A THESIS FOR THE DEGREE OF

MASTER OF SCIENCE IN FOOD AND NUTRITION

The Effect of 12-week Weight Management Program on Clinical Characteristics, Dietary Intakes, and Immune and Inflammatory Responses of the Young Obese

12 주간의 체중조절 프로그램이 젊은 비만인의 임상 및 식생활 특성과 면역 및 염증 지표에 미치는 영향

August, 2012

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이 논문을 생활과학석사 학위논문으로 제출함

2012 년 5 월

서울대학교 대학원

식품영양학과

이 애 진

이애진의 생활과학석사 학위논문을 인준함

2012 년 6 월

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Abstract

The Effect of 12-week Weight Management Program on Clinical Characteristics, Dietary Intakes, and Immune and Inflammatory Responses of the Young Obese

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Objectives: We investigated the effect of a 12-week weight management program on clinical characteristics, dietary intakes, and immune and inflammatory responses of young and otherwise healthy obese adults. Also, we tried to find the traits of those who lost more weight in order to identify the key factors leading to a successful weight loss.

Subjects: One hundred fifty four subjects aged between 19 and 45 years old were screened using questionnaires, and seventy subjects were included in the 12-week weight management program. Subjects were divided into two weight groups based on their BMI. Subjects without 3-day dietary record at baseline were excluded from study I; forty seven obese (BMI > 25) and 21 normal weight subjects (BMI 18.5~23) were included, and 44 obese and 19 normal weight subjects completed the program. Forty obese and 19 normal weight subjects with complete dietary record data were included for dietary

intake analysis. In the study II, 49 obese subjects and 21 normal weight subjects were included. Of these, 45 obese and 19 normal weight subjects completed the 12-week program. The protocol was approved by the Seoul National University Institutional Review Board (SNUIRB, IRB NO. 0908/001-007), and written informed consent was obtained from all subjects.

The 12-week weight management program: Obese subjects participated in the 12-week weight management program with nutritional counseling and behavioral modification. Intervention program included 5 group educations and 6 individual counseling sessions at baseline and 2, 4, 6, 10 (individual counseling only), and 12-week time points. Subjects in the normal weight group attended 6 individual counseling sessions for the evaluation of dietary intake and exercise pattern. The overall goal of the study was to lose about 0.5 kg per week by reducing calorie intake by 300~500 kcal per day from estimated energy requirements and by increasing physical activities.

Measurement: Anthropometric and clinical characteristics and serum leptin and high-molecular-weight (HMW) adiponetin concentrations were evaluated. For dietary analysis, 3-day dietary record was used. To examine the cell-mediated immune response, we measured the production of helper T (T_H) 1/ T_H2 cytokines (interferon (IFN)-γ, interleukin (IL)-2, IL-4, and IL-10) and the proliferative response of immune cells in whole blood. The production of inflammatory cytokines (IL-1β, IL-6, and tumor necrosis factor (TNF)-α) by immune cells in whole blood was measured to determine

the inflammatory response. A subpopulation of immune cells was counted to examine the impact of weight loss on the population of immune cells.

Results: At baseline, obese group had significantly higher weight, BMI, waist circumference, and body fat mass compared with normal weight group. Blood pressure, total and LDL cholesterols, serum triglyceride, and serum leptin concentrations were significantly higher in obese subjects compared with normal weight subjects. Intakes of energy, fat, and protein in obese subjects were not significantly different from those in normal weight subjects. The percentage of $CD4^+$ and $CD8^+$ T cells did not differ between two groups. Obese subjects had significantly higher proliferative response to suboptimal concentrations of mitogen compared with normal weight subjects. The production of phytohemagglutinin (PHA)-stimulated T_H cytokines (except IL-10) were significantly higher in obese group as well. After 12 weeks of program, obese subjects lost average of 2.7 kg (3.3%) weight, and BMI, waist and hip circumferences, waist-to-hip-ratio (WHR), and body fat mass decreased. Significant reduction in serum triglyceride and free fatty acids and a tendency of decrease in the prevalence of metabolic syndrome were observed in the obese. Significantly reduced energy, fat, cholesterol, and sodium intakes and a tendency of increase in vitamin C intake in obese subjects were observed with the program. Prevalence of sedentary lifestyle in obese group decreased significantly. Modest but significant weight loss did not lead to change in the percentage of $CD4^+$ and

$CD8^+$ T cells and proliferative response. However, a tendency of increase in PHA-stimulated IL-10 production, significant decrease in lipopolysaccharide (LPS)-stimulated IL-1ß production, and a tendency of reduction in LPS-stimulated TNF-α production were observed with weight loss in the obese. Following characteristics were identified in the subjects who lost more than 6 kg: *1)* more active participation in the intervention, *2)* more realistic weight loss goal setting, and *3)* weight gain occurred during the adulthood.

Conclusion: The 12-week program with nutrition education led to significant reduction in energy, fat, cholesterol, and sodium intakes and a tendency of increase in vitamin C intake in obese subjects. This indicates that obese subjects reduced animal food intake and incorporated a healthier diet through the program. These dietary changes and a decline in the prevalence of sedentary lifestyle seemed to contribute to the weight loss and improvement of lipid profiles in the obese group. While impaired immune function and elevated inflammatory responses resulted from obesity have been reported, we did not find any significant evidence of them. This was likely because the obese subjects in this study were young and otherwise healthy (most of them with a BMI less than 30). Although elevated proliferation and T_H cytokine production did not change with modest weight loss, inflammatory responses in obese subjects were attenuated.

KEY WORDS: obesity, behavioral modification, inflammation, immune function, cytokine, adipokine

Student Number: 2010-23613

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List of Abbreviations

Con A, concanavalin A

HMW, high-molecular-weight

IFN, interferon

IL, interleukin

KDRIs, Dietary Reference Intakes for Koreans

KNHANES, Korean National Health and Nutrition Examination Survey

LPS, lipopolysaccharide

NK cell, natural killer cell

PBMC, peripheral blood mononuclear cells

PHA, phytohemagglutinin

SEM, standard error of the mean

T_H cytokines, helper T cytokines

TNF, tumor necrosis factor

WHR, waist-to-hip-ratio

I. INTRODUCTION

1. Background

Obesity has a great number of negative health, social, and economic consequences, as evidenced by the higher mortality and morbidity rates among overweight and obese individuals than lean people. Obese people have a 50 to 100% increased risk of premature death from all causes compared to individuals with a healthy weight (Bagchi, 2007). In particular, obesity is associated with an increased risk of cardiovascular diseases, diabetes mellitus, dyslipidemia, and certain cancers (Stroebe, 2008; Calle et al., 1999).

The metabolic disturbances of obesity are accompanied by altered adipokine production which is directly related to adipocyte size. Abnormally expanded adipose tissue showed a high release of pro-inflammatory molecules which contribute to inflammation (Awad et al., 2010; Monteiro et al., 2010; Shoelson et al., 2007; Trayhurn et al., 2007). Diet-induced obesity influences the state of adipose tissue macrophages from an M2 polarized state that protects adipocytes from inflammation to an M1 pro-inflammatory state leading to insulin resistance as well (Awad et al., 2010). As a result, obesity leads to chronic low-grade inflammation (Bruun et al., 2003; Ferrante, 2007; Kuo et al., 2011). Also, obesity is known to impair the function of T cells in humans, which is associated with higher incidence of infection in obese people (Karlsson et al., 2010; Marti et al., 2001).

Modest weight reduction can significantly reduce the risk of these serious health conditions. Weight loss in overweight and obese individuals improves physical, metabolic, and endocrinological complications (Bagchi, 2007). While lots of strategies for preventing obesity have been introduced, many of them were not useful for long term practice due to some side effects caused by excessive energy restriction or imbalance in macronutrients. For example, high-fat and low-carbohydrate diets (e.g., Atkins' diet) might result in ketosis and elevation in LDL cholesterol concentration (Clifton, 2008; Freedman et al, 2001). Among variety of strategies, behavioral modification has been highlighted as a healthy and effective way for weight loss by leading to overall changes in lifestyle.

However, studies which investigated the overall effect of weight management program with a behavioral modification and nutrition education on the clinical characteristics, dietary intakes, and immune and inflammatory responses of the young obese are limited.

2. Objectives of the study

We investigated the effect of the 12-week weight management program with behavioral modification and nutrition education on the clinical characteristics, dietary intakes, and immune and inflammatory responses of the young obese (most of them were in their 20's and with a BMI less than 30 kg/m^2).

In the study I, objective of the study was to investigate the effect of the 12-week program on clinical characteristics and dietary intakes of the young obese. Also, the study was conducted to find the traits of those who lost more weight in order to identify the key factors leading to a successful weight loss, given that subjects who within the same study group achieved a wide range of weight loss.

In the study II, objective of the study was to determine the influence of obesity and weight loss through the 12-week program on cell-mediated immune and inflammatory responses of the young obese.

Ⅱ. Literature Review

1. Behavioral factors that contribute to obesity

Genetic factors contribute to the development of obesity, and some individuals have more risk for gaining weight than others with the same level of energy surplus. However, considering the rapid increases in obesity, it must have been due to environmental changes or most likely due to an interaction of genetic dispositions with environmental changes and behavioral factors (Stroebe, 2008). Excessive calorie intake above daily energy requirements is necessary for the development of obesity, but it is a mistake to assume that simple overeating is responsible for obesity (Goldstein, 1999). In this sense, efforts have been made to identify dietary patterns, nutrient intake and composition, and other behavioral factors associated with obesity.

As for nutrient composition, consumption of diets high in fat, especially rich in animal-fat, seems to be associated with obesity. Obese college women consumed fat-rich foods such as *galbi* and *samgyopsal* more frequently compared with underweight and normal weight college women despite no significant difference in energy intake (Choi et al., 2008). High fat foods can increase food consumption, because they are usually more palatable and require lower chewing and swallowing time than other foods. Also, they are more energy-dense foods because of their high energy content. Moreover, the cost of storing excess fat is only about 3% of ingested energy, whereas the cost of synthesizing fat from carbohydrate or protein and storing it is more than 20% of ingested energy (Goldstein, 1999).

Similarly, consumption of animal protein has been suggested to be linked

to obesity. According to data from a 1999-2004 National Health and Nutrition Examination Survey, meat consumption was related to higher risk for obesity among US adults (Wang et al., 2009). Higher proportion of energy consumed from animal protein seemed to be associated with the risk for obesity in 2,470 women from the southwestern United States (Murtaugh et al., 2007). Generally main source of animal protein is meat, and fat content in meat is fairly high.

Some studies reported that high consumption of simple sugar, especially in sugar-sweetened beverages led to obesity (Hauner et al., 2012; Tate et al., 2012).

Certain eating patterns, such as skipping breakfast and lunch and larger self-reported portion sizes of main meals were related to obesity (Berg et al., 2009). The relationship between obesity and eating frequency is not conclusive; while one study reported that eating more than three times a day was positively associated with excessive energy intake (Howarth et al., 2007), the other study showed that eating frequency was not associated with obesity (Mills et al., 2011). Snacking seems to be linked to obesity. Self-reported between-meal snacking was identified as a major factor in the development of obesity in a Spanish cohort study, which followed up for 4.6 years (Bes-Rastrollo et al., 2010). Snack consumption and energy intake from snacks were higher in obese adolescent girls in Korea (Yoon et al., 2010).

Eating diverse foods has been reported to increase the risk of obesity as well. Diverse dietary pattern was associated with increased risk of being overweight and obese in Mexico (Flores et al., 2010). Obese college women were eating more diverse foods compared with underweight and normal

weight college women (Choi et al., 2008).

In addition to dietary factors, sedentary lifestyle has been suggested to result in obesity. Inactive individuals had increased waist circumference compared with active individuals in an 11.4-year follow-up study, which included 21,729 men and women (Arsenault et al., 2010). Walking distance declined significantly in obese men compared with lean men in 7082 male participants of the National Walkers' Health Study (Williams, 2008).

2. Behavioral modification

Behavioral treatment relies on the functional analysis of behavior to identify events that are associated with inappropriate eating, exercise, or thinking habits (Goldstein, 1999). Standard behavioral modification for weight loss has three main components: dietary modification, regular exercise to increase energy expenditures, and behavioral therapy, which are integral to lifestyle modification (Burke et al., 2011). Behavioral therapy usually includes multiple components, such as keeping food and activity record for self-monitoring, nutritional education, stimulus control, and slowing eating (Foster et al., 2005). Patients not only have to learn how to self-monitor their calorie intake and exercise behavior; they also need to learn how to change these behaviors. Thus, nutrition education is one important component of most behavioral approaches to the treatment of overweight and obesity (Stroebe, 2008).

Among variety of strategies, behavioral modification has been highlighted as a healthy and effective way for weight loss because it can lead to overall changes in lifestyle. Comparing three approaches to the treatment of overweight and obesity (lifestyle modification, pharmacotherapy, and bariatric surgery), the most effective approach to lose weight was lifestyle modification. Pharmacotherapy and bariatric surgery had better outcomes when augmented by lifestyle treatment compared with either approach alone (Burke et al., 2011).

3. Obesity and immune function

Specific immunity is provided primarily by two classes of lymphocytes, B and T lymphocytes (or T cells), which recognize antigens through specific receptors on their cell membranes.

T cells mediate the actions that make up cell-mediated immunity (Stallone, 1994). On antigen recognition, naïve T cells differentiate into several functional classes of effector T cells that are specialized for different activities (Murphy et al, 2008). When $CD8^+$ T cells (cytotoxic T cells) recognize virus-infected cells, the $CD8^+$ effector mechanisms are triggered. Lytic mechanism induces killing of the virus-infected cell; non-lytic mechanism results in secretion of cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α, which reduce the probability of cell infection and viral production (Elemans et al., 2012). On the contrary, $CD4^+$ T cells (helper T cells) cannot kill infected host cells or pathogens. Rather, they help other immune cells—they activate and direct other immune cells. They are essential in B cell antibody class switching, in the activation and growth of $CD8^+$ T cells, and in maximizing bactericidal activity of phagocytes such as macrophages (Harrington et al., 2005).

Obesity is known to impair the function of T cells in humans, which is associated with higher incidence of infection in obese people (Karlsson et al., 2010; Marti et al., 2001). Studies have indicated that obesity can result in suppression of mitogen-induced lymphocyte proliferation. Obese subjects

with a mean BMI of 33.2 kg/m^2 showed significantly lower Con A or PHA-induced lymphocyte proliferation compared with nonobese subjects (Nieman et al., 1999). The obese with a mean BMI of 38.4 kg/m^2 had lower responses of peripheral blood lymphocytes to T cell mitogen compared with nonobese humans, and weight loss seemed to reverse lower proliferation of the obese (Tanaka et al., 1993).

The impact of obesity on alterations in T cell subpopulation is not conclusive. Both an increased frequency of $CD4^+$ T cells (O'Rourke et al., 2005) and a reduction in the number of $CD4^+$ T cells (Tanaka et al., 2001) in obese people were reported. Obese subjects had either decreased $CD8^+$ T cells (O'Rourke et al., 2005; Tanaka et al., 2001) or no difference in the number of $CD8^+$ T cell subsets (Nieman et al., 1999) compared with the nonobese subjects.

The $CD4^+$ helper T (T_H) cells are classified according to differences in cytokine production into a T helper type 1 (T_H1) system and a T_H2 system. The balance between T helper (T_H) 1 / T_H2 responses plays an important role in regulation of immune response. In the immune responses of the T_H1 system, cytokines such as IFN-γ and interleukin 12 (IL-12) are produced to activate macrophages at the site of inflammation for host defense against intracellular pathogens. By contrast, the T_H2 types, which produce cytokines such as IL-4 or IL-10, play the central role in allergic reactions. Also, T_H2 cells have very different effector functions, as they are essential for clearing

extracellular organisms like parasites and helminthes. While uncontrolled T_H1 responses against self-antigens can lead to the development of autoimmunity, uncontrolled T_H2 cells can result in allergic reactions and asthma (Jager et al., 2010; Yoshida et al., 2009).

Obesity has been reported to disturb the T_H1/T_H2 balance, but studies which investigated the impact of obesity on the T_H1/T_H2 balance have not been always consistent. Leptin, which is elevated with increased adiposity, has been suggested to polarize T_H cell response towards a pro-inflammatory T_H1 phenotype (Fantuzzi, 2009; La Cava et al., 2004; Lord et al., 1998). Weight loss in morbid obese subjects (mean BMI 42.8 kg/m^2) led to significant decrease in the number of pro-inflammatory T_H1 cells (Viardot et al., 2010). On the other hand, some studies reported increased risk of T_H2 biased disease such as allergy and asthma in obese people (Colombo et al., 2008; Hersoug et al., 2007). Symptom of asthma in obese people with a BMI more than 30 kg/m^2 was relieved with weight loss through 12-month lifestyle intervention (Ma et al., 2010).

4. Obesity and inflammatory responses

Adipose tissue plays a major role in the low-grade inflammatory state associated with obesity, which contributes to the pathogenesis of several chronic disease including cardiovascular disease and diabetes mellitus via inflammatory pathways (Awad et al., 2010).

Adipocyte size appears to be a key regulator of adipokine production and adipose tissue function. Abnormally expanded adipose tissue showed a higher release of pro-inflammatory molecules, such as leptin, TNF-α, IL-6, and IL-1ß than normal adipose tissue. This imbalance of more pro-inflammatory and fewer anti-inflammatory adipokines in combination with chronic activation of the innate immune system contributes to the chronic low-grade inflammation in obesity (Awad et al., 2010; Monteiro et al., 2010; Shoelson et al., 2007; Trayhurn et al., 2007).

Macrophages in adipose tissue are likely to contribute to the production of inflammatory mediators either alone or in concert with adipocytes (Bagchi, 2007). Adipose tissue contained only 5% to 10% macrophages in lean individuals, but diet-induced weight gain caused significant infiltration of macrophages, with macrophages constituting up to 60% of all adipose tissue cells (Graziani et al., 2011). The number of infiltrated macrophages positively correlated with BMI and adipocyte size in human abdominal subcutaneous adipose tissue (Awad et al., 2010). Macrophages have been recently characterized as two types with a more pro-inflammatory M1 or a

more anti-inflammatory M2 phenotype. In obesity, adipose tissue macrophages alter the state from an M2 polarized state to an M1 pro-inflammatory state (Awad et al., 2010).

Obese people showed significantly higher production of inflammatory cytokines such as IL-6 and TNF-α (Ackermann et al., 2011; Tanaka et al., 2001; Ziccardi et al., 2002). Obese healthy premenopausal women with a mean BMI of 37.0 kg/m^2 showed significantly higher serum IL-6 and TNF-α concentrations compared with nonobese subjects (Ziccardi et al., 2002). In contrast, these elevated inflammatory cytokines in obese people seemed to decrease with weight loss. A low to very low calorie diet and behavior modification led to significant decrease in adipose TNF-α in obese subjects with weight loss (Kern et al., 1995). Serum TNF-α and IL-6 concentrations in healthy premenopausal women aged 25 to 44 years old decreased significantly through weight loss (Kern et al., 1995; Ziccardi et al., 2002).

5. Adipokines and cytokines

Adipose tissue is a complex and active secretory organ that both sends and receives signals that modulate energy expenditure, appetite, insulin sensitivity, endocrine and reproductive functions, bone metabolism, inflammation, and immunity. Leptin and adiponectin are considered to be the primary adipocytokines because they appear to be produced primarily by adipocytes. TNF-α, IL-6, MCP-1, visfatin, and PAI-1 are expressed in adipocytes as well as activated macrophages and/or other immune cells. The potential roles of leptin, adiponectin, resistin, and visfatin as mediators linking adipose tissue, inflammation, and immunity have been recently reviewed (Shoelson et al., 2007).

Leptin

Leptin is a bioactive substance found in adipose tissue that controls food intake and energy expenditure. It also has atherogenic and growth properties. In humans, adiposity and gender are major determinants of circulating leptin concentrations (Awad et al., 2010). Human obesity is associated with elevated leptin concentrations, which have been proposed to have a role in insulin resistance and metabolic syndrome. There may be a direct link between hyperleptinaemia and increased cardiovascular disease risk (Van Gaal et al., 2006). Although the mechanistic link between obesity and diminished immune memory is not clear, a main factor that ties together

obesity, inflammation, and immune cell function is leptin resistance associated with the obese state (Karlsson et al., 2010).

Leptin exerts direct modulating effects on activation, proliferation, maturation, and production of inflammatory mediators in a variety of immune cells, including lymphocytes, natural killer (NK) cells, monocytes/ macrophages, dendritic cells, neutrophils and eosinophils. The observation that administration of leptin to aged mice improved peripheral T cell receptor diversity indicates that leptin can significantly affect the peripheral T lymphocyte compartment (Fantuzzi, 2009).

Also, leptin may bias T-cell responses towards a pro-inflammatory phenotype (T_H 1). The addition of leptin induced the production of large amounts of IFN-γ and suppression of IL-4 production in a dose-dependent manner. Leptin increased both proliferation and IL-2 production as well (Lord et al., 1998).

Adiponectin

Adiponectin is a relatively abundant and approximately 30-kDa plasma protein secreted specifically from adipose tissue. It is found in multimeric complexes in the circulation at relatively high concentrations in healthy human subjects (Byrne, 2005).

Unlike other adipokines, adiponectin is decreased in obesity, diabetes and other insulin-resistant states (Awad et al., 2010; Van Gaal et al., 2006). The

plasma concentration of adiponectin markedly decreased, especially in visceral obesity (Hersoug et al., 2007). Adiponectin has important anti-atherogenic, antidiabetic and anti-inflammatory properties (Bruun et al., 2003). In light of this, the reduced circulating concentrations of adiponectin in visceral adiposity are known to contribute not only to insulin resistance and dysglycaemia but also to the endothelial vascular dysfunction that is characteristic of the metabolic syndrome (Awad et al., 2010; Byrne, 2005).

Studies indicated that adiponectin had an anti-inflammatory effect on endothelial cells through the inhibition of TNF-induced adhesion-molecule expression. In addition, adiponectin-deficient mice had higher levels of expression of mRNA encoding TNF in adipose tissue and higher TNF concentrations in plasma compared with adiponectin-sufficient mice. Adiponectin also induced the production of important anti-inflammatory cytokines, such as IL-10 (Hersoug et al., 2007; Tilg et al., 2006).

There are three types of adiponectin in blood: a trimer (known as low-molecular-weight adiponectin); a hexamer, which consists of two trimers linked by a disulphide bond (known as middle-molecular-weight adiponectin); and a high-molecular-weight 12- to 18-mer (Tilg et al., 2006). Among them, high-molecular-weight (HMW) adiponectin is considered to be a strong predictor of metabolic syndrome due to its bioactivity (Okamoto et al., 2006; Tilg et al., 2006).

However, the direct interaction of the two main adipokines is not well

understood and this might have important implications in understanding the role of these adipokines in obesity-associated disorders (Tilg et al., 2006).

Cytokines

Cytokines are soluble molecules that are involved in intercellular communication and are produced by a wide variety of cells in the body. They comprise several subfamilies, including interferons (IFN), interleukins (IL), tumor necrosis factors (TNF), transforming growth factors (TGF), colony-stimulating factors, and chemokines. Cytokines mediate several fundamental biological processes, including body growth, adiposity, lactation, hematopoiesis, as well as inflammation and immunity. However, they are also implicated in various pathologies, such as atherosclerosis and rheumatoid arthritis (Braunersreuther et al., 2012).

Inflammatory cytokines

1) Tumor necrosis factor α (TNF-α)

TNF-α is an inflammatory mediator secreted by several inflammatory cell types, including monocyte/macrophages, neutrophils, and T-cells, but also by many other tissues, such as the endothelium, adipose tissue, or neuronal tissue (Braunersreuther et al., 2012). It is now well established that TNF-α is

an adipokine with multiple biological functions including cell proliferation and death, metabolism, inflammation, and immune function (Awad et al., 2010). Also, there appears to be a hierarchy of cytokines within adipose tissue with TNF-α playing a pivotal role in relation to the production of several cytokines and other adipokines. For example, TNF-α is a key regulator of the synthesis of IL-6 (Trayhurn et al., 2007).

At the molecular level, TNF-α contributes to insulin resistance through the inhibition of insulin-stimulated glucose uptake and lipoprotein lipase activity in white adipose tissue and insulin-stimulated glucose uptake and fatty acid metabolism in muscle (Awad et al., 2010).TNF-α is an important mediator of insulin resistance in obesity and diabetes (Hotamisligil et al., 1996). Studies have reported increases in serum TNF-α in human subjects with insulin resistance (Shoelson et al., 2007).

TNF-α seems to be associated with obesity as well. Increased expression and secretion of TNF-α from white adipose tissue has been reported in obese and insulin-resistant humans and rodents. In contrast, Body weight reduction in obese humans was associated with lower white adipose TNF-α mRNA expression and improved insulin sensitivity (Awad et al., 2010; Kern et al., 1995).

2) Interleukin-6 (IL-6)

IL-6 is a multi-functional adipokine that regulates immune responses and metabolism as well as the growth and differentiation of a variety of cell

types (Awad et al., 2010). IL-6 was among the first to be implicated as a predictor or pathogenic mediator of insulin resistance and cardiovascular disease (Braunersreuther et al., 2012; Shoelson et al., 2007). In patients undergoing bariatric surgery, decreased IL-6 concentrations were associated with weight loss and insulin resistance improvement (Kopp et al., 2003).

3) Interleukin-1β (IL-1β)

IL-1β is a member of the interleukin 1 cytokine family, which is produced by activated macrophages as a proprotein. This cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. IL-1β together with IL-6 concentrations reportedly predicted risk for type 2 diabetes mellitus in humans better than either cytokine alone (Shoelson et al., 2007).

Helper T (T_H) 1/ T_H2 cytokines

The $CD4^+$ helper T (T_H) cells are classified according to differences in cytokine production into a T helper type 1 (T_H1) and a T_H2 system. The T_H1 cells, which are important for host defense against intracellular pathogens and induction of delayed type hypersensitivity responses, secrete cytokines such as interferon (IFN)-γ and interleukin 2 (IL-2). The IFN-γ is involved in the stimulation of the phagocytic response from macrophages, while IL-2 promotes the proliferation and differentiations of $CD8^+$ T cells (Jager et al.,

2010; Marti et al., 2001; Yoshida et al., 2009).

T_H2 cytokines such as IL-4, IL-5, and IL-13 play the central role in allergic reactions (Jager et al., 2010). IL-10 is considered as an anti-inflammatory cytokine that regulates the inflammation in several organs and tissues in physiological or pathological situations, and it inhibits T cell-, monocyte-, and macrophage-mediated functions (Braunersreuther et al., 2012). The T_H2 cytokine IL-4 strongly inhibited T_H1 differentiation and, vice versa, the T_H1-cytokines IL-12 and IFN-γ inhibited T_H2 differentiation (Jager et al., 2010).

1) Interferon- γ (IFN-γ)

Production of IFN-γ is a function of T cells and of natural killer (NK) cells. Both in humans and in mice, IFN-γ synthesis has been observed in T cells of the cytotoxic/suppressor phenotype. IFN-γ preferentially inhibits the proliferation of T_H2 but not T_H1 cells, indicating that the presence of IFN-γ during an immune response will result in the preferential proliferation of T_H1 cells. Also, IFN-γ is one of the natural B-cell differentiation factors. IFN-γ promotes proliferation of activated human B cells and, in cultures of human B cells, can act synergistically with IL-2 to enhance immunoglobulin light-chain synthesis (Thomson, 1994).

2) Interleukin-2 (IL-2)

Although numerous cytokines have been described to functions as T-cell

growth factors, IL-2 is widely considered to play a pivotal role under most circumstances in T-cell proliferation. The spectrum of its recognized biological activity has expanded significantly to include direct effects on the growth and differentiation not only of T cells but also of B lymphocytes, NK cells, monocytes, and macrophages (Thomson, 1994).

3) Interleukin-4 (IL-4)

Both IL-2 and IL-4 were found to trigger human intrathymic pre-T cells to proliferate in the absence of mitogen. IL-4 can act as authentic T-cell growth factor independent of IL-2. IL-1, IL-6 and TNF all enhance IL-2-induced thymocyte proliferation, whereas only IL-6 augments IL-4-induced proliferation. IL-4 plays important roles in B-cell activation and proliferation as well. IL-4 antagonizes the B-cell growth-promoting effect of IL-2 (Thomson, 1994).

4) Interleukin-10 (IL-10)

IL-10 is produced by macrophages and other cell types, in addition to the T cells from which it was originally identified. As observed for many other cytokines, IL-10 mediates several functions on multiple cell types. IL-10 inhibits several macrophage functions, including presentation of antigen to T_H1 cells, cytokine synthesis and some microbicidal activities. In contrast, IL-10 generally enhances or stimulates mast cells and B cells (Thomson, 1994).

Ⅲ. Study 1

Effect of 12-week weight management program with behavioral modification on clinical characteristics and dietary intake of the young obese and the contributing factors to successful weight loss

INTRODUCTION

Obesity has become a pandemic which leads to many health problems, and Korean young adults are no exception. According to the Korean National Health and Nutrition Examination Survey (KNHANES) conducted in 2010, prevalence of obesity was 20.5% in those aged between 19 and 29, and 31.0% in their 30's.

Young adults, particularly aged between 18 and 25, are vulnerable to obesity due to dramatic lifestyle changes, such as setting up their own lifestyle, reduced physical activity, and declines in overall-diet quality (Nelson et al., 2008). Obesity in young age may lead to serious health problems with advancing ages. Young people with higher BMI have a significantly higher risk for coronary heart disease than slender old has, and the risk for coronary heart disease increases by 3.3% for women and by 3.6% for men for every 1% increase in BMI above a desirable BMI

(Anderson et al., 2001). In light of this, weight control could be more vital in the earlier stages of life for the prevention and management of metabolic syndrome (Kim et al., 2011).

Among variety of strategies, behavioral modification has been highlighted as a healthy and effective way for weight loss because it can lead to overall changes in lifestyle. A total of 110 obese women aged 50 to 75 years old lost average of 10.2% of initial weight through 6-month lifestyle intervention using a deficit of 500~1000 kcal diet and individual counseling (Milsom et al., 2011). After 1-year lifestyle modification with a diet of 1200~ 1500 kcal per day and group educations, a total of 55 middle-aged obese subjects accomplished a weight loss of 6.7 kg (Wadden et al., 2005). Twenty four-month intervention which provided group education and individual counseling sessions led to reduction of 5.1 kg in 138 middle-aged obese patients with at least one cardiovascular disease risk factor (Appel et al., 2011).

However, studies which investigated the overall impact of behavioral modification on clinical characteristics and dietary intake of young and otherwise healthy adults are limited. It has been reported that subjects under 40 years of age (Dalle Grave et al., 2005; Finkler et al., 2011; Moroshko et al., 2011) and those without obesity-related complications were more likely to drop out from weight loss intervention (Inelmen et al., 2005). These results indicated that the young and otherwise healthy obese might be less

motivated. Often, weight-loss interventions resulted in a wide range of weight-loss achievements even within the same study group (Finkler et al., 2011). Therefore, understanding key factors that contribute to a better compliance and successful weight loss would help with the development of strategies for more effective weight loss intervention.

In this study, we investigated the effect of a 12-week weight management program with a behavioral modification on clinical characteristics and dietary intakes of young and otherwise healthy obese adults. The program included dietary and physical activity aspects and intensive group education and individual counseling sessions for effective behavioral modification. We tried to find the traits of those who lost more weight in order to identify the key factors leading to a successful weight loss.

SUBJECTS AND METHODS

Subjects

The study was conducted from September 2009 to June 2011. One hundred fifty four subjects aged between 19 and 45 years old were screened using questionnaires, and 86 subjects were excluded from the study (**Figure 1**).

The questionnaires were two types; one was to classify subject with high BDI (Beck Depression Inventory) score, and the other was to identify subject who had chronic disease or took dietary supplements or medications known to affect serum lipid profiles and immune functions. The subjects who met the following criteria were excluded: taking nonsteroidal anti-inflammatory drugs such as aspirin, vitamin E, fish oil, allergy medicine, anticoagulant, multivitamin, and other dietary supplements or medications known to affect serum lipid profiles and immune functions on a regular basis; were pregnant or planning to be pregnant; receiving hormone therapy after menopause; with arthritis, autoimmune disease, cancer, asthma, atopic disease, endocrine, hepatic, renal, thyroid, or cardiac dysfunction. Subjects who did not submit a 3-day dietary record at baseline and with BMI between 23 and 25 kg/m^2 were excluded from the study as well.

The protocol was approved by the Seoul National University Institutional Review Board (SNUIRB, IRB NO. 0908/001-007), and written informed consent was obtained from all subjects.

Sixty eight subjects were included in the study and divided into two weight groups based on their BMI. Forty seven subjects with a BMI over 25 kg/m^2 were assigned to obese group (BMI higher than 25 kg/m^2 is classified as obese in Korea) and 21 subjects with BMI between 18.5 and 23 kg/m^2 were assigned to normal weight group. Of these, 63 subjects (44 obese and 19 normal weights) completed the study, and 59 subjects (40 obese and 19 normal weights) with complete dietary record data were included for dietary intake analysis.

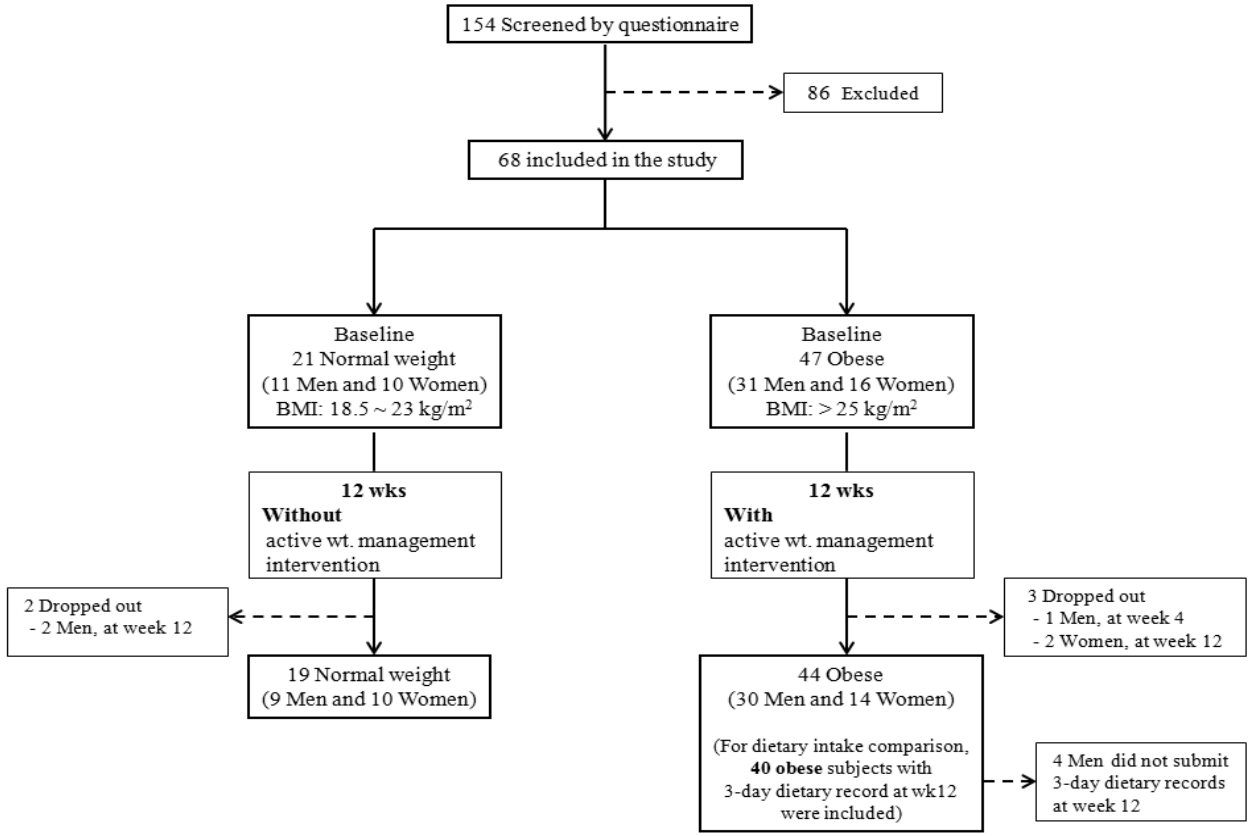

FIGURE 1. Study design and subjects of study I

A 12-week weight management program

At baseline, all subjects received information about the aims and schedule of the study. Obese subjects participated in a 12-week weight management program with nutritional counseling and behavioral modification. Intervention program included 5 group educations and 6 individual counseling sessions at baseline and 2, 4, 6, 10 (individual counseling only), and 12-week time points. Subjects in the normal weight group were asked to keep their usual eating and exercise pattern. They did not attend the group educations and only attended 6 individual counseling sessions for the evaluation of dietary intake and exercise pattern.

Topics of the group educations for obese subjects were: *1)* planning weight loss and how to write diet record (week 0), *2)* how to lose weight in a healthy way (week 2), *3)* understanding the food exchange lists and food labeling (week 4), *4)* portion size control (week 6), and *5)* strategies to maintain weight loss (week 12). During individual counseling sessions, the registered dietitian evaluated 3-day dietary record and reinforced to incorporate low-fat, low-sugar, low-salt, high-fiber, and low energy density foods into their diets. The obese were also encouraged to exercise regularly and to modify undesirable eating behaviors such as late night snacking or binge eating.

The overall weight reduction goal of the program was to lose about 0.5 kg per week by reducing calorie intake by 300~500 kcal per day from

estimated energy requirements and by increasing physical activities. Mean estimated energy requirements using adjusted body weights were 1,715 kcal/d for obese men and 1,390 kcal/d for obese women, which were 83.3% of daily energy requirements for both obese men and women. A personalized goal was set for each obese subject following an individual interview with a registered dietitian at the baseline. Subjects in the normal weight group were asked to keep their usual dietary intake and physical activity to maintain body weight.

Food exchange lists from the Korean Diabetic Association were used for the meal planning. Recommended servings of each food group based on caloric goal and amount of carbohydrate, protein, and fat were provided to each subject. In average, energy provided from carbohydrate, protein, and fat were 60%, 21% and 19% (of energy), respectively, in the individual plan. This recommendation was within acceptable macronutrient distribution ranges (AMDR) for Koreans, in which carbohydrate is 55~70%, fat is 15~25%, and protein is 7~20% of total calorie intake.

Assessment of dietary intake

All subjects were asked to provide 3-day dietary record (2 weekdays and 1 weekend) at baseline, 2, 4, 6, 10, and 12-week time points. The intake and amount of food consumed was confirmed with individual interviews using pictures of the food in actual size. Analysis of nutrient intake was done

using CAN-Pro 3.0, a nutritional analysis program developed by the Korean Nutrition Society. To evaluate changes in dietary intake, 3-day dietary record at baseline and week12 were used. Average of 3-day dietary intake was used as intake of each subject.

Anthropometric measurement

Body weight, waist and hip circumferences, and fat mass were measured using InBody 520 (Biospace, Korea). Standing height without shoes was measured using a stadiometer. BMI and WHR were calculated from the height and body weight and waist and hip circumferences, respectively.

Blood pressure measurement

Blood pressure was measured using an automatic blood pressure monitor (Jawon Medical, Korea) after subjects rested for more than 10 minutes.

Blood samples

Blood for biochemical analysis and serum free fatty acid and adipokines determinations was collected in serum separator tubes (BD vacutainer® SST, Becton Dickinson, Flanklin Lakes, NJ) after a 12-hour fasting. The serum was separated by centrifugation and stored at - 70°C until analysis.

Biochemical analysis

Blood glucose, total cholesterol, HDL cholesterol, and triglyceride concentrations were measured using Cobas Integra® 400 plus (Roche, Switzerland). LDL cholesterol was measured by elimination and an enzymatic assay in Green Cross Reference Lab (Korea).

Nonesterified fatty acids in serum were measured using SICDIA NEFAZYME (Shinyang Chemical Co.,Ltd., Korea). Serum leptin and HMW-adiponectin concentrations were determined using ELISA kits (Millipore, Billerica, MA) according to the manufacturer's instructions. The HMW-adiponectin was measured after pretreatment of serum samples with digestion solution to remove hexameric and trimeric adiponectins.

Statistical analysis

Data were analyzed using SPSS software (version 19.0; SPSS Inc., Chicago, IL). Variables were examined for normality, and not normally distributed values were analyzed using non parametric test.

Baseline characteristics except dietary intakes between normal weight and obese groups were tested using Student *t* test; baseline dietary intakes were tested using 2-way ANOVA in order to evaluate the effect of sex and weight group.

For the investigation of the effects of the 12-week program on anthropometric and clinical characteristics among obese subjects, subjects

were divided into three subgroups based on the magnitude of weight loss: weight loss < 3 kg, weight loss 3~6 kg, and weight loss > 6 kg.

ANOVA or Kruskal-Wallis test was performed to evaluate the overall effect of weight management program on anthropometric and clinical characteristics among normal weight group and three obese subgroups. Duncan's multiple-range test or Mann-Whitney U test with Bonferroni correction was used for multiple comparisons.

To test for changes in anthropometric and clinical characteristics and dietary intakes before and after 12-week weight management program, we used paired *t* test or Wilcoxon's signed-rank test. McNemar's test was used to examine the change in the proportion of subjects diagnosed with metabolic syndrome between before and after weight management program within each weight group.

In order to identify key factors for successful weight loss, we used chi-square test for categorical variables and Student *t* test for continuous variables of obese subgroups. Data are reported as mean $\pm$ SEM. Significance was set at $P < 0.05$.

RESULTS

General characteristics of subjects at the baseline

The general characteristics of subjects at baseline are shown in **Table 1**. The mean age of the normal weight group was 26.4 $\pm$ 1.1 years and that of the obese group was 29.9 $\pm$ 1.3 years. Although we tried to match demographic characteristics other than BMI between two groups, subjects in the obese group were older than those in the normal weight group. However, most of normal weight and obese subjects were in their 20's.

The mean heights of the obese and normal weight groups were similar for both men and women. Weight and BMI of obese group were significantly higher than those of normal weight group as subjects were assigned to the groups according to the BMI. Waist circumference of obese group was also significantly higher than that of normal weight group.

The obese group had significantly higher total cholesterol, LDL cholesterol, and triglyceride concentrations than the normal weight group. Systolic and diastolic blood pressures were also significantly higher in obese group. On the other hand, obese subjects had significantly lower HDL cholesterol than normal weight subjects. There was no significant difference in fasting blood glucose between the two groups.

TABLE 1

General characteristics of subjects at baseline[1]

Characteristics	Normal weight (BMI 18.5-23 kg/m^2)	Obese (BMI >25 kg/m^2)
Subjects	21	47
Men [n (%)]	11 (52.4)[2]	31 (65.9)
Women [n (%)]	10 (47.6)	16 (34.0)
Age (year)	26.4 ± 1.1[4]	29.9 ± 1.3*
Men	25.6 ± 1.3	29.8 ± 1.5
Women	27.3 ± 1.8	30.1 ± 2.4
Height (cm)		
Men	174.2 ± 1.7	174.0 ± 0.7
Women	160.7 ± 1.4	162.3 ± 1.1
Weight (kg)		
Men	67.3 ± 1.9	85.2 ± 1.3*
Women	54.3 ± 1.3	73.6 ± 2.5*
BMI (kg/m^2)	21.6 ± 0.3	28.1 ± 0.4*
Men	22.1 ± 0.4	28.1 ± 0.4*
Women	21.0 ± 0.3	27.9 ± 0.9*
Waist circumference (cm)		
Men	77.5 ± 1.4	93.1 ± 1.0*
Women	73.3 ± 2.4	90.5 ± 2.7*
Hip circumference (cm)		
Men	91.6 ± 1.4	102.4 ± 0.8*
Women	92.7 ± 4.3	104.3 ± 2.4*
WHR		
Men	0.847 ± 0.006	0.908 ± 0.005*
Women	0.794 ± 0.013	0.866 ± 0.011*
Fat mass (kg)		
Men	13.1 ± 1.2	24.5 ± 1.0*
Women	15.3 ± 0.7	27.6 ± 2.0*
Total cholesterol (mg/dL)	166.2 ± 7.0	196.0 ± 4.8*
Men	157.8 ± 9.1	199.4 ± 6.5*
Women	175.4 ± 10.6	189.4 ± 6.3
LDL cholesterol (mg/dL)	92.1 ± 7.3	122.1 ± 4.2*
Men	85.5 ± 9.4	124.7 ± 5.5*
Women	99.5 ± 11.3	116.9 ± 6.6
HDL cholesterol (mg/dL)	61.1 ± 2.5	51.1 ± 1.9*
Men	59.1 ± 3.5	49.2 ± 2.2*
Women	63.3 ± 3.5	54.7 ± 3.4

Triglyceride (mg/dL)	82.7 ± 5.0	137.5 ± 14.4*
Men	84.8 ± 5.2	154.2 ± 21.1*
Women	80.4 ± 9.2	105.1 ± 6.4*
Total: HDL cholesterol	2.81 ± 0.16	4.06 ± 0.18*
Men	2.75 ± 0.21	4.28 ± 0.24*
Women	2.87 ± 0.25	3.63 ± 0.23*
LDL:HDL cholesterol	1.59 ± 0.15	2.55 ± 0.13*
Men	1.52 ± 0.20	2.69 ± 0.16*
Women	1.66 ± 0.23	2.27 ± 0.19#
Glucose (mg/dL)	93.1 ± 1.9	95.6 ± 1.5
Men	95.8 ± 2.4	96.9 ± 1.9
Women	90.0 ± 2.8	93.1 ± 2.5
Systolic BP (mm Hg)	119.8 ± 2.6	129.9 ± 2.0*
Men	126.9 ± 3.2	132.6 ± 2.2
Women	112.0 ± 2.4	124.6 ± 3.7*
Diastolic BP (mm Hg)	70.6 ± 2.0	77.5 ± 1.6*
Men	73.0 ± 2.4	77.9 ± 1.6#
Women	68.0 ± 3.3	76.9 ± 3.5

[1] BP, blood pressure; LDL:HDL cholesterol, ratio of LDL to HDL cholesterols; Total:HDL cholesterol, ratio of total to HDL cholesterols; WHR, waist-to-hip ratio. Data are reported as mean ± SEM. Significance was tested by independent *t* test.

*: Significant difference between two weight groups at baseline ($P < 0.05$).

#: A tendency of difference between two weight groups at baseline ($P < 0.1$).

[2] Percentage of subjects in each weight group (all such values). Percentages may not add up to 100% due to rounding.

Changes in anthropometric characteristics after 12-week weight management program

There was a significant decrease in weight (2.7 ± 0.4 kg, $P < 0.001$), BMI (0.9 ± 0.1 kg/m^2, $P < 0.001$), waist (2.6 ± 0.4 cm, $P < 0.001$) and hip circumference (1.9 ± 0.3 cm, $P < 0.001$), WHR (0.008 ± 0.001, $P < 0.001$), and fat mass (2.4 ± 0.3 kg, $P < 0.001$) in the obese after weight management program participation (**Table 2**). There was no significant change in weight, BMI, waist circumference, hip circumference, and fat mass during 12-week period in the normal weight group.

TABLE 2

Comparison of anthropometric characteristics before and after 12-week program[1]

Characteristics	Normal weight (n=19)		Obese (n=44)	
	Before[2]	After[3]	Before[2]	After[3]
Subjects				
Men [n (%)]	9 (47.4)[4]		30 (68.2)	
Women [n (%)]	10 (52.6)		14 (31.8)	
Weight (kg)	60.1 ± 1.8	60.1 ± 1.9	81.5 ± 1.4*	78.9 ± 1.3†
Men	66.6 ± 2.1	66.4 ± 2.2	84.7 ± 1.2*	81.7 ± 1.3†
Women	54.3 ± 1.3	54.2 ± 1.4	74.8 ± 2.8*	72.6 ± 2.7†
BMI (kg/m^2)	21.5 ± 0.3	21.5 ± 0.3	28.1 ± 0.4*	27.1 ± 0.4†
Men	22.1 ± 0.5	22.0 ± 0.4	28.0 ± 0.4*	27.0 ± 0.4†
Women	21.0 ± 0.3	21.0 ± 0.3	28.2 ± 1.0*	27.4 ± 0.9†
Waist (cm)	75.1 ± 1.5	75.0 ± 1.6	92.4 ± 1.1*	89.8 ± 1.1†
Men	77.1 ± 1.7	76.9 ± 1.8	92.7 ± 0.9*	90.0 ± 0.9†
Women	73.3 ± 2.4	73.2 ± 2.6	91.7 ± 2.9*	89.2 ± 2.9†
Hip (cm)	92.0 ± 2.3	91.6 ± 2.4	103.1 ± 1.0*	101.2 ± 0.9†
Men	91.1 ± 1.6	90.9 ± 1.7	102.2 ± 0.7*	100.5 ± 0.7†
Women	92.7 ± 4.3	92.2 ± 4.3	105.2 ± 2.7*	102.8 ± 2.5†
WHR	0.819 ± 0.010	0.820 ± 0.009	0.895 ± 0.006*	0.886 ± 0.006†
Men	0.846 ± 0.008	0.846 ± 0.007	0.907 ± 0.005*	0.896 ± 0.005†
Women	0.794 ± 0.012	0.797 ± 0.012	0.871 ± 0.012*	0.867 ± 0.013
Fat mass (kg)	14.1 ± 0.8	14.0 ± 0.8	25.4 ± 1.0*	23.0 ± 1.0†
Men	12.9 ± 1.3	12.9 ± 1.3	24.0 ± 0.9*	21.3 ± 0.9†
Women	15.3 ± 0.7	15.0 ± 0.8	28.4 ± 2.3*	26.6 ± 2.2†

[1] Hip, hip circumference; Waist, waist circumference; WHR, waist-to-hip ratio. Data are reported as mean ± SEM.

[2] Significance was tested by independent *t* test.

*: Significant difference between two weight groups at baseline ($P < 0.05$).

[3] Variables were examined for normality, and values not normally distributed were analyzed using non parametric test. Significance was tested by paired *t* test or Wilcoxon's signed-rank test based on normality; significance of

weight, BMI, hip circumference, and fat mass were determined by Wilcoxon's signed-rank test.

†: Significant change during 12-weeks ($P < 0.05$).

4 Percentage of subjects in each weight group (all such values).

Changes in clinical characteristics after 12-week weight management program participation

Changes in clinical characteristics during 12-weeks are shown in **Table 3**. Since the magnitude of weight loss among obese subjects varied, subjects in the obese group were divided into three subgroups based on the magnitude of weight loss to further determine the impact of the magnitude of weight loss on clinical parameters. Considering that the goal of this study was to lose 0.5 kg per week, we divided obese groups based on the level of goal achievement: < 3 kg wt loss (< 50%), 3~6 kg wt loss (50~100%), and > 6 kg wt loss (> 100%).

The 12-week weight management program participation resulted in significant decrease in triglyceride (31.5 $\pm$ 13.6 mg/dL, $P = 0.012$), ratios of total to HDL cholesterols (0.43 $\pm$ 0.15, $P = 0.024$) and LDL to HDL cholesterols (0.22 $\pm$ 0.11, $P < 0.001$), and serum free fatty acid (40.2 $\pm$ 23.5 mEq/L, $P = 0.028$) in obese group. Also, diastolic blood pressure tended to decrease (2.6 $\pm$ 1.4 mm Hg, $P = 0.066$), while HDL cholesterol tended to increase (2.9 $\pm$ 1.5 mg/dL, $P = 0.061$) after weight management program participation in obese group.

Decrease in LDL cholesterol was not significant in both the normal weight and obese group. However, the large weight loss group (> 6 kg wt loss) showed significant decrease in LDL cholesterol (20.2 $\pm$ 6.6 mg/dL, $P = 0.029$).

Mean decrease in blood glucose concentration was not significant in the obese group (2.3 ± 1.6 mg/dL, $P = 0.127$). However, a significant decrease in blood glucose concentration was observed in the large weight loss group (13.5 ± 7.4 mg/dL, $P = 0.027$). Although the normal weight group showed significant decrease in glucose (5.3 ± 1.5 mg/dL, $P = 0.004$), it was mostly due to high glucose concentration of few subjects at baseline.

Obese subjects had significantly higher baseline serum leptin concentration compared with normal weight subjects, and a significant decrease in serum leptin concentration in the intermediate (3~6 kg wt loss, 1.69 ± 0.63 ng/mL, $P = 0.017$) and the large weight loss groups (3.71 ± 1.48 ng/mL, $P = 0.046$) were observed. On the contrary, serum leptin concentration (0.77 ± 0.35 ng/mL, $P = 0.004$) in normal weight group increased significantly despite no significant weight change in normal weight group. Serum HMW-adiponectin concentration at baseline were not different between two groups and did not change with weight loss.

TABLE 3

Comparison of clinical characteristics before and after 12-week program[1]

Characteristics	Normal weight (n=19)		Obese (n=44)	
	Before[2]	After[3]	Before[2]	After[3]
Subjects				
Men [n (%)]	9 (47.4)[4]		30 (68.2)	
Women [n (%)]	10 (52.6)		14 (31.8)	
Systolic BP (mm Hg)	117.8 ± 2.8	118.4 ± 1.7	130.3 ± 2.0*	127.7 ± 2.0
Men	125.1 ± 3.5	123.4 ± 3.4	132.2 ± 2.2	130.3 ± 2.3
Women	112.0 ± 2.4	115.5 ± 2.5	126.1 ± 3.8*	122.4 ± 3.6
Diastolic BP (mm Hg)	70.2 ± 2.5	68.3 ± 1.7	78.1 ± 1.6*	75.5 ± 1.5§
Men	73.0 ± 2.8	69.0 ± 2.0	77.9 ± 1.6	75.6 ± 1.7
Women	68.0 ± 3.3	68.5 ± 2.5	78.6 ± 3.8#	75.5 ± 3.3
Total CHOL (mg/dL)	168.2 ± 7.6	166.1 ± 5.6	197.9 ± 4.8*	192.0 ± 5.1
Men	160.3 ± 11.0	165.4 ± 9.2	198.7 ± 6.7*	193.0 ± 6.8
Women	175.4 ± 10.6	166.7 ± 7.2	196.3 ± 5.1#	189.7 ± 7.0
LDL CHOL (mg/dL)	93.1 ± 8.0	92.7 ± 5.9	124.2 ± 4.2*	123.6 ± 4.7
Men	85.9 ± 11.6	90.0 ± 8.7	124.7 ± 5.7*	124.1 ± 6.3
Women	99.5 ± 11.3	95.1 ± 8.4	123.1 ± 5.7#	122.6 ± 6.2
HDL CHOL (mg/dL)	62.7 ± 2.4	63.9 ± 3.1	50.5 ± 1.9*	53.4 ± 1.6§
Men	61.9 ± 3.6	64.1 ± 5.5	48.2 ± 2.0*	54.1 ± 1.7†
Women	63.3 ± 3.5	63.6 ± 3.7	55.4 ± 3.9	51.9 ± 3.6
Triglyceride (mg/dL)	82.7 ± 5.6	86.9 ± 6.9	139.9 ± 15.3*	108.4 ± 6.5†
Men	85.3 ± 6.4	95.4 ± 10.6	155.0 ± 21.8#	107.7 ± 8.7†
Women	80.4 ± 9.2	78.0 ± 8.5	107.6 ± 7.1*	109.9 ± 9.3
Glucose (mg/dL)	92.1 ± 1.9	86.7 ± 1.4†	95.9 ± 1.6	93.5 ± 1.5
Men	94.3 ± 2.7	89.3 ± 1.9	97.0 ± 2.0	93.2 ± 1.6§
Women	90.0 ± 2.8	84.4 ± 1.9†	93.6 ± 2.8*	94.3 ± 3.5
Total:HDL CHOL	2.77 ± 0.17	2.67 ± 0.13	4.14 ± 0.18*	3.71 ± 0.13†
Men	2.66 ± 0.25	2.68 ± 0.21	4.33 ± 0.24	3.66 ± 0.16†
Women	2.87 ± 0.25	2.70 ± 0.19	3.74 ± 0.24*	3.82 ± 0.22

LDL:HDL CHOL	1.57 ± 0.16	1.53 ± 0.13	2.62 ± 0.13*	2.40 ± 0.11†
Men	1.46 ± 0.24	1.48 ± 0.18	2.73 ± 0.17	2.37 ± 0.14†
Women	1.66 ± 0.23	1.57 ± 0.17	2.39 ± 0.20*	2.48 ± 0.18
FFA (mEq/L)	198.2 ± 25.4	169.5 ± 20.5	227.8 ± 15.7	187.6 ± 18.3†
Men	167.3 ± 15.9	179.4 ± 28.9	205.5 ± 18.6	197.3 ± 25.2
Women	226.0 ± 45.5	160.6 ± 30.1	273.9 ± 25.6	167.4 ± 21.0†
Leptin (ng/mL)	4.08 ± 0.74	4.85 ± 0.85†	7.74 ± 1.11*	6.61 ± 0.90
Men	1.66 ± 0.42	2.19 ± 0.34	4.94 ± 0.57*	3.60 ± 0.59†
Women	6.26 ± 0.91	7.24 ± 1.14	13.48 ± 2.65*	12.85 ± 1.43
Adiponectin (μg/mL)	6.67 ± 1.41	9.47 ± 2.95	9.04 ± 1.45	7.63 ± 0.93
Men	6.78 ± 2.12	7.81 ± 2.64	9.51 ± 2.01	7.56 ± 1.13
Women	6.57 ± 2.00	10.95 ± 5.20	8.04 ± 1.56	7.77 ± 1.67

[1] BP, blood pressure; CHOL, cholesterol; FFA, free fatty acids; LDL:HDL CHOL, ratio of LDL to HDL cholesterols; Total:HDL CHOL, ratio of total to HDL cholesterols. Data are reported as mean ± SEM.

[2] Significance was tested by independent *t* test.

*: Significant difference between two weight groups at baseline ($P < 0.05$).

#: A tendency of difference between two weight groups at baseline ($P < 0.1$).

[3] Variables were examined for normality, and values not normally distributed were analyzed using non parametric test. Significance was tested by paired *t* test or Wilcoxon's signed-rank test based on normality; significance of triglyceride, glucose, Total:HDL cholesterol, LDL:HDL cholesterol, serum leptin, and serum adiponectin were determined by Wilcoxon's signed-rank test.

†: Significant change during 12-weeks ($P < 0.05$).

§: A tendency of change during 12-weeks ($P < 0.1$).

[4] Percentage of subjects in each weight group (all such values).

Comparison of difference in clinical characteristics among subgroups divided by the magnitude of weight loss after 12-week weight management program

The overall effect of the 12-week program on normal weight group and three subgroups in obese subjects for clinical characteristics is presented in **Table 4**.

Thirty six percent of obese subjects achieved at least 50% of the weight reduction goal by losing more than 3 kg. Twenty three percent of obese subjects lost 3~6 kg (4.0 $\pm$ 0.3 kg and 5.1% wt loss, n = 10), and 14% of obese subjects lost more than 6 kg (8.0 $\pm$ 0.8 kg and 9.1% wt loss, n = 6).

Decrease in glucose (13.5 $\pm$ 7.3 mg/dL) and ratio of LDL to HDL cholesterols (0.85 $\pm$ 0.26) was significant only in the large weight loss group (> 6 kg loss). Also, reduction in triglyceride and serum leptin concentrations was dependent on the magnitude of weight loss. However, changes in serum free fatty acid and adiponectin were not significantly different among subgroups.

TABLE 4

Comparison of difference in anthropometric and clinical characteristics among normal weight and three obese subgroups divided by magnitude of weight loss after 12-week program[1]

	Normal weight (n=19)	Obese (n=44)		
Characteristics		Small (< 3 kg loss)	Intermediate (3~6 kg loss)	Large (> 6 kg loss)
Δ Weight (kg)	- 0.1 ± 0.2[a]	- 1.2 ± 0.2[b]	- 4.0 ± 0.3[c]	- 8.0 ± 0.8[d]
Δ BMI (kg/m^2)	- 0.1 ± 0.1[a]	- 0.4 ± 0.1[a]	- 1.4 ± 0.1[b]	- 2.7 ± 0.3[c]
Δ Waist (cm)	- 0.1 ± 0.3[a]	- 1.2 ± 0.3[a]	- 3.7 ± 0.3[b]	- 7.4 ± 1.1[c]
Δ Hip (cm)	- 0.4 ± 0.3[a]	- 0.9 ± 0.3[a]	- 2.7 ± 0.3[b]	- 5.1 ± 0.9[c]
Δ WHR	0.002 ± 0.001[a]	- 0.004 ± 0.001[a]	- 0.012 ± 0.002[b]	- 0.023 ± 0.003[c]
Δ Fat mass (kg)	- 0.2 ± 0.2[a]	- 1.3 ± 0.3[a]	- 3.1 ± 0.4[b]	- 6.4 ± 0.8[c]
Δ Systolic BP (mm Hg)	0.6 ± 2.3	- 1.4 ± 2.4	- 6.4 ± 3.7	- 1.7 ± 4.9
Δ Diastolic BP (mm Hg)	- 1.9 ± 2.0	- 1.2 ± 1.6	- 6.3 ± 2.8	- 2.8 ± 5.3
Δ Total CHOL (mg/dL)	- 2.1 ± 4.6	- 4.5 ± 4.2	- 3.5 ± 8.2	- 16.5 ± 10.0
Δ LDL CHOL (mg/dL)[2]	- 0.4 ± 3.4	2.0 ± 3.9	4.1 ± 8.0	- 20.2 ± 6.6
Δ HDL CHOL (mg/dL)	1.2 ± 2.3	2.1 ± 1.9	2.3 ± 3.4	7.7 ± 3.4
Δ Glucose (mg/dL)	- 5.3 ± 1.5[a]	- 0.7 ± 1.3[a]	- 0.1 ± 3.0[a]	- 13.5 ± 7.3[b]
Δ Triglyceride (mg/dL)[3]	3.5 ± 7.7	- 26.5 ± 20.2	- 33.2 ± 16.2	- 52.3 ± 22.5
Δ Total:HDL CHOL	- 0.08 ± 0.11	- 0.36 ± 0.19	- 0.30 ± 0.31	- 1.00 ± 0.40
Δ LDL:HDL CHOL	- 0.04 ± 0.09[a]	- 0.12 ± 0.11[a]	- 0.10 ± 0.28[a]	- 0.85 ± 0.26[b]
Δ FFA (mEq/L)	- 28.7 ± 26.2	- 45.6 ± 30.0	- 48.1 ± 46.3	- 2.5 ± 74.6
Δ Leptin (ng/mL)	0.77 ± 0.35[a]	- 0.35 ± 0.92[ab]	- 1.69 ± 0.63[b]	- 3.71 ± 1.48[b]
Δ Adiponectin (μg/mL)	2.80 ± 2.11	- 1.92 ± 1.53	- 1.30 ± 1.93	0.78 ± 1.16

[1] BP, blood pressure; CHOL, cholesterol; FFA, free fatty acids; Intermediate, 3~6 kg weight loss group (n=10); Large, > 6 kg weight loss group (n=6); LDL:HDL cholesterol, ratio of LDL to HDL cholesterols; Small, < 3 kg weight loss group (n=28); Total:HDL cholesterol, ratio of total to HDL

cholesterols; WHR, waist-to-hip ratio. Δ indicates difference in characteristics of subjects after the 12-week program (12 week level – Baseline level). Variables were examined for normality, and values not normally distributed were analyzed using non parametric test. Significance was tested by ANOVA or Kruskal-Wallis test. Significance of waist circumference, systolic and diastolic blood pressure, total cholesterol, LDL cholesterol, and HDL cholesterol were determined by ANOVA. Duncan's multiplerange test or Mann-Whitney *U* test with Bonferroni correction was used to determine the difference among subgroups. Data are reported as mean ± SEM.

[2] A tendency of overall difference was observed ($P = 0.083$).

[3] To determine difference among subgroups, Mann-Whitney *U* test with Bonferroni correction was used. However, we did not find any significant difference among subgroups, although overall effect was significant.

Change in the prevalence of metabolic syndrome after 12-week weight management program participation

None of the subjects in normal weight group had metabolic syndrome at the baseline and 12-week time point, whereas 36% of obese subjects were classified as having metabolic syndrome at the baseline (**Table 5**). After 12-week program, prevalence of metabolic syndrome in the obese group tended to decrease (from 36.4% to 20.4%, $P = 0.092$). Decrease in the prevalence of metabolic syndrome was significant in obese men (from 40.0% to 13.3%, $P = 0.021$).

TABLE 5

Change in the prevalence of metabolic syndrome before and after 12-week program[1]

	Normal weight (n=19)			Obese (n=44)		
	Before [n (%)]	After [n (%)]	*P* value	Before [n (%)]	After [n (%)]	*P* value
Metabolic syndrome[2]						
With	0 (0.0)[3]	0 (0.0)	N/A	16	9	0.092
Men	—	—	—	12 (75.0)	4 (44.4)	0.021
Women	—	—	—	4 (25.0)	5 (55.5)	NS

[1] A McNemar test was used to determine significance of a change in the number of subjects with metabolic syndrome after 12-week program. Significance could be calculated only in obese group because none of the normal weight subjects had metabolic syndrome (N/A, not available).

[2] The criteria used for the diagnosis for metabolic syndrome are:

Waist circumference > 90 cm in men and > 85 cm in women;

Triglyceride concentration > 150 mg/dL; HDL cholesterol < 40 mg/dL in men and < 50 mg/dL in women; Systolic blood pressure > 130 mm Hg or diastolic blood pressure > 85 mm Hg; Fasting glucose > 100 mg/dL.

Meeting more than three of these five criteria was diagnosed with metabolic syndrome.

[3] Percentage of subjects in each weight group at each week (all such values). Percentages may not add up to 100% due to rounding.

Comparison of dietary intakes before and after 12-week weight management program participation

Energy, macronutrients, and cholesterol

Energy, macronutrients, and cholesterol intakes estimated by 3-day dietary record are presented in **Table 6**. The percentage of intakes of protein, fiber, and cholesterol compared with Dietary Reference Intakes for Koreans (KDRIs) were calculated according to age and sex of each subject. While intakes of energy, protein, fat, carbohydrate, and cholesterol in obese subjects were higher than those of normal weight subjects regardless of sex, there was no statistically significant difference. Compared with KDRIs, both groups consumed higher amount of protein and cholesterol but lower amount of fiber.

There was a significant decrease in dietary intakes of energy (270.7 $\pm$ 120.9 kcal, $P = 0.031$), fat (16.8 $\pm$ 5.9 g, $P = 0.007$), and cholesterol (142.4 $\pm$ 38.4 mg, $P = 0.001$) in obese group with 12-week program. Significant decrease in percent energy from fat (3.3 $\pm$ 1.4%, $P = 0.027$) and a tendency of decrease in animal fat intake (9.0 $\pm$ 4.8%, $P = 0.069$) in the obese group were observed. Decline in cholesterol intake was significant in all obese subgroups (Data not shown). No significant changes in intake of protein, carbohydrate, and fiber were observed in the obese group. It seemed that obese subjects lost their weight by reducing energy and fat intakes, while maintaining intakes of other macronutrients. Dietary intake of protein in the

normal weight group decreased significantly due to a tendency of reduction in protein intake in normal weight women (18.2 $\pm$ 7.5g, $P = 0.059$).

TABLE 6

Comparison of energy, macronutrients, and cholesterol intakes estimated by 3-day diet record before and after 12-week program[1]

	Normal weight (n=19)		Obese (n=40)	
	Before[2]	After[3]	Before[2]	After[3]
Subjects				
Men [n (%)]	9 (47.4)[4]		26 (65.0)	
Women [n (%)]	10 (52.6)		14 (35.0)	
Energy (kcal)	1855.3 ± 100.5	1739.4 ± 128.0	2175.4 ± 115.7	1904.8 ± 77.7†
Men	1957.0 ± 186.1	1961.8 ± 193.7	2342.9 ± 147.1	2051.6 ± 99.4§
Women	1763.7 ± 93.1	1539.2 ± 151.1	1864.5 ± 161.0	1632.1 ± 87.3
Protein (g)	77.4 ± 4.7	63.0 ± 4.6†	92.6 ± 5.6	83.0 ± 5.6
Men	80.0 ± 7.9	69.8 ± 5.8	99.1 ± 7.2	91.7 ± 7.8
Women	75.1 ± 5.8	56.9 ± 6.7§	80.7 ± 8.0	66.8 ± 4.4
% of energy	16.9 ± 0.8	14.7 ± 0.7†	17.1 ± 0.4	17.8 ± 1.3
% of KDRIs	149.5 ± 8.9	121.3 ± 8.7	175.2 ± 10.1	156.4 ± 9.8
Animal protein (g)	45.4 ± 3.9	32.9 ± 3.8	56.7 ± 4.2	47.5 ± 4.7
Fat (g)	62.4 ± 4.8	58.7 ± 6.6	77.5 ± 5.1	60.6 ± 3.5†
Men	69.4 ± 8.8	67.2 ± 8.3	82.6 ± 6.5	64.4 ± 4.5†
Women	56.1 ± 4.2	50.9 ± 9.9	67.9 ± 7.9	53.6 ± 5.5
% of energy	29.9 ± 1.2	28.9 ± 1.6	31.7 ± 1.0	28.4 ± 1.1†
Animal fat (g)	34.4 ± 4.1	32.1 ± 5.1	41.6 ± 3.7	32.6 ± 2.5§
Carbohydrate (g)	238.2 ± 15.1	236.5 ± 15.1	268.8 ± 13.6	245.4 ± 11.6
Men	244.4 ± 27.2	264.7 ± 25.2	287.8 ± 17.7	264.1 ± 15.8
Women	232.6 ± 16.2	211.1 ± 14.2	233.5 ± 17.7	210.9 ± 10.7
% of energy	51.6 ± 1.9	55.8 ± 1.9†	50.0 ± 0.9	51.9 ± 1.4
Fiber (g)	15.2 ± 1.2	15.5 ± 1.1	17.9 ± 1.0	17.8 ± 0.9
Men	14.2 ± 2.0	15.3 ± 1.9	18.1 ± 1.3	17.8 ± 1.1
Women	16.1 ± 1.6	15.7 ± 1.3	17.6 ± 1.5	17.8 ± 1.8
% of KDRIs	69.3 ± 6.1	70.3 ± 5.2	77.8 ± 4.3	77.4 ± 4.4
Cholesterol (mg)	388.0 ± 38.2	317.7 ± 44.7	433.1 ± 30.2	290.7 ± 22.0†
Men	391.2 ± 53.0	331.0 ± 59.9	440.0 ± 36.3	320.6 ± 28.9†
Women	385.2 ± 57.4	305.7 ± 68.5	420.3 ± 55.5	235.2 ± 28.2†
% of KDRIs	129.3 ± 12.7	105.9 ± 14.9	144.4 ± 10.1	96.9 ± 7.3†

[1] Four obese men did not submit 3-day diet record at week12. KDRIs, Dietary Reference Intakes for Koreans. Data are reported as mean ± SEM.

[2] Significance was tested by 2-way ANOVA.

[3] Variables were examined for normality, and values not normally distributed were analyzed using non parametric test. Significance was tested by paired *t* test or Wilcoxon's signed-rank test. Significance of protein intake and percent calories from protein were determined by Wilcoxon's signed-rank test.

†: Significant change during 12-weeks ($P < 0.05$).

§: A tendency of change during 12-weeks ($P < 0.1$)

[4] Percentage of subjects in each weight group (all such values).

Vitamins and minerals

Dietary vitamin and mineral intakes assessed by 3-day dietary record are shown in **Table 7**. At baseline, obese subjects consumed significantly higher amount of vitamin B2, B6, niacin, phosphorus, and sodium compared with normal weight subjects. Subjects in both groups consumed excessive amount of phosphorus and sodium and lower levels of vitamin C, folate, and calcium compared with KDRIs.

Decrease in consumption of vitamin B2 (0.19 $\pm$ 0.85 mg, P = 0.032), vitamin E (3.5 $\pm$ 1.2 mg, P = 0.005), phosphorus (158.9 $\pm$ 73.5 mg, P = 0.037), zinc (1.8 $\pm$ 0.8 mg, P = 0.010), and sodium (624.0 $\pm$ 271.1 mg, P = 0.027) was significant in obese group after 12-week program participation. Tendency of increase in vitamin C intake (14.8 $\pm$ 8.0 mg, P = 0.071) and significant decrease in sodium intake (624.0 $\pm$ 271.1 mg, P = 0.027) in obese group suggested that they incorporated healthier diet during 12-week program.

While vitamin B2, vitamin E, phosphorus, and zinc intakes decreased significantly in the obese group, the intake levels still met KDRIs. Calcium (79% in men and 72% in women) and iron intakes (86%, only in women) in obese subjects remained lower than KDRIs, however, intakes of these nutrients by the subjects in this study were similar to Koreans in their 20's, according to Korean National Health and Nutrition Examination Survey (KNHANES) in 2010.

TABLE 7

Comparison of vitamin and mineral intakes estimated by 3-day diet record before and after 12-week program[1]

	Normal weight (n=19)		Obese (n=40)	
	Before[2]	After[3]	Before[2]	After[3]
Subjects				
Men [n (%)]	9 (47.4)[4]		26 (65.0)	
Women [n (%)]	10 (52.6)		14 (35.0)	
Vitamin A (μgRE)	679.8 ± 69.0	743.5 ± 111.0	864.0 ± 95.0	784.3 ± 84.9
Men	664.3 ± 137.7	873.4 ± 162.3	834.3 ± 132.9	841.5 ± 125.2
Women	693.8 ± 53.6	626.6 ± 150.0	919.2 ± 117.9$^{\#}$	677.9 ± 66.5†
% of KDRIs	98.1 ± 9.7	105.9 ± 15.6	121.8 ± 13.2	109.4 ± 11.3
Vitamin B1 (mg)	1.21 ± 0.13	1.14 ± 0.10	1.45 ± 0.10	1.29 ± 0.08§
Men	1.35 ± 0.27	1.19 ± 0.14	1.60 ± 0.13	1.40 ± 0.09
Women	1.07 ± 0.08	1.09 ± 0.15	1.17 ± 0.13	1.10 ± 0.11
% of KDRIs	104.8 ± 11.1	97.5 ± 8.7†	123.8 ± 8.3	111.5 ± 6.4†
Vitamin B2 (mg)	1.06 ± 0.06	1.00 ± 0.09	1.36 ± 0.08*	1.17 ± 0.07†
Men	1.03 ± 0.13	1.12 ± 0.11	1.44 ± 0.11$^{\#}$	1.26 ± 0.10
Women	1.09 ± 0.05	0.89 ± 0.13	1.21 ± 0.10	1.00 ± 0.07§
% of KDRIs	80.5 ± 5.2	74.7 ± 6.5	97.8 ± 5.6	84.0 ± 4.7†
Vitamin B6 (mg)	1.85 ± 0.12	1.75 ± 0.12	2.46 ± 0.16*	2.54 ± 0.41
Men	2.02 ± 0.21	1.75 ± 0.17	2.49 ± 0.16	2.32 ± 0.17
Women	1.71 ± 0.11	1.74 ± 0.18	2.40 ± 0.38	2.93 ± 1.14
% of KDRIs	127.9 ± 7.6	120.7 ± 8.4	168.1 ± 11.4	174.0 ± 29.1
Niacin (mg)	16.4 ± 1.1	14.3 ± 1.1§	20.5 ± 1.4	20.6 ± 1.9
Men	18.9 ± 1.7	16.1 ± 0.7	22.9 ± 1.9	50.7 ± 0.9
Women	14.2 ± 0.9	12.7 ± 1.9	16.2 ± 1.3	16.4 ± 1.3
% of KDRIs	109.4 ± 6.2	95.3 ± 7.2§	133.4 ± 8.5	124.6 ± 8.3
Vitamin C (mg)	70.8 ± 8.5	67.7 ± 7.5	74.4 ± 5.2	89.2 ± 8.1§
Men	66.6 ± 15.6	68.6 ± 9.6	74.6 ± 6.7	86.4 ± 9.1
Women	74.5 ± 8.7	67.0 ± 11.9	74.0 ± 8.6	94.5 ± 16.4
% of KDRIs	70.8 ± 8.5	67.7 ± 7.5	74.4 ± 5.2	89.2 ± 8.1§
Folate (mg)	195.2 ± 17.2	195.6 ± 14.8	238.0 ± 17.2$^{\#}$	237.9 ± 18.9
Men	166.9 ± 24.9	213.5 ± 25.9	242.4 ± 23.1$^{\#}$	243.6 ± 26.6
Women	220.7 ± 21.9	179.5 ± 15.3	229.9 ± 25.0	227.3 ± 22.8
% of KDRIs	48.8 ± 4.3	48.9 ± 3.7	59.5 ± 4.3	59.5 ± 4.7

Vitamin E (mg)	16.0 ± 1.6	15.7 ± 1.7	18.9 ± 1.3	15.4 ± 1.0†
Men	15.9 ± 2.3	17.7 ± 3.3	20.8 ± 1.8	16.5 ± 1.4†
Women	16.1 ± 2.3	13.9 ± 1.4	15.3 ± 1.0	15.5 ± 1.1
% of KDRIs	147.2 ± 15.1	142.7 ± 14.5	166.3 ± 10.2	136.5 ± 8.4†
Calcium (mg)	499.6 ± 42.6	460.9 ± 44.5	609.5 ± 55.2	550.3 ± 36.3
Men	413.3 ± 62.6	497.2 ± 63.2	645.8 ± 78.1	592.8 ± 50.6
Women	577.2 ± 48.5	428.3 ± 63.7†	542.1 ± 61.9	471.2 ± 38.1
% of KDRIs	72.8 ± 6.7	66.1 ± 6.3	85.2 ± 7.5	76.8 ± 4.8
Phosphorus (mg)	954.6 ± 58.6	859.8 ± 65.3	1172.7 ± 67.3*	1013.8 ± 49.5†
Men	947.7 ± 102.6	927.8 ± 83.3	1240.9 ± 89.0#	1076.9 ± 66.5
Women	960.9 ± 68.0	798.6 ± 98.7	1046.1 ± 93.0	896.5 ± 59.9
% of KDRIs	136.4 ± 8.4	122.8 ± 9.3†	167.5 ± 9.6	144.8 ± 7.1†
Iron (mg)	15.0 ± 1.9	12.2 ± 1.1	16.4 ± 1.2	14.2 ± 1.2§
Men	14.9 ± 3.4	11.5 ± 1.0	17.3 ± 1.6	15.2 ± 1.7
Women	15.1 ± 2.2	12.9 ± 1.8	14.7 ± 1.8	12.4 ± 1.2
% of KDRIs	127.4 ± 18.3	102.0 ± 8.5	149.4 ± 12.4	129.7 ± 12.3
Zinc (mg)	10.1 ± 1.6	7.5 ± 0.6	10.8 ± 0.7	9.0 ± 0.5†
Men	9.4 ± 1.0	8.1 ± 0.9	11.7 ± 0.9	9.8 ± 0.7†
Women	10.7 ± 2.9	7.0 ± 0.8	9.0 ± 0.7	7.7 ± 0.5
% of KDRIs	115.4 ± 19.8	84.8 ± 6.3	118.9 ± 6.8	99.9 ± 5.3†
Sodium (mg)	3360.7 ± 250.4	3103.1 ± 270.5	4371.5 ± 239.6*	3747.5 ± 213.8†
Men	3494.9 ± 384.5	3598.2 ± 307.0	4718.3 ± 316.7*	3946.0 ± 263.4†
Women	3239.8 ± 341.5	2657.6 ± 395.0	3727.5 ± 291.2	3379.0 ± 358.0
% of KDRIs	168.0 ± 12.5	155.2 ± 13.5	218.6 ± 12.0	187.4 ± 10.7†
Potassium (mg)	2193.9 ± 123.2	2063.5 ± 171.9	2594.1 ± 124.9#	2492.1 ± 114.7
Men	2068.9 ± 218.7	2230.4 ± 291.0	2756.8 ± 168.5*	2524.9 ± 144.8
Women	2306.4 ± 128.0	1913.4 ± 198.2†	2292.0 ± 146.6	2431.1 ± 193.6
% of KDRIs	62.7 ± 3.5	59.0 ± 4.9	74.1 ± 3.6	71.2 ± 3.3

[1] Four obese men did not submit 3-day diet record at week12. KDRIs, Dietary Reference Intakes for Koreans. Data are reported as mean ± SEM.

[2] Significance was tested by 2-way ANOVA.

*: Significant difference between two weight groups at baseline ($P < 0.05$).

#: A tendency of difference between two weight groups at baseline ($P < 0.1$).

[3] Variables were examined for normality, and values not normally distributed

were analyzed using non parametric test. Significance was tested by paired *t* test or Wilcoxon's signed-rank test based on normality. Significance of intakes of vitamin A, vitamin B6, folate, iron, and zinc were determined by Wilcoxon's signed-rank test.

†: Significant change during 12-weeks ($P < 0.05$).

§: A tendency of change during 12-weeks ($P < 0.1$).

4 Percentage of subjects in each weight group (all such values).

Change in the prevalence of sedentary lifestyle before and after 12-week program

In order to investigate the prevalence of sedentary lifestyle, we used individual counseling reports at baseline and 12-week time points. Based on a criterion in the KNHANES, sedentary lifestyle was set as less than 60 minutes per week of physical activity (**Table 8**).

At baseline, the prevalence of sedentary lifestyle did not differ between two groups. However, significant decrease in the prevalence of sedentary lifestyle in obese groups was observed after 12-week weight management program participation.

TABLE 8

Change in the prevalence of sedentary lifestyle before and after 12-week program[1]

	Normal weight (n=17)			Obese (n=43)		
	Before [n (%)]	After [n (%)]	*P* value	Before [n (%)]	After [n (%)]	*P* value
Sedentary lifestyle[2]						
Yes	5 (29.4)	6 (35.3)	NS	18 (41.9)	7 (16.3)	0.013
Men	2 (40.0)	2 (33.3)	NS	12 (66.7)	2 (28.6)	0.006
Women	3 (60.0)	4 (66.7)	NS	6 (33.3)	5 (71.4)	NS

[1] A McNemar test was used to determine significance of a change in the number of subjects with sedentary lifestyle after weight management program.

[2] Sedentary lifestyle: < 60 min per week of physical activity.

Comparison of characteristics of obese subgroups divided by the magnitude of weight loss

We tried to identify the factors that contributed to the difference in weight loss as the magnitude of weight loss varied among obese subjects. In order to collect information on the contributing factors to successful weight loss, we used individual counseling reports at baseline. Information regarding weight change history, family history of obesity and chronic diseases, goal weight, and weight control experience were collected. According to weight change history, subjects who became obese after high school graduation were classified as adult obesity. Since all subjects in the large weight loss group (> 6 kg wt loss) became obese in the adulthood, participated in all the individual counseling sessions, and submitted all 3-day dietary record, statistical analysis between the large and the small weight loss group (< 3 kg wt loss) was impossible. Therefore, we compared the small weight loss group with the intermediate and large weight loss groups combined (≥ 3 kg wt loss).

Characteristics of the small and the intermediate-large weight loss groups are shown in **Table 9**. A proportion of subjects who became obese in the adulthood was significantly higher in the intermediate-large weight loss group than that in small weight loss group (93.7% vs. 46.4%, $P = 0.003$).

The 12-week goal weight set by subjects at baseline was not different between the small (< 3 kg wt loss) and the intermediate-large weight loss

groups (≥ 3 kg wt loss). However, when we compared the small (< 3 kg wt loss) and the large weight loss groups (> 6 kg wt loss), the 12-week goal weight of the large weight loss group (> 6 kg wt loss) tended to be higher compared with the small weight loss group (75.3 ± 2.3 kg vs. 68.5 ± 1.7 kg, $P = 0.070$). Baseline body weight of the small weight loss group was not different from that of the intermediate-large weight loss group. Adjusted body weight, which was used for assessment of energy requirement, did not differ between the small and the large weight loss groups (67.1 ± 1.1 kg vs. 70.8 ± 2.1 kg, $P = 0.181$) as well.

Subjects in the intermediate-large weight loss group were more diligent in submitting 3-day dietary record than those in the small weight loss group (0.69 ± 0.31, $P = 0.008$). In addition, they tended to attend individual counseling more often than those in the small weight loss group (0.26 ± 0.14, $P = 0.074$). All subjects in the large weight loss group, in particular, attended all individual counseling sessions. This suggests that more active involvement in the weight management program led to more successful weight loss.

TABLE 9

Comparison of characteristics of obese subgroups divided by the magnitude of weight loss[1]

Characteristics	Small (n=28)	Intermediate-Large (n=16)	*P* value[2]
Subjects			NS
Men [n (%)]	18 (64.3)	12 (75.0)	
Women [n (%)]	10 (35.7)	4 (25.0)	
Adult obesity[3]			0.003
Yes [n (%)]	13 (46.4)[4]	15 (93.7)	—
No [n (%)]	15 (53.6)	1 (6.3)	—
12-week goal weight (kg)[5]	68.5 ± 1.7[6]	72.3 ± 2.1	NS
Baseline weight (kg)	80.1 ± 1.9	82.7 ± 2.3	NS
Adjusted weight (kg)[7]	67.1 ± 1.1	68.9 ± 1.6	NS
Individual counseling attendance[8]	4.7 / 5.0	4.9 / 5.0	0.074
Dietary record submission[8]	4.3 / 5.0	4.9 / 5.0	0.008

[1] Intermediate, 3~6 kg weight loss group (n=10); Large, > 6 kg weight loss group (n=6), Small, < 3 kg weight loss group (n=28).

[2] Chi-square test was used for categorical variables. Student *t* test was used for continuous variables.

[3] Those who became obese after graduating from high school were classified as adult obesity. Information on onset of obesity was collected through individual interview at baseline.

[4] Percentage of subjects in each weight group (all such values).

[5] Obese subjects set 12-week goal weight (kg) on their own in the baseline interview.

[6] Data are reported as mean ± SEM.

[7] Adjusted weight (kg) was calculated as follows: IBW + (baseline weight – IBW) * 0.25; IBW, Ideal body weight, was determined based on BMI=22.

[8] Numerator reflects average of actual number of times and denominator means expected number of times. Obese group were required to attend individual counseling and to submit 3-day dietary record at baseline and 2, 4, 6, 10, and 12-week time points.

DISCUSSION

In this study, we examined the effect of the 12-week weight management program with group education and individual counseling sessions on clinical characteristics and dietary intakes in young obese adults, most of them in their 20's. Obese subjects lost average of 2.7 kg (3.3%) weight, and BMI, waist and hip circumferences, WHR, and fat mass decreased. While the magnitude of weight loss seemed somewhat modest compared to the goal, it was similar to what has been reported in studies by others (Dansinger et al., 2005; Lang et al., 2011; Wadden et al., 2005). In addition, 36% of obese subjects lost more than 3 kg (average of 5.5 kg and 6.6% wt loss).

Significant improvement of lipid profiles, decreased triglyceride and increased HDL cholesterol, in obese group was observed despite the modest weight loss. On the contrary, only the large weight loss group (> 6 kg wt loss) showed significant decrease in serum glucose. These results are consistent with the results from the studies by Lang et al (Lang et al, 2011) and Dansinger et al. (Dansinger et al. 2005). Average of 3.2% reduction in body weight through 8-week intervention with exercise and nutrition education did not lead to reduction in serum glucose concentration despite the improvement of blood lipid profiles (Lang et al., 2011). The 2-month calorie restriction resulted in 3.5 kg weight loss and reduction in cardiac risk factors but no change in glucose concentration (Dansinger et al., 2005). It seemed that lipid profiles are more sensitive to modest weight loss than

blood glucose concentration.

Leptin and adiponectin are adipose tissue-specific proteins, and obese people are reported to show higher leptin and lower adiponectin concentrations (Klempel et al., 2011; Van Gaal et al., 2006). In the present study, serum leptin in obese group decreased significantly in both the intermediate (5.0% wt loss) and the large weight loss groups (9.1% wt loss). This is consistent with the finding that at least 5% of weight loss is required to decrease leptin concentration in obese people (Klempel et al., 2011; Varady et al., 2009). On the contrary, serum HMW-adiponectin concentration at baseline was not different between two groups. Finding that there was no significant correlation between plasma adiponectin and BMI in healthy population (Kuo et al., 2011) is consistent with the results observed in this study. No change in serum HMW-adiponectin following weight loss might indicate that leptin is more sensitive and adiponectin is less sensitive marker to moderate weight reduction. The 10-week hypoenergetic (- 600 kcal/day) diets in obese but otherwise healthy subjects led to significant reduction in leptin secretion but no significant change in adiponectin concentration with 7.5% weight loss (Arvidsson et al., 2004).

It seemed that significant reduction of energy and fat intakes contributed to weight loss in obese group in this study. Significantly positive correlation between changes in percent calories from animal fat and BMI in obese group was observed (r = 0.325, P = 0.041), and this is consistent with the

results from the study by Field et al. Percent of calories from animal fat had positive association with weight gain in 41,518 women in a Nurses' Health study (Field et al., 2007). Obese subjects appeared to reduce animal food intake and incorporated a healthier diet during weight management program participation since significant decrease in cholesterol and sodium intakes and a tendency of increase in vitamin C intake were observed. This may be the effect of nutrition education; obese subjects who were asked to keep calorie restriction (1200-1600 kcal) and received group education reduced average of 244 kcal per day and intakes of carbohydrate, total and saturated fat, and cholesterol (Dansinger et al., 2005). Also, nutritional education seemed to be effective for improvement of lipid profiles, since lipid profiles and fat intake showed significantly positive association (Data not shown).

In this study, significant decrease in vitamin B2, phosphorus, and zinc intakes in obese group was observed. Considering that dairy foods are main sources of vitamin B2, significant decrease in vitamin B2 may be due to low consumption of dairy foods. Lower calcium intake compared with KDRIs in obese group may support this supposition. Reduced zinc intake seems to be associated with reduced intake of animal foods in obese group. However, the intake levels of all these nutrients still met KDRIs or were similar to the intake levels of those in their 20's or 30's in KNHANES 2010. Decreased phosphorus intake might be explained by the reduced intake of processed foods by the obese subjects, given that processed foods usually contain high

quantity of phosphorus. The 12-week program with nutrition education and personalized nutrition guideline does appear to help obese subjects to incorporate healthier eating patterns.

Sedentary lifestyle has been associated with obesity. Inactive individuals had greater waist circumference compared with active individuals in 21,729 men and women in a 11.4-year follow-up study (Arsenault et al., 2010). We observed significant decrease in the prevalence of sedentary lifestyle (less than 60 min per week of physical activity) in the obese group (from 41.9% to 16.3%, P = 0.013). Given that combining dietary approaches with physical activity is the most effective method for reducing weight gain (Field et al., 2010), obese subjects in our study are expected to maintain reduced weight for a long time since behavioral modification has occurred.

Following traits were observed in the subjects who lost more than 6 kg: *1)* more active participation in the weight management program, *2)* more realistic weight loss goal setting, and *3)* weight gain occurred during the adulthood. Higher attendance at individual counseling sessions and better compliance in submitting dietary record have been reported to result in more successful weight loss and better clinical outcomes. Higher levels of attendance and food record completion appeared to increase odds of achieving 5% weight loss (Bartfield et al., 2011). Given that participation can mediate the relationship between autonomous motivation and weight loss (Webber et al., 2010), those who lost more weight might have been

more motivated. Significantly more dietary record submissions in those who lost more weight was observed ($P = 0.008$), and the number of attendance at individual counseling sessions tended to be higher ($P = 0.074$) as well in those lost more weight. In light of this, submitting 3-day dietary record seemed to be a more sensitive indicator of autonomous motivation than the attendance. This is likely because keeping a dietary record plays a role in self-monitoring (Foster et al., 2005), the centerpiece of behavioral modification for weight loss, by helping self-assessment and providing evaluative information (Burke et al., 2011; Mockus et al., 2011; Wilde et al., 2007).

While establishing a goal is useful for enhancing adherence for chronic disease management (Pearson, 2012), unrealistic expectation would lead to higher dropout from intervention (Dalle Grave et al., 2005; Moroshko et al., 2011). In this study, while adjusted body weight which was a guideline for assessment of energy requirement did not differ between the large weight loss group (> 6 kg wt loss) and the small weight loss group (< 3 kg wt loss), the large weight loss group tended to set higher 12-week goal. Given that subjects set the 12-week goal on their own, higher 12-week goal weight might imply more realistic goal setting. Considering relationship between unrealistic expectation and higher possibility of drop out (Dalle Grave et al., 2005; Moroshko et al., 2011), continuous participation seemed to mediate between realistic goal setting and successful weight loss.

Age of onset of obesity has been regarded as a predictor of dropout (Moroshko et al., 2011) rather than as a key factor for successful weight loss. In this study, those who became obese in the adulthood achieved more successful weight loss despite no difference in the family history of overweight. This result was likely due to ingrained poor lifestyle habits in those who were obese since the childhood. Those who were obese since the childhood are more likely to have deeply rooted behavioral factors related to weight gain such as specific dietary pattern. Of young adults in the highest or lowest intake quintiles for 13 nutrients at year 0, over 60% of them remained in the same or adjacent quintiles at year 7 despite, which seemed to be due to ingrained dietary habits (Dunn et al., 2000).

There are some limitations to this study. While we tried to match factors other than BMI between the obese and normal weight subjects, mean age of obese group was significantly higher compared with normal weight group. Even so, most of normal weight and obese subjects were in their 20's. Extrapolation of our results to a more heterogeneous population might be limited since the subjects included in our study were highly educated and homogenous population. However, it would reflect the effect of behavioral modification in otherwise healthy young adults with a minimal influence from other confounding factors.

In conclusion, the 12-week weight management program participation led to significant improvements in anthropometric and clinical characteristics

through significant decrease in energy and fat intakes and sedentary life style.

Ⅱ. Study 2

Effect of 12-week weight management program with behavioral modification on immune and inflammatory responses in the young obese

INTRODUCTION

The prevalence of obesity has increased in Korean young adults. Obesity can lead to various metabolic and immunologic changes, including low-grade long-term inflammation. Inflammatory cytokines such as IL-6, TNF-α, and IL-1ß have been suggested to contribute to inflammation (Monteiro et al., 2010; Shoelson et al., 2007; Trayhurn et al., 2007). Obese people showed significantly higher production of inflammatory cytokines (Ackermann et al., 2011; Tanaka et al., 2001; Ziccardi et al., 2002), and many obesity-related diseases such as cardiovascular disease or type 2 diabetes seemed to be associated with inflammation (Bruun et al., 2003; Ferrante, 2007; Kuo et al., 2011). These elevated inflammatory cytokines in obese subjects seemed to decrease with weight loss (Kern et al., 1995; Ziccardi et al., 2002).

In addition, obesity is known to impair the function of T cells in humans, which is associated with higher incidence of infection in obese people (Karlsson et al., 2010; Marti et al., 2001). Studies have indicated that obesity can cause suppression of mitogen-induced lymphocyte proliferation

(Nieman et al., 1999; Tanaka et al., 1993) and alteration in T cell subpopulation (Karlsson et al., 2010; O'Rourke et al., 2005; Tanaka et al., 2001). Weight loss in the obese with a mean BMI of 38.4 kg/m^2 seemed to reverse lower responses of peripheral blood lymphocytes to T cell mitogen compared with nonobese humans (Tanaka et al., 1993). The impact of obesity on alteration in T cell subpopulation is not conclusive. While O'Rourke et al (O'Rourke et al., 2005) reported an increased frequency of $CD4^+$ T cells in obese subjects, Tanaka et al (Tanaka et al., 2001) reported a reduction in the number of $CD4^+$ T cells in the obese. Obese subjects had either decreased $CD8^+$ T cells (O'Rourke et al., 2005; Tanaka et al., 2001) or no difference in the number of $CD8^+$ T cell subsets (Nieman et al., 1999) compared with the nonobese.

The balance between T_H1/T_H2 responses plays an important role in regulation of immune response. Obesity has been reported to disturb the T_H1/T_H2 balance, but results from the studies which investigated the effect of obesity on the T_H1/T_H2 balance have not been always consistent. Leptin, which is elevated with increased adiposity, has been suggested to polarize T_H cell response towards a pro-inflammatory T_H1 phenotype (Fantuzzi, 2009; La Cava et al., 2004; Lord et al., 1998). Viardot et al (Viardot et al., 2010) reported that weight loss in morbid obese subjects (mean BMI 42.8 kg/m^2) led to significant decrease in the number of pro-inflammatory T_H1 cells. On the other hand, some studies reported increased risk of T_H2 biased

disease such as allergy and asthma in obese people (Colombo et al., 2008; Hersoug et al., 2007). The finding by Ma et al (Ma et al., 2010) that symptom of asthma in obese people with a BMI more than 30 kg/m^2 was relieved with weight loss through 12-month lifestyle intervention supports obesity-related T_H2 bias.

Previous studies which investigated the impact of obesity on immunologic and inflammatory responses in humans were mostly conducted in subjects with a BMI of more than 35 kg/m^2. However, fewer than 5% of adults had a BMI more than 30 kg/m^2 in Korea, according to KNHANES 2010. Also, obesity-induced immunologic change appeared to be dependent on the magnitude of obesity (Kueht et al., 2009). In this sense, the investigation of immunologic changes in an obese population with a BMI less than 30 kg/m^2 and effect of weight loss in these population would be helpful for understanding of the influence of obesity on the immune response and for applying to Korean.

In this study, we investigated the effect of weight loss through 12-week weight management program with nutritional counseling and behavioral modification on cell-mediated immune function and inflammatory responses of the young and otherwise healthy obese (most of them with a BMI less than 30 kg/m^2). To examine the cell-mediated immune response, we measured the production of helper T (T_H) 1/ T_H2 cytokines and the proliferative response of immune cells. The production of inflammatory

cytokines by immune cells was measured to determine the inflammatory response. A subpopulation of immune cells was counted to examine the impact of weight loss on the population of immune cells.

SUBJECTS AND METHODS

Subjects

The study was conducted from September 2009 to June 2011. A total of 154 subjects aged between 19 and 45 were screened using questionnaires, and 84 subjects were excluded from the study (**Figure 2**).

The questionnaires were two types; one was to classify subject with high Beck Depression Inventory (BDI) score, and the other was to identify subject who had chronic disease or took dietary supplements or medications known to affect serum lipid profiles and immune functions. The subjects who met the following criteria were excluded: taking nonsteroidal anti-inflammatory drugs such as aspirin, vitamin E, fish oil, allergy medicine, anticoagulant, multivitamin, and other dietary supplements or medications known to affect serum lipid profiles and immune functions on a regular basis; were pregnant or planning to be pregnant; receiving hormone therapy after menopause; with arthritis, autoimmune disease, cancer, asthma, atopic disease, endocrine, hepatic, renal, thyroid, or cardiac dysfunction. Subjects with BMI between 23 and 25 kg/m^2 were excluded from the study as well.

The protocol was approved by the Seoul National University Institutional Review Board (SNUIRB, IRB NO. 0908/001-007), and written informed consent was obtained from all subjects.

Seventy subjects were included in the study and divided into two weight

groups based on their BMI. Forty nine subjects with a BMI over 25 kg/m^2 were assigned to obese group (BMI higher than 25 kg/m^2 is classified as obese in Korea) and 21 subjects with BMI between 18.5 and 23 kg/m^2 were assigned to normal weight group. Of these, 64 subjects (45 obese and 19 normal weights) completed the study.

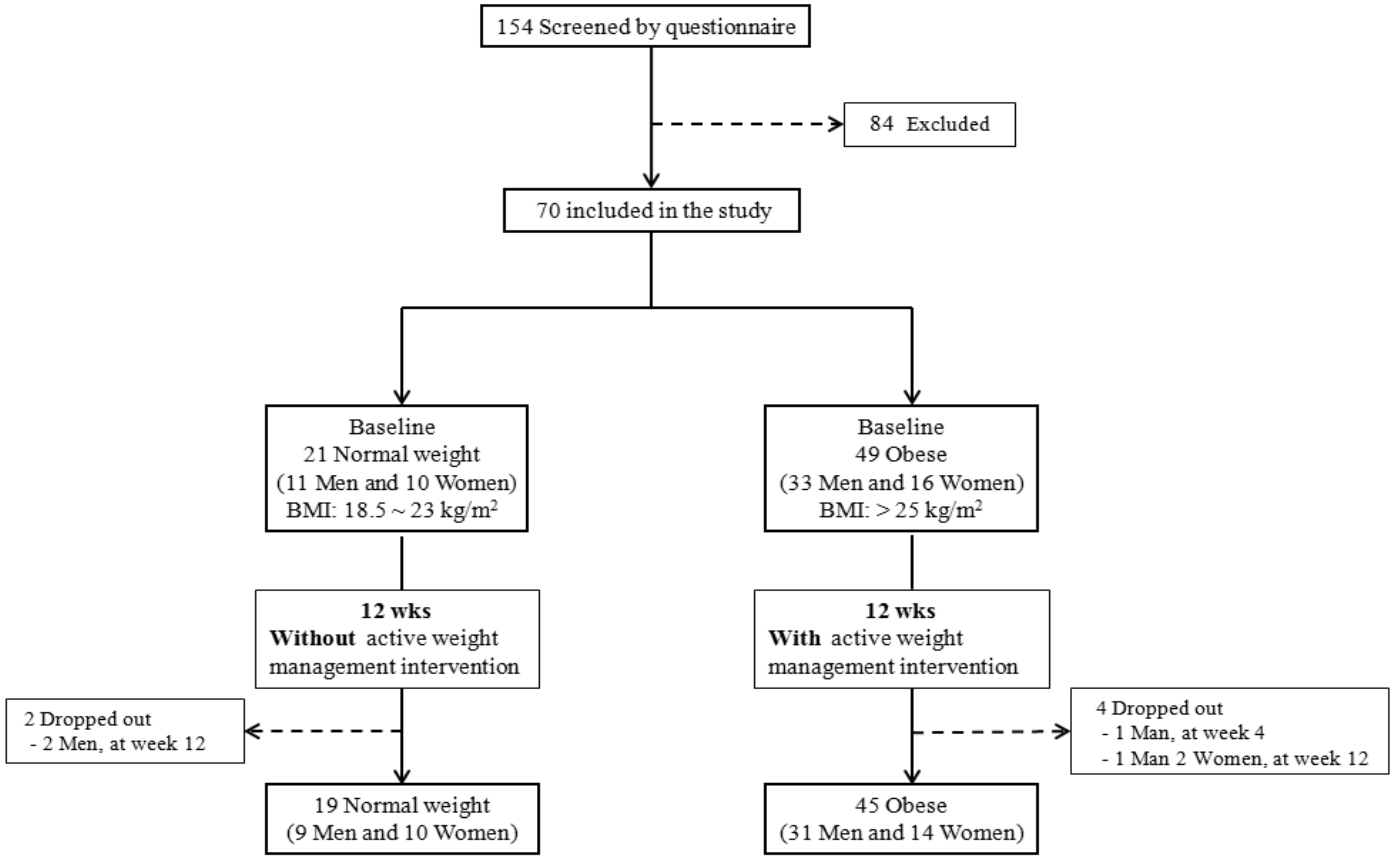

FIGURE 2. Study design and subjects of study II

A 12-week weight management program

At baseline, all subjects received information about the aims and schedule of the study. Obese subjects participated in a 12-week weight management program with nutritional counseling and behavioral modification. Intervention program included 5 group educations and 6 individual counseling sessions at baseline and 2, 4, 6, 10 (individual counseling only), and 12-week time points. Subjects in the normal weight group were asked to keep their usual eating and exercise pattern. They did not attend the group educations and only attended 6 individual counseling sessions for the evaluation of dietary intake and exercise pattern.

Topics of the group educations for obese subjects were: *1)* planning weight loss and how to write diet record (week 0), *2)* how to lose weight in a healthy way (week 2), *3)* understanding the food exchange lists and food labeling (week 4), *4)* portion size control (week 6), and *5)* strategies to maintain weight loss (week 12). During individual counseling sessions, the registered dietitian evaluated 3-day dietary record and reinforced to incorporate low-fat, low-sugar, low-salt, high-fiber, and low energy density foods into their diets. The obese were also encouraged to exercise regularly and to modify undesirable eating behaviors such as late night snacking or binge eating.

The overall weight reduction goal of the program was to lose about 0.5 kg per week by reducing calorie intake by 300~500 kcal per day from

estimated energy requirements and by increasing physical activities. Mean estimated energy requirements using adjusted body weights were 1,715 kcal/d for obese men and 1,390 kcal/d for obese women, which were 83.3% of daily energy requirements for both obese men and women. A personalized goal was set for each obese subject following an individual interview with a registered dietitian at the baseline. Subjects in the normal weight group were asked to keep their usual dietary intake and physical activity to maintain body weight.

Food exchange lists from the Korean Diabetic Association were used for the meal planning. Recommended servings of each food group based on caloric goal and amount of carbohydrate, protein, and fat were provided to each subject. In average, energy provided from carbohydrate, protein, and fat were 60%, 21% and 19% (of energy), respectively, in the individual plan. This recommendation was within acceptable macronutrient distribution ranges (AMDR) for Koreans, in which carbohydrate is 55~70%, fat is 15~25%, and protein is 7~20% of total calorie intake.

Anthropometric measurement

Body weight, waist circumferences, and fat mass were measured using InBody 520 (Biospace, Korea). Standing height without shoes was measured using a stadiometer. BMI was calculated from the height and body weight.

Blood samples

Blood from subjects who fasted overnight was collected for immune response measurements in blood collection tubes (BD vacutainer®, Becton Dickinson, Flanklin Lakes, NJ) containing sodium heparin (158 USP U). Blood samples were kept at room temperature and used within 2 hours. Blood for complete blood cell analysis was collected using K_2EDTA tubes (BD vacutainer®, Becton Dickinson). Blood for measuring serum adipokines was collected in serum separator tubes (BD vacutainer® SST, Becton Dickinson), and the serum was separated by centrifugation and stored at - 70°C prior to measurement.

Isolation of mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood by centrifugation through a Histopaque (density, 1.077 g/mL; Sigma, St Louis, MO). Peripheral blood mononuclear cells were removed from the interface and washed twice with RPMI 1640 (Lonza, Walkersville, MD) supplemented with 100,000 U/L penicillin (Gibco, Carlsbad, CA), 100 mg/L streptomycin (Gibco), 2 mmol/L $_L$-glutamine (Gibco), and 25 mmol/L HEPES (Sigma) (complete RPMI). Cells were resuspended in complete RPMI and counted by the trypan blue exclusion method. Cells were resuspended at appropriate concentrations in complete RPMI for analysis of subpopulations by flow cytometric analysis.

Complete blood count and flow cytometric analysis

A complete blood count including white blood cells (WBCs), red blood cells, and platelets were obtained using a blood cell counter (H-8; SEAC, Italy).

Flow cytometric analysis was done as described (Meydani et al., 1993) using the following antibodies: anti-CD3 FITC (clone HIT3a; BD Pharmingen, San Diego, CA), anti-CD4 APC (clone RPA-T4), anti-CD8 PE (clone HIT8a), anti-CD19 PE (clone HIB19), and anti-CD56 PE (clone B159). Lymphocyte subpopulations stained with fluorescence-activated cell sorter antibodies were analyzed with a flow cytometer (FACScalibur; Becton Dickinson).

Lymphocyte proliferation

Lymphocyte proliferation was measured by [^{3}H] thymidine incorporation after stimulation with T-cell mitogens using a modified whole blood assay (Bloemena et al., 1989). Heparinized whole blood was diluted 1/5 (vol/vol) with complete RPMI. One hundred microliters of the diluted blood was stimulated with concanavalin A (Con A) (Sigma, St Louis, MO) or phytohemagglutinin (PHA) (Sigma) and incubated in 96-well round-bottom plates (200 μL of final culture volume; Nunc, Roskilde, Denmark) for 72 hours at 37°C in an atmosphere of 5% CO_2 and 95% humidity. Final concentrations of mitogens were 5, 25, and 50 μg/mL for Con A (Sigma)

and 1, 5, and 50 μg/mL for PHA. Each well was pulsed with 0.5 μCi of [^{3}H] thymidine (Perkin Elmer, Boston, MA) in 20 μL for the last 4 hours of the incubation. Cells were harvested onto glass microtiter filter paper using a cell harvester (MicroBeta FilterMate-96 Harvester; Perkin Elmer), and radioactivity incorporation was counted in a liquid scintillation counter (MicroBeta2 Plate Counter; Perkin Elmer). The results are reported as corrected disintegrations per minute (dpm), which is the mean dpm of mitogen-stimulated cultures minus the mean dpm of cultures without mitogens.

Interleukin 2, IL-4, IL-10, and interferon γ production

Heparinized whole blood was diluted 1/3.6 (vol/vol) with complete RPMI. Diluted whole blood (900 μL) was stimulated with 100 μL of either Con A (25 μg/mL final concentration) or PHA (20 μg/mL final concentration) solutions and incubated in 24-well flat-bottom plates (BD falcon, Becton Dickinson, Flanklin Lakes, NJ) for 48 hours at 37°C in an atmosphere of 5% CO_2 and 95% humidity.

Cell-free supernatants were collected and stored at -70°C for later analysis. Protein concentrations of IL-2, IL-4, IL-10, and interferon (IFN) γ were measured by an enzyme-linked immunosorbent assay (ELISA) using BD OptEIA (BD Pharmingen) according to the manufacturer's instructions.

Interleukin 1ß, IL-6, and tumor necrosis factor α production

Heparinized whole blood was diluted 1/3.6 (vol/vol) with complete RPMI. Diluted whole blood (900 μL) was cultured with 100 μL of lipopolysaccharide (LPS) (0.01 or 1 μg/mL final concentration) solution and incubated in 24-well flat-bottom plates for 24 hours at 37°C in an atmosphere of 5% CO_2 and 95% humidity.

Cell-free supernatants were collected and stored at -70°C for later analysis. IL-1ß, IL-6, and tumor necrosis factor (TNF) α concentrations were measured by an ELISA using BD OptEIA (BD Pharmingen) according to the manufacturer's instructions.

Measurement of serum adipokines

Serum leptin and high-molecular-weight (HMW) adiponectin concentrations were determined using ELISA kits (Millipore, Billerica, MA) according to the manufacturer's instructions. The HMW- adiponectin was measured after pretreatment of serum samples with digestion solution to remove hexameric and trimeric adiponectins.

Statistical analysis

Data were analyzed using SPSS software (version 19.0; SPSS Inc., Chicago, IL). Variables not normally distributed were analyzed using non parametric test. Student's *t* test was used to test for differences in

immunologic parameters between the obese and normal weight groups. To test for changes in immunologic parameters before and after 12-week weight management program, we used paired *t* test or Wilcoxon's signed-rank test based on normality. For the investigation of the effects of the 12-week program on immunologic parameters among obese subjects, subjects were divided into three subgroups based on the magnitude of weight loss: weight loss < 3 kg, weight loss 3~6 kg, and weight loss > 6 kg. Data are reported as mean $\pm$ SEM. Significance was set at $P < 0.05$.

RESULTS

Change in anthropometric characteristics after 12-week weight management program

There were no significant differences in age and height between the obese and normal weight group at baseline. The mean age of the normal weight group was 26.4 ± 1.1 years and that of the obese group was 29.5 ± 1.2 years. The average heights of the obese and normal weight groups were similar for both men (174.2 ± 1.7 cm and 173.9 ± 0.7 cm, respectively) and women (160.7 ± 1.4 cm and 162.3 ± 1.1 cm, respectively). Weight and BMI of obese group were significantly higher than those of normal weight group as we recruited subjects according to the BMI. Waist circumference of obese group was also significantly higher than that of normal weight group. Two normal weight subjects (2 men) and 4 obese subjects (2 obese men and 2 obese women) dropped out during the program (**Figure 2**).

We observed a significant decrease in weight (from 81.5 ± 1.4 kg to 78.8 ± 1.3 kg, $P < 0.001$), BMI (from 28.0 ± 0.4 kg/m^2 to 27.1 ± 0.4 kg/m^2, $P < 0.001$), waist circumference (from 92.3 ± 1.1 cm to 89.7 ± 1.1 cm, $P < 0.001$), and fat mass (from 25.3 ± 1.0 kg to 23.0 ± 1.0 kg, $P < 0.001$) in the obese after weight management program participation. Changes in weight, BMI, waist circumference, and fat mass during 12-week period in the normal weight group were not significant.

Change in lymphocyte subpopulation after 12-week weight management program participation

The percentages of lymphocyte subpopulations are presented in **Table 10**. The percentage of $CD4^+$ and $CD8^+$ T cells did not differ between two groups at baseline, and no significant changes in that of $CD4^+$ and $CD8^+$ T cells in both groups were observed at 12-week time point. At 12-week time point, the percentage of B cells in both groups increased significantly, but the magnitude of increase in percentage of B cells did not differ between two groups (2.9% $\pm$ 0.9% in normal weight group vs. 1.9% $\pm$ 0.8% in obese group, $P = 0.448$). Similarly, while we observed decrease in the percentage of natural killer cells in normal weight and obese groups ($P = 0.091$, $P = 0.023$, respectively), the magnitude of decrease in the percentage of NK cell between two groups was not significant (4.1% $\pm$ 2.2% in normal weight group vs. 3.9% $\pm$ 1.6% in obese group, $P = 0.964$).

TABLE 10

Comparison of the lymphocyte subpopulation before and after 12-week program[1]

Lymphocyte subpopulation	Normal weight n= 15		Obese n= 39	
	Before[2]	After[3]	Before[2]	After[3]
$CD4^+$ T cell (% gated)	31.1 ± 3.0[2]	29.3 ± 2.2	33.4 ± 1.9	31.7 ± 1.8
$CD8^+$ T cell (% gated)	18.9 ± 1.7	19.1 ± 2.0	19.0 ± 1.3	18.3 ± 1.3
B cell (% gated)	6.8 ± 0.6	9.7 ± 1.3†	9.2 ± 0.8*	11.1 ± 0.9†
NK cell (% gated)	18.7 ± 2.0	14.6 ± 2.3§	20.7 ± 1.7	16.7 ± 1.3†

[1] NK cell, natural killer cell; percent (%) gated, percent (%) of total lymphocytes. If the number of PBMC was insufficient to be analyzed all types of lymphocyte, we analyze $CD4^+$ T and $CD8^+$ T cells first. Data are reported as mean ± SEM.

[2] Significance was tested by independent *t* test.

*: Significant difference between two weight groups at baseline ($P < 0.05$).

[3] Significance was tested by paired *t* test.

†: Significant change during 12-weeks ($P < 0.05$).

§: A tendency of change during 12-weeks ($P < 0.1$).

Changes in the lymphocyte proliferative response of whole blood and white blood cell counts after 12-week weight management program

Changes in the lymphocyte proliferative response of whole blood are shown in **Table 11**. At baseline, obese subjects showed higher lymphocyte proliferative responses to suboptimal concentrations of PHA compared with normal weight group (P = 0.004 for PHA at 1 μg/mL, P = 0.047 for PHA at 50 μg/mL). However, there was no significant difference in proliferative response to optimal concentration of Con A or PHA. After 12-week weight management program participation, there was no significant change in the lymphocyte proliferative response in obese subjects. In normal weight group, a significant increase in proliferative response to PHA at 1 μg/mL was observed despite no significant weight change.

The total number of white blood cells (WBC), red blood cells (RBC), and platelets determined by differential cell counts are presented in **Table 12.** WBC and RBC counts were higher in obese group compared with normal weight group (P = 0.051, P = 0.040, respectively). The 12-week weight management program participation resulted in a significant decrease in the number of WBC, RBC, and platelets in obese group.

Since we measured the proliferative response of whole blood, we adjusted the proliferation using WBC counts in order to exclude the effect of WBC counts. When the ratio of proliferative response of whole blood to WBC counts was compared, no significant changes in the ratio in both groups

were observed (Data not shown). As for proliferation of PBMC, increased PBMC proliferation in both groups during 12-week period was observed, but the magnitude of change did not differ between two groups (Data not shown).

TABLE 11

Comparison of the lymphocyte proliferative response of whole blood before and after 12-week program ($\times 10^3$ corrected dpm)[1]

	Normal weight (n= 19)		Obese (n= 45)	
Mitogen	Before[2]	After[3]	Before[2]	After[3]
Con A				
5 μg/mL	37.4 ± 8.2	39.1 ± 6.2	36.0 ± 3.3	36.8 ± 4.6
25 μg/mL	64.2 ± 11.9	67.2 ± 10.1	63.8 ± 6.3	69.3 ± 7.7
50 μg/mL	45.6 ± 10.2	53.6 ± 9.7	56.3 ± 5.3	60.5 ± 7.2
PHA				
1 μg/mL	3.9 ± 1.3	6.9 ± 1.6†	11.7 ± 2.2*	9.3 ± 2.1
5 μg/mL	83.9 ± 17.8	96.3 ± 15.5	86.4 ± 8.8	90.2 ± 10.9
50 μg/mL	28.1 ± 3.6	36.8 ± 5.7	39.5 ± 3.3*	36.0 ± 2.8

[1]Lymphocyte proliferation was measured by [^{3}H] thymidine incorporation during the last 4 hours of a 72-hour incubation after stimulation of diluted whole blood (1/5 vol/vol dilution) with T-cell mitogens (final concentrations were 5, 25, and 50 μg/mL for Con A and 1,5, and 50 μg/mL for PHA) at 37°C in an atmosphere of 5% CO_2 and 95% humidity. The results are reported as corrected disintegrations per minute (dpm), which is the mean dpm of mitogen-stimulated cultures minus the mean dpm of cultures without mitogens. Data are reported as mean ± SEM.

[2] Significance was tested by independent *t* test.

*: Significant difference between two weight groups at baseline ($P < 0.05$).

[3] Variables were examined for normality, and values not normally distributed were analyzed using non parametric test. Significance was tested by paired *t* test or Wilcoxon's signed-rank test; significance of the lymphocyte proliferative response to Con A at 50 μg/mL and PHA at 1 μg/mL was

tested by Wilcoxon's signed-rank test.

†: Significant change during 12-weeks ($P < 0.05$).

TABLE 12

Comparison of white blood cells, red blood cell, and platelets counts before and after 12-week program[1]

	Normal weight (n=19)		Obese (n=45)	
Blood cells	Before[2]	After[3]	Before[2]	After[3]
WBC ($\times 10^3$ cells/μL)	5.71 ± 0.41	5.84 ± 0.51	6.62 ± 0.24[#]	6.20 ± 0.24[†]
RBC ($\times 10^6$ cells/μL)	4.70 ± 0.12	4.62 ± 0.12	4.98 ± 0.07*	4.87 ± 0.07[†]
PLT ($\times 10^3$ cells/μL)	257.8 ± 12.8	256.2 ± 14.3	253.9 ± 9.0	240.0 ± 8.2[†]

[1] PLT, platelets; RBC, red blood cells; WBC, white blood cells. Data are reported as mean ± SEM.

[2] Significance was tested by independent *t* test.

*: Significant difference between two weight groups at baseline ($P < 0.05$).

[#]: A tendency of difference between two weight groups at baseline ($P < 0.1$).

[3] Significance was tested by paired *t* test.

[†]: Significant change during 12-weeks ($P < 0.05$).

Change in interleukin 2, IL-4, IL-10, and IFN-γ production after 12-week weight management program participation

The production of IL-2, IL-4, IL-10, and IFN-γ in whole blood culture is presented in **Table 13**. Concentrations of PHA-stimulated IL-2, IL-4, and IFN-γ were significantly higher in obese subjects compared with normal weight subjects (P = 0.039 for IL-2, P = 0.028 for IL-4, and P = 0.034 for IFN-γ). At 12-week time point, there was no significant change in the production of these cytokines (IL-2, IL-4, and IFN-γ) in both groups. However, a tendency of increase in production of PHA-stimulated IL-10 in obese group was observed (788.3 $\pm$ 378.9 pg/mL, P = 0.060).

TABLE 13

Comparison of the production of T_H1 and T_H2 cytokines by whole-blood culture before and after 12-week program[1]

	Normal weight (n=19)		Obese (n=45)	
Cytokines	Before[2]	After[3]	Before[2]	After[3]
IL-2 (ng/mL)				
Con A at 25 μg/mL	2.33 ± 0.42	2.79 ± 0.47	2.27 ± 0.32	2.08 ± 0.26
PHA at 20 μg/mL	8.47 ± 1.33	8.20 ± 1.41	12.61 ± 1.10*	12.37 ± 1.12
IFN-γ (ng/mL)				
Con A at 25 μg/mL	227.8 ± 64.5	190.1 ± 48.8	158.6 ± 30.6	223.1 ± 42.4
PHA at 20 μg/mL	165.4 ± 25.0	162.4 ± 40.8	215.5 ± 19.8*	211.0 ± 26.0
IL-4 (pg/mL)				
Con A at 25 μg/mL	65.8 ± 10.9	56.0 ± 10.7	67.3 ± 11.1	57.5 ± 6.8
PHA at 20 μg/mL	87.5 ± 10.6	91.3 ± 11.1	125.8 ± 13.3*	130.5 ± 13.3
IL-10 (pg/mL)				
Con A at 25 μg/mL	555.8 ± 82.2	429.5 ± 53.9[§]	418.6 ± 45.7	488.3 ± 45.5
PHA at 20 μg/mL	5533 ± 632	6423 ± 858	6181 ± 475	6970 ± 632[§]

[1] Heparinized whole blood was diluted 1/3.6 (vol/vol) with complete RPMI. Diluted whole blood (900 μL) was stimulated with 100 μL of either Con A (25 μg/mL final concentration) or PHA (20 μg/mL final concentration) solutions and incubated in 24-well flat-bottom plates for 48 hours at 37°C in an atmosphere of 5% CO_2 and 95% humidity. Cell-free supernatants were collected and stored at -70°C for later analysis. Protein concentrations of interleukin (IL)-2, IL-4, IL-10, and interferon (IFN) γ were measured by an enzyme-linked immunosorbent assay (ELISA) using BD OptEIA (BD Pharmingen) according to the manufacturer's instructions. Data are reported as mean ± SEM.

[2] Significance was tested by independent *t* test.

*: Significant difference between two weight groups at baseline ($P < 0.05$).

[3] Variables were examined for normality, and values not normally distributed were analyzed using non parametric test. Significance was tested by paired *t* test or Wilcoxon's signed-rank test; only the production of IL-10 stimulated with PHA at 20 μg/mL was tested by paired *t* test.

§: A tendency of change during 12-weeks ($P < 0.1$).

Change in interleukin 1ß, IL-6, and TNF-α production after 12-week weight management program participation

The production of LPS-stimulated inflammatory cytokines is shown in **Table 14**. Production of IL-1ß by whole blood stimulated with LPS at 1 μg/mL in obese group tended to be higher compared with normal weight subjects (P = 0.082). The production of IL-1ß, IL-6, and TNF-α without stimulation did not differ between two groups both at baseline and 12-week time point (Data not shown). Obese group showed a significant decrease in production of IL-1ß by whole blood stimulation with LPS at 0.01 μg/mL (0.15 $\pm$ 0.09 ng/mL, P = 0.049) and a tendency of decrease in TNF-α production after program participation (0.06 $\pm$ 0.17 ng/mL, P = 0.092). As for IL-6, only the large weight loss group (> 6 kg wt loss, n=6) showed significant decrease in IL-6 production by whole blood stimulated with LPS at 0.01 μg/mL (from 25.6 $\pm$ 6.9 ng/mL to 12.4 $\pm$ 3.3 ng/mL, P = 0.046). Significantly negative correlations between difference in the production of PHA-stimulated IL-10 and LPS-stimulated IL-1ß and IL-6 in obese group were observed (**Figure 3, Figure 4**). These results suggested that obese subjects with increased IL-10 production were likely to show decreased production of IL-6 and IL-1ß after the program. On the contrary, the production of inflammatory cytokines in normal weight group did not change.

TABLE 14

Comparison of the production of inflammatory cytokines by whole-blood culture before and after 12-week program[1]

	Normal weight (n=19)		Obese (n=45)	
Cytokines	Before[2]	After[3]	Before[2]	After[3]
IL-1ß (ng/mL)				
LPS at 0.01 μg/mL	0.90 ± 0.21	0.75 ± 0.18	0.85 ± 0.07	0.67 ± 0.07[†]
LPS at 1 μg/mL	1.60 ± 0.43	1.83 ± 0.52	2.23 ± 0.27[#]	2.45 ± 0.29
IL-6 (ng/mL)				
LPS at 0.01 μg/mL	23.5 ± 2.5	20.4 ± 3.0	23.9 ± 1.7	21.7 ± 2.1
LPS at 1 μg/mL	50.5 ± 5.8	50.7 ± 6.9	52.8 ± 3.0	56.5 ± 3.6
TNF-α (ng/mL)				
LPS at 0.01 μg/mL	1.18 ± 0.21	0.98 ± 0.18	1.18 ± 0.13	1.13 ± 0.16[§]
LPS at 1 μg/mL	3.04 ± 0.54	3.33 ± 0.57	3.15 ± 0.36	3.16 ± 0.34

[1] Heparinized whole blood was diluted 1/3.6 (vol/vol) with complete RPMI. Diluted whole blood (900 μL) was stimulated with 100 μL of lipopolysaccharide (LPS) (0.01 or 1 μg/mL final concentration) solution and incubated in 24-well flat-bottom Plates for 48 hours at 37°C in an atmosphere of 5% CO_2 and 95% humidity. Cell-free supernatants were collected and stored at -70°C for later analysis. Protein concentrations of IL-2, IL-4, IL-10, and interferon (IFN) γ were measured by an enzyme-linked immunosorbent assay (ELISA) using BD OptEIA (BD Pharmingen) according to the manufacturer's instructions. Data are reported as mean ± SEM.

[2] Significance was tested by independent *t* test.

[#]: A tendency of difference between two weight groups at baseline ($P < 0.1$).

[3]Variables were examined for normality, and values not normally distributed were analyzed using non parametric test. Significance was tested by paired *t* test or Wilcoxon's signed-rank test; the production of IL-1ß stimulated with LPS at 1 μg/mL and TNF-α stimulated with LPS at 0.01 and 1 μg/mL were tested by Wilcoxon's signed-rank test.

†: Significant change during 12-weeks ($P < 0.05$).

§: A tendency of change during 12-weeks ($P < 0.1$)

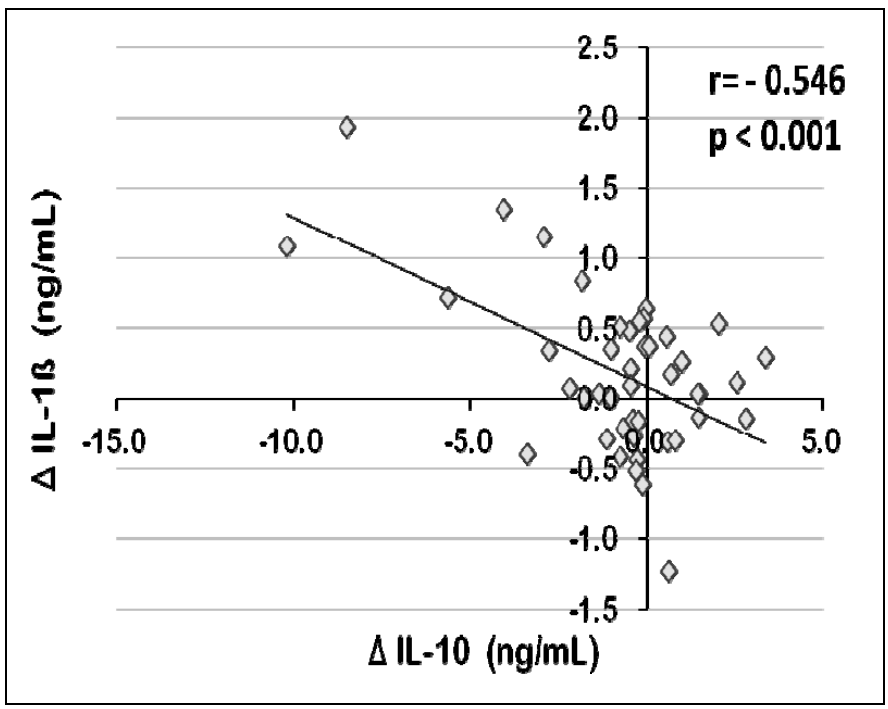

FIGURE 3

The correlation between difference in PHA-stimulated IL-10 production and LPS-stimulated IL-1ß production in obese group

Pearson correlation was used.

Δ indicates difference in characteristics of subjects after a 12-week weight management program (Baseline level – 12 week level). Positive number indicates a decrease in levels.

IL-10 stimulated with PHA at 20 μg/mL.

IL-1ß stimulated with LPS at 0.01 μg/mL.

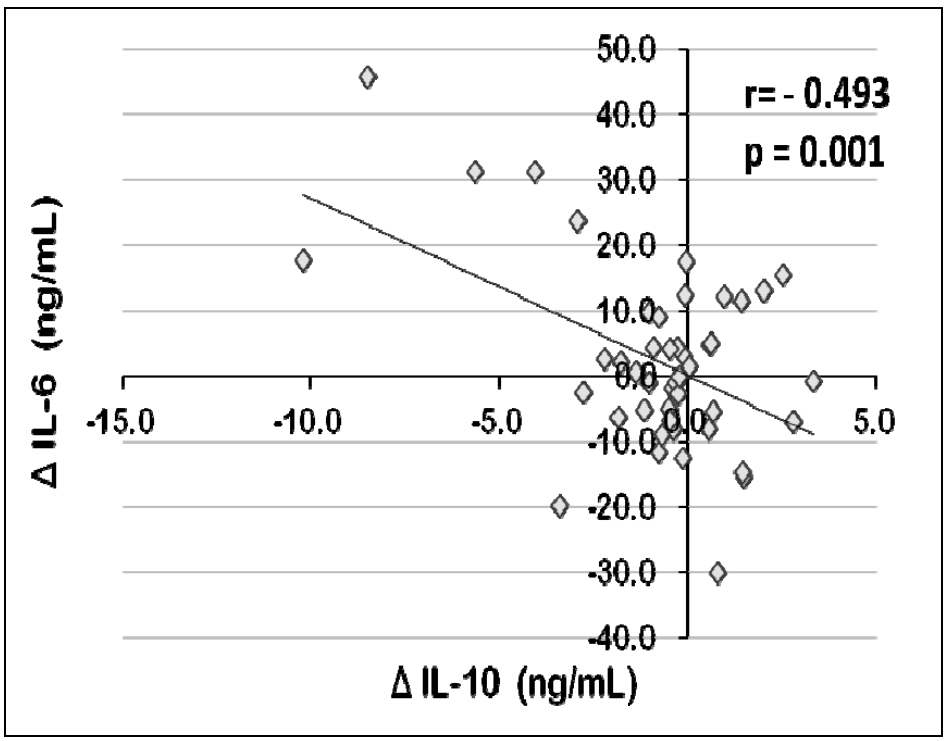

FIGURE 4

The correlation between difference in PHA-stimulated IL-10 production and LPS-stimulated IL-6 concentrations production in obese group

Pearson correlation was used.

Δ indicates difference in characteristics of subjects after a 12-week weight management program (Baseline level – 12 week level). Positive number indicates a decrease in level.

IL-10 stimulated with PHA at 20 μg/mL.

IL-6 stimulated with LPS at 0.01 μg/mL.

Change in serum leptin and adiponectin after 12-week weight management program participation

At baseline, both obese men and women had significantly higher serum leptin concentration compared with normal weight men and women (1.66 ± 0.42 ng/mL in normal weight men vs. 4.94 ± 0.57 ng/mL in obese men, P = 0.014; 6.26 ± 0.91 ng/mL in normal weight women vs. 13.48 ± 2.65 ng/mL in obese women, P = 0.016). While correlations between leptin and anthropometric characteristics including BMI (r = 0.312, P = 0.039), hip circumference (r = 0.318, P = 0.035), and body fat mass (r = 0.572, P < 0.001) in obese subjects were significant, leptin concentration did not show a correlation with concentrations of inflammatory cytokines TNF-α, IL-1ß, and IL-6 in the obese. Serum HMW-adiponectin concentration did not differ between two groups (6.78 ± 2.12 μg/mL in normal weight men vs. 9.51 ± 2.01 μg/mL in obese men, P = 0.485; 6.57 ± 2.00 μg/mL in normal weight women vs. 8.04 ± 1.56 μg/mL in obese women, P = 0.564). Correlations between adiponectin and anthropometric characteristics were not significant, however, significantly negative correlation between adiponectin and IL-6 production stimulated with LPS at 0.01 μg/mL was observed (r = - 0.321, P = 0.034).

There was no significant decrease in serum leptin in overall obese group with 12-week program participation (from 7.74 ± 1.09 ng/mL to 6.61 ± 0.90 ng/mL, P = 0.167). However, when we divided obese subjects into three

subgroups based on the magnitude of weight loss, serum leptin concentrations in the intermediate (3~6 kg wt loss, from 6.45 $\pm$ 0.94 ng/mL to 4.76 $\pm$ 1.03 ng/mL, P = 0.017) and the large weight loss groups (> 6 kg wt loss, from 8.16 $\pm$ 3.43 ng/mL to 4.45 $\pm$ 2.22 ng/mL, P = 0.046) decreased significantly. Serum leptin in normal weight group increased significantly (from 4.08 $\pm$ 0.74 ng/mL to 4.85 $\pm$ 0.85 ng/mL, P = 0.022) despite no significant weight change in normal weight group. Correlations between difference in leptin concentration and anthropometric characteristics (body weight, BMI, WHR, waist circumference, and body fat mass) in the obese were significantly positive after the program participation (Data not shown). However, we did not observe any significant correlation between difference in leptin concentration and inflammatory cytokines TNF-α, IL-1ß, and IL-6 in obese subjects. Change in serum HMW-adiponectin concentration in both groups and correlations between difference in HMW-adiponectin and anthropometric characteristics or inflammatory cytokines in obese subjects were not significant.

DISCUSSION

In this study, we examined the effect of weight loss through 12-week weight management program with group education and individual counseling sessions on cell-mediated immune function and inflammatory responses in the young and otherwise healthy obese, many of them in their 20's (68%). While obese subjects lost body weight significantly (2.7 kg and 3.3% loss, $P < 0.001$), cell-mediated immune function did not change. However, we observed significant decrease in production of inflammatory cytokines in obese subjects with weight loss.

In order to evaluate cell-mediated immune response, we measured PBMC subpopulation and whole blood proliferative responses to Con A or PHA. Alteration in lymphocyte subpopulation associated with obesity has been reported in humans (Karlsson et al., 2010; O'Rourke et al., 2005; Tanaka et al., 2001), which resulted in higher rate of infections in obese people (Karlsson et al., 2010; Marti et al., 2001). In the present study, however, we did not observe any evidence of alteration in the percentage of $CD4^+$ and $CD8^+$ T cells in obese subjects both at baseline and week 12. This is inconsistent with previous studies; O'Rourke et al (O'Rourke et al., 2005) reported an increased frequency of $CD4^+$ T cells and decreased $CD8^+$ T cells, and Tanaka et al (Tanaka et al., 2001) reported a decline in both $CD4^+$ and $CD8^+$ T cells in the obese. Difference in BMI may partly explain these results. The mean BMI of obese subjects in the study of Tanaka et al

(Tanaka et al., 2001) was 38.4 kg/m^2 and the study of O'Rourke et al (O'Rourke et al., 2005) was 48.0 kg/m^2, but mean BMI of subjects in this study was 28.0 kg/m^2. Unlike T cells, significant changes in the percentage of B and NK cell were observed during 12-week program, but the magnitude of change was not different between two groups. Correlations between difference in leptin concentration and the percentage of NK cell was not significant, while obese subjects showed significant decrease in leptin which is a regulator of NK cell development and activation (Tian et al., 2002). This is likely because obese subjects in our study were overweight or obese, but otherwise healthy individuals. The finding by Meyers et al (Meyers et al., 2008) that there was no significant associations between leptin and NK cytotoxicity in obese subjects with a mean BMI of 30.2 kg/m^2 might support our results.

Although suppression of mitogen-induced lymphocyte proliferation associated with obesity has been reported (Nieman et al., 1999; Tanaka et al., 1993), evidence of immunosuppression in obese subjects was not observed in this study. Obese group showed higher proliferative responses to PHA at 1 and 50 μg/mL at baseline compared with normal weight group. It is consistent with a study by Boissonneault et al (Boissonneault et al., 1994) that there were significantly higher response of splenic T cells to Con A and PHA in young and adult obese mice compared with their lean counterparts, which suggested more accelerated maturation of T cell in the young obese.

Considering that most of subjects in this study were in their 20's, higher baseline proliferative responses in the young obese might be due to accelerated maturation of T cell. The proliferative response in obese subjects remained unchanged after weight loss. Taken together, obesity does not seem to suppress the cellular immune response in otherwise healthy young obese adults with a BMI less than 30 kg/m^2, and modest weight loss does not appear to affect their cell-mediated immune function.

Higher baseline WBC counts in obese subjects decreased significantly with weight loss, which is consistent with previous studies (Dixon et al., 2006; Garanty-Bogacka et al., 2011; Johannsen et al., 2010; McGuire et al., 2011). Given that higher WBC counts is a risk factor for cardiovascular disease (Bovill et al., 1996; Brown et al., 2001), reduction in WBC counts with weight loss *per se* has a significant clinical meaning.

The T_H1/T_H2 balance, which plays an important role in regulation of the immune response, can be disturbed by obesity. Leptin, which is elevated with increased adiposity, has been reported to cause bias toward pro-inflammatory T_H1 phenotype (Fantuzzi, 2009; La Cava et al., 2004; Lord et al., 1998). On the contrary, increase in leptin concentration in obese people seems to result in skewing towards a T_H2 cytokine profile (Hersoug et al., 2007). In this study, however, both PHA-stimulated T_H1 (IL-2 and IFN-γ) and T_H2 (IL-4) cytokine production in obese subjects were significantly higher compared with normal weight subjects. Higher production of IL-2, a

growth factor of T cell (Crispin et al., 2009), may be associated with higher proliferation to suboptimal concentration of PHA in the obese; significantly positive correlations between PHA-stimulated IL-2 production and proliferative response to suboptimal concentration of PHA were observed (Data not shown). Unlike other T_H cytokines, the production of IL-10 in obese subjects was comparable to normal weight subjects in this study, while decreased IL-10 concentration in obese individuals has been reported by others (Hersoug et al., 2007; McGuire et al., 2011). This is likely because obese subjects in our study were healthy and without signs of insulin resistance, given that decreased IL-10 production might be associated with the type 2 diabetes mellitus (Shoelson et al., 2007). Modest weight loss did not lead to decrease in upregulated T_H cytokines (IL-2, IFN-γ, and IL-4). The finding that IFN-γ production from mitogen-stimulated whole blood culture remained unchanged with weight loss through bariatric surgery (Tussing-Humphreys et al., 2011) is consistent this result. PHA-stimulated IL-10 production in obese group tended to increase (P = 0.060). Also, significantly negative correlations between difference in the production of IL-10 and inflammatory cytokines IL-1ß and IL-6 in obese group were observed. In this regard, increased production of PHA-stimulated IL-10 might be due to an anti-inflammatory property of IL-10 (McGuire et al., 2011; Shoelson et al., 2007). Taken together that there were decrease in inflammatory cytokines and increase in anti-inflammatory cytokine IL-10 in

obese subjects, modest weight loss seemed to attenuate inflammatory responses in the young obese.

Low-grade long-term inflammation has been suggested to cause many obesity-related disorders (Bruun et al., 2003; Ferrante, 2007; Kuo et al., 2011). Pro-inflammatory adipokines and cytokines such as leptin, IL-6, TNF-α, and IL-1ß released by excessive adipose tissue seemed to contribute to inflammation (Monteiro et al., 2010; Shoelson et al., 2007; Trayhurn et al., 2007). Ziccardi et al (Ziccardi et al., 2002) reported that obese healthy premenopausal women with a mean BMI of 37.0 kg/m^2 showed significantly higher serum IL-6 and TNF-α concentrations compared with nonobese subjects. Production of TNF-α stimulated with LPS in vitro was significantly higher in obese subjects with a mean BMI of 38.4 kg/m^2 than that in nonobese subjects (Tanaka et al., 2001). In our study, however, only LPS-stimulated IL-1ß in obese group tended to be higher compared with normal weight group (LPS at 1 μg/mL, $P = 0.082$). Also, we did not find any significant correlation between baseline inflammatory cytokines and anthropometric parameters in obese group. Considering that mean BMIs of obese subjects in most of the previous studies were over 30 kg/m^2, obesity does not seem to increase inflammatory responses in otherwise healthy young adults with a BMI less than 30 kg/m^2. LPS-stimulated IL-1ß and TNF-α in obese group decreased after weight management program participation, which is similar with the results from previous studies by

others. Kern et al (Kern et al., 1995) reported that weight loss through a low to very low calorie diet and behavior modification led to significant decrease in adipose TNF-α in obese subjects. Ziccardi et al (Ziccardi et al., 2002) showed that serum TNF-α and IL-6 in healthy premenopausal women aged between 25 to 44 years decreased significantly with weight loss. Significant decrease in LPS-stimulated IL-6 in the large weight loss group (> 6 kg wt loss; 8.0 kg and 9.1% loss) only is consistent with the review by Klempel et al. (Klempel et al., 2011). At least over 8% of weight loss appeared to be required to reduce plasma IL-6 concentration. While one study reported that 7.5% weight loss in obese subjects led to significant decrease in leptin concentrations (Arvidsson et al., 2004), the other study showed that 4% weight loss in obese subjects did not result in change of leptin concentrations (Peairs et al., 2008).

Leptin and adiponectin are adipose tissue-specific proteins, and obese people show higher leptin and lower adiponectin concentrations compared with nonobese people (Fantuzzi, 2009; Klempel et al., 2011; Van Gaal et al., 2006). Leptin controls food intake and regulation of immune and inflammatory responses (Fantuzzi, 2009). Decreased adiponectin concentration in obese people appears to contribute to insulin resistance and atherosclerosis, and adiponectin is reported to interact with the production of adipose tissue-derived cytokines such as IL-6 and TNF-α (Bruun et al., 2003). In this study, both obese men and women had significantly higher

baseline serum leptin concentration compared with normal weight counterparts. Significantly positive correlations between baseline anthropometric parameters and leptin concentration were observed as well. However, serum HMW-adiponectin concentration did not differ between two groups, and adiponectin concentration and anthropometric parameters did not have any correlation. Kuo et al (Kuo et al., 2011) also reported that there was no significant correlation between plasma adiponectin and BMI in healthy population. The obese subjects in our study were healthy and their fasting glucose and lipid profiles were still within reference range. Taken together, leptin seems to be an early indicator of obesity, but adiponectin is not. With weight loss, significant reduction in leptin concentration was observed in both the intermediate (3~6 kg wt loss; 4.0 kg and 5.0% loss) and the large weight loss groups (> 6 kg wt loss; 8.0 kg and 9.1% loss). The finding that at least 5% of weight loss is required to decrease leptin concentration in obese people (Klempel et al., 2011; Varady et al., 2009) supports this result. Decrease in leptin concentration and reduction of all anthropometric parameters had significantly positive correlations. On the contrary, serum HMW-adiponectin concentration in obese subjects was comparable to normal weight subjects. This is likely because adiponectin is less sensitive to modest weight loss than leptin does (Klempel et al., 2011). Obese but otherwise healthy subjects lost 7.5% of baseline weight through hypoenergetic diets (- 600 kcal/day) during 10-weeks, which led to

significant reduction in leptin concentration but there was no significant change in adiponectin concentration (Arvidsson et al., 2004).

Collectively, the lack of differences in adiponectin concentration and inflammatory responses between two groups may be due to the characteristics of obese subjects in our study: young, otherwise healthy, and with a BMI less than 30 kg/m^2. Nevertheless, significant decrease in leptin concentration, WBC counts, and inflammatory responses in the obese was observed with weight loss.

Since the subjects included in our study were highly educated and homogenous population, the extrapolation of our results might be limited. However, factors other than BMI between the obese and normal weight subjects were matched. Therefore, the results of this study might reflect the impact of obesity and weight loss on immunologic and inflammatory responses with a minimal influence from other confounding factors.

In conclusion, this study suggests that obesity with a BMI less than 30 kg/m^2 in otherwise healthy young adults does not significantly impair cell-mediated immune function. We did not find any significant evidence of dysregulation of the inflammatory response as well. Modest weight loss does not seem to affect cell-mediated immune function, but inflammatory responses were attenuated.

Ⅳ. CONCLUSION

In this study, we investigated the effect of the 12-week weight management program with nutritional counseling and behavioral modification on clinical characteristics, dietary intakes, and cell-mediated immune and inflammatory responses of the young and otherwise healthy obese (most of them with a BMI less than 30 kg/m^2). Anthropometric and clinical characteristics, 3-day dietary record, adipokines, PBMC subpopulation, whole blood lymphocyte proliferative response, T_H1/T_H2 cytokines (IFN-γ, IL-2, IL-4, and IL-10), and inflammatory cytokines (IL-1β, IL-6, and TNF-α) were evaluated. We tried to find the traits of those who lost more weight in order to identify the key factors leading to a successful weight loss as well. Results of this study were as follows.

1) Seventy subjects aged between 19 and 45 years were included in the 12-week weight management program and divided into two weight groups based on their BMI. Subjects without 3-day dietary record at baseline were excluded from study I; forty seven obese (BMI > 25) and 21 normal weight subjects (BMI 18.5~23) included, and 44 obese and 19 normal weight subjects completed the program. Forty obese and 19 normal weight subjects with complete dietary record data were included for dietary intake analysis. In the study II, 49 obese subjects and 21 normal weight

subjects participated in the program. Of these, 45 obese and 19 normal weight subjects completed the 12-week program.

2) There was a significant decrease in weight, BMI, waist and hip circumference, waist-to-hip-ratio (WHR), and body fat mass in the obese with the program. Thirty six percent of obese subjects achieved at least 50% of the weight loss goal by losing more than 3 kg during 12 weeks. Twenty three percent of obese subjects lost 3~6 kg (4.0 $\pm$ 0.3 kg and 5.1% wt loss, n = 10), and 14% of obese subjects lost more than 6 kg (8.0 $\pm$ 0.8 kg and 9.1% wt loss, n = 6).

3) At baseline, blood pressure, total and LDL cholesterol, and serum triglyceride were significantly higher in obese subjects compared with normal weight subjects. The 12-week weight management program resulted in significant decrease in triglyceride, ratios of total to HDL cholesterols and LDL to HDL cholesterols, and serum free fatty acid in obese group. Also, diastolic blood pressure tended to decrease, while HDL cholesterol tended to increase in obese group.

4) Thirty six percent of obese subjects were classified as having metabolic syndrome at baseline. After 12-week weight management program, prevalence of metabolic syndrome in the obese group tended to decrease. Decrease in the prevalence of metabolic syndrome was significant in obese men.

5) At baseline, intakes of energy, fat, and protein in obese subjects were not significantly different from those in normal weight subjects. There was a significant decrease in dietary intakes of energy, fat, and cholesterol in obese group with weight management program. Significant decrease in percent calories from fat and a tendency of decrease in animal fat intake in the obese group were observed as well. While vitamin B2, vitamin E, phosphorus, and zinc intakes decreased significantly in the obese group, the intake levels still met KDRIs.

6) Obese subjects had significantly higher baseline serum leptin concentration compared with normal weight subjects. The intermediate (3~6 kg wt loss) and the large weight loss groups (> 6 kg wt loss) showed a significant decrease in serum leptin concentration. On the contrary, baseline serum HMW-adiponectin concentration was not different between two groups as well as did not change with weight loss.

7) The percentage of $CD4^+$ and $CD8^+$ T cells did not differ between two groups at baseline, and no significant changes in that of $CD4^+$ and $CD8^+$ T cells in both groups were observed.

8) At baseline, obese subjects presented higher whole blood lymphocyte proliferative responses to suboptimal concentrations of PHA compared with normal weight group. After 12-week weight management program, no significant change in lymphocyte proliferative responses in obese group was observed.

9) The production of PHA-stimulated IL-2, IL-4, and IFN-γ were significantly higher in obese subjects compared with normal weight subjects. At 12-week time point, we did not observe any significant change in the production of these cytokines (IL-2, IL-4, and IFN-γ) in both groups. However, a tendency of increase in the production of PHA-stimulated IL-10 in obese group was observed. Given that significant negative correlation between difference in IL-10 and inflammatory cytokines IL-1β and IL-6, it was likely because IL-10 is an anti-inflammatory cytokine.

10) At baseline, the production of IL-1ß stimulated with LPS at 1 μg/mL in obese group tended to be higher compared with normal weight group. Obese group showed a significant decrease in IL-1ß (stimulated with LPS at 0.01 μg/mL) and a tendency of decrease in TNF-α (stimulated with LPS at 0.01 μg/mL) with weight management program.

11) Following traits were observed in the subjects who lost more than 6 kg: *1)* more active participation in the weight management program, *2)* more realistic weight loss goal setting, and *3)* weight gain occurred during the adulthood.

The 12-week weight management program with nutrition education led to a significant reduction in energy, fat, cholesterol, and sodium intakes and a tendency of increase in vitamin C intake in obese subjects, which implies that obese subjects reduced animal food intake and introduced a healthy

diet. These dietary changes and a decline in the prevalence of sedentary lifestyle seemed to contribute to weight loss and improvement of lipid profiles in obese group.

While impaired immune function and elevated inflammatory responses resulted from obesity have been reported, we did not find any significant evidence of them. The percentage of $CD4^+$ and $CD8^+$ T cells did not differ between two groups. The production of PHA-stimulated IL-2 and whole blood proliferative responses to suboptimal concentrations of PHA were significantly higher in obese group compared with normal weight group. Also, inflammatory responses in obese subjects were comparable to those in normal weight subjects despite elevated leptin concentration. These results were likely because obese subjects in this study were young and otherwise healthy (most of them with a BMI less than 30 kg/m^2). Activated proliferation and upregulated production of T_H cytokines did not change through modest weight loss in the obese. However, inflammatory responses in obese subjects were attenuated with weight loss; increase in anti-inflammatory cytokine IL-10 and decrease in inflammatory cytokines IL-1ß and IL-6 were observed.

There are some limitations to this study. We did not investigate physical activity thoroughly; we relied on individual counseling reports, not specific questionnaires for physical activity evaluation. Also, compliance of each subject during 12 weeks did not evaluated; we compared 3-day dietary

record and individual counseling reports at baseline and 12-week time points only. Lastly, the extrapolation of our results might be limited, since the subjects included in our study were highly educated and homogenous population; most of obese subjects were in their 20's and with a BMI less than 30 kg/m^2. However, factors other than BMI between the obese and normal weight subjects were matched. Therefore, the results of this study might reflect the impact of obesity and weight loss on immune and inflammatory responses with a minimal influence from other confounding factors.

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국문초록

12 주간의 체중조절 프로그램이 젊은 비만인의 임상 및 식생활 특성과 면역 및 염증 지표에 미치는 영향

서울대학교 대학원 식품영양학과

이 애 진

연구 목적: 본 연구는 12 주간의 체중조절 프로그램이 젊은 비만인의 임상 및 식생활 특성과 면역 및 염증 반응에 미치는 영향을 확인하기 위하여 수행되었다. 그와 함께, 체중 감소 정도에 따른 특성을 비교함으로써 성공적인 체중 감소에 기여하는 요인을 확인하고자 하였다.

피험자: 총 154 명의 만 19 세 이상 45 세 미만의 성인이 모집되었고, 설문지를 통해 70 명의 피험자가 연구에 포함되었다. 피험자의 BMI 에 따라 비만군 (BMI > 25) 및 정상 체중군 (BMI 18.5~23)의 두 군으로 피험자들을 나누었다. 연구 1 에는 0 주차 식사일지를 제출하지 않은 피험자를 제외한 비만군 47 명과 정상 체중군 21 명의 피험자가 포함되었다. 비만군 44 명과 정상 체중군 19 명의 피험자가 12 주 프로그램을 완료하였고, 이 중 0 주 및 12 주차의 식사일지를 모두 제출한 비만군 40 명과 정상 체중군 19 명이 식생활 특성의 분석에 포함되었다. 연구 2 에는 비만군 49 명과 정상 체중군 21 명의 피험자가 포함되었고, 비만군 45 명과 정상 체중군 19 명의 피험자가 12 주 프로그램을 완료하

였다. 본 연구는 서울대학교 생명윤리 심의위원회에서 승인을 받았다 (IRB NO. 0908/001-007).

12 주 체중조절 프로그램: 비만군을 대상으로 0, 2, 4, 6, 10 (그룹 교육 제외) 및 12 주에 걸쳐 총 5 번의 그룹 교육 및 6 번의 개별 영양 상담을 실시하였다. 정상 체중군은 식이 및 운동 패턴을 평가하기 위한 6 번의 개별 면담에 참여하였다. 프로그램의 목표는 하루 필요 열량 추정량보다 300~500 kcal 를 적게 먹음과 동시에 운동량을 늘림으로써 주당 0.5 kg 를 감량하는 것이었다.

측정 지표: 체중 및 임상지표를 측정하였고, 3 일간의 식이기록지 (3-day dietary record)를 분석하여 에너지와 영양소섭취 실태를 알아보았다. 아디포카인 중 렙틴과 아디포넥틴을 혈청에서 측정하였다. 비만군과 정상 체중군의 면역기능을 확인하기 위하여 FACS(Fluorescence activated cell sorter)를 이용한 말초혈액단핵구(Peripheral blood mononuclear cell, PBMC)의 subpopulation 분석과 전혈(whole blood)에서의 림프구 증식반응 및 T_H1/T_H2 사이토카인(IFN-γ, IL-2, IL-4 및 IL-10) 측정을 수행하였다. 비만군과 정상 체중군의 염증반응 수준을 알아보기 위하여 전혈에서의 염증성 사이토카인 (IL-1β, IL-6 및 TNF-α) 생성을 측정하였다.

결과: 연구 시작 시점에서, 비만군은 정상 체중군에 비해 유의적으로 높은 체중 지표, 수축기 및 이완기 혈압, 총 콜레스테롤, LDL 콜레스테롤, 혈청 중성지방 및 혈청 렙틴 수치를 보였다. 비만군과 정상 체중군의 열량, 단백질 및 지질 섭취량의 차이는 없었다. $CD4^+$ 및 $CD8^+$ T cell 비율에는 군 간의 차이가 없던 반면, suboptimal 농도의 PHA 에 대한 비만군에서의 림프구 증식반응이 정상 체중군에 비해 유의적으로 높았다. PHA 로 자극한 IFN-γ, IL-2 및 IL-4 의 농도 역시 비만군이

정상 체중군에 비해 유의적으로 높았다. 12 주간의 프로그램 후, 비만군에서 체중(2.7 kg, 3.3%)을 비롯한 모든 체중 지표와 혈청 중성지방 및 유리지방산이 유의적으로 감소하였다. 그와 함께 대사증후군(metabolic syndrome)에 해당되었던 비만군의 비율이 감소하는 경향이 관찰되었다. 식사일지 분석 결과, 비만군에서 열량, 총 지질 섭취량 및 지질의 열량 기여 비율, 콜레스테롤 섭취량 및 나트륨 섭취량이 유의적으로 감소한 반면, 비타민 C 섭취가 증가하는 경향이 있었다. 비만군의 비활동적 생활양식(sedentary lifestyle) 비율 역시 유의적으로 감소하였다. 유의적인 체중 감소에도 불구하고 $CD4^+$ 및 $CD8^+$ T cell 의 비율과 mitogen 에 대한 비만군의 림프구 증식반응에는 변화가 없었다. 그러나 비만군에서 PHA 로 자극한 IL-10 농도의 증가 경향 및 LPS 0.01 μg/mL 로 자극한 IL-1ß 농도의 유의적인 감소와 TNF-α 농도의 감소 경향이 관찰되었다. 체중 감소 정도에 따라 비만군을 3 kg 미만 감소, 3~6 kg 감소, 6 kg 이상 감소군으로 분류한 뒤 비교한 결과 *1)* 적극적인 연구 참여, *2)* 현실적인 목표 체중 설정 및 *3)* 성인이 된 이후의 체중 증가가 성공적인 체중감소에 기여한 것으로 확인되었다.

결론: 12 주 프로그램은 비만군의 열량, 지질, 콜레스테롤 및 나트륨 섭취량을 유의적으로 감소시킴과 동시에 비타민 C 섭취 증가를 이끌었다. 이는 12 주 프로그램 기간 동안에 비만군이 동물성 식품의 섭취를 줄이고 보다 건강한 식이를 먹기 시작했음을 의미한다. 비만군의 체중 감소 및 임상 지표 개선은 이와 같은 식이 변화 및 비활동적 생활양식(sedentary lifestyle)의 비율 감소 때문으로 사료된다. 비만으로 인해 면역 기능이 저하되고 염증반응이 증가하였다는 선행 연구들이 있으나, 본 연구에서는 그러한 결과들이 발견되지 않았다. 이는 본 연구의 비만군이 비만 외의 다른 질병을 가지고 있지 않은 젊은 성인이면서 대부분

30 미만의 체질량지수(BMI)를 가지고 있었기 때문으로 판단된다. 비만군의 체중 감소는 비록 활성화된 림프구 증식반응 및 T_H 사이토카인 생성에는 영향을 미치지 못하였으나, 비만군의 염증반응을 완화시켰다.

주요어: 비만, 행동 수정, 염증, 면역기능, 사이토카인, 아디포카인

학번: 2010-23613