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Editorial

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Current Controversies around Carbohydrate Restriction and the Risk of High-Protein Diets

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LOW CARBOHYDRATE DIET AND FRUCTOSE-RICH CORN SYRUP

Recently, a Low carbohydrate (LCH) diet has been recommended by many doctors to control hyperglycemia and overweight. Unlike a traditional calorie-restricted diet, a carbohydrate-restricted diet typically contains less than 15% of the total energy intake from carbohydrates and about 30% from proteins. High glycemic index carbohydrates are the only cause of the glucose spike, so the main benefit of a LCH diet is not to cause postprandial hyperglycemia, which is considered to be the most serious risk factor for arteriosclerosis in diabetic patients. Life With Diabetes[1] says that all absorbable carbohydrate foods turn to glucose in the blood, while fats and proteins do not, at least directly. Compared to a calorie-restricted diet, a carbohydrate-restricted diet accelerates fat metabolism yielding to ketogenic energy and helps gluconeogenesis in the liver, resulting in a more effective control of weight.

The recent movie "That Sugar Film" seems to expose the dangers of eating sugar for the society. Inspired by "Super Size Me", the antecedent of "That Sugar Film", Gameau relates how he experienced on his own body for 60 days, and indulged in taking healthy foods containing sugar. The experiment caused fatty liver, an excess of 10 cm of visceral fat around his waist, mood swings, and metabolic changes which could lead to coronary disease. Gameau actually consumed the typical Australian amount of 40 teaspoons of sugar (160 g) a day, maintained physical exercise, took the same amount of kilojoules as in his usual diet, and only ate food items perceived to be healthy. The latter include cereal, smoothies, muesli bars, and low-fat yoghurt. For Gameau, the worst effects of the diet were on his cognition, mood and ability to concentrate.

Food companies are convincing people that these foods might actually be good for them. At the same time, these products are replete with cheap additives, and premium prices are charged to make consumers believe that they are purchasing something healthy. In 2015, World Health Organization (WHO) recommended to reduce the intake of free sugars throughout the life course.[2] For both adults and children, WHO recommends reducing the intake of free sugars to less than 10% of total energy intake. WHO suggests a further reduction of the intake of free sugars to below 5% of the total energy intake. However, the problem is not only caused by refined sugars, but also by syrup hidden in processed foods. The sweetness of fructose is 1.5 times stronger than sucrose. Because high fructose corn syrup is cheap and easy to handle, it is frequently added to many industrial foods.

The average dietary intake of fructose, largely derived from sweeteners based on high-fructose corn syrup, has been estimated to increase by 20-40% over the last three decades. Compared to glucose, fructose is more potent in the stimulation of de novo hepatic lipogenesis and Very Low Density Lipoprotein (VLDL) secretion, which subsequently impact on systemic energy metabolism and insulin sensitivity. Fructose is absorbed by enterocytes through Glucose Transporter or Fructose Transporter (GLUT5), a fructose-specific hexose transporter, and reaches the liver through the portal vein. In the liver, fructose enters the glycolytic pathway downstream of phosphofructokinase, a rate-limiting enzyme of the glycolysis, and generates carbons for the synthesis of fatty acids and triglycerides. Fructose intake also activates the expression of lipogenic genes, which involves the induction of Sterol Regulatory Element Binding Proteins (SREBP), particularly SREBP1c, a major transcriptional regulator of lipogenic

gene expression.[3] High-fructose corn syrup is certainly not a healthy alternative to sucrose.[4]

Recently, sweetened fruit (soft) drinks have received considerable attention as popular high-energy beverages potentially related to the prevalence of obesity among young children. Wright et al[5] performed a secondary analysis of the data from the National Health and Nutrition Examination Survey (NHANES) 1999-2002. Twenty-four percent of the children were overweight or at risk for overweight, and more than 80% children drank fructose-rich high-calorie drinks.

Thus, the sources of excessive intake of fructose and glucose are mostly processed foods, fruit drinks, and soda, where sweeteners are added to cause taste addiction. Fruit sugar (fructose, the sweeter half of sucrose or cane sugar) is poisonous to the liver in sustained large quantities. Excessive intake of apple juice and other fruit juices is not part of a healthy diet. Abid et al[6] reported that the consumption of soft drinks is associated with fatty liver disease, independently of the presence of a metabolic syndrome.

HYPERKETONEMIA FROM LOW CARBOHYDRATE DIET

Low-carbohydrate diet relies on alternate energy sources for the human body. Some specialized cells, for example in the brain, retina, gonadal germinal epithelium, and erythrocytes require glucose as the primary energy source. Carbohydrate-restriction diet increases the concentration of β-hydroxy butyrate (BHB) and other ketones in the blood. Cahill[7] studied the glucose metabolism of people who voluntarily fasted for 40 days. He reported that in starving human adults, BHB and aceto-acetate were produced in the liver from long-chain fatty acids and released into the blood. BHB can rise to approximately 6 mM during starvation, but newly produced amounts of acetyl-CoA from fat cannot be metabolized in the Krebs cycle and it is diverted towards ketone body synthesis. The reference range of BHB is less than 0.4-0.5 mmol/L in healthy persons, but it may exceed 1 mmol/L as a consequence of a carbohydrate-restricted diet.[8]

Glucose, BHB, and aceto-acetate are used as energy sources for the brain of people put on a low-carbohydrate diet. However, the brain requires 80 g glucose a day by gluconeogenesis, with the following daily synthesis: 15-20 g glucose from lactic acid and glycerol, 20 g from pyruvate reuse, 35-40 g from ketone bodies, and 10-11 g from the degradation of proteins. The liver accounts for two-fifths, and the kidney for three-fifths of the glucose production.[9] A low-carbohydrate diet inevitably requires high-fat high-protein diet. An example of high-fat diet is the keton formula in which 70% of energy comes from fat, and 30% from proteins. The keton formula is usually given for 2 years to epileptic children, and long-term effects are unknown. The effects of dietary composition on energy expenditure during the maintenance of weight loss have been shown in recent studies.[10]

RISKS OF HIGH PROTEIN, LOW CARBOHYDRATE DIET

Ebbeling et al[10] conducted a cross-over study on the effects of three different diet regimens administered for 4 weeks: isocaloric low-fat diet (60% of energy from carbohydrates, 20% from proteins); low-glycemic index diet (40% from carbohydrates, 20% from proteins); and very low-carbohydrate diet (10% from carbohydrates, 30% from proteins). Compared with the baseline prior to weight loss, the resting and total energy expenditure were increased in the very low-carbohydrate/high protein group. Urinary corticosteroids and C-reactive protein were also high. These observations would suggest that metabolic changes caused by a 3-weeks intake of low-carbohydrate/high protein diet resulted in stress and inflammation of the body.

Altogether, the above data suggest that longer-term human studies are necessary to determine the ideal balance between major nutrients, carbohydrate, protein and fat associated with a healthy longevity. The low carbohydrate/high-protein diet used in the Swedish women's cohort study showed that low carbohydrate/high protein diets are associated with an increased risk of cardiovascular diseases after an average of 15.7 years of follow-up.[11] The longest follow-up study is the US physician and nurse's cohorts, in which 44,548 males and 85,168 females were followed up for 20 years and 12.6 years respectively.[12] The total number of deaths among males was 8678, including 2746 cardiovascular deaths and 2960 cancer cases. Those among females were 1255 total deaths, 2458 cardiovascular deaths and 5780 cancer cases. The relative risk of high protein low carbohydrate diet was estimated at 1.23 for total deaths, 1.14 cardiovascular deaths, and 1.28 cancers. Other studies have shown similar trends.[13,14] The risk of chronic kidney diseases has not been described, but the risk of high-protein diet for kidney has been shown in many studies. We conducted a cross-sectional study on Chronic Kidney Disease (CKD) patients with very low protein diet (protein <0.5 g/kg/day). We found that they remained healthy for more than 6.7 years on average without clinical manifestation, starting from more than 5-6 mg serum creatinin/dl.[15]

IDEALLY BALANCED HEALTHY DIET AND DIETARY HABITS

Solon-Biet et al[16] compared three regimens varying in protein to carbohydrate ratio under both Calorie Restriction (CR) and

ad libitum conditions. These diets were classified as low-protein (5%), Low-protein high-carbohydrate (LPHC), medium-protein (33%), medium-carbohydrate and high-protein (60%), low-carbohydrate. Fat content was fixed at 20% of the total energy intake for all three diets. Ad libitum LPHC diets offered similar benefits to CR in terms of levels of insulin, glucose, lipids, and Homeostatic model assessment (HOMA), despite an increased energy intake. CR on LPHC diets did not provide additional benefits relative to ad libitum LPHC.

Whereas HPLC diets do not sustain optimal cardio-metabolic health in later ages, it is important to note that nutritional requirements change with age, and higher P:C diets are required to support reproduction rather than to sustain a maximal lifespan.[17,18] You can prevent very severe health risks by improving your diet with an appropriate carbohydrate content, by exclusively eating natural foods with plenty of green vegetables, and by avoiding meat. Most of us know more or less what we should eat in order to feel well and have the weight which suits us. Fruits and vegetables should be part of our everyday meals. And whatever we eat should conform with our hunger and satiety.

Nettleton JA et al[19] recently reported a meta-analysis investigating associations between healthy diet, fasting glucose, insulin levels, and genetic loci associated with glucose homeostasis. They utilized data from 15 USA and European cohort studies comprising 51,289 persons without diabetes to test whether genotype and diet interact to influence glucose or insulin concentration. Genome-wide association studies focusing on genomic regions of diabetes and obesity did not show statistically significant associations.

Thus, dietary and other lifestyle habits are important for a healthy live. We all know these basic rules, but many of us are intoxicated by the food industry and so called ‘specialist’ messages. Rational thinking and emotions are disconnected . Brownell et al[20] argue in favour of a tax system that could promote good nutrition and help the nation recover health care costs associated with the consumption of sugar-sweetened beverages. Such integrative approaches should be effective to make our world a healthier place.

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Case Report

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The Use of *Urtica dioica* (Stinging Nettle) as a Blood Sugar Lowering Herb: A Case Report and a Review of the Literature

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ABSTRACT

Introduction: Medicinal plants have been used in traditional medicine to manage blood sugar levels in patients with diabetes, but only a few of them have received scientific investigation. Many patients tend to self-medicate with herbal supplements, based on information they obtain from various sources.
Case Summary: A 57 year-old African-American male with diabetes had been prescribed metformin. He started on his own taking Stinging Nettle concurrently with metformin, which led to hypoglycemia. He then stopped taking metformin and continued with the herb. His morning fasting blood sugar stayed at less than 120 mg/dL. Because of accessibility, several months later he then discontinued taking Stinging Nettle, at which time his blood glucose level climbed up to 140-160 mg/dL. At this point, he saw his healthcare provider who put him back on metformin, and his blood glucose was well managed after that.
Conclusion: A systematic literature evaluation on Stinging Nettle showed some evidence of the blood sugar lowering effect of the plant. The patient in this case may have benefited from this property of the plant. Considering poor regulation and the possible variation of herbal supplement products in the market, routine use of Stinging Nettle should not be encouraged. However, there is some evidence on the blood lowering property of Stinging Nettle.

KEYWORDS: *Urtica dioica*; Stinging nettle; Diabetes.

ABBREVIATIONS: PPAR: Peroxisome Proliferator-Activated Receptor; TZDs: Thiazolidinediones; SGOT: Serum Glutamic Oxaloacetic Transaminase; HbA1c: Glycated hemoglobin; IL-6: Interleukin 6; TNF-alpha: Tumor Necrosis Factor-alpha; hs-CRP: High Sensitive C-Reactive protein.

INTRODUCTION

Diabetes mellitus affects over 250 million people worldwide and is expected to affect some 380 million by 2025.[1] Each year another 7 million people develop diabetes. The first line treatment for type 2 diabetes is diet, weight control and physical activity. If blood glucose level remains high despite a trial of these lifestyle measures, then medications are usually advised. Although, there are many effective drugs available on the market, the majority of persons with type 2 diabetes eventually fail to respond to a commonly used first-line oral medication (e.g. metformin). There are several categories of drugs for type 2 diabetes, including sulfonylureas, biguanides, thiazolidinediones, meglitinides, dipeptidyl peptidase IV, insulin, etc. Finding an effective alternative oral treatment to avoid administration of exogenous insulin and/or other therapies by daily needle injection would be desirable. Some persons with diabetes continue to self-medicate with alternative products such as herbs and other supplements.

The blood glucose lowering effect of Stinging Nettle has been noted in old writings.

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Recently, some investigations have reported on the hypoglycemic effect of *Urtica dioica*, but so far, the mechanism of this effect has not been deduced. Some studies show that it may work as a secretagogue, or as a Peroxisome Proliferator-Activated Receptor (PPAR) agonist. PPAR agonists are drugs that lower blood glucose level by enhancing insulin secretion by Langerhans Islets.

CASE REPORT

A 57 year-old African-American contacted our drug information center regarding the use of Stinging Nettle in lowering his blood sugar. The caller admitted to having a history of diabetes mellitus and had been on metformin 500 mg twice daily for a few years prior. His other medications included aspirin 81 mg daily, metoprolol 50 mg twice daily, and atorvastatin 40 mg once daily. He started using Stinging Nettle about a year or so ago previously. He prepared a tea by boiling the fresh leaves in hot water and straining out the leaves. He consumed the hot tea once daily in the morning. After experiencing a few hypoglycemic episodes, he stopped taking metformin, but continued taking the herb. His follow up self-reported average morning fasting blood glucose was less than 120 mg/dL. After about 9 months, he moved to another city and stopped taking the herb because he was not able to get the fresh leaves anymore. Within 2 months, his blood glucose level started to rise to a range of 140-160 mg/dL. At the time of his call to our center, he was put back on metformin. The patient stated that his current metformin dosage regimen at the time he contacted us was 500 mg three times daily, which resulted in good blood sugar control. In a follow up call in preparation for this publication, the patient also admitted that after he moved to the new city, he has not been exercising as much. No other lab data or medical record was available for review. We now provide a critical review of the published literature to assess the potential therapeutic value of Stinging Nettle as a natural product with blood glucose lowering properties.

DISCUSSION

Urtica dioica L. (Family: Urticaceae)[2]

Stinging Nettle is a small plant that has fine hairs on the leaves and stems. The scientific name for the plant is *Urtica dioica*. The genus name Urtica comes from the Latin verb urere that means, "*to burn*" because of these stinging hairs. The species name dioica means "*two houses*" because the plant usually contains either male or female flowers. The species is divided into six subspecies, five of which have many hollow stinging hairs called trichomes on the leaves and stems, which act like hypodermic needles, injecting histamine, serotonin, and choline that produce a stinging sensation when they come into contact with humans and other animals.[3] The plant has been used for hundreds of years as a diuretic and to treat painful muscles and joints, eczema, and arthritis. Today, many people use Stinging Nettle to treat benign prostatic hyperplasia. Stinging nettle products are usually made from the leaves and stems, and sometimes from the roots. (Figure 1)

Figure 1: *Urtica dioica* (stinging nettle).

LITERATURE REVIEW

Medicinal plants have been a repository of a wide variety of biologically active compounds for many centuries but are still largely unexplored.[4] More than 400 traditional plants have been recorded with antidiabetic effects, but very few of these traditional plants have received proper scientific or medical investigation.[5] It is estimated that today, plant materials are present in, or have provided models for development of about half of the Western drugs.[6] Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low cost herbal drugs are prescribed widely even when their biologically active compounds are unknown.[7]

Several studies suggest that the Stinging nettle works as a PPAR gamma agonistic and alpha-glucosidase inhibitory agent.[8,9] The two most common receptor targets for a number of PPAR agonist marketed drugs are PPAR-alpha and PPAR-gamma receptors. PPAR-alpha receptors are the main target for fibrate drugs used in reducing triglycerides, while PPAR-gamma receptors are the main target of the drug class of Thiazolidinediones (TZDs) used for blood glucose lowering in persons with diabetes mellitus.

Pancreatic α-amylase and intestinal α-glucosidase are enzymes that play major roles in the digestive system in catalyzing starch by hydrolyzing the α-1,4-glucoside linkages. The inhibition of these enzymes significantly decreases the digestion and uptake of carbohydrates, thereby decreasing the postprandial blood glucose level in persons with non-insulin dependent diabetes mellitus.[8] Drugs such as acarbose, miglitol and voglibose are currently used as α-glucosidase and α-amylase inhibitors. The main drawback of these drugs is that their hypoglycemic effect is lower than that of other oral antidiabetic agents, including sulfonylureas. They are therefore recommended as add-on therapy only. Another drawback of these agents is their side effects such as abdominal distention, bloating, flatulence and possibly diarrhea if not titrated up slowly.[10] It has been suggested that the gastrointestinal effects might be caused by the excessive inhibition of the pancreatic α-amylase, leading to the abnormal bacterial fermentation of undigested carbohydrates in the colon.[11] It has been postulated that natural products such as Stinging Nettle that have been shown to possess a low inhibitory effect against α-amylase and high inhibitory activity against

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α-glucosidase can be used as an effective means to reduce postprandial hyperglycaemia with minimal adverse effects.[12]

One of the most recent studies published in the use of Stinging Nettle in lowering blood sugar was done by Kianbakht, et al.[9] The authors conducted a randomized double-blind placebo-controlled clinical trial to evaluate the effects of taking Stinging Nettle leaf extract (one 500 mg capsule every 8 hours for 3 months) combined with the conventional oral antihyperglycemic drugs.[9] The authors evaluated the effect of the extracts on the blood levels of fasting glucose, postprandial glucose, Glycated hemoglobin (HbA1c), creatinine, Serum Glutamic Oxaloacetic Transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and systolic and diastolic blood pressures. The clinical trial included 46 patients in the treatment arm and 46 patients in the placebo group. The results demonstrated that the extract significantly lowered the blood levels of fasting glucose. It was also shown that it decreases the 2-hour postprandial glucose level and HbA1c. However, there was no significant effect on the other parameters compared with placebo. The authors concluded that these results demonstrated that Stinging Nettle is safe and may have a beneficial effect on glycemic control in patients with advanced type 2 diabetes mellitus that typically require insulin therapy.

Another recent study in Iran on Stinging Nettle leaves has shown evidence that the plant may have potential in antidiabetic therapy.[13] In this study, the Stinging Nettle consisted of freeze-dried extract from 100 g of powdered dried leaves in 100 ml of water. The results showed time- and concentration-dependent inhibition of α-amylase. According to the authors, the Stinging Nettle extracts showed the same inhibitory pattern as that of acarbose, a known α-glycosidase inhibitor, which is only one among a class of drugs with similar activities. However, drugs in this class also act as strong competitive inhibitors of α-amylase. Acarbose, for example, is a well-known, natural product produced by several species of Actinoplanes. This compound has a pseudosugar ring and the glycosidic nitrogen linkage that mimics the transition state for the enzymatic cleavage of glycosidic bond and hence competitively inhibits α-amylase.[14] In this study, the authors reported that a 0.4 mg/ml of Stinging Nettle leaf extract demonstrated a 60% inhibition of α-amylase activity. The level of inhibition was also time-dependent. The inhibitory effect increased from 40% at 5 minutes to 60% at 30 minutes.

A study in 2011 by Namazi, et al. evaluated the effect of hydroalcoholic extract of Stinging Nettle on insulin sensitivity and some inflammatory indicators on a cohort with type 2 diabetes.[15] Diabetes is a metabolic disorder that is strongly associated with micro-complications, such as retinopathy, nephropathy, and neuropathy and macro-complications including cardiovascular risk. Inflammation is a potential risk factor for diabetic complications particularly cardiovascular disease. These anti-inflammatory indicators were measured in this study along with insulin sensitivity. The study was a randomized double-blind clinical trial and included 50 men and women with type 2 diabetes. The study was done over 8 weeks. The authors adjusted the study participants for age, sex and duration of diabetes, and then randomly assigned them into two groups, an intervention and a control group. The treatment groups received 100 mg/kg body weight nettle extract or placebo in three portions a day for 8 weeks. The parameters measured included Interleukin 6 (IL-6), Tumor Necrosis Factor-alpha (TNF-alpha), high sensitive C-Reactive protein (hs-CRP), and fasting insulin concentration. The researchers calculated Insulin Sensitivity, at the beginning and the end of the study. After 8 weeks, IL-6 and hs-CRP showed a significant decrease in the intervention group compared to the control group ($p<0.05$). The findings showed that the hydro-alcoholic extract of Stinging Nettle lowered the inflammatory markers, IL-6 and hs-CRP, in patients with type 2 diabetes after eight weeks intervention.

A study by Ahngarpour, et al. showed the effect of hydro-alcoholic extract of Stinging Nettle on fructose-induced insulin resistance rats.[16] Forty male Wistar rats were randomly divided into five groups: 1) Control; 2) Fructose; 3) Extract 50; 4) Extract 100; and 5) Extract 200. The control group received vehicle. The fructose and extract groups received fructose 10% for eight weeks. The extract groups received single daily injection of 50, 100 or 200 mg/kg/day of extract for the two weeks. The results showed that the extract groups had a significant reduction in serum glucose and insulin levels. The study also showed a reduction in LDL. Leptin and LDL/HDL ratio. The authors concluded that Stinging Nettle extract decreases serum glucose, and thus may be useful for treatment of type 2 diabetes. They also speculated that Stinging Nettle might improve metabolic syndrome by the positive effect shown on lipid profile and also by lowering effect on leptin levels.

SAFETY AND ADVERSE EFFECTS

Stinging Nettle is relatively a safe plant if used appropriately. The major adverse effect that has been documented in animal studies is that it lowers blood pressure and heart rate.[17] Those with heart conditions should seek the advice and supervision of a health practitioner to determine if the herb is suitable for their condition. Nettle has been documented to have diuretic effects. Thus, chronic use of this plant may be contraindicated in various medical conditions where diuretics are not advised. Because of the herb's diuretic effects, it may enhance the effect of blood pressure medications including ACE inhibitors, beta-blockers, or calcium channel blockers. It can also increase the effects other diuretics, including thiazides and loop diuretics, thus raising the risk of dehydration and electrolyte disturbances.[18]

Other occasional side effects include mild stomach upset, fluid retention, sweating, diarrhea, and hives or rash (mainly from topical use). It is important to be careful when handling the nettle plant because touching it can cause an allergic rash. Stinging Nettle should never be applied to an open

wound. Because, Stinging Nettle can alter the menstrual cycle and may contribute to miscarriage, pregnant women should not use Stinging Nettle.

LIMITATIONS

Detailed medical history and complete demographic data of the patient were not collected. The amount of Stinging Nettle leaves used by the patient each time to prepare tea was not obtained. In the literature reviewed in this paper, there are no reported large multi-center and placebo-controlled studies on the benefits of the Stinging Nettle in patients with diabetes. However, based on the studies reviewed, the plant may have some potential benefits in this patient population. Further studies involving a large number of patients are required to confirm the benefits.

BENEFITS

If patients choose to use Stinging Nettle for management of diabetes on their own initiative, they should be encouraged to monitor their blood sugar very closely. In addition, they should also be advised to notify their physician of such use. The herb is also rich in vitamins A and C, iron, potassium, manganese, and calcium. It has a flavor similar to spinach and cucumber when cooked. In its peak season, Stinging Nettle contains up to 25% protein, dry weight, which is high for a leafy green vegetable.[19] Soaking Stinging Nettles in water or cooking removes the stinging chemicals from the plant, which allows them to be handled and eaten without injury.

CONCLUSION

Following a call to our drug information center by a patient regarding the use of Stinging Nettle for treating diabetes, we conducted a review of the literature on medicinal properties of Stinging nettle. Previously published small human clinical trials suggest that the plant may be considered for investigation as a natural product source of a novel as-yet-unidentified active compound with glucose lowering activity.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

CONSENT

The patient has provided written permission for publication of the case details.

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Review

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Diabetes in the Northwest Territories

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INTRODUCTION

An estimated 2.7 million (7.6%) Canadians were living with diabetes in 2012.[1] Internationally, Canada has the fourth highest rate of diabetes, behind Mexico, the United States and Portugal.[2] The prevalence of diabetes in Canada has doubled since 2000, and is expected to keep increasing.[2] The Northwest Territories (NWT) is one of three territories located in the most Northern part of Canada. In the NWT, the prevalence of diabetes was estimated to be 5.5% in 2008/2009.[3] Approximately 200 new cases of diabetes are diagnosed each year in the NWT, contributing to the increasing prevalence of diabetes across the territory.[3] Aboriginal populations are at a disproportionately higher risk of developing diabetes, post adoption of a more Westernized culture.[4] With 51% of NWT's population of 43623 identifying as Aboriginal, coupled with an aging population, the burden of diabetes in the NWT is expected to increase in the coming years.[5]

To help combat the anticipated growth in the burden of disease in the NWT, specific screening and diagnosis clinical practice guidelines for type 2 diabetes were developed in 2014 in consultation with the Canadian Diabetes Association (CDA).[6] According to the guidelines, a diagnosis of diabetes is made if any one of three tests for diabetes has a positive result: Fasting Plasma Glucose (FPG), two hour 75 g Oral Glucose Tolerance Test (OGTT) and Glycated hemoglobin (A1C) (Table 1). Screening for diabetes is implemented based on risk for developing diabetes, established from an NWT adapted Canadian Diabetes Risk Questionnaire (CANRISK) assessment.[7] These adapted guidelines recommend annual screening starting at age 30 for those at high risk, every two years starting at age 30 for those at moderate risk, and every three years starting at age 40 for those at low risk.

Test	Result required for diagnosis
Fasting plasma glucose (FPG)	≥7.0 mmol/L
2-hour plasma glucose in 75 g oral glucose tolerance test (OGTT)	≥11.1 mmol/L
Glycated hemoglobin (A1C)	≥6.5%

Table 1: Required results for a diabetes diagnosis, based on NWT screening and diagnosis guidelines.

In addition to the implementation of the 2014 NWT Type 2 Diabetes Screening Diagnosis Clinical Practice Guidelines, a territory-wide diabetes registry has been legislated and will make diabetes a notifiable disease to the Department of Health and Social Services (DHSS) beginning on January 1, 2016. For this registry to be implemented effectively, accurate territory-wide prevalence rates were needed. In the winter of 2015, a prevalence review for the previous three year period (2012-2014) was undertaken.

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METHODS AND ANALYSIS

To ensure the highest capture rate of diabetes, both lab data and community level data were used. Lab data included all FPG, OGTT and A1C tests performed between 2012 and 2014. Community level data was obtained from a nurse in charge for smaller communities, a chronic disease management nurse for regional centers, and from the Electronic Medical Record (EMR) in communities where installed, for the 33 communities across the NWT. Specifically, nurses in the community health centers provided line lists of those living with diabetes in their community, whereas the EMR data extracts were used as the source of data for the 2 communities where it was installed. An individual was considered a case of diabetes if any one of the three lab tests was positive or if they were included on any of the community lists of individuals with diabetes. Due to the reliance on lab data, it was impossible to differentiate between type 1 and type 2 diabetes.

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 22 and Microsoft Excel 2010. Crude prevalence rates were calculated for the seven health authorities in the NWT as well as by age group. Age standardized prevalence rates were calculated for all other variables of interest using the 1991 Canadian Standard Population.[8] Standard errors and 95% confidence intervals were calculated for all variables.

RESULTS

The crude prevalence for 2014 was 6.7%. The age standardized prevalence for 2014 was 7.5%. The average age of the 2908 prevalent cases in 2014 was 58 years, with a range of 4 to 95 years. The average age of female cases was 57 years whereas it was 59 years in males. The oldest age group, 65 and older, had the highest prevalence rate at 31.6%. Rates decreased accordingly with decreasing age, with the youngest age group, 0-24 years, having the lowest prevalence of 0.3%. The 55-64 year old group had a prevalence of 18.9%, 45-54 had 10.5%, 35-44 years had 4.5% and 25-34 years had a prevalence of 1.6%. Males had an age standardized prevalence rate of 8.0%, while females had a rate of 6.9%.

NWT communities were grouped into three categories to describe community types: Yellowknife City, Regional Centres (Hay River, Fort Smith, and Inuvik, population size range: 2536 to 3689), and Small Communities (all remaining NWT communities, population size range: 71 to 2039).Regional Centres had the highest age standardized prevalence at 9.7%, followed by Yellowknife at 7.3% and Small Communities had the lowest prevalence at 6.2%.

Results were age standardized by specific ethnicity (Dene, Metis, Inuit and Non-Aboriginal). Metis people had the highest prevalence of diabetes at 10.12%, followed by Non-Aboriginal people at 7.8%, Dene people at 7.3% and Inuit people had the lowest prevalence at 5.3%. Non-Aboriginal males had the highest prevalence of diabetes at 9.2%, followed by Aboriginal females at 7.8%. Aboriginal males had a prevalence rate of 6.8% and Non-Aboriginal females had the lowest rate at 6.1%.

LIMITATIONS

While the data review provided accurate prevalence estimates, it is only a starting point for a territory wide diabetes registry. There were several limitations with the data and considerations in implementing the new diabetes registry. Firstly, there was no way to differentiate between Type 1, Type 2, or gestational diabetes from the lab data. The prevalence rates presented represent all diabetes in the NWT and are not limited to Type 2 diabetes. Additionally, without a date of diagnosis, it was impossible to calculate incidence or evaluate trends in prevalence. The implementation of the diabetes registry will eliminate some elements of this as diagnosis date will be made available.

While the combination of lab data and community level data provided a robust estimate of diabetes prevalence, it is still possible that cases of diabetes were missing from the data, especially if they were never screened or tested or don't have an NWT healthcare number (non-residents were excluded from the analysis). It is also possible that the threshold of any one positive test is too low, thereby falsely including identifying some non-diabetics as having diabetes. Finally, while the active resident list provided the most up to date community data, it is possible that the population in the NWT is highly mobile and therefore geographic distribution would be misrepresented in the data.

CONCLUSIONS

These results provide evidence of the high risk groups for targeting future diabetes programming, including prevention and management. The information from this prevalence review will be used to inform implementation of a territory wide diabetes registry and help establish where diabetes programming could best focus across the NWT. Diabetes prevalence rates have been increasing nationally and this is also the case in the NWT. The new diabetes registry will allow us to further monitor screening compliance with the new guidelines and better determine rates for pre-diabetes as well as incident diabetes cases. It will also allow further delineation of type of diabetes mellitus (Type 1, Type 2, other specified, unspecified) as per ICD 10 classification (E10-E14). The steps being taken will help better inform programs and services in order to reduce the burden of disease from diabetes in the NWT, reduce strain on the health care system and improve quality of life for those living with diabetes.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Review

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Clinical and Experimental Evidence of Hypoglycemic Neuropathy

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ABSTRACT

When compared with the extensive research on hypoglycemic impacts on Central Nervous System (CNS) and cardiovascular system, the effects of hypoglycemia on the Peripheral Nervous System (PNS) have not been investigated as thoroughly. Epidemiologic data and risk factors for hypoglycemic neuropathy are still lacking. Interestingly, hyperglycemia mainly results in the damage of sensory and autonomic nerve fibers, whereas hypoglycemia predominantly leads to the development of motor neuropathy. Most clinical features are concluded from patients with insulinoma, and neuropathology has shown axonal degeneration in large myelinated fibers. Experimental animal models support the clinical and histopathological findings. The exact pathophysiological mechanisms of hypoglycemic neuropathy remain elusive. The influence of hypoglycemia on peripheral nervous system warrants further investigations.

KEYWORDS: Hypoglycemic neuropathy; Hypoglycemia; Peripheral nervous system.

ABBREVIATIONS: CNS: Central Nervous System; PNS: Peripheral Nervous System; NCV: Nerve Conduction Velocity; IIH: Insulin-Induced Hypoglycemia; DPN: Diabetic Peripheral Neuropathy; DM: Diabetes Mellitus; HbA1c or A1C: Glycated hemoglobin; LDL-cholesterol: Low-Density Lipoprotein-cholesterol; HDL-cholesterol High-Density Lipoprotein-cholesterol.

INTRODUCTION

Hypoglycemia is a condition primarily affecting diabetic patients treated under excessive medication, such as insulin or other hypoglycemic agents.[1-3] Other causes of hypoglycemia include insulinoma, poor intake, infections, liver, and kidney diseases.[1-3]

The incidence of hypoglycemia varies considerably among studies by means of using different biochemical criteria to define an event.[4,5] The symptoms of hypoglycemia vary between individuals. Neurogenic (autonomic) symptoms include tremor, palpitations, hunger, and cold sweating. Neuroglycopenic symptoms often include behavioral changes, confusion, seizure, coma, and death.[4] Compared to the abundant and extensive data on hypoglycemia effects on the Central Nervous System (CNS) and cardiovascular system, there is little evidence in humans and experimental animal studies toward hypoglycemic effects on the Peripheral Nervous System (PNS).[6,7]

Diabetic Peripheral Neuropathy (DPN) is the commonly reported vascular complication affecting as many as 50% of patients with Type 1 and Type 2 Diabetes Mellitus (DM).[4,5,8] The

common risk factors for DPN include diabetic duration, Glycated hemoglobin (HbA1c or A1C).[9] Recently, cardiovascular risk factors, such as elevated blood pressure, hyper-triglyceridemia, Low High-Density Lipoprotein-cholesterol (HDL-cholesterol), High Low-Density Lipoprotein-cholesterol (LDL-cholesterol), and decreased estimated glomerular filtration rate also appear to be related to newly diagnosed Diabetic Peripheral Neuropathy (DPN) in type 2 DM independent of HbA1c.[9-12] Moreover, with diverse patterns of DPN in diabetes, it is possible that the development of DPN is not only influenced by hyperglycemia but by other factors, such as hypoglycemia, as well. Therefore, it is important to identify the role that hypoglycemia plays in DPN. In this article, we first introduce the clinical characteristics of hypoglycemic neuropathy. Then, we provide the evidence from animal studies. After which, we discuss the key issues of pathogenesis. Lastly, key points regarding hypoglycemic neuropathy are summarized in the conclusion.

CLINICAL EVIDENCE OF HYPOGLYCEMIC NEUROPATHY

There are very few reports on the effects of hypoglycemia on the human PNS. In 1946, Silfverskiöld reported hypoglycemia related motor symptoms in insulinoma patients.[13] In 1956, Mulder, et al. presented similar cases and proposed the motor symptoms may be related to neuritis by abnormal high insulin levels.[14] Afterwards, more and more patients with insulinoma were reported.[15-19] In 1982, Jaspan, et al. reported a case and reviewed previously reported twenty-eight cases with insulinoma and frequent hypoglycemia.[20] The average age of the cases was 38 year old with a mild male predominance. The most typical presentation started with obvious distal paraesthesia with or without significant sensory loss, followed by motor-predominant distal symmetric peripheral neuropathy with obvious muscle atrophy. Unlike the usual pattern of diabetic polyneuropathy, the upper extremities are generally more involved than the lower ones, but foot drop can occur frequently. Moreover, neuropsychiatric symptoms with fluctuated or episodic confusion were easily noted. The onset and course of neuropathy varied. Most patients ran sub-acute or chronic polyneuropathy pattern in 3-6 months. Some patients got acute polyneuropathy after coma recovery. But these patients also experienced many hypoglycemia episodes before.[20] Thus, whether severe hypoglycemia alone is sufficient to lead to polyneuropathy was still questionable. After removal of the insulinomas, sensory symptoms tend to regress greatly but definite improvement in motor weakness was uncommon.[13-20] Nerve Conduction Velocity (NCV) study showed distal symmetric predominantly axonal motor neuropathy from most of their reports.[13-20] There was also electromyographic evidence for denervation of skeletal muscles.[15,16] Normal cerebrospinal fluid protein suggested peripheral axonal injury rather than dorsal root ganglion involvement.[10-12] With respect to neuropathology, axonal degeneration in large myelinated fibers and neurogenic atrophy in muscle had been shown in peripheral nerves of insulinoma patients.[21-23]

In contrast, other researchers revealed that insulinoma related hypoglycemia neuropathy affected both motor and sensory fibers or mainly the sensory symptoms in mice and humans.[19,24] This is the same situation of diverse presentations of neuropathy in diabetic patients. Some diabetic patients show mainly sensory disturbances and/or autonomic dysfunctions, others show mixed sensorimotor symptoms or motor problems predominant. It is possible that some of the clinical findings are not exclusively the consequence of hypoglycemia but also in combination of hyperglycemia and hypoglycemia states.[12] Hypoglycemic effect may contribute to the diverse pattern of diabetic peripheral neuropathy in humans. Further studies are needed to investigate the co-existence effects of hypoglycemia in diabetic neuropathy.

EVIDENCE FROM ANIMAL STUDY

Investigators have used various experimental animal models, such as healthy and diabetic rats or mice to elucidate hypoglycemic neuropathy.[10-12] Normal and diabetic rats underwent experimental hypoglycemia with excessive insulin injection exhibit either abnormal NCV or nerve structure change with a combination of Wallerian-type axonal de-and regeneration.[25-27] The pathological change indicates that hypoglycemia affects the neuron rather than the Schwann cell and the axonal degeneration affects large myelinated fibers preferentially.[28-30] Furthermore, motor axons are more severely damaged than sensory axons in the peripheral nerve trunks.[28-30] Both the duration and the severity of the hypoglycemia can influence the occurrence of neuropathy.[29,30] These findings of animal studies are highly compatible with hypoglycemic neuropathy reported by Jaspan.[20]

When looking at the rats, pathological changes of hypoglycemic neuropathy at the nerve trunk level are much more obvious than at the spinal root level, including ventral horn and dorsal root ganglion.[26-31] This discovery is again compatible with cerebrospinal fluid and pathologic results in human beings.[10-12]

The investigators also studied the Insulin-Induced Hypoglycemia (IIH) effects on non-diabetic and diabetic rats by analysis of tibial nerves. They demonstrated that the Wallerian-type axonal degeneration happens only in treated diabetic rats, especially more severe in the daily insulin injection group. Other investigators studied hyperglycemic rats, and selectively gave them either small or high doses of insulin to the point of hypoglycemia, or just left them remained hyperglycemic state without insulin.[32] In the hypoglycemic rats, loss of large myelinated fibers and decreased NCVs were noted, while the other groups had mainly sensory fiber abnormalities.[32,33]

PATHOGENESIS OF HYPOGLYCEMIA NEUROPATHY

IIH is one of the most common forms of hypoglycemia in diabetes. Thus, it is also the most frequently aroused research interest to explain the pathophysiology of hypoglpycemic neuropathy.[11,12] In addition, histopathological findings induced by IIH are similar to those findings in cases of hypoglycemic

agents, other than insulin or insulinoma, in human.[10,12] It indicates that pathogenesis of hypoglycemia neuropathy induced by IIH does not represent a species-specific effect.[10-12] We highlight the key issues herein, and more detailed descriptions of pathogenesis of hypoglycemia neuropathy induced by IIH have been described elsewhere in another review article.[12]

Mechanisms Involved in the Pathogenesis are Complex and Multifactorial

Because hypoglycemia is involved, energy depletion appears to be a likely mechanism in IIH induced hypoglycemia neuropathy; however, other mechanisms such as ischemia, might also play an important role as well.[10-12]

The depletion of energy within neurons may result in altered intraneural concentrations of various metabolites and lead to axonal degeneration seen during IIH.[12] Furthermore, neuron axons and Schwann cells are not only physical neighbours but also influence and support each other.[34,35] Hence, the myelin breakdown seen on Schwann cells during IIH may be caused directly by ATP depletion or caused indirectly by ATP depletion in neurons.[10-12] Local ischemia caused by decreased nerve blood flow leads to local hypoxia, which may result in axonal degeneration and myelin breakdown.[36,37] This phenomenon caused by ischemia change corresponds with energy deprivation as the cause of the changes in IIH related neuropathy.[12,34]

However, it is somewhat difficult at present to explain why large diameter myelinated motor fibers are particular vulnerable to IIH only based on mechanisms of energy deprivation or nerve ischemia.[12] If energy deprivation or nerve ischemia is the only mechanism involved in pathogenesis, it may be expected that small myelinated axons, which have higher per-volume metabolic rates than large myelinated axons, are more susceptible to oxygen deprivation or ischemia.[12,35] Since the opposite appears to be true, it is reasonable to hypothesize that the underlying mechanism involved in the pathogenesis of IIH related hypoglycemic neuropathy is complex and multifactorial.

Hypoglycemia Or Hyperinsulinemia is the Main Cause of PNS Changes

Previous studies have considered insulin to be a major cause in PNS images.[26-31] However, growing evidence suggests that insulin is beneficial for PNS function and serves as a promotor of axonal regeneration after injuries.[10-12] On the contrary, hypoglycemia during normal insulin levels causes axonal degeneration in the PNS.[12] Thus, the present available data seems to support that hypoglycemia, not hyperinsulinaemia, plays a more important role in IIH peripheral neuropathy.[10-12] It raises the question of whether hypoglycemia rather than hyperinsulinaemia or a combination of the two is the main cause of peripheral nerve injury. Further studies are needed to clarify this important issue.

In conclusion, the mechanisms involved in the pathogenesis remain poorly understood. Deeper understanding in pathogenesis from basic research is needed.

CONCLUSION

Clinical observational studies in human suggest that large myelinated motor fibers appear to be vulnerable to hypoglycemia. This is different from the common pattern of diabetic polyneuropathy with predominant sensory and autonomic neuropathy. Experimental animal models confirm the clinical observations. Future studies are needed to investigate the effects of frequency, severity and duration of hypoglycemia events on the progression, and the outcomes of hypoglycemic neuropathy in clinical setting. More experimental studies are also needed to provide mechanistic insights into the pathophysiology of hypoglycemic effects toward PNS.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

DISCLOSURES

All authors report no disclosures.

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Research

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Changes of Fat Volume and Adipocytokines by the Randomized Intervention Program for Obesity Control Program (SCOP)

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ABSTRACT

Adipocytokines are bioactive substances synthesized and secreted by fat cell. Previous studies have reported an association between weight loss and adipocytokines. However, these studies are inconsistent and they have not clarified the relationship between weight regain and changes in circulating levels of adipocytokines. In this study, we analyzed the relationship between weight and fat volume changes and adipocytokines. The subjects were 235 obese people recruited in the Saku Control Obesity Program (SCOP). Participants were randomly assigned to either immediate (Group A) or delayed (Group B, control group). Group A participants were followed for another two years after completion of the one year intervention. As controls, Group B participants received the same intervention as Group A after a delay of one year. Then they were followed up for one year. The intervention consisted in a one-year lifestyle program to induce weight loss, based on a cognitive-behavioral approach. After the first year of the study, body weight, Body Mass Index (BMI), body fat and abdominal fat areas were significantly lower in group A participants, compared to controls. After the intervention, leptin levels were significantly lowered both in men and women. After one year follow-up, both men and women re-gained about 1.5 kg body weights on average. BMI, waist circumference, fat areas by Computed Tomography (CT) and Glycated hemoglobin (HBA_{1c}) significantly increased during the follow-up period. The change of adipocytokine levels by analysis of the quartile of body weight decrease and regain revealed that increased adiponectin and decreased leptin was noteworthy for weight reduction, while increase of leptin influenced the weight regain. In conclusion, our results suggest that leptin could have broad effects on the distribution of fat tissues and on lipid metabolism. Leptin inversely associated with adiponectin, which in turn was necessary to decrease body weight. In particular, leptin decreased remarkably in the process of weight reduction, and its increase seemed to be related in weight regain. The observed increase of adiponectin seemed to be induced by reduction in fat volume.

KEYWORDS: Adiponectin; Leptin; Biomarker; RCT; Obesity control; Human.

ABBREVIATIONS: SCOP: Saku Control Obesity Program; BMI: Body Mass Index; CT: Computed Tomography; HBA_{1c}: Glycated hemoglobin; CHD: Coronary Heart Disease; T2D: Type 2 Diabetes; TNF-α: Tumor Necrosis Factor-alpha; FFAs: Free Fat Acids; MetS: Metabolic syndrome; TFA: Total Fat Area; SFA: Subcutaneous Fat Area; VFA: Visceral Fat Area; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; TG: Triglyceride; SRL: Special Reference Laboratories; HMW: High Molecular Weight; ELISA: Enzyme-linked immunosorbent assay; ANOVA: Analysis of variance; SPSS: Statistical Pack-

age for the Social Science; CRP: C-reactive protein; RCT: Randomised Controlled Trial; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FFA: Free Fatty Acid; AMPK: AMP-activated protein kinase.

INTRODUCTION

Overweight and obese individuals are at a greater risk for developing Coronary Heart Disease (CHD), Type 2 Diabetes (T2D), and certain type of cancer compared with their normal-weight counterparts.[1,2] Weight loss can reduce these risks, but weight maintenance after weight loss is difficult to achieve.[3-5]

Adipose tissue has been considered to be the energy storage tissue, but recent studies have shown that fat cells synthesize and secrete various bioactive substances called adipocytokines, such as leptin, adiponectin, Tumor Necrosis Factor-alpha (TNF-α), Free Fat Acids (FFAs), resistin and angiotensinogen.[6] Most of the studies on adipocyte-derived cytokines have so far focused on the two adipocytokines, adiponectin and leptin, because of their role in the regulation of the metabolic homeostasis.[7-9]

Adiponectin is associated with insulin sensitivity and atherosclerosis. Despite the fact that adiponectin is secreted by the adipose tissue, plasma levels are lower in individuals with obesity, insulin resistance and T2D.[10-12]

Leptin inhibits food intake, stimulates energy expenditure, and regulating immune function.[13] In humans, serum leptin levels are positively correlated with obesity, T2D, hypertension and Metabolic syndrome (MetS).[14,15] Therefore, it is generally considered that obese people are associated with a state of resistance to the effects of leptin.[16,17]

Thus, adiponectin and leptin play an important role on obesity and metabolic disorders. Previous studies have reported an association between weight loss and adipocytokines.[18] However, these studies are inconsistent and they have not clarified the relationship between weight regain and changes of adipocytokines.[19-21] For example, Ambeba et al[19] conducted a 24-month weight loss trial and reported that adiponectin increased with weight reduction and decreased with weight regain. On the other hand, Bluher et al[20] reported continued increase in adiponectin levels with both weight loss and weight regain. They also reported that leptin levels decreased with weight reduction and increased with weight regain. In addition, Crujeiras et al[21] reported that leptin continued to decrease throughout weight reduction and regain. Using the effective intervention program for obesity control developed by our group, a nested randomized intervention trial in human dock examinees was designed to evaluate the multiple metabolic changes and factors involved in weight reduction. Plasma levels of adiponectin, leptin and other biomarkers could be analyzed in relationship with the body weight reduction and changes in fat volume.

SUBJECTS AND METHODS

Study Subjects

This study was performed as part of the Saku Control Obesity Program (SCOP). The outline of SCOP has been described previously.[22] The SCOP study protocol initially included 235 Japanese obese subjects (116 men and 119 women) recruited from the database of medical checkup record of Saku Central Hospital Human Dock Center, Nagano, Japan. The study participants had medical checkups since 2000 and were aged 40-64 years old, with a body mass index (Body Mass Index (BMI): kg/m^2)) greater than 28.3 (the upper 5 percentile of all examinees). They were asked to participate in an intervention program for weight control, i.e. SCOP. Exclusion criteria were: psychiatric disease; physical conditions (i.e., morbid hepatic or renal dysfunction; cardiovascular disease such as heart failure, stroke, and transient ischemic attacks); patients who were under treatment for obesity or any treatment known to affect eating or weight. Participants were randomly assigned to either immediate (Group A) or delayed intervention (Group B, control group). After guidance with written and oral information, including the purpose of the study, assurance of refusal, and confidentiality of personal information, written informed consent was obtained from all participants. The study protocol was approved by the Ethics Committee of the National Institute of Health and Nutrition.

Study Design

Participants were given a one-year lifestyle intervention program for weight loss, based on a cognitive-behavioral approach.[23-32] The program was conducted at the Saku Central Hospital Human Dock Center from July, 2006. The participants received individual counseling (30 minutes) from a registered dietician, and group sessions about effective physical activity (20 minutes) by exercise instructors. Body composition parameters were measured at baseline and at 1, 3, 6 and 9 months. The integrative interventions have been reported in detail previously.[23-32] Group A participants were followed for two years after one year intervention, without any period. Clinical and biological parameters were assessed at baseline (0 month), after the end of intervention (12 month) and during the follow-up (24 month) (Figure 1).

As controls, Group B participants received the same intervention as Group A after a delay of one year. Then they were followed up for one year. They received precise health check-up at 0 month, 12 month, 24 month and 36 month in the same way as Group A participants (Figure 1).

The height and weight of the subjects were measured with an automatic scale (Tanita, BF-220, Tokyo, Japan). Their BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured twice at the umbilicus level while the subject was in a standing position,

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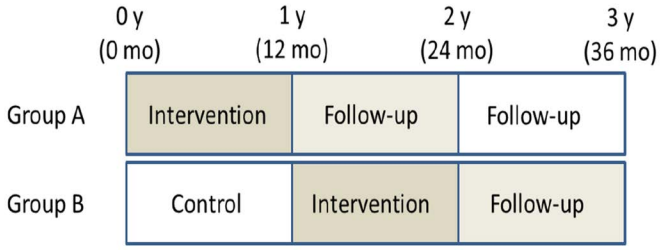

Figure 1: Study design.

using a fiberglass measuring tape. The average measurement was used for the analysis. Blood pressure was measured while the subject was in a sitting position using a validated automated blood pressure monitor (ES-H55; Terumo, Tokyo, Japan). Total fat areas were assessed by a computed tomography scan at the level of the umbilicus, with the subjects in the supine position. The Total Fat Area (TFA), Subcutaneous Fat Area (SFA) and Visceral Fat Area (VFA) were calculated using commercially available software (Fat Scan; N2 System Corp, Osaka, Japan). Following an overnight fast, blood samples were collected at the time of each health checkup at the Saku Human Dock Center. High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C), Triglyceride (TG), HbA_{1c} and other blood and biochemical values were analyzed at the clinical laboratory of the Saku Central Hospital.

Adipocytokines (adiponectin, leptin, TNF-α, FFA) were measured using laboratory testing services provided by Special Reference Laboratories (SRL) Inc. (Tokyo, Japan). The High Molecular Weight (HMW) form of adiponectin (μg/mL) was measured by Enzyme-linked immunosorbent assay (ELISA) with a detection limit of 0.18 μg/mL. Leptin (ng/mL) was measured by a radioimmunoassay (Human Leptin RIA Kit, LINCO Research, St. Charles, MO, USA) with a sensitivity of 0.5 ng/mL.

Prevalence of metabolic abnormality, hypertension, dyslipidemia and diabetes mellitus, was determined according to Japanese diagnostic criteria for metabolic syndrome. The cut-off values defining metabolic abnormalities were established at 130/85 mmHg for high blood pressure; ≥150 mg/dL triglyceride and/or <40 mg/dL HDL cholesterol for dyslipidemia; and 110 mg/dL for high blood glucose; or patients being treated for hypertension, dyslipidemia, and/or diabetes .

Data Analysis

Statistical analysis was focused on the effect of intervention by Randomised Controlled Trial (RCT) design so that we compared Group A as intervention group and Group B as control group. Furthermore, we analyzed degree of rebound after intervention by combined group A and B data (Group A+B). Data are presented as mean±SD. Adipocytokines and C-reactive protein (CRP) were log-transformed to fit normality or linearity assumption for statistical analysis. The serum level of adipocytokines and CRP are presented the median and range (25 percentile and 75 percentile). Independent sample t-test was used to compare intervention group and control group in baseline and 1 year later. Spearman correlations were used to test the associations between body composition and blood data. In Group A+B, paired t-test was used to compare end point of intervention and follow-up. Weight reduction was calculated as the difference between before and after the intervention. Weight regain was calculated as the difference during follow-up period. Weight reduction and regain values are separated quartile groups, respectively. The significance of differences among that quartile were analyzed with one-way Analysis of variance (ANOVA) followed by Bonferroni and Games-Howell post hoc test. Statistical analysis was performed by Statistical Package for the Social Sciences (SPSS) version 20.0 software (SPSS Inc., Chicago, IL, USA).

Results

Effect of the Intervention by RCT

Baseline characteristics for men and women are displayed in Table 1. The mean age of the study population was 53.8 years for men and 54.7 years for women. BMI was 30.2±3.1 kg/m^2 for men and 31.0±3.0 kg/m^2 for women, the waist circumference was 100.0±6.5 cm and 103.2±8.1 cm, and the visceral fat area was 157±48 cm^2 and 129±47 cm^2 (mean±SD) (Table 1).

After one year of intervention we observed a 5% body weight reduction (6.1 kg in men and 4.0 kg in women in average) in 51% participants, 10% reduction in about one fourth participants. The changes of body weight, BMI, fat% in body composition, waist circumference, fat area by CT, and selected biochemical data by group are shown in Table 2. After the intervention, body weight, BMI, body fat and abdominal fat areas were significantly less than those of controls.

Adipocytokines, lipid, glucose and HbA_{1c} levels of intervention and control groups are shown in Table 3. Leptin was significantly lowered than control in both men and women after the intervention. Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) improved after the intervention. Only in women, TNF-α was significantly increased by intervention (Table 3).

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n	unit	116	119
Age	years	53.8±6.5	54.7±6.4
Height	cm	168.4±6	155.1±5.7
Weight	kg	85.7±10.4	74.8±9.4
BMI	kg/m^2	30.2±3.1	31.0±3.0
Waist circumference	cm	100.0±6.5	103.2±8.1
Body fat	%	28.8±4.1	40.7±5.4
Total fat area	cm^2	404±111	468±111
Sq fat area	cm^2	247±91	339±90
Vis fat area	cm^2	157±48	129±47
Total cholesterole	mg/dl	204±29	217±39
HDL-cholesterole	mg/dl	50±10	56±12
LDL-cholesterole	mg/dl	121±28	131±33
Triglyceride	mg/dl	171±120	151±81
FFA	mEq/L	0.5±0.2	0.6±0.2
TNF-α	pg/ml	1.1(0.9-1.4)	1.2(0.9-1.4)
Leptin	ng/mL	6.7(4.9-9.2)	18.0(13.6-25.3)
Adiponectin	μg/mL	2.2(1.6-3.6)	4.8(3.5-7.4)
CRP	mg/dl	0.12(0.07-0.18)	0.11(0.07-0.24)
SBP	mmHg	133±15	136±18
DBP	mmHg	82±13	83±12
Fasting glucose	mg/dl	111±26	114±27
HBA_{1C}	%	5.8±1.1	5.9±1.2

Mean±SD. Adipocytokines and CRP showed median and range (25 percentile and 75 percentile).

Table 1: Baseline data of the participants.

At baseline health examination revealed the prevalence of dyslipidemia was 64.3% and 40.4% in men and women, high blood pressure was 69.6% and 67.3%, and high blood glucose was 35.7% and 38.5%. The prevalence were improved 19% in both sex in dyslipidemia, 21% and 11% in men and women in high blood pressure, 5% and 30% in high blood glucose, respectively.

Effect of Intervention with Group A+B

Effect of intervention in fat tissues and adipocytokines: BMI and fat area usually showed good correlations in both males and females (Table 4). TG correlated with visceral fat and Free Fatty Acid (FFA). After the intervention TG showed strong positively correlation with leptin and negatively correlation with adiponectin than before the intervention, except for adiponectin in women. Adiponectin and leptin inversely correlated only in men. Leptin was widely interrelated among BMI and various fat areas, but not with TG, FFA and HbA_{1c} in women before the intervention and FFA and HbA_{1c} after the intervention. In men leptin and TG was significantly correlated. TNF-α correlated with visceral fat, TG, FFA, leptin, and HbA_{1c} in women before the intervention, but after the intervention only TG and HbA_{1c} remained. In men TNF-α did not show any association with the above variables.

The change of weight, adiponectin and leptin between before and after the intervention is shown by the quartile of body weight reduction (Figure 2). Leptin decrease and increase of adiponectin was noticed in upper quartile of body weight reduction by intervention both men and women.

Weight regain during follow-up period: Weight, BMI, waist circumference, HBA_{1c}, TFA and VFA were significantly increased at the end of follow-up in Group A+B analysis. Both men and women gained about 1.5 kg body weight gain on average. However, these the end of follow-up values remained significantly

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	A: Intervention group		B: Control group			
	M(n=56)	F(n=52)	M(n=49)		F(n=52)	
Age	53.7±6.7	55.0±6.6	53.9±6.3		54.5±6.2	
Height	167.9±5.9	155.1±5.7	168.9±6.0		155.2±5.7	
Body wt 0y	84.2±8.5	74.5±8.4	87.3±12.1		75.2±10.3	
Body wt 1y	79.3±8.7	70.7±9.3	87.5±13	**	75±11	*
Body wt difference 0_1y	5.0±5.1	3.8±3.7	-0.2±2.8	**	0.2±2.5	**
BMI 0y	29.8±2.3	31.0±2.9	30.6±3.8		31.1±3.0	
BMI 1y	28.1±2.5	29.4±3.4	30.5±4.2	**	31.0±3.2	*
BMI difference 0_1y	1.7±1.8	1.6±1.5	0±1.0	**	0.1±1.0	**
Body fat 0y	28.5±3.6	39.7±5.2	29.2±4.6		41.8±5.4	*
Body fat 1y	26.8±4.4	37.8±5.9	29.6±5.1	**	41.8±5.8	**
total fat 0y	393±82	467±98	417±137		468±123	
sq fat 0y	243±66	343±80	253±114		335±100	
abd fat 0y	150±48	125±47	164±48		133±47	
total fat 1y	333±81	402±93	397±104	**	455±108	**
sq fat 1y	207±62	302±76	238±96	*	326±88	
abd fat 1y	126±46	100±38	159±48	**	130±45	**
total fat difference 0_1y	60±64	65±57	19.7±57.9	**	13.2±44.0	**
sq fat difference 0_1y	36±38	41±44	14.3±32.6	**	9.7±30.7	**
abd fat difference 0_1y	24±33	24±26	5.4±33.3	**	3.5±22.4	**

Significantly different between intervention and control groups.

*:$P<0.05$,**:$P<0.01$

Sq: subcutaneous, abd: abdominal.

Table 2: Baseline and post intervention data of the participants by the arm.

lower than those before the intervention (Table 5).

Leptin value at the end of follow-up showed significantly lower than the end of intervention for men and women. Adiponectin and TNF-α was significantly decreased at the end of follow-up than at the end of intervention both men and women (Table 5).

The change of weight and adipocytokine levels for weight regain groups between follow-up periods is shown by the quartile of body weight regain (Figure 3). Leptin was significantly higher in upper quartile of weight regain.

DISCUSSION

In this study, we achieved a 5 percent body weight loss through a one-year lifestyle intervention program using a behavioral approach. In parallel with the weight reduction, many metabolic syndrome-related factors significantly improved. During the intervention, energy source, crops and diary decreased, while the intake of green yellow vegetables increased.[27] Eating speed improved and physical activity also increased compared to the control group.[32] Especially in Group A, women increased their daily physical activity and improved their irregular eating habits during the follow-up period. Eating motivation could be changed through the cognitive-behavior approach.

Following encouragements from trainers, physical activity seemed to be more frequent during the intervention period. This did not last during the follow-up period, while dietary habits seemed to be maintained even after intervention. Altered dietary habit could contribute to the later life. As body weight significantly increased at the end of follow-up examination compared to the weight at the end of the intervention program, fixation of

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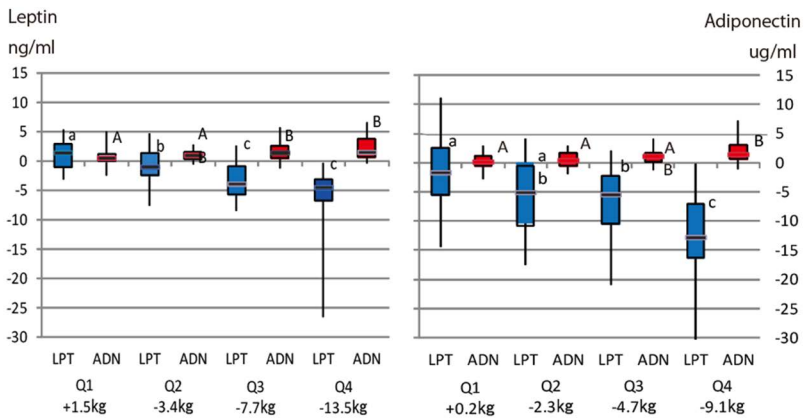
	A: Intervention group		B: Control group			
	M(n=56)	F(n=52)	M(n=49)		F(n=52)	
Triacylglycerol 0y	170±98	131±61	173±141		172±94	**
Triacylglycerol 1y	143±74	118±58	159±72		164±87	**
LDL cholesterol 0y	123±29	128±32	119±28		134±35	
LDL cholesterol 1y	124±31	129±26	123±29		130±30	
HDL cholesterol 0y	48±11	56±12	52±9		56±12	
HDL cholesterol 1y	50.3±12.7	57.2±13	48.7±8.8		53.5±12	
FFA 0y	0.53±0.17	0.60±0.20	0.48±0.20		0.55±0.22	
FFA 1y	0.43±0.17	0.50±0.18	0.40±0.17		0.50±0.17	
TNFα0y	1.1(0.9-1.5)	1.2(0.9-1.4)	1.2(1.0-1.4)		1.2(0.8-1.4)	
TNFα1y	1.3(1.1-1.5)	1.4(1.1-1.7)	1.3(1.1-1.5)		1.1(1.0-1.4)	**
Leptin 0y	6.7(4.2-9.1)	17.7(13.2-23.5)	6.9(5.2-9.4)		18.9(13.9-25.7)	
Leptin 1y	5.7(3.8-8.8)	15.7(10.7-20.7)	8.3(5.7-11.4)	**	22.3(16.2-29.3)	**
Adiponectin 0y	2.2(1.5-3.6)	5.1(4.1-8.4)	2.3(1.7-3.7)		4.3(2.5-5.6)	**
Adiponectin 1y	3.2(2.1-5.9)	6.8(5.1-10.2)	2.7(2.0-4.5)		5.5(3.8-8.6)	*
CRP 0y	0.11(0.08-0.18)	0.10(0.06-0.21)	0.10(0.06-0.21)		0.13(0.08-0.26)	
CRP 1y	0.07(0.05-0.14)	0.08(0.04-0.13)	0.08(0.04-0.13)		0.14(0.07-0.24)	
SBP 0y	132±15	133±16	134±15		139±19	
SBP 1y	126±14	127±17	134±18	*	135±18	*
DBP 0y	81±14	81±11	83±12		84±12	
DBP 1y	79±11	78±12	85±13	*	85±12	*
Fasting blood glucose 0y	111±28	112±30	112±23		115±25	
Fasting blood glucose 1y	109±24	110±36	116±20		112±19	
HbA_{1c} 0y	5.7±1.1	5.9±1.2	6.0±1.1		6.0±1.2	
HbA_{1c} 1y	5.5±0.8	5.6±1.0	5.8±0.8	*	5.7±0.8	

Significantly different between intervention and control groups.

*:P<0.05,**:P<0.01

FFA: Free Fatty Acid, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure.

Table 3: Changes of adipocytokine, serum lipids and some biochemial data by arm.



The distinct of character shows statistically significant difference (p<0.05; A-B and a-b;, p<0.01 a-c).

Figure 2: Leptin (blue) and adiponectin (red) concentration after intervention by quartile of body weight loss.

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1y end / baseline	BMI 1y	total fat 1y	sq fat 1y	vis fat 1y	TG 1y	FFA 1y	HBA_{1C} 1y	L_leptin 1y	L_adipo 1y	L_TNF 1y	**after the intervention**
BMI 0y		.873**	.807**	.461**	.278**	.195	.281**	.622**	-.336**	-.026	BMI 1y
total fat 0y	.846**		.852**	.635**	.293**	.164	.279**	.738**	-.219*	-.095	total fat 1y
sq fat 0y	.817**	.859**		.136	.091	.145	.101	.632**	-.106	-.064	sq fat 1y
vis fat 0y	.266**	.495**	-.021		.421**	.097	.378**	.464**	-.257**	-.085	vis fat 1y
TG 0y	.125	.088	-.043	.246*		.233*	.122	.429**	-.436**	-.039	TG 1y
FFA 0y	-.006	.032	.015	.037	.311**		.062	.129	-.192	-.141	FFA 1y
HBA_{1C} 0y	.185	.128	.008	.236*	.124	.127		.241*	-.147	.038	HBA_{1C} 1y
L_leptin 0y	.608**	.668**	.618**	.255**	.227*	-.036	.166		-.356**	-.028	L_leptin 1y
L_adipo 0y	-.128	-.067	.018	-.162	-.275**	-.244*	-.105	-.080		-.081	L_adipo 1y
L_TNF 0y	.059	.013	.076	-.103	-.094	-.014	.026	.079	-.108		L_TNF 1y
baseline	BMI 0y	total fat 0y	sq fat 0y	vis fat 0y	TG 0y	FFA 0y	HBA_{1C} 0y	L_leptin 0y	L_adipo 0y	L_TNF 0y	

Men(n=102)

*:P<0.05,**:P<0.01

1y end / baseline	BMI 1y	total fat 1y	sq fat 1y	vis fat 1y	TG 1y	FFA 1y	HBA_{1C} 1y	L_leptin 1y	L_adipo 1y	L_TNF 1y	**after the intervention**
BMI 0y		.846**	.777**	.535**	.253*	.175	.185	.620**	-.057	.040	BMI 1y
total fat 0y	.802**		.924**	.622**	.253*	.103	.189	.622**	-.127	.019	total fat 1y
sq fat 0y	.722**	.904**		.274**	.128	.031	.049	.546**	-.039	-.046	sq fat 1y
vis fat 0y	.477**	.588**	.186		.372**	.196	.375**	.445**	-.238*	.141	vis fat 1y
TG 0y	.077	.092	-.054	.314**		.137	.212*	.260**	-.150	.245*	TG 1y
FFA 0y	.227*	.118	.033	.208*	.153		.275**	.000	.072	.157	FFA 1y
HBA_{1C} 0y	.022	.055	-.107	.328**	.217*	.382**		.139	-.260**	.254*	HBA_{1C} 1y
L_leptin 0y	.550**	.549**	.462**	.387**	.126	-.062	-.061		-.150	.118	L_leptin 1y
L_adipo 0y	.054	.072	.125	-.072	-.192	.008	-.171	-.050		-.112	L_adipo 1y
L_TNF 0y	.145	.210*	.032	.421**	.248*	.250*	.268**	.218*	-.202*		L_TNF 1y
baseline	BMI 0y	total fat 0y	sq fat 0y	vis fat 0y	TG 0y	FFA 0y	HBA_{1C} 0y	L_leptin 0y	L_adipo 0y	L_TNF 0y	

Women(n=98)

*:*P*<0.05,**:*P*<0.01

Table 4: Correlation analysis between fat and adipocytokines.

good health habit would be very difficult without decision of individuals.

Decrease and increase of fatty tissue should be a basis of body weight change. Adipose tissue is a major source of energy for the human body. It is also a source of major adipocytokines, adiponectin and leptin.[6] In this study, leptin changed markedly in the process of weight reduction and regain. Adiponectin decreased according to the weight reduction, but not changed between weights regain quartile. Leptin seemed to be highly sensitive to weight changes than adiponectin.

A few studies have explored the relationship between weight change and changes of adipocytokines, but results are inconsistent.

Leptin inhibits food intake, stimulates energy expenditure, and regulating immune function.[13] In humans, serum leptin levels are positively correlated with obesity, T2D, hypertension and Metabolic syndrome (MetS).[14,15] Leptin levels increase in obesity and subcutaneous fat has been a major determinant of circulating leptin levels. The net action of leptin is to inhibit appetite, stimulate thermogenesis, enhance fatty acid oxidation,

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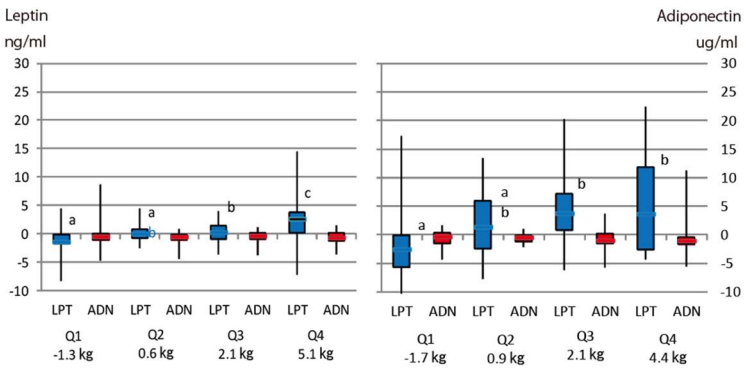
		Men(n=102)			Women(n=98)		
		End intervention	End follow-up		End intervention	End follow-up	
		mean±SD	mean±SD	t	mean±SD	mean±SD	t
Weight	kg	79.9±10.1	81.5±10.3	**	70.7±10.1	72.0±10.2	**
BMI	kg/m^2	28.2±3.0	28.8±3.1	**	29.3±3.4	29.9±3.4	**
Waist circumference	cm	95.8±8.6	97.5±8.4	**	99.1±9.4	100.7±9.2	**
Total fat area	cm^2	333±95	366±103	**	403±100	425±99	**
Sq fat area	cm^2	204±74	213±75	**	302±82	307±84	
Vis fat area	cm^2	129±50	153±61	**	102±40	118±44	**
Total cholesterol	mg/dl	194±34	196±31		210±27	213±33	
HDL-cholest	mg/dl	52±11	52±12		57±12	57±12	
LDL-cholest	mg/dl	118±30	121±29		129±26	133±30	
Triglyceride	mg/dl	132±76	143±79		117±58	120±54	
Leptin	ng/mL	4.9(3.3-7.4)	5.5(3.8-7.7)	*	14.0±(8.4-19.0)	15.0±(10.2-21.7)	**
Adiponectin	µg/mL	3.4(2.2-5.6)	2.9(1.8-5.0)	**	6.3±(4.8-9.6)	5.6±(4.1-8.4)	**
TNF-α	pg/ml	1.3(1.1-1.5)	1.2(1.0-1.5)	*	1.3±(1.0-1.6)	1.2±(1.0-1.3)	*
FFA	mEq/L	0.5±0.2	0.4±0.2		0.5±0.2	0.5±0.2	
CRP	mg/dl	0.07(0.04-0.15)	0.08(0.04-0.18)		0.08(0.04-0.13)	0.09±(0.04-0.16)	
SBP	mmHg	128±15	128±14		128±18	126±16	
DBP	mmHg	80±11	80±11		80±11	77±10	**
Fast glucose	mg/dl	108±22	108±20		109±29	108±23	
HBA_{1C}	%	5.6±0.9	5.7±0.8	*	5.7±0.9	5.8±0.9	*

Significantly different between end of intervention and follow-up.

*:P<0.05,**:P<0.01

Table 5: Changes of body weight and biomarkers during follow-up.



The distinct of character shows statistically significant difference (p<0.05; A-B and a-b;, p<0.01 a-c).

Figure 3: Difference of leptin (blue) and adiponectin (red) concentration during follow-up period by quartile of body weight gain.

decrease glucose, and reduce body weight and fat. Our results suggest that leptin change associated with weight reduction and regain, and other effects on metabolic markers.

Weight loss significantly elevates plasma adiponectin levels. We measured HMW-adiponectin in this study. Adiponectin is present in serum as a trimer, hexamer, or high molecular weight form. HMW adiponectin is closely related to coronary artery disease and weight reduction, it affects insulin-sensitizing.[33,34] Moreover, HMW-adiponectin contributes to the activation of AMP-activated protein kinase (AMPK), to the suppression of endothelial cell apoptosis, and to cytostatic activity.[35,36] We propose that early reports describing variable effects of adiponectin should be interpreted with caution, as HMW adiponectin is a more reliable assay reflecting the active form of adiponectin. A reduction in adiponectin has previously been associated with insulin resistance, dyslipidemia, and atherosclerosis in humans.

TNF-α significantly increased by intervention in women even though body weight and fat area decreased. At the end of follow-up, TNF-α significantly decreased than at the end of intervention both in men and women even though body weight and fat area increased. TNF-α is secreted from not only fat cells but also macrophages. Thus, the changes of TNF-α may be influenced by other factors independently from body weight, obesity or fat cell. It may need further study in the future.

Ambeba et al[19] conducted the 24 month weight loss trial. They reported that adiponectin increased with weight reduction and decreased with weight regain. These results are similar to our observations. On the other hand, Bluher et al[20] reported an increase in adiponectin with weight loss. However, there was a continued increase in adiponectin levels with weight regain. There are several differences between Bluher's and our study. First, we conducted separate statistical analysis between sexes, while they analyzed aggregate data. Furthermore, most of the participants were men (86%). Bluher et al[20] conducted a 2-year intervention for weight loss, using calorie or carbohydrate diet restrictions.

Our study has several limitations. We could set control group in intervention phase by cross-over design, as Group B participants waited their intervention for one year as a control group of A. However, follow-up period of Group A could not be combined to control group of intervention, because they trailed the effect of intervention. It was impossible to set monthly or yearly wash out period, so this problem should be elaborated in the future epidemiological design of human study. So, there was no control group in follow-up phase. Secondly, the relationship between dietary and physical activity was not sufficiently connected to the adipocytokine changes. Individual health conditions, different absorption capacities, and different intestinal microbiota could affect the metabolism of individuals and their predisposition to obesity or leanness.[37] Further studies are necessary to better integrate individual holistic variables.

In conclusion, our results suggest that leptin should have broad effects among fat tissues and lipid metabolism. Leptin inversely associated with adiponectin, which in turn was necessary to decrease body weight. In particular, leptin decreased remarkably in the process of weight reduction, and its increase seemed to be related in weight regain. Increase of adiponectin seemed to be induced by the reduction of fat volume.

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CONFLICTS OF INTEREST

The authors declare that they has no conflicts of interest.

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