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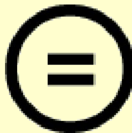

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A Thesis for the Degree of Master of Science

A Novel Variety of Pepper (*Capsicum annuum* L.) Improves Insulin Resistance in High-Fat Diet-Fed Mice

고지방식이 섭취 마우스 모델에서
신품종 고추의 인슐린 저항성 개선 효능 규명

February, 2016

By

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Abstract

Chili pepper (*Capsicum Annuum* L.) is one of the main food in South Korea and many kinds of pepper have been consumed. It has been well known that a variety of phytochemicals such as capsaicin, lutein, and quercetin in pepper have anti-diabetic effects. Therefore, I developed a new variety of pepper as a representative pepper in Korea, which has not only cultivation stability and high quality but also anti-diabetic effect. By screening a total of 6,000 new varieties with excellent characteristics in reliability and quality, 'Hana 4176' was selected. Mice were fed chow diet (CD) or high-fat diet (HD) with or without 0.83% of a novel variety of pepper (NV) for 33 weeks. Insulin tolerance test (ITT) showed that the level of blood glucose of NV group was decreased as CD group. Western blot assay showed that NV effectively increased phosphorylation of Akt especially in liver and

muscle indicating NV improved insulin signaling. I found that mRNA levels of F4/80, CD68, MCP-1, and CCR2 which are macrophage-related marker genes were decreased in the white adipose tissues of NV group compared to HD group. Additionally, decreased mRNA level of SREBP1c, FAS, and ACC2 in NV group compared to HD group indicated that lipid synthesis was reduced by NV administration in the liver. The mRNA level of UCP3 and CPT-1b was increased in NV group compared to HD group and this showed that fatty acid oxidation was increased by NV administration in the skeletal muscle. However, these effects of NV group was not mainly attributed to reduced body weight increase. Consequently, NV had protective effect on HD-induced insulin resistance in each organs such as white adipose tissue, liver, and muscle. Therefore, this implies that developed new functional variety prevents type 2 diabetes as a food.

Keywords: Chili pepper (*Capsicum annuum* L.); Novel variety; Insulin resistance; Inflammation; lipid metabolism

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Ⅰ. Introduction

Chili pepper is native to South America but it is generally used for food flavoring nowadays. Moreover, it has been used for medicinal and culinary purposes for a long time. *Capsicum annuum* is mainly cultivated species of the spice *Capsicum* [1, 2]. The major compounds of chili pepper are pungent capsaicinoids (capsaicin, dihydrocapsaicin), antioxidant vitamins (ascorbic acid, vitamin E), carotenoids (beta-carotene, beta-cryptoxanthine), organic acids and minerals [3, 4].

The components of chili pepper has been well-known for type 2 diabetes [5]. Capsaicinoids, major pungent components, have been studied that they attenuate metabolic dysfunction by controlling adipose tissue inflammation [6]. Carotenoids which are natural pigments such as α-, β-carotene, lutein, and lycopene have been known for association with type 2 diabetes [7]. Lutein which is one of the

carotenoids has been studied that it is able to use a treatment with insulin for diabetes [8]. In addition, quercetin attenuates oxidative stress and inflammation related to type 2 diabetes [9, 10]. Therefore, pepper has a lot of relevance with type 2 diabetes.

Even though chili pepper has a several of phytochemicals which has anti-diabetic effects, breeding representative functional variety of pepper with high quality and stability as well as anti-diabetic effect has been less studied. In this study, a lot of new varieties of pepper with these features were developed. Before examination of anti-diabetic effect, 24 kinds of pepper were analyzed [11]. Among them, a novel variety of pepper, 'Hana 4176' which has cultivation stability, high quality (taste, red color, functionality etc.) was elected. A novel variety of pepper was used in this study to establish anti-diabetic effect. This pepper was compared with a normal pepper 'Muhanjilju (MJ)'.

Type 2 diabetes Mellitus (T2D) is a chronic metabolic disorder

and it has been increasing steadily all over the world [12]. Almost 177 million people worldwide have diabetes and it is likely to be two times more by 2030 [13]. The main causes of T2D is insulin resistance which makes cells fail to respond to insulin due to impaired insulin receptor (IR) activation or disrupted insulin-stimulated glucose signaling pathway [14]. Akt protein clearly plays an important role in insulin-stimulated glucose uptake [15].

There are some main causes for insulin resistance including macrophage-related inflammation in adipose tissue and ectopic lipid accumulated in other tissues such as liver and skeletal muscle [16, 17]. First of all, high-fat diet leads adipose tissue to release monocyte chemotactic protein 1 (MCP-1) and macrophage goes into the adipose tissue by capturing MCP-1 to C-C chemokine receptor type 2 (CCR2) [18]. Therefore, in adipose tissue of insulin resistance state, F4/80 and CD68 as a macrophage marker were increased [19]. When ectopic lipid

accumulated in liver and muscle, lipid synthesis marker such as SREBP1c, FAS and ACC2 were increased [20] and fatty acid oxidation markers such as UCP3 and CPT-1b were decreased [21].

In the present study, I selected one variety of pepper among 6000 kinds by experiments. Then I examined the protective effect of a novel variety of pepper (NV) on HD-induced insulin resistance in mice. Additionally, I investigated the alteration of insulin resistance causing factors on each major organs such as liver, muscle, and adipose tissue.

Ⅱ. Materials and methods

2.1. Chemicals and reagents

A novel variety of pepper (NV; gene number BP1188236) and Muhanjilju (MJ) pepper were cultivated and grinded from Hana Jongmyo (Anseong, Korea). The antibody against phospho-Akt (Ser473) was obtained from Cell Signaling Technology (Danvers, MA, USA). The antibody against Akt and F4/80 were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). The enzyme-linked immunosorbent assay (ELISA) kits for insulin was purchased from ALPCO (Salem, NH, USA) and for adiponectin was purchased from R&D systems (Minneapolis, MN, USA). Triglyceride (TG) was analyzed with triglyceride reagent, free glycerol reagent and treolin which are purchased from Sigma-Aldrich (Saint Louis, MO, USA). Non-esterified fatty acid (NEFA) was analyzed with LabAssay™ NEFA

kit from Wako (Osaka, Japan).

2.2. Animal diets

A chow diet (CD) group mice were allowed free access to normal CD containing 4.0% (wt/wt) total fat (Rodent NIH-31 Open Formula Auto, Zeigler Bros., Gardners, PA, USA) and water. A high-fat diet (HD) group and sample group mice were also allowed free access to HD containing 60.0% (wt/wt) total fat (TD.06414 – Adjusted Calories Diet–60/Fat, Harlan Laboratories, Madison, WI, USA). 0.83% of chili pepper powder was used to make blended pellets for each sample groups. A muhanjilju (MJ) group and a novel variety (NV) group were received the blended pellets processed by Daehan Biolink (Eumseong, Korea).

2.3. Animals

Female C57BL/6J mice (13-week-old) were purchased from

Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed in climate-controlled quarters (23 ± 3℃, 50 ± 10% humidity) with a 12-h light-dark cycle. Mice (n=20) were divided into four different groups (n=5 each group): a CD, a HD, a HD with 0.83% of Muhanjilju pepper powder (MJ) and a HD with 0.83% of novel variety of pepper powder (NV). All diet were provided in the form of pellets for experiment period. Mice were given free access to food and water. Body weight and food intake were monitored on a weekly basis. Mice were maintained in accordance with the rules and regulations of the Animal Care Ethical Committee of the Institute of Laboratory Animal Resources (ILAR) of Seoul National University, Korea (Case Number: SNU-140513-7-1).

2.4. Insulin resistance diagnostic tests

The 4-5 hour-fasted mice were prepared to measure blood glucose. Insulin tolerance test (ITT) is one of the diagnostic tests to

examine insulin resistance. Mice were injected 0.75 U/kg body weight insulin and measured blood glucose at 0, 15, 30, 60, 90, and 120 min of point time. Measured blood glucose was compared and quantified area under curve (AUC). End of the whole experiment, fasted mice blood was collected to measure plasma glucose and insulin concentration in lithium heparin-coated capillary tube (Kent scientific, Torrington, CT, USA) and centrifuged (1,000 x g, 4℃, 10 min). Taken supernatant was stored at -20℃. The homeostatic model assessment-insulin resistance (HOMA-IR) is another test for insulin resistance. The result was calculated followed by equation; glucose (mg/dL) x insulin (mU/L) / 22.5.

2.5. Tissue sample preparation

At the end of the experiment, the 4-5 hour-fasted animals were injected 1 U/kg body weight insulin. 15-20 minutes after injection, mice

were sacrificed by isoflurane (Piramal, PA, USA). Liver, white adipose tissue (WAT), and gastrocnemius muscle (GAS) were dissected in order. All tissues were snap-frozen in liquid nitrogen and stored at -80℃ for RNA and protein analysis or fixed in 4% formaldehyde solution.

2.6. Western blot assay

Tissues were lysed with RIPA buffer (Cell Signaling Technology) added 1 mM Phenylmethylsulfonyl fluoride (PMSF, Sigma-Aldrich) and 10 mM Sodium fluoride (NaF, Sigma-Aldrich) and centrifuged (24,100 x g, 4℃, 10 min) to remove debris and fat. Protein level was verified using Protein Assay Reagent (Bio-Rad, Hercules, CA, USA). Protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Amersham Protran 0.2 Nitrocellulose (NC) membrane (GE Healthcare Life Sciences, London, UK). Membranes were blocked in 5% Bovine

Serum Albumin (BSA; Bovogen, East Keilor, VIC, Australia) and incubated with specific primary antibody (Cell signaling Technology) followed by HRP-conjugated secondary antibody (Santa Cruz Biotechnology). The protein bands were visualized using a chemiluminescence detection kit (GE Healthcare Life Sciences).

2.7. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from tissues with an Ambion® RNA isolation kit (Ambion, Huntingdon, UK). The amount of RNA level was confirmed using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was synthesized with a PrimeScript™ 1st strand cDNA Synthesis Kit (Takara, Kyoto, Japan) and quantitative real-time polymerase chain reaction (qRT-PCR) was conducted using specific primers and SYBR Green Master Mix (Bio-Rad) with a Bio-Rad CFX96 real-time PCR detection system (Bio-Rad).

The forward and reverse primers for each transcript were as follows (Bioneer, Daejeon, Korea): mouse β-actin mRNA was 5’ - TGT CCA CCT TCC AGC AGA TG - 3’ and 5’ - AGC TCA GTA ACA GTC CGC CT - 3’; mouse CCR2 mRNA was 5’ - ATC CAC GGC ATA CTA TCA ACA TC - 3’ and 5’ - CAAA GGC TCA CCA TCA TCG TAG - 3; mouse F4/80 mRNA was 5’ - CTT TGG CTA TGG GCT TCC AGT C - 3’ and 5’ - GCA AGG AGG ACA GAG TTT ATC GTG - 3; mouse CD68 mRNA was 5’ - AGC TCC CTT GGG CCA AAG - 3’ and 5’ - AGG TGA ACA GCT GGA GAA AGA ACT - 3; mouse MCP-1 mRNA was 5’ - CCA CTC ACC TGC TGC TAC TCA T - 3’ and 5’ - TGG TGA TCC TCT TGT AGC TCT CC - 3; mouse SREBP1c mRNA was 5’ - CTG GCA CTA AGT GCC CTC AAC - 3’ and 5’ - GCC ACA TAG ATC TCT GCC AGT GT - 3; mouse FAS mRNA was 5’ - TTG CCC GAG TCA GAG AAC C - 3’ and 5’ - CGT CCA CAA TAG CTT CAT AGC - 3; mouse ACC2 mRNA was 5’ - ACG AGC ACA CAC AGT

CCA TG - 3’ and 5’ - GAT GAC CTC TGG ATG TTC TT - 3; mouse UCP3 mRNA was 5’ - ATG CTG AAG ATG GTG GCT CA - 3’ and 5’ - TTG CCT TGT TCA AAA CGG AG - 3; mouse CPT-1b mRNA was 5’ - TCG GGG CTG GTC CTA CAC TT - 3’ and 5’ - TCA CCT GGG CTA CAC GGA GA - 3. β-actin was using as a target transcript relative to calculate a ratio of the target gene expression by using the amplification program.

2.8. Tissue staining

To measure the levels of F4/80 in tissue, Immunohistochemistry (IHC) was performed. Mouse skin sample were fixed with 10% neutral-buffered formalin, and embedded in paraffin. Serial sections (4 μm) were then mounted onto slides. Slides were incubated in 0.3% hydrogen peroxide and blocked using 5% normal goat serum for 30 min. After blocking, slides were incubated with F4/80 antibody (Santa cruz) at 4℃

overnight. Next, they were incubated with biotinylated secondary antibody (Vector Labs, CA, USA) and developed using an avidin-biotin complex kit (Vector Labs, CA, USA). The reaction was visualized with 3,3'-diaminobenzidine tetrahydrochloride hydrate solution (Vector Labs, CA, USA) and counterstained with hematoxylin. The expression of F4/80 in mouse tissue sections were then examined under 400 x magnification using an Olympus AX70 light microscope.

To observe the morphology of tissues, Hematoxylin and eosin (H&E) staining was performed. Mouse skin samples were fixed with 10% neutral-buffered formalin, and put in paraffin. Serial sections (4 μm) were stuffed onto slides. After deparaffinizing, tissue sections were re-hydrated and stained with hematoxylin solution for 5 minutes. After this step, slides were washed and stained in counterstain in eosin Y solution for 30 seconds. Next, the slides were dehydrated through 95% alcohol and washed in absolute alcohol, 5 minutes each. Lastly, the

slides were incubated in xylene. Tissue sections were examined at 400× magnification using an Olympus AX70 light microscope (Tokyo, Japan).

2.9. Liver lipid extraction

To quantify the amount of lipid in liver tissue, liver lipid was extracted. Liver tissue was weighed and moved in 2 mL tube. 1mL of 3mM $CaCl_2$ was added and tissue was homogenized for 3 min. All contents were removed to weighed 50 ml conical tube and added 1.5 mL of 3 mM $CaCl_2$. 10 mL of forch solution was added and mixed for 30 min. These tubes were centrifuged at 3000 rpm for 2 min. After separating layer, lower part was collected and dry organic solvent overnight. Lastly, 1 mL of triton and ethanol (1:3) solution was added to the lipid and dissolve it.

2.10. Lipid quantitative analysis

To quantify the amount of TG in serum and liver tissue, TG assay was examined. Free glycerol reagent (Sigma-Aldrich, Saint Louis, MO, USA) was mixed with triglyceride reagent (Sigma-Aldrich, Saint Louis, MO, USA) (1:4) that called triglyceride working reagent (TWR). 4 μl of specimen was mixed with 200 μl of TWR and incubated at 37℃ for 5 min. After incubation, this solution was measured by spectrophotometer at 550 nm.

To quantify the amount of NEFA in plasma, NEFA assay was examined. The analysis was followed by protocol of kit (Wako, Japan).

2.11. Statistical analysis

Data were expressed as means ± standard error of mean (SEM). The groups were compared using one-way or two-way analysis of variance with the Duncan test as post-hoc analysis by SPSS 22.0 software (SPSS, Chicago, IL, USA) or using Student's t-test. A

probability value of $p<0.05$ was used as the standard for statistical significance.

Ⅲ. Results

3.1. NV improves insulin sensitivity on HD-fed mice

To identify the effect of NV on HD-induced insulin resistance in mice, insulin tolerance test (ITT) was performed. This data indicated that HD-fed mice showed significantly higher blood glucose compared to CD-fed mice that means HD-fed mice had insulin resistance. But, NV-fed mice effectively decreased blood glucose in response to 0.75 U/kg insulin and its area under curve (AUC) compared to HD-fed mice. But, MJ-fed mice had no improvement even though it had been fed pepper sample (Fig 1A-B). This indicates that NV-fed mice are insulin sensitive than HD-fed mice. For these reasons, HD-fed mice showed high glucose level in plasma significantly compared to CD-fed mice after fasting 4-5 hrs but NV-fed mice showed low glucose level compared to HD-fed mice (Fig 1C). Also, HD-fed mice showed high insulin level in plasma significantly compared to CD-fed mice after

fasting 4-5 hrs but NV-fed mice showed low insulin level compared to HD-fed mice (Fig 1D). In addition, Homeostatic model assessment-Insulin resistance (HOMA-IR) value which is one of the methods to diagnose insulin resistance was effectively decreased in NV-fed mice compared to HD-fed mice (Fig 1E). These results indicate that NV increases insulin sensitivity compared to MJ- and HD-fed group.

Figure 1

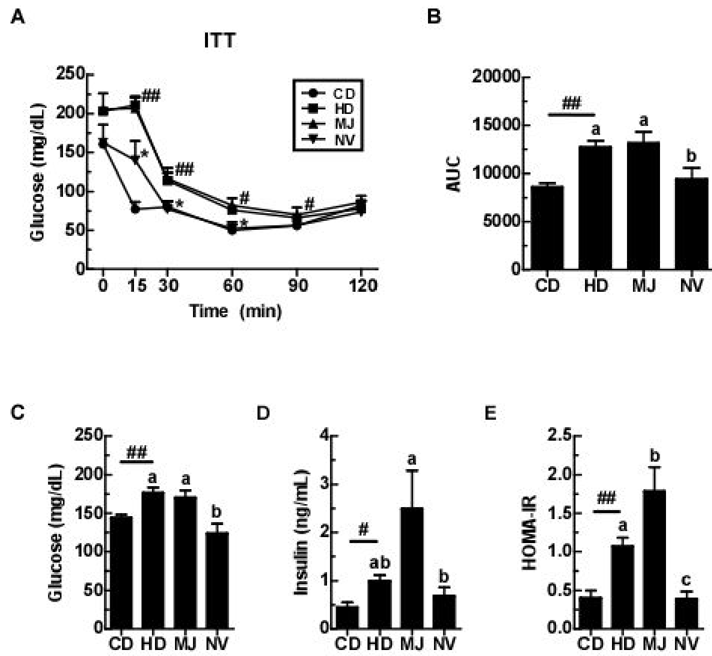


Figure 1. Effect of NV on HD-induced insulin resistance in mice.

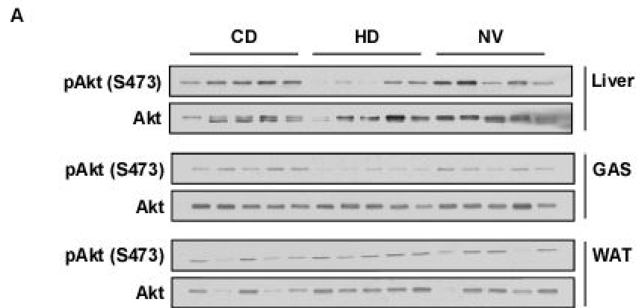
(A-B) After 33 weeks, insulin tolerance test (ITT) between 0-60 min. of time points and its area under curve (AUC). NV-fed mice decreased in blood glucose compared to HD- and MJ-fed mice. (C) Fasting glucose after 4-5 hrs was also lower in NV-fed mice compared to HD- and MJ-fed mice. (D) Fasting insulin after 4-5 hrs was lower in NV-fed mice compared to MJ-fed mice. (D) HOMA-IR after 4-5 hrs was significantly lower in NV-fed mice than HD- and MJ-fed mice. Value are expressed as means ± SEM. ##, $p < 0.01$ and #, $p < 0.05$, indicates significant differences between CD-fed mice and HD-fed mice, * or letters (a-c) and $p < 0.05$, indicates significant differences between HD-fed mice and samples treated mice.

3.2. NV increases phosphorylation of Akt in liver and GAS but not in WAT

To regulate blood glucose level, insulin stimulates Akt phosphorylation (Ser473) in the cells of liver, gastrocnemius muscle (GAS) and White adipose tissue (WAT) [22]. To investigate effects of NV on insulin signaling, western blot assay was performed. These results showed that HD-fed group decreased pAkt protein expression compared to CD-fed group but NV-fed group increased pAkt protein expression compared to HD-fed group in liver and GAS. (Fig 2A)

Also, quantitative pAkt per Akt ratio showed that NV-fed group improved pAkt/Akt ratio compared to HD-fed group in liver and GAS. However, NV had no effect on phosphorylation of Akt in WAT (Fig 2B). These results suggest that NV protects HD-induced insulin resistance in liver and GAS but not WAT.

Figure 2



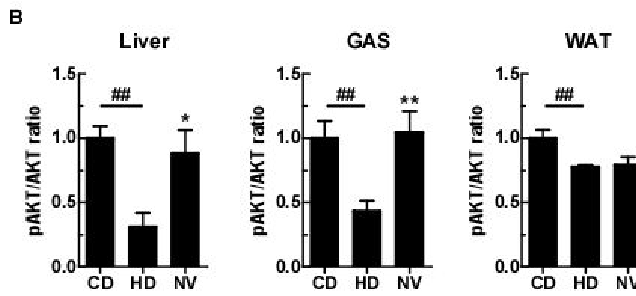


Figure 2. Effect of NV on Akt phosphorylation in tissues.

(A) Expression of pAkt was determined by protein immunoblot assay. Phosphorylation of Akt in NV-fed mice had higher expression than HD-fed mice in liver and GAS. (B) The ratio of pAkt/Akt improved in liver and GAS of NV-fed mice compared to HD-fed mice. Value are expressed as means ± SEM. ##, $p < 0.01$ and #, $p < 0.05$, indicates significant differences between CD-fed mice and HD-fed mice, * or letters (a-c) and $p < 0.05$, indicates significant differences between HD-fed mice and samples treated mice.

3.3. NV reduces macrophage infiltration in WAT

It is known that macrophage infiltration is occurred by high fat related eating habits and it affects to insulin resistance on other tissues [23]. To investigate the effect of NV on HD-induced macrophage infiltration in WAT, IHC of F4/80 was performed. This staining data showed that HD-fed group increased macrophage compared to CD-fed group but NV-fed group reduced macrophage compared to HD-fed group (Fig 3A). Also, real-time quantitative PCR was tasked to examine the mRNA level in WAT. F4/80 was 6 times up-regulated in HD-fed group compared to CD-fed group, but NV-fed group was 83% down-regulated compared to HD-fed group (Fig 3B). CD68 was 6 times higher in HD-fed group than CD-fed group, but NV-fed group was 59% down-regulated compared to HD-fed group (Fig 3C). MCP-1 was 12.5 times increased in HD-fed group compared to CD-fed group, but NV-fed group was 76% down-regulated compared to HD-fed group (Fig 3D).

CCR2 was 4.5 times up-regulated in HD-fed group compared to CD-fed group, but NV-fed group was 66% down-regulated compared to HD-fed group (Fig 3E). Overall, these results indicate that NV reduces HD-induced macrophage infiltration.

Figure 3

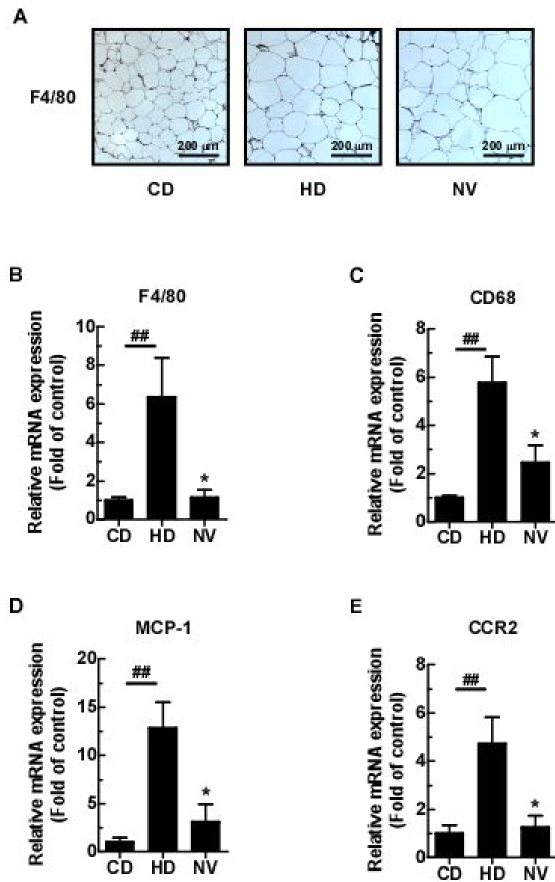


Figure 3. Effect of NV on macrophage infiltration in WAT

(A) IHC for visualizing F4/80 level which appears dark brown color. This data showed that HD-fed mice had more brown color than NV-fed mice. (B-E) Each mRNA level was analyzed by Real-time quantitative PCR. F4/80, CD68, MCP-1 and CCR2 were down-regulated in NV-fed mice than HD-fed mice. Value are expressed as means ± SEM. ##, $p < 0.01$ and #, $p < 0.05$, indicates significant differences between CD-fed mice and HD-fed mice, * or letters (a-c) and $p < 0.05$, indicates significant differences between HD-fed mice and samples treated mice.

3.4. NV ameliorates lipid accumulation and synthesis in liver

Liver is one of major organs for insulin resistance [24]. To investigate the physiology of liver, H&E staining was performed (Fig 4A). The H&E data showed that lipid droplet was dramatically increased in HD-fed group compared to CD-fed group but it was reduced in NV-fed group compared to HD-fed group. Also hepatic lipid was extracted, TG was analyzed. TG was 1.5 times increased in CD-fed group compared to HD-fed group but NV-fed group was decreased as a level of CD-fed group (Fig 4B). To examine lipid metabolism, real-time quantitative PCR was performed to check the mRNA level in liver. SREBP1c was 10 times up-regulated in HD-fed group compared to CD-fed group, but NV-fed group was 80% down-regulated compared to HD-fed group (Fig 4C). FAS was 5 times higher in HD-fed group than CD-fed group, but NV-fed group was 92% down-regulated compared to HD-fed group (Fig 4D). ACC was 3 times increased in HD-fed group

compared to CD-fed group, but NV-fed group was 66.7% down-regulated compared to HD-fed group (Fig 4E). These results indicate that NV suppresses lipogenesis in liver.

Figure 4

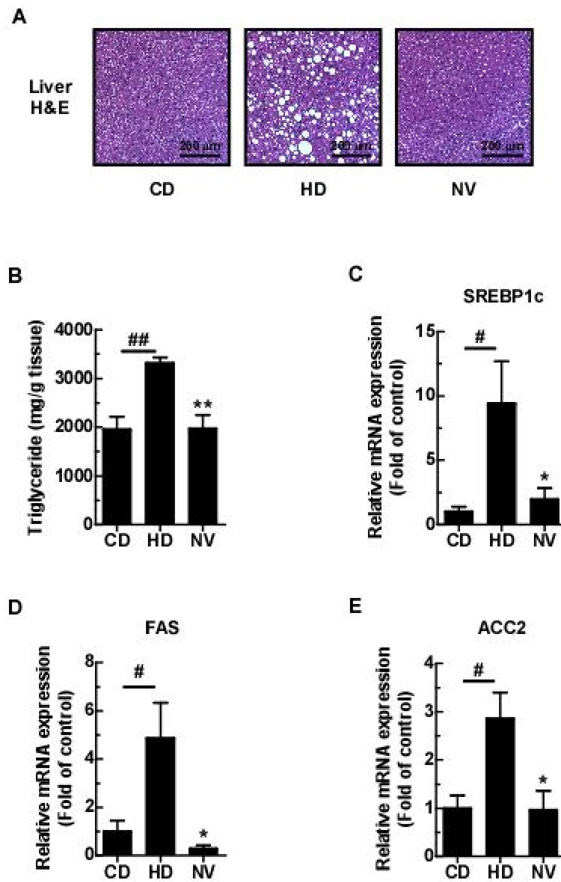


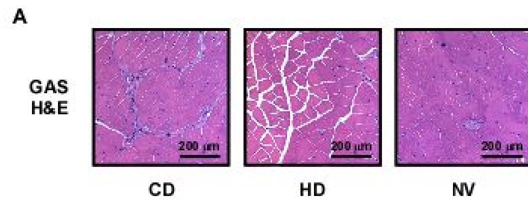
Figure 4. Effect of NV on lipogenesis in liver

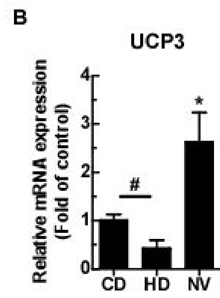
(A) H&E staining for visualizing liver tissue showed that NV-fed mice had less lipid droplet than HD-fed mice. (B) TG from liver tissue extract was examined that NV-fed mice significantly decreased compared to HD-fed mice. (C-E) Each mRNA level was analyzed by Real-time quantitative PCR. SREBP1c, FAS and ACC2 was down-regulated in NV-fed mice compared to HD-fed mice. Value are expressed as means ± SEM. ##, $p < 0.01$ and #, $p < 0.05$, indicates significant differences between CD-fed mice and HD-fed mice, * or letters (a-c) and $p < 0.05$, indicates significant differences between HD-fed mice and samples treated mice.

3.5. NV improves fatty acid oxidation in GAS

Muscle is the most major organ for glucose uptake [25]. To investigate the physiology of skeletal muscle, H&E staining was performed (Fig 5A). Skeletal muscle is known for fatty acid oxidation [26]. To examine fatty acid oxidation, real-time quantitative PCR was conducted to experiment on the mRNA level in GAS. UCP3 was 2 times down-regulated in HD-fed group compared to CD-fed group, but NV-fed group was 5 times up-regulated compared to HD-fed group (Fig 5B). CPT-1b was not changed between HD-fed group and CD-fed group, but NV-fed group was 3.5 times up-regulated compared to HD-fed group (Fig 5C).

Figure 5





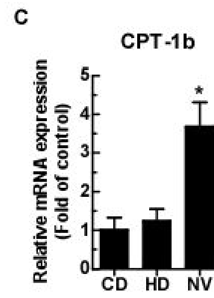


Figure 5. Effect of NV on fatty acid oxidation in GAS

(A) H&E staining for visualizing GAS tissue. (B-C) Each mRNA level was analyzed by Real-time quantitative PCR. UCP3 and CPT-1b was significantly up-regulated in NV-fed mice than HD-fed mice. Value are expressed as means ± SEM. ##, $p < 0.01$ and #, $p < 0.05$, indicates significant differences between CD-fed mice and HD-fed mice, * or letters (a-c) and $p < 0.05$, indicates significant differences between HD-fed mice and samples treated mice.

3.6. NV does not reduce body weight increase

Obesity is a major reason for insulin resistance [27]. To identify the role of obesity, body weight was measured every weeks and this data showed that HD-fed mice significantly increased compared to CD-fed mice but NV-fed mice had no significant effect on weight loss (Fig 6A). Also, food intake of NV-fed mice was more than HD-fed mice (Fig 6B). These results indicated that NV-fed mice consumed diet not less than HD-fed mice. Also, H&E staining showed that NV-fed mice had no significant effect on adipocyte size (Fig 6C). To investigate lipid amount in plasma, several plasma analysis was performed. TG, NEFA and Adiponectin had no change among groups (Fig 6D-F). These results indicate that NV does not play a pivotal role in weight loss.

Figure 6

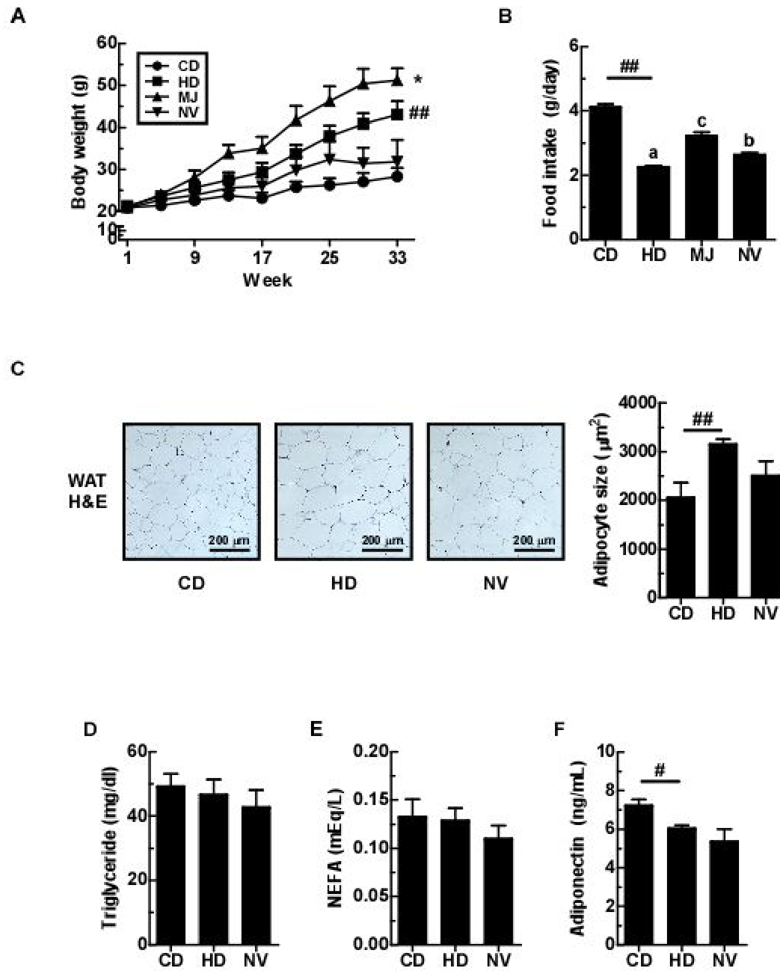


Figure 6. Effect of NV on obesity

Body weight and food intake were measured every weeks. (A) NV-fed mice did not reduce body weight gain. (B) Food intake was not less in NV-fed group than HD-fed group. (C) H&E staining was performed in WAT and adipocyte size was measured by Image J that there was no significantly difference between HD- and NV-fed groups. (D) TG assay, (E) NEFA assay and (F) adiponectin assay which were performed in plasma showed that NV had no significantly improvement compared than HD control. Value are expressed as means ± SEM. ##, $p < 0.01$ and #, $p < 0.05$, indicates significant differences between CD-fed mice and HD-fed mice, * or letters (a-c) and $p < 0.05$, indicates significant differences between HD-fed mice and samples treated mice.

Ⅳ. Discussion

In this present study, 6000 kinds of new pepper variety were tested and selected considering such as stability, red color, pungent taste, functionality. 'Hana 4176 (NV)', as a final elected pepper, was used as a sample in order to examine anti-diabetic effects. NV-fed mice was better to control blood glucose than HD-fed mice. Even NV-fed mice had improvement on blood glucose control compared to MJ-fed mice. So, this data demonstrates that NV is able to representative pepper which has not only high stability and quality but also outstanding preventive effect on insulin resistance.

During the animal study, although obesity has been widely known as one of the major reason for insulin resistance [28], I found that mice had no weight loss effect but blood glucose was improved in ITT data. In adipose tissue, the size of adipocytes did not changed in

NV-fed group compared to HD-fed group. However, infiltration of macrophage in adipose tissue is known as major causing factors of insulin resistance secreting inflammatory cytokines such as TNF-alpha, IL-1beta and IL-6 [29] which circulate locally or wholly of the body [30]. Therefore, inhibitory effect of NV on insulin resistance is mainly caused by reducing macrophage infiltration into adipose tissue, irrespective of body weight.

Ectopic lipid means that cells store triglycerides within non-adipose tissue and it causes insulin resistance [31]. Liver phenotype of NV-fed group by H&E staining had less triglycerides than HD-fed group. Moreover, the expression of lipogenesis-related genes such as SREBP1c, FAS and ACC2 in liver, and of fatty acid oxidation-related genes such as UCP3 and CPT-1b in muscle were improved indicating that NV has an effect on blocking ectopic fat storage. Consequently, phosphorylation of Akt of NV-fed group was increased in liver and muscle, but NV-fed

group had no improvement compared to HD-fed group.

In conclusion, a novel variety of pepper protects against HD-induced insulin resistance in mice. It is widely known that chili pepper has a weight loss effect [37], but body weight did not significantly decrease in NV-fed mice compared to HD-fed mice. However, food intake was lower in HD-fed mice than MJ- and NV-fed mice. In the previous study, NV was proved to its high pungent taste and carotenoids [11]. It could be explained that this novel variety of pepper promotes energy expenditure. In further study, it needs to confirm which compounds of NV have an important role in each tissue. Also, GC/MS should be performed to analysis different components of NV compared to MJ. Taken together, these results indicate that NV could have potential agents for preventing HD-induced insulin resistance, largely T2DB. Therefore, candidate phytochemicals should be tested *in vitro* to elucidate therapeutic target of insulin resistance.

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VI. 국문 초록

고추는 한국을 대표하는 식품 중 하나로서, 고추를 이용한 많은 식품들이 존재하여 섭취가 되고 있다. 고추의 다양한 생리활성 성분들은 특히 당뇨에 효능이 있다고 알려져 왔다. 따라서 한국의 대표 고추 품종으로써 재배안정성, 고품질, 항당뇨 기능성을 모두 갖춘 고추 품종을 개발하고자 이 연구를 진행하였다. 총 6000여 개의 신품종으로부터 재배안정성 및 품질에서 우수한 특성을 가진 신품종 고추 ‘하나 4176’을 선별하여 본 연구에 활용하였다. 33주간 고지방식이를 먹인 쥐와 고지방식이에 0.83%의 고춧가루가 함유된 식이를 먹인 쥐를 비교한 결과, ITT 실험 시 신품종 고추를 먹인 쥐에서 인슐린에 반응하여 현저한 혈당 저하 효능을 확인하였다. Western blot을 통하여 인슐린 신호의 대표 단백질인 Akt의 인산화가 증가됨을 확인하였다. 인슐린 저항성의 주요 원인인 염증을 지방조

직에서 IHC 염색과 mRNA 수준을 확인한 결과, 대식세포 인자인 F4/80와 CD68, 그리고 대식세포를 유인하는 MCP-1과 이것의 수용체인 CCR2 또한 현저히 감소하였다. 인슐린 신호가 개선된 간과 근육에서의 효능을 확인 한 결과, SREBP1c, FAS 그리고 ACC2 mRNA 수준과 더불어 지방간이 감소한 결과를 확인하여 간에서 지질 합성 및 축적을 억제한 것을 확인하였다. 또한, 근육에서는 UCP3와 CPT1b의 mRNA 수준을 유의적으로 증가시키면서 지방산의 연소를 증가시킨 것을 확인하였다. 하지만 비만에는 큰 영향이 없었음을 추가로 제시하였다. 본 연구 결과를 통해 신품종 고추는 고지방식이로 유도되는 인슐린 저항성을 각 조직 별로 억제하여 식품으로써 제2형 당뇨를 예방하는 기능성 식품 품종을 육성하였음을 밝혔다.