regulated the Protein Expression Levels Related to the Adipogenesis Mechanism in Adipocytes

The results of the protein expression level measurements related to the mechanism of suppressing adipogenesis are shown in Figure 1. The phospho-CREB/CREB ratio was significantly decreased in the IGBP 2:1 50, 100, and 200 μg/mL groups (12.79 ± 0.26, 6.73 ± 0.74, and 6.26 ± 1.09, respectively) in a dose-dependent manner compared to that of the C group (18.58 ± 2.44) (Figure 1B). On the other hand, MAPK expression significantly increased in the IGBP 2:1 50, 100, and 200 μg/mL groups.
(0.33 ± 0.06, 0.35 ± 0.03, and 0.34 ± 0.03, respectively) relative to the C group (0.15 ± 0.02) (Figure 1C). The C/EBPβ expression significantly decreased in the IGBP 2:1 50, 100, and 200 µg/mL groups (0.40 ± 0.02, 0.10 ± 0.00, and 0.09 ± 0.00, respectively) compared to the C group (0.61 ± 0.02) (Figure 1D). The C/EBPα expression level also significantly decreased in the IGBP 2:1 50, 100, and 200 µg/mL groups (0.14 ± 0.02, 0.11 ± 0.02, and 0.06 ± 0.01, respectively) in a dose-dependent manner relative to the C group (0.30 ± 0.02) (Figure 1E). Finally, the PPARγ expression significantly decreased in the IGBP 2:1 50, 100, and 200 µg/mL groups (0.22 ± 0.03, 0.12 ± 0.01, and 0.08 ± 0.00, respectively) in a dose-dependent manner compared to that of the C group (0.30 ± 0.02) (Figure 1F).

3.3. The IGBP Mixture Regulated the Protein Expression Levels Related to the Adipogenic Gene in Adipocytes

The results of the protein expression level measurements related to the adipogenic gene are shown in Figure 2. The FABP4 expression significantly decreased in the IGBP 2:1 200 µg/mL group (0.32 ± 0.04) compared to that of the C group (0.48 ± 0.04) (Figure 2A). The adiponectin expression level significantly increased in the IGBP 2:1 50, 100, and 200 µg/mL groups (0.73 ± 0.04, 0.69 ± 0.02, and 0.66 ± 0.02, respectively) compared to that of the C group (0.43 ± 0.01) (Figure 2B). Conversely, the leptin expression significantly decreased in the IGBP 2:1 100 and 200 µg/mL groups (0.20 ± 0.02 and 0.10 ± 0.01, respectively) compared to that of the C group (0.24 ± 0.01) (Figure 2C). Finally, the G6Pase expression level also significantly decreased in the IGBP 2:1 50, 100, and 200 µg/mL groups (0.36 ± 0.05, 0.23 ± 0.01, and 0.10 ± 0.00, respectively) in a dose-dependent manner relative to the C group (0.57 ± 0.04) (Figure 2D).

**Figure 2.** Effects of the IGBP 2:1 mixture on adipogenesis-related proteins in 3T3-L1 adipocytes: (A) FABP4 expression, (B) adiponectin expression, (C) leptin expression, (D) G6Pase expression. NC, normal control; C, differentiated control; Met, metformin 1 mM; IG200, IG 200 µg/mL; BP200, BP 200 µg/mL; IGBP 2:1 (50, 100, or 200 µg/mL). The resulting values are shown as mean ± standard deviation (SD; n = 3), and the different superscript letters (alphabet) indicate significance at p < 0.05.
3.4. The IGBP Mixture Regulated the Protein Expression Levels Related to GLUT4 Signaling in Adipocytes

The results of the protein expression level measurements related to GLUT4 signaling in the lipogenesis mechanism are shown in Figure 3. The p-IRS1/IRS1 ratio significantly increased in the IGBP 2:1 50, 100, and 200 µg/mL groups (0.51 ± 0.08, 0.63 ± 0.18, and 0.86 ± 0.05, respectively) in a dose-dependent manner compared to that of the C group (0.26 ± 0.05) (Figure 3A). The p-PI3K/PI3K ratio also significantly increased in the IGBP 2:1 50, 100, and 200 µg/mL groups (1.06 ± 0.07, 1.47 ± 0.10, and 4.31 ± 0.23, respectively) in a dose-dependent manner relative to that of the C group (0.26 ± 0.01) (Figure 3B). In addition, the p-AKT/AKT ratio significantly increased in the IGBP 2:1 50, 100, and 200 µg/mL groups (2.37 ± 0.40, 3.47 ± 0.03, and 6.69 ± 1.30, respectively) in a dose-dependent manner compared to that of the C group (1.21 ± 0.28) (Figure 3C). Finally, the GLUT4 expression significantly increased in the IGBP 2:1 100 and 200 µg/mL groups (0.33 ± 0.04 and 0.38 ± 0.03, respectively) in a dose-dependent manner compared to that of the C group (0.11 ± 0.00) (Figure 3D).

3.5. The IGBP Mixture Regulated the Protein Expression Levels Related to Lipogenesis Mechanism in Adipocytes

The results of the protein expression level measurements related to the lipogenesis mechanism are shown in Figure 4. The p-AMPK/AMPK ratio significantly increased in the IGBP 2:1 200 µg/mL group (4.38 ± 0.77) compared to that of the C group (0.88 ± 0.08) (Figure 4A). Conversely, the SREBP1c
expression significantly decreased in the IGBP 2:1 50, 100, and 200 µg/mL groups (0.46 ± 0.01, 0.37 ± 0.02, and 0.22 ± 0.03, respectively) in a dose-dependent manner relative to that of the C group (2.22 ± 0.02) (Figure 4B). The p-ACC/ACC ratio significantly increased in the IGBP 2:1 50, 100, and 200 µg/mL groups (2.03 ± 0.54, 1.38 ± 0.21, and 5.12 ± 0.83, respectively) compared to that of the C group (0.20 ± 0.02) (Figure 4C). The FAS expression level significantly decreased in the IGBP 2:1 50, 100, and 200 µg/mL groups (0.17 ± 0.02, 0.12 ± 0.03, and 0.11 ± 0.03, respectively) in a dose-dependent manner relative to that of the C group (0.47 ± 0.01) (Figure 4D). Finally, the LPL expression significantly decreased in the IGBP 2:1 50, 100, and 200 µg/mL groups (0.27 ± 0.03, 0.21 ± 0.02, and 0.14 ± 0.02, respectively) in a dose-dependent manner compared to that of the C group (0.99 ± 0.00) (Figure 4E).

Figure 4. Effects of the IGBP 2:1 mixture on lipogenesis-related proteins and fatty acid (FA) levels in 3T3-L1 adipocytes: (A) p-AMPK/AMPK expression ratio, (B) SREBP1c expression, (C) p-ACC/ACC expression ratio, (D) FAS expression, (E) LPL expression, and (F) intracellular FA levels. NC, normal control; C, differentiated control; Met, metformin 1 mM; IG200, IG 200 µg/mL; BP200, BP 200 µg/mL; IGBP 2:1 (50, 100, or 200 µg/mL). The resulting values are shown as mean ± standard deviation (SD; n = 3), and the different superscript letters (alphabet) indicate significance at p < 0.05.

### 3.6. The IGBP Mixture Inhibited the FA Levels in Adipocytes

The intracellular FA levels of the Met group (176.98 ± 31.23 nmol/µL) significantly decreased by 40.3% compared to that of the C group (296.52 ± 33.65 nmol/µL). Likewise, the FA levels of the IG200 (193.22 ± 8.53 nmol/µL) and BP200 (254.80 ± 5.16 nmol/µL) groups decreased by 34.8% and 14.1%, respectively, compared to that of the C group. Regarding the IGBP 2:1 mixture, the FA levels of the 50, 100, and 200 µg/mL groups decreased by 16.1%, 37.8%, and 47.6%, respectively, compared to that of the C group (Figure 4F).

### 3.7. The IGBP Mixture Inhibited the Lipid Accumulation in Adipocytes

Upon measuring lipid accumulation by performing Oil Red O staining, we observed that the OD of the C group (1.34 ± 0.05) significantly increased compared to that of the NC group (0.08 ± 0.01). Furthermore, the OD value of the Met (1.01 ± 0.04), IG200 (0.60 ± 0.02), and BP200 groups (1.10 ± 0.05) significantly decreased relative to the C group. In the case of the IGBP 2:1 mixture, the values of the 50, 100, and 200 µg/mL groups (0.88 ± 0.02, 0.57 ± 0.03, and 0.29 ± 0.03, respectively) decreased in a dose-dependent manner compared to that of the C group (Figure 5A,B). As a result of measuring the TG content, we found that the value of the C group (5.73 ± 0.34 mM) significantly increased relative to that
of the NC group (1.33 ± 0.68 mM). On the other hand, in the Met (2.35 ± 0.45), IG200 (2.06 ± 0.51 mM), and BP200 groups (2.82 ± 0.48), it significantly decreased compared to that of the C group. In the case of the IGBP 2:1 group, the values of the 50, 100, and 200 µg/mL groups (3.31 ± 0.56, 2.12 ± 0.15, and 1.36 ± 0.63, respectively) decreased in a dose-dependent manner compared to that of the C group (Figure 5C).

Figure 5. Effects of the IGBP 2:1 mixture on lipid accumulation, triglyceride (TG) levels, and glycerol release in 3T3-L1 adipocytes: (A) Oil Red O staining, (B) optical density of lipid accumulation, (C) TG levels, and (D) glycerol release. NC, normal control; C, differentiated control; Met, metformin 1 mM; IG200, IG 200 µg/mL; BP200, BP 200 µg/mL; IGBP 2:1 (50, 100, or 200 µg/mL). The resulting values are shown as mean ± standard deviation (SD; n = 3), and the different superscript letters (alphabet) indicate significance at p < 0.05.

3.8. The IGBP Mixture Increased the Glycerol Release in Adipocytes

Upon measuring the glycerol release, we observed that in the Met group (0.32 ± 0.03 µg/mL), it significantly increased by 66.63% compared to that in the C group (0.0.19 ± 0.05 µg/mL). In the case of the IG200 (0.55 ± 0.03 µg/mL) and BP200 (0.47 ± 0.03 µg/mL) groups, the values increased relative to those of the C group. Regarding the IGBP 2:1 mixture, the glycerol release values in the 50, 100, and 200 µg/mL groups (0.63 ± 0.07, 0.80 ± 0.06, and 1.01 ± 0.01 µg/mL, respectively) increased in a dose-dependent manner compared to that of the C group, and the synergistic effect of IG and BP was shown (Figure 5D).

4. Discussion

Obesity, which is characterized by overweight, is heavily influenced by changing social, economic, lifestyle, and nutritional conditions, and populations of all ages, including adults, the elderly, infants, and adolescents, are at risk of developing obesity-related diseases [26,27]. In addition, a society that focuses on physical appearance has naturally led to an interest in dietary control and functional health foods for weight loss, and research on supplements that help control weight to meet these needs is also actively being conducted [28–30]. IG, which is rich in vitamin C and minerals, has long been used as a herbal medicine in ancient Indian Ayurvedic medicine and has recently been found to contain active substances, such as gallic acid and ellagic acid [12,13]. BP, which is rich in dietary fiber and antioxidants, is a cultivated plant in many parts of the world and has recently been identified to possess active substances, such as saponarin and lutonarin [18–20]. IG and BP have been reported to affect lipid metabolism individually, but their synergistic effects are unknown. Our study aimed to determine whether an IGBP mixture exerts effects on the adipogenesis and lipogenesis mechanisms of differentiated 3T3-L1 cells. In a previous study, we confirmed that the IGBP mixture showed a lipolytic effect on mature adipocytes, and the cell viability experiment also showed no toxicity up to the maximum concentration (200 µg/mL) used in this experiment. Regarding the mixing ratio of the IGBP mixture, the ratios that were most effective in the ratio determination test conducted before the.
start of this experiment were selected first, and the 2:1 (IG:BP) ratio was ultimately selected to account for the available supply of raw material.

To clarify the effect of the IGBP 2:1 mixture on the adipogenesis and lipogenesis mechanisms in mature adipocytes, we measured the expression and activity levels of adipogenesis- and lipogenesis-related proteins in 3T3-L1 adipocytes. Adipogenesis is the process by which the differentiation of pre-adipocytes to mature adipocytes takes place [31]. It begins with a decrease in the cAMP level. This increases the activation of CREB and affects the expression of transcription factors, such as PPARγ and C/EBPα [32]. Adipogenesis is regulated by several signaling pathways or transcription factors that are master regulators in the final stage of differentiation [33]. PPARγ, a ligand-activated transcription factor, can induce the expression of C/EBPα, which subsequently mediates the expression levels of adipogenesis-related genes, including FABP4, adiponectin, leptin, and G6Pase [9,10]. In the present study, the expression levels of cAMP in adipogenic-induced adipocytes decreased. Although the phosphorylation of MAPK has not been confirmed, it is assumed that MAPK was phosphorylated and converted to an active form. In addition, it was confirmed that the expression levels of C/EBPβ, C/EBPα, and PPARγ increased in the adipocyte differentiation model. Thus, the IGBP 2:1 mixture increased the cAMP expression levels compared to the differentiation-inducing group, reduced the activity of CREB, and decreased the expression levels of C/EBPβ, C/EBPα, and PPARγ. In the case of MAPK expression, further research will be required in vivo, but it can be inferred that the effect of IGBP will reduce the conversion of MAPK to the phosphorylated form. In addition, we confirmed that the expression levels of FABP4, adiponectin, leptin, and G6Pase genes related to adipogenesis were regulated.

Among the various lipogenesis mechanisms, the GLUT4 signaling pathway also plays an important role. GLUT4, a glucose transporter, regulates glucose homeostasis and lipogenesis by regulating insulin signaling [34]. The phosphorylation of IRS-1 and PI3K activates AKT and Tre-2/USP6, BUB2, cdc16 domain family member 1 (TBC1D1) phosphorylation to induce glucose uptake [35,36]. In the present study, the IGBP 2:1 mixture increased the phosphorylation of IRS1 compared to the C group, as well as increased the phosphorylation of PI3K. In addition, AKT was activated and converted to its phosphorylated form, and the expression of GLUT4 increased by more than two-fold relative to the C group, thereby increasing the influx of glucose into cells to suppress lipogenesis induction. In addition, although additional studies are needed regarding insulin resistance, these results are considered to have potential as substances capable of controlling blood sugar by suppressing insulin resistance.

In the lipogenesis mechanism, SREBP1c regulates FA synthesis and activates lipogenesis-related factors, including ACC and FAS [37]. ACC has generally been described in previous research as the rate-limiting enzyme system in de novo FA synthesis [38]. ACC is an enzyme that is responsible for the production of malonyl-CoA, which is the intermediate substrate required for chain extension by FAS, the multifunctional enzyme [39]. In addition, SREBP1c can stimulate the ligand for the nuclear receptor and activate PPARγ transcription [40,41]. AMPK acts as a sensor of cellular energy that regulates lipid metabolism, including anabolism and catabolism. Activated AMPK can inhibit lipogenesis by down-regulating SREBP1c protein expression [42,43]. In addition, LPL secreted from adipocytes is involved in FA synthesis and lipoprotein metabolism [44]. In this cell model for inducing differentiation with an adipogenic cocktail, the activity of AMPK decreased and the expression of SREBP1c increased. In addition, the activity of ACC was inhibited and the FAS, LPL, and FA levels increased. The IGBP 2:1 mixture induced the reduction of SREBP1c by converting AMPK to the active type compared to the C group. In addition, treatment with the IGBP 2:1 mixture led to the expression of the active form of ACC, induced reductions in the FAS and LPL expression levels, and decreased the adipocyte FA level in a dose-dependent manner. These results affected reductions in lipid accumulation and TG content and led to increased glycerol release from the adipocytes (Figure 6). We expect that the anti-obesity activity of the IGBP mixture was due to ellagic acid and saponarin. Ellagic acid and saponarin have been found to be detected in the component analysis of the IGBP mixture (data not shown), and this result requires a little further validation and is expected to be active in animal studies as well.
Figure 6. Effects of the IGBP 2:1 mixture on the adipogenesis and lipogenesis mechanisms in 3T3-L1 adipocytes. cAMP, cyclic adenosine monophosphate; MAPK, mitogen-activated protein kinase; C/EBP, CCAAT/enhancer-binding protein; PPARγ, peroxisome proliferator-activated receptor gamma; FABP4, fatty-acid-binding protein 4; G6Pase, glucose 6-phosphatase; SREBP1c, sterol regulatory element-binding protein-1c; IRS1, insulin receptor substrate-1; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; GLUT4, glucose transporter type 4; AMPK, AMP-activated protein kinase; ACC, acetyl coenzyme A carboxylase; FAS, fatty acid synthase; LPL, lipoprotein lipase.

5. Conclusions

In conclusion, the IGBP 2:1 mixture regulated the expression of adipogenesis-related proteins by reducing the expression of transcription factors in the early stage of differentiation at the cellular level and regulated the expression of proteins involved in GLUT4 signaling to inhibit lipogenesis. In addition, related factors in the lipogenesis mechanism were regulated through acetyl-CoA, while FA and TG production, as well as glycerol release, were also ultimately regulated. Our previous study confirmed the lipolytic effect of the IGBP 2:1 mixture on adipocytes and this study proved that it inhibited adipogenesis and lipogenesis. Therefore, if the anti-obesity effect of the IGBP 2:1 mixture is demonstrated in future animal experiments, it may present potential as a functional health food that can reduce weight and maintain a healthy body.

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