The effect of Red-yeast-rice on serum lipid profile and glucose control in subjects with impaired fasting glucose or impaired glucose tolerance

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A Master's Thesis Submitted to
the Department of Graduate Program in Science for Aging
and the Graduate School of Yonsei University
in partial fulfillment of the requirements for
the degree of Master in Science for Aging

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January 2008

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January 2008

감사의 글

지난 2년 동안 대학원의 생활은 제 자신의 발전을 위해 값진 것들을 배우고 느낄 수 있었던 소중한 시간이었습니다. 부족하나마 논문이 완성되기까지 따뜻한 격려를 주신 많은 분들께 지면을 통해 그 감사의 마음을 전합니다.

무엇보다 대학원 생활을 잘 마칠 수 있도록 따뜻한 격려와 관심, 사랑을 주신 아빠와 다 큰 딸에게 아침마다 사랑의 도시락을 싸주신 엄마께 진심으로 존경과 사랑의 마음을 전합니다. 누나에게 아낌없는 조언으로 든든한 버팀목이 되어준 듬직한 나의 동생 태근이에게도 고마움과 사랑의 마음을 전합니다. 내 생애 최고의 선물인, 세상 누구보다 소중한 가족에게 사랑의 마음과 함께 이 작은 결실을 바칩니다.

임상영양학에 대한 배움의 기회를 주시고 학문에 대한 진정한 열정을 가르쳐 주신 이종호 교수님께 감사드립니다. 바쁘신 와중에도 항상 인자한 웃음으로 신경써 주시는 장양수 교수님께 감사드립니다. 언제나 매력적인 목소리로 강의해 주시고 학생의 입장에서 배려해 주시는 자상하신 정지형 선생님, 유쾌한 강의로 해주시는 언변의 마술사 조홍근 선생님께도 감사의 마음을 전합니다. 학부시절 영양학이 얼마나 중요한 학문인가 가르쳐 주시고 자상하게 지도하여 주신 손숙미, 김석신, 고경희, 오명숙 교수님들께도 너무 감사합니다.

학문적으로, 인간적으로도 많이 배우고 싶은 오연언니와 나의 석사학위 논문을 지도하며 함께 신경써주신 지영언니, 함께 생활하며 도시락을 통해 정이든 예쁜 아기의 엄마가 된 지숙언니, 카스리마와 소탈한 성격이 매력적인 멋쟁이 수정언니, 언제나 밝은 미소로 노화과학을 이끌어 주신 정임언니, 힘들 때 정신적으로 의지할 수 있게 도와주신 유머와 재치를 겸비한 예정언니와 순수하고 해맑은 미소를 가진 상큼 발랄 혜진언니께도 깊은 감사를 드립니다. 항상 톡톡 튀고 거침없

는 말솜씨로 즐겁게 해준 현양언니와 아기 같은 하얀 피부를 가진 어여쁜 진경언니에게도 감사의 마음을 전합니다. 함께한 시간이 짧았지만 항상 웃음으로 반겨준 윤지숙 언니, 깜짝 결혼발표와 함께 10월의 신부가 된 선배이자 친구인 정현이에게도 감사의 마음을 전합니다.

어리숙한 저를 관심과 열의를 가지고 항상 곁에서 지켜봐주신 선배들, 지금은 졸업하여 각 자의 길에서 최선을 다하는 현진언니, 오수현언니, 계영언니, 미진언니, 유미언니, 박수현언니, 민지언니에게 고마움을 전합니다. 그리고 각 자의 길을 가기위해 최선을 다하는 후배들... 생년월일시까지 똑같은 사주친구 승현이, 파티클 미인라인을 이어나갈 당차고 여성스러운 주리, NO 실험 파트너이자 엉뚱함이 매력적인 귀여운 소연이, 씩씩하게 최선을 다하는 모습이 아름다운 파티클 미인라인 민아, 아기자기한 미모를 소유한 여성스러운 윤경이, 아기 코알라를 닮은 씩씩하고 야무진 지원이, 무뚝뚝함과 전혀 어울리지 않는 상큼 발랄함으로 나를 놀래킨 민주언니, 존재자체만으로도 애교가 넘쳐흐르는 애교만점 영민언니, 곁에서 응원을 아낌없이 해준 고마운 정호오빠. 소중한 인연으로 만나서 기쁘고 앞으로 행복하길 바랍니다.

단란한 가족과 같은 포근한 노과연 식구들... 짧은 유머로 큰 기쁨을 안겨준 유쾌하고 마음이 따뜻한 수혁오빠, 세심한 배려와 수줍음 많은 속 깊고 정 많은 마음이 잘 맞는 승원오빠, 차가운 이미지와 달리 재밌고 귀여운 개구쟁이 강원선배, 나의 뒤를 이어 멋지게 과대를 이끌어준 카메라맨 태원선배, 칵테일과 잘 어울리는 봉준오빠, 노과모임의 정예멤버로 돈독해진 여성스럽고 성격 좋은 지혜, 언니처럼 편하게 대해주신 이세영선생님, 영양분야에 관심이 많아 학생으로서 배울점이 많은 이기호 선생님, 김경철 선생님. 푸근하고 자상하신 신경균 선생님, 깔끔한 인상과 지적이신 핸섬가이 오세연 선생님, 여동생처럼 착하고 편한 현주, 카리스마 지희. 첨단 3인방의 청일점이자 나와 시내언니의 흑기사가 되어준 착하고 인자한 범식님. 소중한 인연으로 좋은 추억 많이 만들어 준 노과연 식구들에게 감사의 마음을 전하며, 모두가 행복하길 바랍니다.

그리고 우리 동기들... 희노애락을 함께하며 나의 버팀목이 되어준 센스쟁이 시내언니, 보라색코트가 잘 어울리는 재치만점의 귀여운 소연이, 동기 중 유일한 동

생이자 깜찍 발랄한 효희, 2년간 일산 MI 스터디를 함께한 까칠하지만 속마음은 따뜻한 주연언니, 4월의 예비신부 여진언니, 빨간색이 잘 어울리는 물란 닮은 주영이, 단발머리가 잘 어울리는 해맑은 미소의 소유자 소의언니와 함께 힘들고 즐겁고 지칠 때마다 함께 할 수 있어서 항상 큰 힘이 될 수 있었습니다... 잊지 못할 우리 8명. 사회에 나가서도 각자의 길에서 최선을 다하자..^^ 아자아자 화이팅!!

이른 아침부터 따뜻한 미소로 함께 해주신 김문경 선생님. 아래학기가 없어서 힘들었지만, 선생님과 함께 이야기할 수 있는 시간이 많아서 즐거웠습니다. 선생님 항상 행복하세요. 그리고 순환기 내과 의사선생님들 (양주영 선생님, 김병극 선생님, 전동운 선생님, 오성진 선생님)과 간호사 선생님들께도 감사의 마음을 전합니다.

나의 소중한 친구들... 고등학교 때부터 지금까지 변함없는 종로파 현주, 진숙, 선아와 정미, 화현이, 철호. 가톨릭대학교 1학년 때부터 자칭 여인천하라 부르며 함께 지낸 아동학과 서진, 주영, 현정이와 의류학과 수연이, 식품영양학과 선아, 은진, 현진, 수희, 그리고 학과 학생회 활동을 하며 친해진 은경, 소영, 유리, 수혜, 현라. 병원실습을 할 수 있도록 도와준 남궁신아 언니. 학교생활에서 가장 뜻깊은 추억을 만들어준 동아리 7기 동기들... 길용오빠, 승애, 민우, 재현이, 경용이, 진주, 준례, 혜연이. 모두들 나의 곁에 소중한 친구로 있어줘서 고맙고 행복합니다. 마지막으로 2년간의 대학원 생활동안 친구보다 가까운 곳에서 나를 응원해준 신이에게 감사의 마음을 전합니다.

2008년 1월

강 미 란

CONTENTS

List of Figures

List of Tables

ABSTRACT

The effect of Red-yeast-rice on serum lipid profile and glucose control in subjects with impaired fasting glucose or impaired glucose tolerance

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This study aimed to evaluate the effect of red-yeast-rice dietary supplementation on cholesterol-lowering and glucose control in subjects with impaired fasting glucose or impaired glucose tolerance. We conducted a double-blind, placebo-controlled study with 3 groups ; placebo, low dose group (Test Ⅰ: red yeast rice 210.0mg/capsule, 2.52g/day) and high dose group (Test Ⅱ: red yeast rice 420.0mg/capsule, 5.04g/day),

which were randomly assigned to subjects with impaired fasting glucose or impaired glucose tolerance. We measured serum lipid profile (total-, LDL-, HDL- cholesterol, triglyceride) and glucose control related parameters (glucose, 2h OGTT, HOMA-IR, insulin, FFA) before and after the supplementation. Both Test Ⅰ and Test Ⅱ groups had significant improvement in LDL cholesterol and atherogenic index(AI) compared with placebo group($p<0.05$). Additionally, total and HDL cholesterol improved significantly in Test Ⅱ group compared with placebo group($p<0.05$). Fasting glucose decreased in test groups and increased in placebo group after intervention. However, it was not significant differences. In subjects which fasting blood glucose is more than 110mg/dl, fasting glucose had a tendency to decrease in test Ⅱ group($p<0.1$) and HbA1c had significant decrease in Test Ⅰ group, while insulin and HOMA-IR had a tendency to increase in placebo group after intervention. Mean changes of glucose related parameters compared with placebo group did not show significant differences. In conclusion, subjects with impaired fasting glucose or impaired glucose tolerance significantly improved in serum lipid profile by red yeast rice supplementation without serious side effects. These are more effective in the case of a high dose. The effects of red yeast rice supplementation on glucose control were insignificant. In addition, Long-term clinical studies need to be performed with larger population number of subjects to determine the effects of red yeast rice on the insulin resistance in the subjects with IGT or IFT and Type 2 DM.

Key words : Red yeast rice, Blood glucose, Impaired glucose tolerance
Impaired fasting glucose, Serum lipid profile

1. Introduction

Recently, the sharp increase of type 2 DM prevalence and CVD prevalence caused by type 2 DM were shown the main problem of health. The number of people with type 2 diabetes is estimated to increase rapidly within the next 25 years (1), with an estimated 42% increase in developed countries. In developed countries, the prevalence of overweight and obesity is increasing rapidly (2) because of reduced physical activity and overeating. This causes a rapid increase in the prevalence of diabetes (2,3). According to KNSO (The korea national statistically office), the number of people aged over 65 years in korea will increase without parallel in the world from about 14.4% in 2019 to 34.4% in 2050 (4). Accordingly it is considered that type 2 DM incidence also will increase rapidly.

Of the healthy men without type 2 DM, 0-16% had an IFG (Impaired fasting glucose) 6.1mmol/L-6.9mmol/L, and 6.9-17.8% had an IGT (Impaired glucose tolerance) with diagnosed an FPG (Fasting plasma glucose) <6.1mmol/L on glucose tolerance test, and 2-h PG 7.8mmol/L-11.0mmol/L and 0.5-9.7% had a type 2 diabetes with diagnosed FPG $\geq$ 7.0mmol/L and 2-h PG $\geq$ 11.1mmol/L (5). In 1988, Reaven defined the insulin resistance syndrome (syndrome X) which consists of insulin resistance, hyperinsulinemia, obesity, glucose intolerance, hypertriglyceridemia, reduced high density lipoprotein (HDL)-cholesterol level, hypertension, coronary arterial diseases, prothrombotic state (e.g., high fibrinogen), and pro-inflammatory state (e.g., elevated high-sensitivity C-reactive protein (h-CRP) in the blood) (6). Blood lipid and lipoprotein levels have been confirmed as important predictors of this pathology (7,8), and more recently, markers of low-grade inflammation have been shown to predict the occurrence of type 2 diabetes (7). Mild-moderate

insulin resistance, a common clinical case, leads to impaired glucose tolerance (IGT), type 2 diabetes mellitus (DM), hypertension and early atherosclerosis (9). Cardiovascular disease (CVD) is the leading cause of death among individuals with type 2 diabetes mellitus, accounting for 40% to 50% of all deaths (10). In these patients, the mortality risk for coronary, cerebrovascular and peripheral vascular disease is 2 fold to 10 fold higher than the nondiabetic population (11-13). Although type 2 diabetes is frequently associated with other cardiovascular risk factors, such as dyslipidemia and hypertention (14,15), it is believed that hyperglycemia per serum is independent risk factor (15). Several studies have now confirmed the increased risk of CVD in patients with impaired glucose tolerance (IGT) even after adjusting for classic risk factors (16-20). Therefore impaired glucose tolerance and impaired fasting glucose need to appropriate blood glucose control because it progress to type 2 DM or is considered the risk factor of CVD.

Monascus spp. have been used as foods and medicines in the Orient for over 1000years. In China and Taiwan, it has been called "Hong Qu", "Hon-Chi", "Anka" or "Ang-kak" using the Chinese or Taiwanese phonetic alphabet. The Japanese use the name "Beni Koji" or "red Koji". In the United States and Europe, it has been called "red rice", "red-mold rice" or "red Chinese rice". Many publications and commercial products use "red yeast rice" which is not an appropriate name for filamentous fungi (21).

Red yeast rice is the fermented product of rice on which red yeast (M. purpureus) has been grown (22). The most important bio-active compound isolated from Monascus is monacolin K, which is identical to the potent cholesterol-lowering, anti-atherosclerotic drug lovastatin, a HMG-CoA reductase inhibitor (21). Many studies have reported that Monacolin K disturb cholesterol synthesis and show anti-cholesterol effect (23-26). Red yeast rice is an extract of cholestin that has been approved by the Korea Food and Drug

Administration (KFDA) as serum cholesterol control (27).

High values of serum lipid increased in risk of type 2 DM (7,28), while Monacolin K, a functional ingredient of red-yeast rice, were observed improvement of cholesterol and decreased to serum triglyceride in patients with DM, impaired fasting glucose and impaired glucose tolerance. Therefore there is being reported the improved effect about glucose metabolism (5,29-31).

On this wise, Monacolin-K, a functional ingredient of red-yeast rice, have been reported that there is an effect of lowing serum blood glucose through animal itself efficacy and reference. In this study, red yeast rice was produced not drug but dietary supplementation (a low dose, a high dose) in the shape of capsule. Therefore, this study examined to confirm the effect on the improvement of blood glucose and serum lipid concentration of red-yeast rice in subjects with impaired fasting glucose or impaired glucose tolerance using a double-blind, placebo-controlled, prospectively randomized design.

Table 1. IDC and ADA criteria (adapted from ref. 32)

HbA1(%)	Average Blood Gucose (mg/dl)	Average glucose con. (mg/dl)	Care condition
6	135	7.5	Normal
7	170	9.5	Low complication
8	205	11.5	
9	240	13.5	High complication
10	275	15.5	
11	310	17.5	
12	345	19.5	

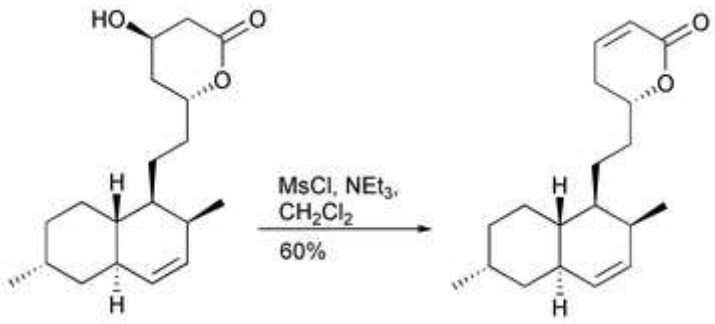
IDC: International Diabetes Center , ADA: American Diabetes Association

Table 2. The care condition about HbA1, average blood glucose and average glucose concentration

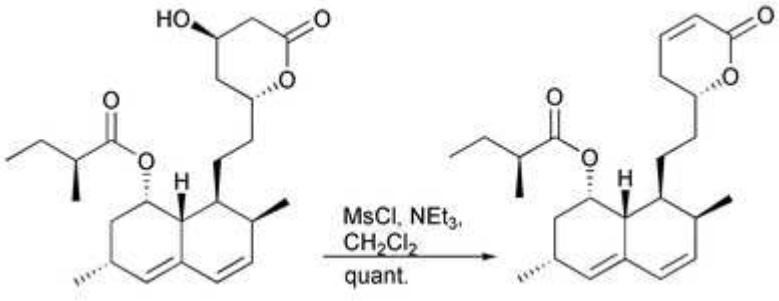
	Fasting glucose (mg/dl)	OGTT (mg/dl)
Normal	110>	140>
Impaired fasting glucose	110-125	140>
Impaired glucose tolerance	126>	140-199
DM	126≤	200≤

2. BACKGROUND

2.1. Structure of Monacolin-K and Lovastatin



Monacolin-K (or Mevinolin) non-activation (lacton form) **Monacolin-K (or Mevinolin) activation (acid form)**



Lovastatin non-activation **Lovastatin activation**

Figure 1. Chemical structure of Monacolin-K, Lovastatin (adapted from ref. 33)

Initially identified from Monascus spp., monacolin K ($C_{24}H_{36}O_5$), also known as mevinolin or lovastatin, is a polyketide, which is structurally identical to lovastatin. In addition to monacolin, there are several other minor monacolins. At least six structurally related monacolins have been identified from the genus Monascus, namely monacolin J, K, L and X, dihydromonacolin K, and dihydromonacolin L. Monacolin K and its hydroxyl acid form would be dehydrated, and converted to dehydromonacolin K at high temperature (80℃), while the monacolin K would be transformed into their corresponding hydroxyl acid forms under the condition of high humidity (92.5%RH, 25℃) (21). Mevinolin in the hydroxy acid form, mevinolinic acid (monascus K acid form), is a potent competitive inhibitor of HMG-CoA reductase. The structure and absolute configuration of mevinolin and its open acid form, mevinolinic acid, determine by a combination of physical techniques (34).

2.2. Evidence for health benefits of Monacolin-K

Besides monacolin K (lovastatin, once the world's largest selling class of cholesterol-lowering drugs), the Monascus products contain many other substance (flavonoids, polyunsaturated fats, pyrrolonic compounds, and so on) with a wide variety of actions. their effects may be more extensive and complex than those of statins alone. It also makes MRP an ideal candidate for the treatment of the metabolic syndrome (35).

2.2.1. Cholesterol-lowering effect

2.2.1.1. The inhibitor of HMG-CoA reductase

Hypercholesterolemia, especially elevated plasma LDL-cholesterol, is a key risk factor leading to the pathogenesis of atherosclerosis (36). Lovastatin (mevinolin) as the inhibitor of HMG-CoA reductase has greatly advanced the development of cholesterol-lowering drugs. Inhibition of hepatic HMG-CoA reductase, the rate-limiting enzyme in the cholesterol bio-synthetic pathway, stimulates the expression of LDL receptor (also called Apo B/E receptor) (37). Increased uptake of LDL through a receptor-mediated pathway reduces plasma LDL-C. Many clinical studies have shown that lovastatin and other statin drugs reduce plasma total cholesterol (TC) and LDL-C. Treatment with lovastatin also reduces plasma triacylglycerols (TG) and increase HDL-C to an extend less than the magnitude of TC and LDL-C lowering. MRP (Monacolin rice product) is used for dietary supplementations with the health claim that the product will lower plasma lipids, especially plasma TC and LDL-C. The MRP supplementations contain a family of naturally occurring statins (monacolins) including monacolin K. There are many commercial products of MRP worldwide. Among them "Cholestin", "Xuezhikang" and "Unchole" have demonstrated a cholesterol -inhibiting effect similar to statins in animal studies and clinical trials (21).

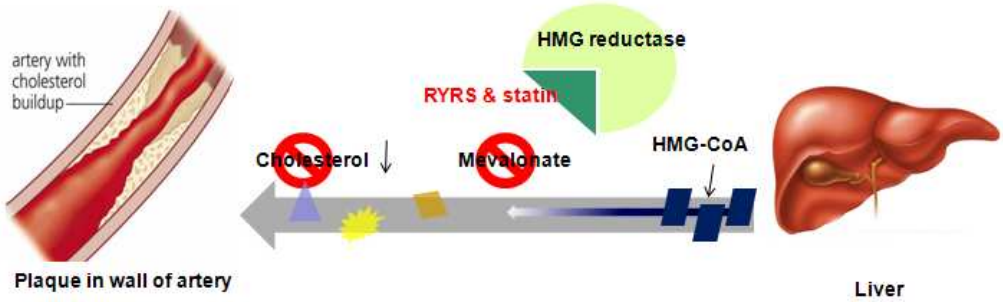


Figure 2. Putting HMG CoA reductase inhibitors into action

*** RYRS (Red Yeast Rice Supplementation)**

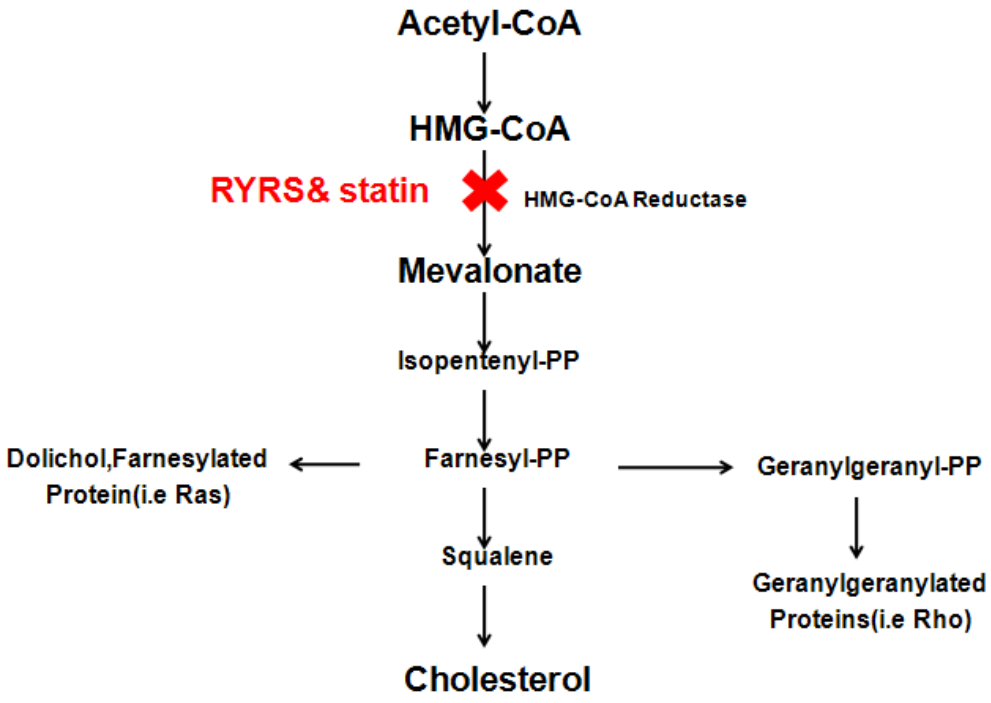


Figure 3. Pathway for cholesterol bio-synthesis (adapted from ref. 38)

*** RYRS (Red Yeast Rice Supplementation)**

2.2.1.2. Animal studies

Long-term feeding effects of M. purpureus-fermented rice (Cholestin) on serum lipids and the severity of atherosclerosis were examined in rabbits fed for 200 days on a semi-purified diet containing 0.25% cholesterol. Total serum cholesterol was 25% and 40% lower, respectively, in rabbits fed 0.4 or 1.35g/kg/day of MRP (Monacolin rice product) compared to those of controls. This treatment also lowered serum LDL-C, serum TG and the atherosclerotic index (ratio of non HDL-C to HDL-C). Although similar reductions of TC, LDL-C and TG were observed, a parallel group of rabbits fed with lovastatin (0.0024g/kg/day) did not have a significantly reduced atherosclerotic index (39). In the rat, orally administered sodium mevinolinate was an active inhibitor of cholesterol synthesis in an acute assay (50% inhibitory dose=46ug/kg). Furthermore, it was shown that mevinolin was an orally active cholesterol-lowering agent in the dog. Treatment of dogs for 3 weeks with mevinolin at 8mg/kg per day resulted in a 29.3±2.5% lowering of plasma cholesterol (34).

2.2.1.3. Clinical studies

Several studies and clinical trials have demonstrated that the intake of Monascus rice products (MRP) significantly decrease TC, LDL-C and TG in subjects, without causing clinically adverse effects in the liver and muscle tissue. The first human trial (40), an 8-week study conducted in china, evaluated the effect of 1.2g/day red yeast rice on 324 hypercholesterolemic adults (total cholesterol above 230mg/dl) who also had elevated LDL (over 130mg/dl) and low HDL (under 40mg/dl) verse controls. Total cholesterol,

LDL cholesterol and triglycerides dropped by 23, 31 and 34%, respectively. Serum HDL levels increased by 20%. The second study (27) included 83 hypercholesterolemic adults on 2.4g red yeast rice daily or placebo. Participants were asked to maintain a diet of 30% fat, 10% SFA and a maximum of 300mg cholesterol daily. After 8-weeks the treatment group had an 18% lower mean total cholesterol level compared to placebo and a 17% drop in total cholesterol baseline. There was also a 23% difference in LDL between the treatment group and the placebo group and a 23% drop in the treatment group, evident at eight weeks. Triglycerides also dropped 16% in the treatment population. The drops in total cholesterol and LDL were consistent at 8 and 12 weeks. There were no changes in HDL levels. Cheng-Chieh Lin, et al (41) was to assess the lipid-lowering effect of M. purpureus Went rice on serum lipids in patients with hyperlipidemia. In this stydy, Monascus purpureus Went rice resulted in significant reductions in LDL-C (30.6%), total cholesterol (23.7%) and triglycerides (13.4%) level from baseline to 4 weeks. These reductions were maintained at 8 weeks. Other study (42) reported mean LDL-C reductions to be 20, 16, 19 and 24% for fluvastatin (80mg), lovastatin (80mg), pravastatin (40mg) and simvastatin (20mg) respectively; mean apolipoprotein B levels were reduced by 16, 19, 16 and 20mg respectively. In this study, 1200mg of M. purpureus Went rice contains a total of 13.9mg statins (contains 11.4mg lovastatin) but reduced LDL-C by 27.7% and apolipoprotein B by 26.0%.

2.2.2. Antihyperglycemic activity

2.2.2.1. Association with Insulin resistance and TG

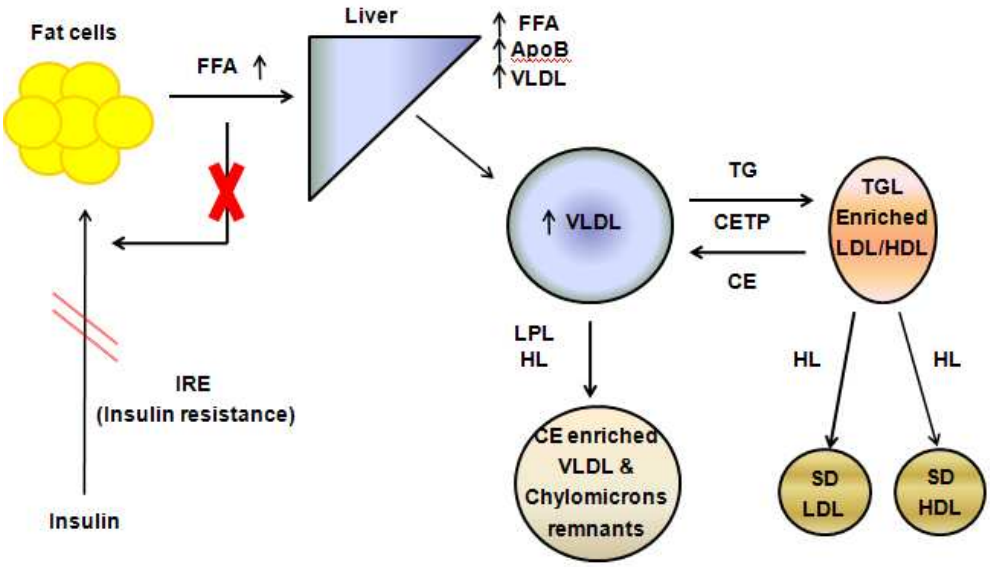


Figure 4. Mechanism relating insulin resistance with lipid concentration. (adapted from ref. 43)

Insulin resistance allowed an increased production and flow of free fatty acids from the abdominal viscera to the liver (44), where these fatty acids accelerate triglyceride production. Such negative effect of insulin resistance is magnified by the occurrence of hyperglycaemia, which also increase VLDL production by the liver (45). On the other hand, elevated plasma triglyceride concentration is responsible for an overactivity of the Randle cycle due to a prevalent lipid oxidation. The latter phenomenon, in turn, has been shown to impair insulin-mediated glucose metabolism in both oxidative and non-oxidative components (46).

2.2.2.2. Animal studies

MRPs produced by fermentation with M. pilosus and M. purpureus were used for antihyperglycemic activity screening in streptozotocin-induced diabetic rats (STZ-diabetic rats) (47) Single oral administration of MRP decreased plasma glucose in STZ-diabetic rats in a dose-dependent manner from 50 to 350mg/kg. These results suggest that oral administration of MRP could decrease hepatic gluconeogenesis to lower plasma glucose in diabetic rats with insulin deficiency. The hypoglycemic effect of MRP was also studied by another group (48). Oral administration of MRP, fermented with M. pilosus and M. purpureus for 90min to fasting Wistar rats resulted in a decrease in plasma glucose in a dose-dependent manner. In parallel to the reduction of plasma glucose, an increase in the plasma level of insulin and C-peptide was also observed. The study also suggests that MRP has an ability to stimulate the release of acetylcholine from nerve synapses, which in turn stimulates muscarinic M(3) receptors in pancreatic cells and augments insulin release to results in a plasma glucose-lowering action.

2.2.2.3. Clinical studies

Xuezhikang contains a family of monacolin-related substances, one of which is a naturally occurring lovastatin. The China Coronary Secondary Prevention Study (CCSPS) (49), is a multi-center trial designed to compare the effects of xuezhikang and placebo on reducing CVD events in 4870 patients who had a history of myocardial infarction. This report presents a detailed subgroup analysis of the 591 diabetic participants. A total of 591 diabetic patients with CHD were randomized to the zeuzhikang group (n=306) and the placebo

group (n=285). During the average 4 years of follow-up, there were 28 case of CHD events (9.2%) in the xuezhikang group and 53 cases (18.6%) in the placebo group. When patients with and without diabetes compare, it seemed likely that patients with diabetes could gain more beneficial effects from xuezhikang treatment with respect to CHD events and total mortality.

3. SUBJECTS AND METHODS

3.1. SUBJECTS

Our study subjects were recruited from a population of men and women between the ages of 18 and 80 years. This study consisted of 85 cases that met diagnosed with impaired fasting glucose (110mg/dl≦fasting blood glucose≦ 126mg/dl) or impaired glucose tolerance (140mg/dl≦2h OGTT≦199mg/dl) or diagnosed with 6%≦ glycosylated haemoglobin (HbA1c) ≦7%. Exclusion criteria that all selected patients met were:

- having a evidence of renal, hepatic or cancer disease;
- having a severe allergies as determined by medical history;
- being previously diagnosed with type 2 DM (fasting blood glucose≥ 126mg/dl or 2h OGTT plasma glucose ≥200mg/dl)
- having a normal lipid profile (total cholesterol, triglyceride, LDL cholesterol);
- taking obesity treatment within last two months (diet or medical)
- attending regular physical program or weight loss of more than 3% within the last 6 months;
- having obesity due to endocrinologic disease (hypothyroid, etc.);

- being on drugs that may affect insulin resistance; for example, taking an angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker group drug;
- cases with acute coronary diseases;
- patients on hormone replacement treatment (HRT) or those using selective estrogen receptor modulator (SERM);
- presence of infection, severe physical or psychological trauma on the day of insulin tolerance test or any recent day, as these may affect the result or the test;

None of them were taking any medication. Written conformed consent was obtained from all subjects and the protocol was approved by the institute of Review Board of Yonsei University.

3.2 STUDY DESIGN

Our study was a double-blind, placebo-controlled, randomized study. All enrolled subjects were screening to HbA1c test, glucose metabolism test (fasting glucose test, OGTT: oral glucose tolerance test) during a week run-in period. At the end of the run-in period, a total of 85 of the 102 randomized subjects were included in the study and 17 subjects were excluded this study according to exclusion criteria. At the end of the follow-up study during 12 weeks, we excluded 21 subjects who were canceled agreement, personal feelings and allergy, finally 64 subjects were left in the follow-study and used in the analysis. All subjects were assigned randomly to Test Ⅰ(a low dose; red yeast rice 210.0mg/capsule, 2.52g/day)(n=21), Test Ⅱ(a high dose; red yeast rice 420.0mg/capsule, 5.04g/day) (n=23), placebo group (lactose 420.0mg)(n=20).(table

3)(table 4) All subjects were taken four by four three times a day after meals during 12 weeks (figure 4). Attending subjects usually maintained lifestyle during this follow-study period. All of the volunteers were instructed by a research dietitian to record for 2 days on weekdays and a day on weekend all food and beverages consumed, preparation method and approximate portion sizes in food diaries at the time of consumption.

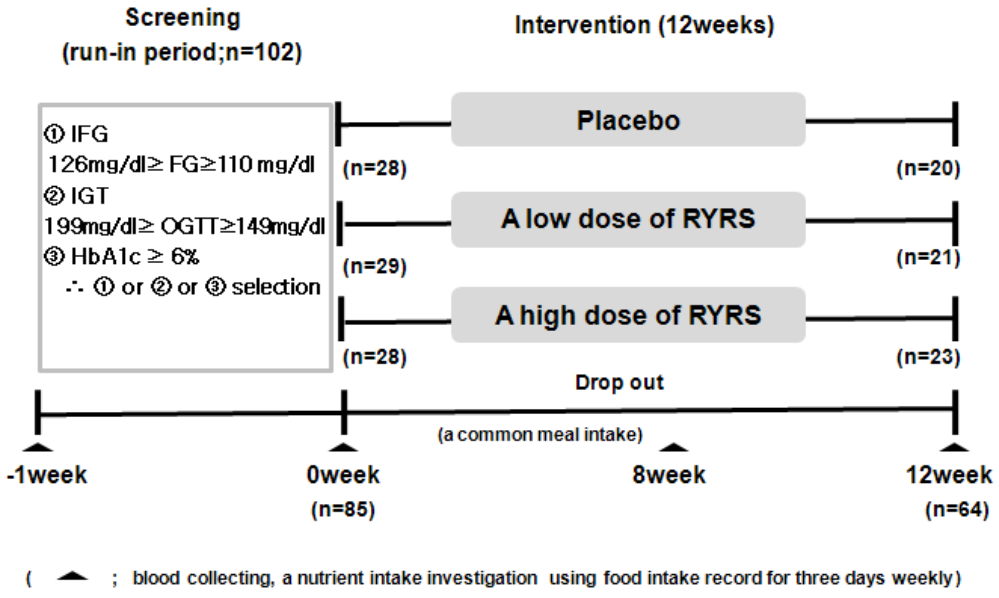


Figure 5. Study design * **RYRP: Red yeast rice supplement**

Table 3. The composition of test products (in 1 capsule)

	Ingredient	Test Ⅰ group (a low dose)	Test Ⅱ group (a high dose)
A main ingredient	Red yeast rice powder	210.0mg	420.0mg
	A lactose agent (lactose 95%, dextrin 5%)	210.0mg	0.0mg
A accessory ingredient	A lactose agent (lactose 95%, dextrin 5%)	77.5mg	77.5mg
Lubricant	Magnesium stearate	2.5mg	2.5mg

Table 4. The composition of placebo products (in 1 capsule)

	Ingredient	Placebo
A main ingredient	A lactose agent (lactose 95%, dextrin 5%)	420.0mg
A accessory ingredient	A lactose agent (lactose 95%, dextrin 5%)	77.5mg
Lubricant	Magnesium stearate	2.5mg

3.3. METERIAL AND METHODS

For laboratory assay, all measurements were done in a single batch at the end of the study, and the laboratory staff was blind to the clinical data.

3.3.1 Anthropometric parameters and Dietary intake and energy expenditure

Body weight and height were measured in the morning, light clothed without shoes to 0 week and 12 week. The body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared. Waist and hip circumference were measured, and waist to hip ratio (WHR) was computed as an indiction of the index of body fat distribution. Waist circumference was measured with a flexible tape midway between the lower rib margin and the iliac crest, and the hip girth was measured at widest part of the hip. Both circumferences were measured in the standing position after normal expiration. Blood pressure was measured from the left arm while the subjects remained seated. An average of three measurements was recorded for each subject. When the systolic blood pressure was greater than or equal to 140mmHg, or the systolic blood pressure was greater than or equal to 90mmHg, they were classified as having hypertension according to the 6th report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. A dietitian gave written and verbal instructions to all subjects in follow-up study on how to comp‘ 0, 8 and 12 weeks visits throughout the whole intervention period and in cross-sectional study given once. On the sheet, subjects were instructed to record the amount of foods. Nutrient intake data were analyzed using Can pro program (Can-pro-2.0). Total energy

expenditure (TEE) (kcal/day) was calculated from activity patterns including basal metabolic rate (BMR), physical activity for 24 hours and specific dynamic action of food. BMR of each subject was calculated with the Harris-Benedict equation.

3.3.2. Fasting blood collection

To reduce the influence of circadian variation, all blood specimens were collected between AM 08:00 and AM 10:00 after the subjects had fasted overnight. Venous blood specimens were collected in EDTA-treated and plain tubes after a 12h fast. The tube were immediately placed on ice until arrived at the laboratory room (within 1~3h). As arrived, the blood were separated by centrifuge and stored at -70℃ until analysis.

3.3.3. Serum glucose and free fatty acid

Glucose was measured by a glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA, U.S.A.). FFA was analyzed with a Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). The glucose criteria, newly developed and modified by the National Diabetes Data Group (NDDG) and the World Health Organization (WHO) Expert Committee on Diabetes Mellitus, were used to categorize subjects as diabetic : fasting glucose $\geq$ 7.0mmol/L(126mg/dl)

3.3.4. Oral glucose tolerance test (OGTT)

75g OGTT was performed in all subjects to establish glucose tolerance status. A 250-350ml solution of 75g anhydrous glucose is administered orally and venous blood is drawn. The test were done at 9:00-10:00h after a 12h overnight fast. Fasting and 2h blood samples be taken. OGTT was measured by Oxidation enzyme.

3.3.5. Insulin, HOMA-IR and HbA1C (Hemoglobin A1c)

Insulin was measured by radioimmunoassays with commercial kits from Immuno Nucleo Corporation (Stillwater, MN, U.S.A). Insulin resistance (IR) was calculated with homeostasis model assessment (HOMA) using the following equation :

HOMA-IR = {fasting insulin (uU/ml)×fasting glucose (mmol/l)}/22.5

HbA1C were measured by Immunoturbidimetric analyzer using turbidimeter.

3.3.6. Serum lipid profile

Serum total cholesterol, LDL cholesterol and triglyceride were measured with commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd.,Tokyo, Japan) After precipitation of serum chylomicrons, low density lipoprotein (LDL) and VLDL using dextran sulfate-magnesium, high density lipoprotein (HDL) cholesterol left in the supernatant was measured with an enzymatic method. LDL cholesterol was estimated indirectly using the Friedwald formula, I.e., LDL cholesterol=total cholesterol-{HDL cholesterol+(triglycerides/5)}, for subjects with serum triglycerides levels <400mg/dl.

3.3.7. Haematology · Chemical blood test

Haematology test were measured by electric resistance method using an Automatic Blood Cell Counter (LC-240A, HORIBA Co., Japan).

Liver function indicator as chemical blood test were measured serum GOT, GPT activity and kidney function indicators were measured by Colormetry using Hitachi 7600-110 (Hitachi, Japan) with total protein, BUN, and creatinine concentration.

3.3.8. Serum TNF-alpha, IL-6 and CRP

Serum tumor necrosis factor-alpha (TNF-α) as measured using an enzyme immunoassay (R&D system, MN, USA). Sample was added to monoclonal anti-TNF-alpha antibody was added. A substrate solution was added to develop color in proportion to the amount of TNF-α bound. The resultant color reaction was read using a Victor2 (Perkin Elmer life sciences Turka, Finland) at 490nm and wavelength correction was set to 650nm. Quantification of TNF-α was performed with using the peak area ratio.

Serum interleukin-6 (IL-6) was added to a monoclonal anti-IL-6 antibody which was pre-coated onto a microplate. After washing away any unbound substances, an enzyme-linked polyclonal anti-IL-6 antibody was added. A substrate solution was added to develop color in proportion to the amount of IL-6 bound. The resultant color reaction was read using a Victor2 (Perkin Elmer life sciences, Turka, Finland) at 450nm and wavelength correction was set to 540nm. Quantification of IL-6 was performed with using the peak area ratio.

Serum high-sensitive C-reactive protein (hs-CRP) levels were measured with an

Express+ autoanalyzer (Chiron Diagnostics Co., Walpole, MA, U.S.A.) using a commercially-available, high-sensitivity CRP-Latex(Ⅱ) X2 kit(Seiken Laboratories Ltd., Tokyo, Japan) that allowed detection of CRP levels as low as 0.001mg/dl and as high as 32mg/dl.

3.3.9. Urinary $PGF_{2\alpha}$ and Plasma MDA

Urine was collected after 12 hour fast in polyethylene bottles containing 1% butylated hydroxytoluene (BHT) before blood collection. The tubes were immediately covered with aluminum foil and stored at -70℃ until extraction. 8-epi-prostaglandin-$F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) was measured using an enzyme immunoassay (Bioxytech 8 Isoprostane™ Assay kit, OXIS International Inc., OR, USA). The resultant color reaction was read using Victor^2 (Perkin Elmer life science, Turka, Finland) at 650nm. Quantification of 8-epi-$PGF_{2\alpha}$ was performed with using the peak area ratio. Urinary creatinine concentrations were determined by the alkaline picrate (Jaffe) reaction, and urinary 8-epi-$PGF_{2\alpha}$ levels were expressed as picograms per milligram creatinine.

Plasma malondialdehyde (MDA) was assayed according to the fluorometric method described by Buckingham. MDA are acts with thiobarbituric acid to form absorption adduct with maximum absorption at 532nm. Absorption measurements were obtained on Wallac Victor 2 1420 multilabel counter (Perkin Elmer life sciences, Turka, Finland).

3.4. STATISTICAL ANALYSIS

All analyses were performed with the use of SPSS version 12.0 for Windows (Statistical Package for the Social Science, SPSS Ins., Chicago, IL, U.S.A.). While the pearson correlation coefficient was used for average, mean+SE, the categorical variables were used for frequency. We compared baseline characteristics between the intervention and control groups by using Paired t-test for continuous variables and chi-square tests for categorical variables. And we compared the difference in changes from baseline to follow-up in dietary nutrient intake, body weight, serum lipid profiles (total-, LDL-, HDL-cholesterol, triglyceride), glucose related parameters (glucose, 2h OGTT, HOMA-IR, insulin, FFA), lipid peroxidation (MDA, $PGF_{2\alpha}$) pro-inflammatory cytokines (serum CRP, TNF-α, IL-6) between the intervention and control groups by using Paired t-tests. In the main analysis, we included only study participants who completed the intervention. Results are expressed as mean±SE. A two tailed value of $P<0.05$ was considered statistically significant.

4. RESULTS

4.1. General Characteristics of the subjects

Study participants age were mean 53.6±1.44 years and mean body mass index 25.0±0.40kg/m^2 and mean fasting blood glucose 130±2.21mg/dl. Each Test group and placebo group did not show differences in age, BMI, and fasting blood glucose on the study onset. The male range of each group did not differ from sex range of each group in Test Ⅰ group (n=15, 71.4%), Test Ⅱ group (n=14, 60.9%) and placebo group (n=10, 50.0%).

4.2. Anthropometric and Blood pressure

Baseline values of each group did not show significant differences between each Test group and placebo in anthropometric. (Table 5) When the pre- and post-treatment parameters of the subjects were compared, Between Test Ⅰ group and placebo group did not show significant changes in body weight (kg), body fat (%), lean body mass (kg) and blood pressure after 12 weeks. (Table 5) In Test Ⅱ group, body weight decreased 66.5±1.90kg to 65.7±1.87kg significantly, (p=0.026) and diastolic blood pressure decreased 84.4±3.35mmHg to 78.0±2.30mmHg significantly (p=0.025) after 12weeks, while the others did not find significant changes. (Table 5)

Table 5. Anthropometric parameters and blood pressure before and after intervention

	Test I (n=21)		Test II (n=23)		Placebo (n=20)	
	Baseline	**12 week**	**Baseline**	**12 week**	**Baseline**	**12 week**
Body weight (kg)	70.60± 2.03	70.10± 2.01	66.50± 1.90	65.70± 1.87*	64.60± 2.84	64.60± 2.94
Body fat (%)	29.10± 1.66	29.40± 1.52	28.70± 1.95	28.80± 1.73	28.80± 1.85	28.30± 1.71
Lean body mass (kg)	50.00± 1.67	49.40± 1.68	47.30± 1.58	46.60± 1.53	46.10± 1.93	45.70±41.83
Systolic BP (mmHg)	135.00± 3.12	135.00± 4.06	133.00± 4.08	1300± 5.15	129.00± 3.21	129.00± 4.01
Diastolic BP (mmHg)	82.00± 2.37	83.20± 1.89	84.40± 3.35	78.00± 2.30*	77.80± 2.20	77.30± 2.51

Mean ± S.E. *$p<0.05$ compared with initial value in each group

4.3. Serum lipid concentration

In placebo group after 12 weeks, serum lipid concentration did not show significant differences. (Table 8) In Test Ⅰ group after 12 weeks, serum cholesterol and LDL cholesterol changes decreased significant levels statistically to -18.1±8.46mg/dl and -23.6±6.55mg/dl respectively compared with baseline (p=0.045, p=0.002), HDL cholesterol increased to 2.80±6.55mg/dl compared with baseline. (p=0.068) (Table 8) (Figure 5) In Test Ⅱ group, serum triglyceride concentration had a tendency to decrease to -23.7±13.0mg/dl, total cholesterol and LDL cholesterol changes decreased significantly to -27.0±3.98mg/dl and -27.0±3.78mg/dl respectively compared with baseline (p<0.001). And HDL cholesterol changes increased significantly to 4.65±2.10mg/dl compared with baseline(p=0.038) after 12 weeks. (Table 8) (Figure 5) The arteries harden index (AI) did not show significant changes in placebo group, while it was improved significantly in both Test Ⅰ group and Test Ⅱ group (p=0.006, p=0.000) after 12 weeks. (Table 8) (Figure 5)

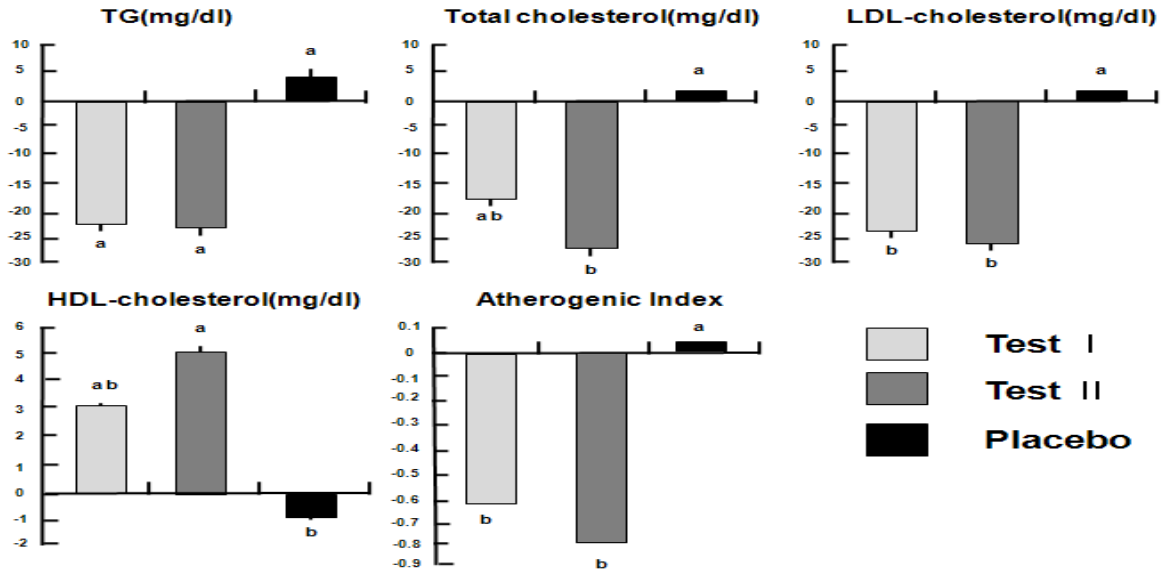


Figure 6. Comparison of changes in triglyceride, total cholesterol, LDL-cholesterol, HDL-cholesterol and atherogenic index among test and placebo groups

Mean±S.E. changed values significantly different ($p<0.05$) are indicated by different letters based on one-way ANOVA

4.4. Glucose control related parameters (Glucose, 2h OGTT, HbA1C, Insulin, HOMA-IR, FFA)

Mean fasting blood glucose changes after 12 weeks decreased to -2.29±4.04mg/dl in Test Ⅰ group, -4.39±2.66mg/dl in Test Ⅱ group, and -1.40±3.96mg/dl in placebo group compared with baseline. Mean fasting blood glucose were more decrease Test Ⅰ,Ⅱ groups than placebo group, while changes were not significant statistically. (Table 6) In addition, In subjects which fasting blood glucose is more than 110mg/dl, blood glucose had a tendency to decrease fasting blood glucose after 12 weeks. (p=0.055) (Table 7)

Blood glucose changes of 2h OGTT decreased to -18.6±12.1mg/dl in Test Ⅰ group, -4.22±12.5mg/dl in Test Ⅱ group. All Test groups were improved 2h OGTT, while placebo group increased blood glucose after 2h OGTT, but these were not significant statistically. (Table 6) In addition, In subjects which fasting blood glucose is more than 100mg/dl, 2h OGTT did not show significant differences in all groups after 12 weeks.

HbA1C changes after 12weeks had a tendency to decrease to -0.13±0.07% in Test Ⅰ group, decrease to -0.06±0.12% in Test Ⅱ group compared with baseline, which did not find significant differences statistically. In placebo group, HbA1C changes increased to 0.02±0.11% compared with baseline, which were not significant differences statistically. (table 6) In addition, in subjects which fasting blood glucose is more than 110mg/dl, HbA1C decreased significantly in Test Ⅰ group after 12 weeks. (p=0.013) (table 7)

In all Test groups after 12 weeks, insulin, HOMA-IR and FFA showed significant differences compared with baseline. (Table 6) In placebo group after 12 weeks, FFA concentration did not show significant differences, while serum insulin concentration and HOMA-IR had a tendency to increase. (p=0.059,

p=0.067) (Table 4) In addition, in subjects which fasting blood glucose is more than 110mg/dl, serum insulin, HOMA-IR and FFA did not show significant changes in all Test groups, while serum insulin concentration had a tendency to decrease compared with baseline (p=0.069), HOMA-IR had a tendency to increase compared with baseline (p=0.087) in placebo group.(Table 7)

4.5 Lipid peroxidation, pro-inflammatory cytokines

Table 8 lists lipid peroxidation, pro-inflammatory cytokines according to before, after intervention of red yeast rice supplement in three groups. The concentration of MDA, the index of lipid peroxidation, had a tendency to decrease in Test Ⅱ group, but did not show significant differences in Test Ⅰ, placebo groups. On the other hand, the concentration of urinary $PGF_{2\alpha}$, the index of lipid peroxidation, had a tendency to increase in placebo group, but did not show significant differences in Test Ⅰ,Ⅱ groups.

The concentration of TNF-α and serum CRP among pro-inflammatory makers did not show significant differences in all groups, while IL-6 concentration had a tendency to increase in placebo group, but did not show significant differences in Test Ⅰ,Ⅱ groups.

Table 6. Glucose metabolism parameters before and after intervention

	Test I (n=21)		Test II (n=23)		Placebo (n=20)	
	Baseline	**12 week**	**Baseline**	**12 week**	**Baseline**	**12 week**
Glucose (mg/dl)	127.00± 4.41	125.00± 5.20	128.00± 2.39	124.00± 3.43	134.00± 4.20	132.00± 5.59
Glucose120′ (mg/dl)	215.00± 16.20	196.00± 17.30	220.00± 10.20	216.00± 13.50	217.00± 17.40	234.00± 17.20
HbA1c (%)	6.50± 0.18	6.37± 0.18∓	6.39± 0.12	6.33± 0.13	6.39± 0.11	6.41±. 0.14
Insulin (IU/ml)	8.58± 0.71	8.56± 0.70	8.56± 1.15	9.16± 1.66	8.01± 0.70	9.62± 1.13∓
HOMA-IR *	2.70± 0.23	2.60± 0.22	2.77± 0.43	2.82± 0.55	2.61± 0.22	3.10± 0.33∓
FFA (uEq/L)	458.00± 44.30	382.00± 32.50	438.00± 36.40	417.00± 28.00	446.00± 39.10	399.00± 35.50

Mean ± S.E. ∓p<0.10 compared with initial value in each group
*** HOMA-IR (Homeostasis model assessment--estimated insulin resistance)={fasting insulin(uU/mL)× fasting glucose (mmol/L)} (26)**

Table 7. Glucose metabolism parameters before and after intervention (subjects with fasting glucose ≥ 110mg/dl)

	Test I (n=17)		Test II (n=21)		Placebo (n=19)	
	Baseline	**12 week**	**Baseline**	**12 week**	**Baseline**	**12 week**
Glucose (mg/dl)	133.00± 4.47	129.00± 5.52	130.00± 2.69	125.00± 3.69∓	135.00± 4.07	133.00± 5.80
Glucose120′ (mg/dl)	224.00± 17.90	201.00± 19.70	223.00± 11.10	218.00± 14.50	221.00± 17.90	238.00± 17.40
HbA1c (%)	6.59± 0.21	6.42± 0.21*	6.45± 0.12	6.38± 0.14	6.42± 0.11	6.42±. 0.14
Insulin (IU/ml)	8.59± 0.83	8.31± 0.81	8.95± 1.23	9.48± 1.81	7.83± 0.72	9.47± 1.18∓
HOMA-IR *	2.80± 0.27	2.62± 0.25	2.92± 0.45	2.94± 0.60	2.60± 0.23	3.09± 0.35∓
FFA (uEq/L)	477.00± 47.40	381.00± 35.50	428.00± 39.00	409.00± 29.00	443.00± 41.20	402.00± 37.40

Mean ± S.E. ∓p<0.10, *p<0.05 compared with initial value in each group
*** HOMA-IR (Homeostasis model assessment--estimated insulin resistance)={fasting insulin(uU/mL)× fasting glucose (mmol/L)} (26)**

Table 8. Lipid peroxidation, pro-inflammatory cytokines before and after intervention

	Test I		Test II		Placebo	
	Baseline	**12 week**	**Baseline**	**12 week**	**Baseline**	**12 week**
MDA (nmol/mL)	11.50± 1.50	9.50± 0.41	14.77± 2.30	10.29± 0.52∓	10.92± 1.21	9.76± 0.51
urinary $PGF_{2\alpha}$ (pg/mg creatinine)	1941.99± 172.99	2114.91± 146.39	1921.32± 188.74	1970.31± 194.78	1546.99± 128.03	1877.14± 205.62∓
TNF-α (pg/mL)	1.12± 0.10	1.00± 0.14	0.93± 0.75	0.84± 0.18	0.93± 0.10	0.81± 0.13
IL-6 (pg/mL)	2.70± 0.23	2.60± 0.22	2.77± 0.43	2.82± 0.55	2.61± 0.22	3.10± 0.67∓
serum CRP (mg/dL)	1.58± 0.35	1.38± 0.39	2.09± 0.70	1.34± 0.44	1.29± 0.57	1.65± 0.36

Mean ± S.E. ∓p<0.10 compared with initial value in each group

4.6 Haematology · Chemical Blood and Urine

The results that analyzing haematology test, RBC (red blood cell), WBC (white blood cell), Hct (hematocrit), Platelet etc., did not show significant differences in all groups after 12 weeks. (Table 9)

When the pre- and post-treatment parameters of the subjects were compared, GOT and GPT concentration decreased significantly ($p=0.045$, $p=0.014$) and serum creatinine concentration decreased significantly too ($p=0.012$) in Test Ⅰ group, which is within normal range. (Table 9) The liver parameters (GOT, GPT) and the renal parameters (BUN, creatinine) did not show significant differences to pre- and post- treatment in Test Ⅱ group and placebo group. (Table 9)

4.7 Total calorie intake · Total energy expenditure

Total calorie intake (TCI) and total energy expenditure (TEE) of a day did not show significant differences in all groups after 12 weeks, but intake ratio of protein (%/TCI/day) had a tendency to decrease in Test Ⅱ group. ($p=0.083$) (Table 10) In placebo group, TCI had a tendency to decrease ($p=0.057$), while TEE, and intake of carbohydrates, fat, protein (%/TCI/day) did not show significant changes. (Table 10) In all groups, intake of cholesterol a day did not show significant changes. (Table 10)

Table 9. Laboratory measurements before and after intervention

	Test I (n=21)		Test II (n=23)		Placebo (n=20)	
	Baseline	12 week	Baseline	12 week	Baseline	12 week
GOT (U/L)	26.80± 2.34	22.60± 0.96*	22.40± 1.30	22.50± 1.00	21.70± 1.81	21.70± 1.20
GPT (U/L)	34.20± 4.97	24.90± 2.19**	25.70± 2.62	23.40± 1.70	23.90± 2.41	24.10± 2.37
Creatinine (mg/dl)	0.93± 0.03	0.87± 0.04*	0.90± 0.02	0.90± 0.03	0.82± 0.03	0.81±. 0.05
BUN (mg/dl)	17.40± 1.33	15.60± 0.86	16.50± 1.01	16.50± 0.95	15.90± 0.99	15.80± 0.95
WBC ($x10^3/\mu l$)	5.77± 0.28	5.66± 0.32	6.10± 0.49	5.89± 0.44	5.83± 0.47	5.59± 0.34
RBC ($x10^3/\mu l$)	4.97± 0.08	5.09± 0.10	4.77± 0.10	4.83± 0.10	4.77± 0.14	4.70± 0.18
Hemoglobin (g/dl)	15.10± 0.31	15.10± 0.31	14.70± 0.26	14.80± 0.26	14.30± 0.31	14.20± 0.34
Hematocrit (%)	45.00± 0.82	45.40± 0.92	43.90± 0.75	44.50± 0.69	43.60± 1.01	43.10± 0.98
Platelet count	226.00± 10.30	219.00± 8.94	248.00± 11.60	233.00± 9.20	210.00± 9.47	212.00± 12.90

Mean ± S.E. *p<0.05, **p<0.01 compared with initial value in each group

Table 10. Daily food intake and total energy expenditure before and after intervention

	Test I (n=21)		Test II (n=23)		Placebo (n=20)	
	Baseline	12 week	Baseline	12 week	Baseline	12 week
TCI[1] (kcal/d)	2451.00± 55.80	2429.00± 56.70	2289.00± 66.80	2264.00± 65.80	2168.00± 87.70	2137.00± 90.00+
TEE[2] (kcal/d)	2249.00± 59.70	2243.00± 59.10	2128.00± 57.10	2122.00± 57.00	2012.00± 72.30	1996.00± 74.20
Carbohydrates (% of TCI)	63.50± 0.41	63.40± 0.40	62.70± 0.58	63.30± 0.52	62.50± 0.60	62.30± 0.57
Protein (% of TCI)	17.00± 0.34	16.80± 0.29	16.90± 0.43	16.20± 0.43+	17.20± 0.43	16.70± 0.46
Fat (% of TCI)	20.20± 0.34	20.50± 0.35	21.00± 0.59	21.50± 0.37	21.10± 0.45	21.10± 0.49
Cholesterol (mg)	309.00± 47.40	329.00± 38.80	278.00± 49.00	368.00± 57.10	262.00± 30.20	219.00± 28.70

Mean ± S.E. +p<0.10 compared with initial value in each group, [1]Total calorie intake, [2]Total energy expenditure

5. Discussion

Monacolin K is a potent inhibitor of HMG-CoA reductase as a natural cholesterol - lowering agent, and is also known as Mevinolin or Lovastatin.(21) Lovastatin dose which has been sold as drug is from 20mg to 80mg a day, but a maximum monacolin K intake as red yeast rice supplementation is far lower than lovastatin as 8mg a day. In this clinical study, used red yeast rice supplementation amount restricted within a monacolin K 8mg/day by a standard of the Korea Food and Drug Administration (KFDA) (27).

High blood lipid and lipoprotein levels increase the risk of diabetes. It has been long known that an increase in serum triglyceride levels in metabolic syndrome increases the risk of impaired glucose tolerance. (7) In this study, we measured serum lipid profile (total-, LDL-, HDL- cholesterol, triglyceride) before and after intervention. Measured results showd significant changes. Both Test Ⅰ and Test Ⅱ groups had significant improvement in LDL cholesterol and atherogenic index(AI) compared with placebo group($p<0.05$). Additionally, total and HDL cholesterol improved significantly in Test Ⅱ group compared with placebo group ($p<0.05$). Therefore, red yeast rice supplementation in subjects with impaired glucose tolerance or impaired fasting glucose is effective to improve serum lipid profile and AI. Especially with a high dose it had a tendency to get better results.

The existing studies mainly have been the clinical studies joining subjects with hyperlipidemia (27,41,41), but this study is meaningful to evaluate the effect of red-yeast-rice dietary supplementation on cholesterol-lowering in subjects with impaired glucose tolerance or impaired fasting glucose whom a control of serum lipid concentration is important. Also, although normal eating pattern have been maintained in this study, serum lipid concentration showed significant results. Here, we found that red yeast rice a potent competitive

inhibitor of HMG-CoA reductase regardless of eating pattern. Cholesterol is a waxy, fat-like material that is found in all parts of the body. It comes from two sources: our liver produces it, and we consume it in meat and dairy products. We supposed that this result establish the fact that monacolin K acts on endogenic cholesterol (51).

In this study, we measured glucose control related parameters (glucose, 2h OGTT, HOMA-IR, insulin, FFA) before and after the supplementation. Measured results did not show significant changes by and large. Fasting glucose decreased in Test groups and increased in placebo group after intervention. which did not show significant difference. In subjects which fasting blood glucose is more than 110mg/dl, fasting glucose had a tendency to decrease in test Ⅱ group($p<0.1$) and HbA1c had significant decrease in Test Ⅰ group, while insulin and HOMA-IR had a tendency to increase in placebo group after intervention. Mean changes of glucose related parameters compared with placebo group did not show significant differences. These results were insignificant to establish a functional food and a effective dose on glucose control of red yeast rice.

In this study, although red yeast rice was insignificant the effect on glucose control, we measured additionally whether red yeast rice influence lipid peroxidation and pro-inflammatory cytokines. First, we measured the concentration of MDA and urinary $PGF_{2\alpha}$, the index of lipid peroxidation. In results, MDA had a tendency to decrease in Test Ⅱ group, while urinary $PGF_{2\alpha}$ had a tendency to increase in placebo group. these results corresponded with a decrease of serum lipid concentration. Low degree inflammatory markers (TNF-α, IL-6 and CRP) were shown to reflect type 2 DM development risk.(7) Inflammatory cytokines secreted by macrophage and T lenfosits modify the endothelial function, smooth muscle cell proliferation, collagen destruction and thrombosis (52,53). TNF-α and IL-6 have been known to inhibit the lipose

activity of lipoprotein and stimulate the liposis in adipose tissue (54,55). TNF-α causes insulin resistance by reducing the insulin signaling and GLUT-4 expression (56). It is argued that progression from central obesity towards insulin resistance can be interrupted by obtaining a decrease in these cytokines via statin treatment. Guclu et al. (57) reported that the increase in insulin resistance which was obtained by using pravastatin can be attributed to the inhibition of the above mentioned pro-inflammatory cytokines, even though not measured. it has been shown in this study that using statin in dyslipidemia treatment of metabolic syndrome cases is a treatment approach having advantageous effects on insulin resistance. Accordingly, we supposed that monacolin K, an ingredient of statin, influence pro-inflammatory. In this study, we measured CRP, TNF-α, IL-6 as pro-inflammatory. In results, The concentration of TNF-α and serum CRP did not show significant differences in all groups, while IL-6 concentration had a tendency to increase in placebo group. We consider that these results were insignificant by reason of a small number.

The existing studies have been not identified that minacolin K of red yeast rice examine whether glucose-lowering related to serum lipid-lowering influence. Therefore in this study, monacolin K as dietary supplementation examined whether glucose control related to a main function of serum lipid-lowering influence, but glucose control related parameters (glucose, 2h OGTT, HOMA-IR, insulin, FFA) did not show significant changes.

In conclusion, subjects with impaired fasting glucose or impaired glucose tolerance significantly improved in serum lipid profile by red yeast rice supplementation without serious side effects. These are more effective in the case of a high dose. The effects of red yeast rice supplementation on glucose control were insignificant. This study focused on monacolin K, but we consider that other ingredients with monacolin k of red yeast rice have effect on

glucose control via results on blood glucose in this study. In addition, longer-term clinical studies should be performed with larger number of subjects to determine the effects of red yeast rice on the insulin resistance in the subjects with impaired glucose tolerance or impaired fasting glucose and type 2 DM.

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국 문 요 약

공복 혈당장애 및 내당능장애자에서 홍국의 섭취가 혈중지질 및 혈당 조절에 미치는 영향

내당능 장애와 공복혈당장애는 장차 당뇨병으로 진행되거나 심혈관 질환을 일으키는 위험인자로 간주되어 적절한 혈당 및 혈중지질수치의 조절이 필요하다. 홍국의 기능성분인 모나콜린-K는 statin계열 약물의 주원료로서 이미 지질 강하제로 잘 알려져 있다. 홍국의 지질 강하제 이외의 다른 효과로서 동물 자체 효력 시험과 문헌 고찰을 통해 혈당 강하 효과가 보고되고 있으므로 이중맹검, 무작위배정, 위약 대조 인체시험을 통하여 홍국의 혈중 지질 및 혈당 개선에 대한 효능을 확인하는 목적으로 본 연구를 실시하였다.

본 연구는 18-80세 사이의 남녀를 대상으로, 스크리닝 시 공복혈당이 110mg/dl 이상 126mg/dl 이하인 공복 혈당 장애이거나 경구 당 부하 검사 2시간 후 혈당이 140mg/dl 이상 199mg/dl 이하인 내당능 장애자이거나 당화혈색소 수치가 6%이상 7%이하인 자로서 심각한 합병증이 없는 사람을 대상으로 하였으며, 홍국 저용량군(Test Ⅰ) 21명, 고용량군(Test Ⅱ) 23명, Placebo 20명으로 총 64명을 대상으로 12주간 검사에 대한 결과분석을 실시하였다. 1차 유효성 지표로 혈중 지질관련 지표인 중성지방, 총 콜레스테롤, LDL 콜레스테롤, HDL 콜레스테롤, Atherogenic index (AI)를 측정하였으며, 2차 유효성 지표로 혈당 지표인 공복 혈당, 경구 당부하 검사 2시간 혈당치, 당화혈색소, 인슐린, HOMA-IR, 유리지방산을 측정하였다.

홍국의 섭취가 혈중 지질 농도에 미치는 영향을 살펴보면, 시험군에서 제재 섭취 전후로 중성지방, 총콜레스테롤, LDL 콜레스테롤, HDL콜레스테롤 수치가 유의적으로 개선되었으며 동맥경화지수도 유의적으로 향상되었다. 또한 홍국의 고용량

군의 총콜레스테롤, HDL 콜레스테롤과 LDL 콜레스테롤 및 동맥경화지수 개선 정도는 Placebo군의 변화량과 비교하여 유의적으로 개선되는 것으로 나타났고, 저용량군의 경우에도 LDL 콜레스테롤과 동맥경화지수 개선 정도가 Placebo군의 변화량과 비교하여 유의적으로 개선되었다. 따라서 홍국의 섭취는 혈중 지질 농도와 동맥경화지수를 개선시키는데 효과적이며, 고용량의 경우 더욱 효과적일 것으로 사료된다. 홍국의 섭취가 혈당조절에 미치는 영향을 살펴보면, 모든 시험군에서 제재 섭취 12주 후 공복혈당이 감소하였고, 시험군이 Placebo군에 비해 더욱 감소하였으나 통계적으로는 유의하지 않았다. 주평가지표인 공복혈당을 기준으로 공복혈당 110mg/dl 이상인 피험자만을 대상으로 당대사 관련 지표를 분석한 결과, 홍국 고용량군에서 공복혈당의 감소경향이 관찰되었고, 홍국 저용량군에서는 당화혈색소의 유의적인 감소 효과를 확인하였으나 Placebo군의 변화량과 비교하여 유의적이지 않았다.

따라서 공복혈당 장애자 또는 내당능 장애자에서 하루 홍국 분말 2.52g (저용량군) 또는 5.04g (고용량군)을 포함한 건강기능식품을 12주간 섭취시킬 경우 혈중 지질 농도와 동맥경화지수의 개선으로 심혈관 질환의 위험도를 낮출 수 있을 것으로 보이며, 고용량군일 경우 더 효과적일 것으로 사료된다. 홍국의 섭취가 혈당조절에 미치는 효과는 미약하여 혈당조절 기능성과 유효용량을 설정하기 위해서는 다수의 피험자를 대상으로 한 장기 임상연구가 후속적으로 더욱 필요할 것으로 사료된다.

핵심되는 말 : 홍국, Monacolin-K, 혈중지질 농도, 이중 맹검/시험군-대조군 실험, 혈당 조절