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Establishment and application of a quick screening method for hypoglycemic compounds

Hsueh-Ling Cheng,
Graduate Institute of Biotechnology

Introduction

According to the data from World Health Organization and the International Diabetes Federation, the number of patients of diabetes mellitus was around 100 ~ 135 million worldwide in 1994-1995. However, it was increased to 246 million in 2007, and 95% of which belonged to type 2 diabetes. It is believed that this amazing rate of increasing is due to the living style and diet of modern life. In USA, the increment of diabetic patients results in financial difficulties for the health insurance system, which is predicted to be bankrupt in 2030. Thus, the prevention and treatment of type 2 diabetes has become an importance issue.

In mammals, blood glucose is raised after a meal. The raised blood glucose provokes the secretion of insulin, which promotes glucose uptake by cells of the body and thus lowers the concentration of blood glucose. In patients of type 2 diabetes, cells do not respond to insulin normally (referred to as insulin resistance) and lose their abilities for insulin-promoted glucose uptake, resulting in hyperglycemia. The prevalence of type 2 diabetes is attributed to high calorie intake and lack of exercise in modern life style. Therefore, weight control and proper exercise is theoretically the major approach for the prevention and treatment of diabetes. Nonetheless, this approach is well known but difficult to be executed, as reflected by the amazing increment of diabetic patients.

Middle-aged individuals and the elderly have decreased rates of metabolism, which cause the difficulty of weight control and bring about the accumulation of body fat that renders them be prone to the development of type 2 diabetes. Some scholars suggested that human beings have evolved this tendency of fat accumulation for efficient storage of energy in order to resist famine in the ancient times. Therefore, in addition to efforts in weight management, some may need the assistance of medicine or health foods for the prevention of diabetes.

The currently available medicines for clinical treatment of diabetes are not able to cure the disease. Some even have side effects. On the other hand, some diabetic patients may adjust to the medicine, resulting in the requirement for increasing dosages for the control of the disease. Thus, scientists are still looking for effective medicine for diabetes. Plants and herbs are important sources for the pursuit of hypoglycemic molecules.

For example, Momordica charantia, also known as bitter gourd or bitter melon, has been widely consumed as a vegetable as well as herbal medicine for diabetic patients in Asia, Africa and South America. The crude extract from the fruit, seed, foliage, or whole plant of M. charantia has been reported effective by many studies in the treatment of diabetes in animal models. However, the exact hypoglycemic constituents in these extracts were not clear due to the lack of an effective assay or screening method.

To analyze the hypoglycemic activity of a compound, a direct assay is animal tests, in which diabetic animal models are fed with the compound and blood glucose levels are checked afterwards to examine hypoglycemic effects. However, the low-throughput property of animal tests limits their application for the screening of bioactive compounds from complicated sources, such as the extracts of plants. Thus, to explore the hypoglycemic components present in the extract of bitter melon or other plants, we tried to develop a cell-based method for the rapid screening of hypoglycemic molecules.

The establishment of a method for quick screening of hypoglycemic molecules

Cell-based methods for the assays of hypoglycemic activities of molecules had been reported in the literature. In these methods, isotope-labeled glucose analog was used to be ingested by cells. The radioactivities of the cells were then determined to evaluate glucose uptake of the cells. However, the safety and handling of radioactive molecules is always a concern. The use of a large amount of radioactive materials should be avoided. Thus, this method is not suitable for high throughput screening. It is usually used for the study of working mechanism of a compound that is known to have a hypoglycemic activity (only a small amount of radioactive glucose analog is needed to be used in this way). Hence, a fluorescence-labeled glucose analog was synthesized as a replacement. Nonetheless, it was found that, due to the high sensitivity of fluorescence, the background interference of this glucose analog was serious, causing significant errors and failure of the experiments. Moreover, the fluorescence-labeled glucose analog is quite expensive that renders it not affordable for the budget if used in high throughput screening. Based on these observations, we decided to develop a new method that can provide simple, safe, quick and inexpensive screening of hypoglycemic molecules from complicated sources.

A mouse liver cell line was treated with a reagent to induce insulin resistance of the cells. Subsequently, the cells were treated with insulin and a sample (a molecule, or an extract or fraction of a

plant, etc.). At 0, 1, 2, 3, 4, 5 hours after the treatment, aliquots of the cell culture medium were withdrawn and assayed for glucose concentrations (Figure 1).

Figure 1. The operation of the quick screening method for hypoglycemic molecules. A, culture of cells for the screening of hypoglycemic molecules; B, rapid screening and analysis for hypoglycemic molecules using 96-well plates.

If the reduction of glucose concentration with time in the medium is similar with that of the control (insulin-resistant cells stimulated with insulin only; Figure 2, resistant+insulin), the sample is considered of no hypoglycemic activity (e.g. resistant+insulin+sample 1 in Figure 2); if the reduction of glucose concentration with time in the medium is obviously faster than that of the control, and is similar with that of normal cells stimulated with insulin (Figure 2, normal+insulin), the sample is considered to have a hypoglycemic activity (e.g. resistant+insulin+sample 2 in Figure 2).

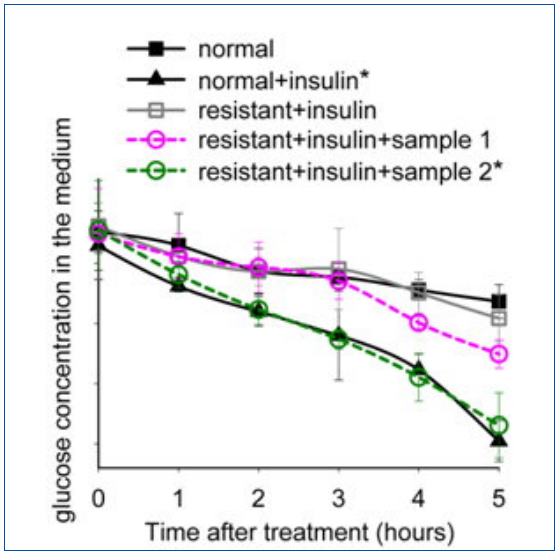

Figure 2. An example of results from the quick screening of hypoglycemic molecules. Normal represents normal cells without any treatment； normal+insulin is normal cells stimulated with insulin; resistant+insulin is insulin-resistant cells stimulated with insulin; resistant+insulin+sample 1 is insulin-resistant cells treated with insulin and sample 1; resistant+insulin+sample 2 is insulin-resistant cells treated with insulin and sample 2. The x-axis indicates the time after sample treatment; glucose concentrations of the media, plotted as the y-axis, are assayed at the time points indicated on the x-axis. *$p < 0.05$ when statistically analyzed against "resistant+insulin". The data reveal that sample 2 possesses a hypoglycemic activity, sample 1 does not.

The application of the screening method for hypoglycemic molecules

The application of the method described above has afforded the identification of several hypoglycemic molecules from the strain of bitter melon that is most commonly consumed among others in Taiwan, and from a new strain of wild bitter melon provided by Hualien District Agricultural Research and Extension Station, Council of Agriculture. Some of these molecules have been filed for patents. Fractions of bitter melon or wild bitter melon that contained these molecules were confirmed to have hypoglycemic effects in vivo in hyperglycemic animal models. The data obtained from the screening of molecules from bitter melon have been published (Journal of Agricultural and Food Chemistry 56, 6835-6843, 2008). This is the first article able to illustrate a systematic screening of hypoglycemic constituents from the crude extract of a plant.

Moreover, we found that Cucurbita moschata contains hypoglycemic molecules as well. Fractions that contain hypoglycemic compounds have been identified by screening. Animal tests have confirmed the hypoglycemic effects of some of these fractions in vivo. Identification of the hypoglycemic constituents of these fractions is being carried out. Meanwhile, a university and industry collaboration project supported by Pingtung Agricultural Biotechnology Park is undertaken, in which this screening method is used to evaluate the hypoglycemic activities of materials provided by the collaborating company, and to identify the hypoglycemic constituents in the materials. This screening method can be applied continuously for the screening of hypoglycemic molecules from various sources.

Preliminary Study on the Toxicity and Recycle ability of Municipal Solid Waste Incineration Ashes

Wu-Jang Huang,
Department of Environmental Engineering and Science

Since 1990, the Taiwan government started to treat the municipal solid waste (MSW) either by incineration and land-filling, in which the incineration is more favor. Until now there are 21 large scale municipal solid waste incineration plants (MSWI) to recycle the valuable metals and energy from wastes. About 80% of original MSW is burned out in the MSWI, the un-combustible parts of MSW have become ash wastes MSWI, for example fly ash (including the scrubber residue and baghouse ash or electrostatic ash) and bottom ash. Figure 1 shows the flow chart of flue gas treatment and ash waste generation units in the MSWI equipped a semi-dry de-SOx and NOx system. Among these wash wastes, fly ash containments dioxins and heavy metals, it is classified as hazardous materials; while the bottom ash is classified as non-hazardous materials and general industrial wastes, due to that it is fully filled the regulation of toxic characteristic leaching procedure (TCLP) test. According to the statically data of Taiwan EPA in 2008, the total reuse amount of MSWI ash wastes was about 52%, most of the resued ash wastes is bottom ash. The MSWI fly ash is difficult for recycling or resues. Table 1 shows the chemical compositions of scrubber residue; results indicate that the scrubber ash has relatively lower calcium content and high silicate content, which is closed to the chemical compositions of high alumina cement. Table 2 summarized the analyzed chemical composition of baghouse ash; results indicate that baghouse ash has high calcium content and relatively lower silicate content. It is closed to the chemical composition of Portland Type I cement.

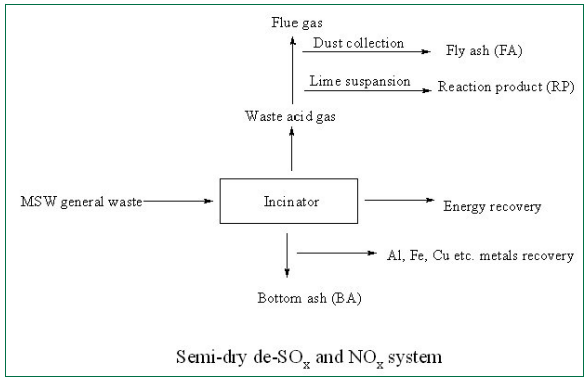

Figure 1 The Flow Chart of Flue Gas Treatment and Ash Wastes Generation Point in the Municipal Solid Waste Incineration Plant Equipped a Semi-dry de-SOx and NOx system

Plant	SiO_2	Al_2O_3	CaO	CaO/SiO_2	CaO/Al_2O_3
1	32.20	12.0	38.45	1.19	3.20
2	20.60	39.31	26.71	1.30	0.68
3	19.60	46.31	32.20	1.64	0.69
4	36.48	32.63	26.25	0.72	0.80
5	21.08	44.71	26.14	1.24	0.58
6	24.28	33.59	38.28	1.58	1.14
7	36.68	24.61	28.16	0.77	1.14

Table 1 Chemical Compositions of Scrubber Residue

Plant	SiO_2	Al_2O_3	CaO	CaO/SiO_2	CaO/Al_2O_3
1	9.26	26.33	55.21	5.96	0.35
2	9.12	16.15	50.75	5.56	0.56
3	17.57	32.30	42.36	2.41	0.54
4	3.12	42.82	41.39	13.27	0.19
5	3.76	37.48	45.65	12.14	0.10
6	6.80	16.40	55.20	8.12	0.41
7	3.91	37.93	42.60	10.90	0.10

Table 2 Chemical Compositions of Baghouse Ash

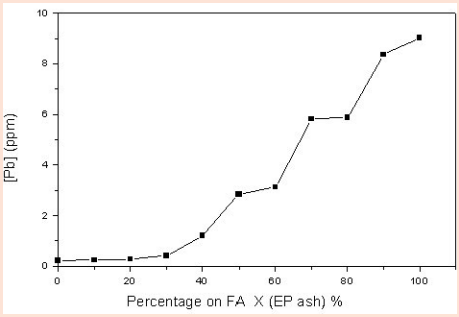

Figure 2 The Leached Concentration of Lead from the Mixtures of Scrubber Residue and Baghouse Ash (FA)

$$[Pb]_{mixture} = [Pb]_{0,FA} \times [0.5 \times (-0.0692) + 0.0263 \times (\frac{CaO\,\%}{SiO_2\,\%})_{mixture}] + [Pb]_{0,EP} \times [-0.0162 + 0.0342 \times (\frac{CaO\,\%}{Al_2O_3\,\%})_{mixture}]$$

Figure 2 demonstrates the leached concentration of lead from the mixed ash of scrubber residue and baghouse ash or electrostatic (EP) ash on TCLP test. It is clearly to see that the leaching behavior of lead is not a linear, it is rather an exponential growth curve. We have developed an empirical equation [1] to describe this curve. The equation can be used to describe several mixed ashes collected from different MSWI plants well.

During the sampling operation of ash wastes in these MSWI plants, the in-site operator often told to us, the so called "MSWI fly ash" is the mixed ash of scrubber residue and Baghouse ash through online mixing on convey tape of ash wastes, so the mixing ratio between these two ashes is always UNKNOWN! From an alternative side-view, the TCLP leaching concentration of MSWI fly ash can be controlled through collection of these two ashes separately and remix in a proper ratio. Regarding our study, the generation amount of baghouse ash is higher than that of scrubber residue, if we mix the scrubber residue and baghouse ash under a ratio of 50 wt%, the baghouse ash will be remained. So to mix the scrubber residue and baghouse ash to pass the TCLP test is not a good idea for long time operation. We shall keep seeking a cheaper technique for the de-toxicfication of baghouse ash directly. Scrubber residue almost meet

the requirement of non-hazardous materials, expected for the leaching of Cr (VI) ions. It is suitable to be classified as general industrial waste as well as bottom ash, and to seek a proper reuse method in the future.

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Ruby of Cereals in Taiwan — the Functional Value of Djulis and Its Development

Pi-Jen Tsai,
Department of Food Science and Technology

Introduction

Djulis (Chenopodium formosanum Koidz.) is a traditional cereal plant in Taiwan. However, the bitterness and browning of the hull make it less acceptable and limit its usage. Very few Djulis products can be seen on the market in recent years. Therefore, the characteristics, functional ability or applications of different varieties of this ethnic crop remained unknown untill we started our investigation 2006. Besides the analysis of its nutrients and functional compounds, we also tried to develop its application in functional foods. Hopefully, Djulis may become an economical crop with high profit.

The general nutrients compounds in Djulis

Results showed that the nutrients in Djulis are much higher than that of general cereal plants. For example, Djulis contain starch over 50% and protein may reach 19.3% (similar to beef). Djulis also possesses dietary fiber around 14-22%, which is 3 or 6 fold greater that of oat or sweet potato. For the fatty acids (FA), more than 70% was unsaturated FA, and poly-unsaturated FA was more than half of the total. They also contain essential amino acid such as lysine, valine and histidine, which are little in common cereals. They are also rich in minerals such as calcium, iron, potassium, magnesium, zinc etc. Among them, the contents of K, Ca and Mg are higher than that in soybean. The rare elements (Se or Ge) may also be found in Djulis. As mentioned above, Djulis is abundant in nutrients and very healthful. The dietary fiber may improve intestine function, prevent constipation, obesity and cardiovascular disease. Moreover, Djulis, being easy to plant and fast growing, may become a good solution for the food crisis owing to its high content of starch and protein.

Table 1, The comparison of the nutriens in Djulis with other foods

Nutrient	Djulis	Sweet potato	Wheat	Rice	Oat	Soybean	Beef
Starch (%)	55.7	28.6	68.4	77.2	66.2	25.3	-
Dietary fiber (%)	17.5	2.4	11.3	0.3	5.1	13.0	-
Protein (%)	17.7	1.0	14.0	7.5	11.5	36.8	19.6
Lipid(%)	3.2	0.3	1.6	0.5	10.1	18.0	21.1
Ca (ppm)	2523	340	290	50	390	1710	90
Fe (ppm)	55.6	5.0	28.0	2.0	32.0	57.0	30.1
Mg (ppm)	2523	280	1380	190	1120	2120	190
Na (ppm)	238	440	20	20	50	220	650
K (ppm)	35280	2900	3350	860	2950	15700	3390
P (ppm)	4607	530	1600	550	1600	3960	2050
Zn (ppm)	24.5	3	26	11	22	20	61

- : not detectable

The functional compounds in Djulis

Various kinds of functional compounds including betanin, superoxide dismutase (SOD), γ - aminobutyric acid (GABA) and polyphenols have been found in the Djulis. Betanin, as we know, may exhibit a powerful antioxidant capacity and act as anticancer or anti-inflammation. Polyphenols, an important antioxidant, may inhibit pathogens, LDL oxidation and arteriosclerosis. Polyphenols are also good in maintaining the elasticity of blood vascular wall and anti-cancer. As to SOD, they act as a powerful antioxidant enzyme, may have anti-inflammation activity, anti-tumor effects and protect the cell to resistant superoxide. GABA, a non-protein amino acid, may act as a major inhibitory neurotransmitter in the central nervous system. Besides modifying blood pressure, it also improves the quality of sleep. Furthermore, the saponin in Djulis may be extracted to use as natural detergent or skin cleaning.

Products developed from Djulis

We start the research project of Djulis (Named as Chenopodium formosanum in 2008) on the behalf of COA (Council of Agriculture, Executive Yuan) since 2006. During this period, we have published 20 papers and about 20 kinds of products. We also applied for 6 patents related to Djulis products. The product of Djulis Yogurt was regarded as an excellent work in the competition [2009 Crazy Idea], held by Industrial Develop Bureau, Ministry of Economical Affairs.

We developed the products in three directions, healthy food, leisure food and facial care products. The products have been developed are as followings: (1). General foods include waffles, puff, moa-chi, oat biscuit, rice roll, fried potato ball and rice cake. (2) Healthy foods include instant Djulis beverage, cereal milk, Djulis capsule, Djulis GABA tea, Djulis probiotics, Djulis SOD and yogurt. (3) Facial care products include clay facial mask and moisturizing essence. (4) Others such as preservative solution for Djulis cut flowers or Djulis pain patch. Since the global market of healthy foods based on cereals keeps growing, we believe that Djulis with its superior nutrients and functional compounds will be highlighted in the future.

Physical Activity Dose-response Effects on Outcomes of Diabetes Mellitus

Chin-Hsing Hsu,
Department of Recreation Sports and Health Promotion

Introduction

Diabetes mellitus is classed as a metabolism disorder. Metabolism refers to the physical and chemical processes involved in the activities of the body. A person with diabetes has a condition in which the quantity of glucose in the blood is too elevated, or called hyperglycemia. This is because the body either does not produce enough insulin, which is one of hormones the body uses to maintain blood glucose levels, produces no insulin, or has cells that do not respond properly to the insulin the pancreas produces. Such mechanism causes too much glucose building up in the blood. As a result, the blood has plenty of glucose; the cells are not getting it for their fundamental energy and growth requirements.

There are several common diabetes symptoms, such as frequent urination, disproportionate thirst, intense hunger, and unusual weight loss. Despite of clinical symptoms, diabetes mellitus can be diagnosed by Fasting Plasma Glucose Test (FPGT) and Oral Glucose Tolerance Test (OGTT) . A person who has been diagnosed as having impaired fasting glycaemia or impaired glucose tolerance and does not have diabetes runs a significantly higher risk of eventually developing Type 2. Understanding blood glucose level ranges is key to both diabetes diagnosis and diabetes

self-management.

According to Bureau of Health Promotion, Taiwan, the age-adjusted prevalence rate of previously diagnosed diabetes ranges from 2.2 to 6.9% and newly diagnosed diabetes appears to be stable at around 4.0%, while the annual incidence rate is about 1.8%. However, nationwide survey reported that 9.2 percent of Taiwans' population was affected by the disease in 2008, up 2.7 percentage points from 2002. In addition, the prevalence of diabetes was estimated 6.5 percent.

Materials and methods

Briefly, in a sample of 210 patients (101 men and 109 women) aged 62.30 ± 9.45, body weight, waist circumference, blood pressure, fasting blood glucose, triglycerides, and HDL cholesterol were measured. In addition, IPAQ (International Physical Activity Questionnaire) short form was assessed by a structured personal interview. The IPAQ short form asks about three specific types of activity undertaken in the four domains introduced above. The specific types of activity that are assessed are walking, moderate-intensity activities and vigorous-intensity activities. The items in the short IPAQ form were structured to provide separate scores on walking, moderate-intensity and vigorous-intensity activity. Computation of the total score for the short form requires summation of the duration (in minutes) and frequency (days) of walking, moderate-intensity and vigorous-intensity activities. Patients were divided into two groups, one was HbA1c under-control group, the other one was HbA1c not-under-control group. Statistical analyses were performed with SPSS. Simple frequencies and descript statistics were used to describe the variables, and logistic regression models were used to analyze the associations physical activity and other variables.

Results

The results were collected at baseline and 12 months after the first counseling session. 10 of the 220 patients did not complete the study because they have changed their lifestyle based on the IPAQ statements.

Levels of physical activity

There were significant differences (P<.01) in systolic blood pressure, fasting blood glucose, and triglycerides between HbA1c under-control group and HbA1c not-under-control group; in addition, the average physical activities between groups did not shown significant difference except for total walking minutes/week (as shown in table 1 & 2).

Group / Testing	HbA1c under-control group N	Mean	SD	HbA1c not-under-control group N	Mean	SD	t-value
BMI	93	26.91	4.48	117	26.41	3.46	.93
WHR	93	.89	.07	117	.92	.07	-1.87
SBP	93	135.82	15.76	117	140.91	20.13	-1.99*
DBP	93	79.34	13.12	117	78.61	12.13	.40
Glucose AC	93	125.72	33.93	117	155.85	53.76	-4.71**
TC	93	167.72	36.31	117	177.83	42.76	-1.82
Triglyceride	93	110.67	52.49	117	135.29	79.45	-2.56**
HDL-C	93	43.72	10.98	117	44.96	15.37	-.65
LDL-C	93	96.3	30.46	117	102.66	34.08	-1.39

Table 1 Physical characteristics and blood biochemical parameters of participants in HbA1c under-control group and not-under-control group

Group / Week	HbA1c under-control group N	Mean	SD	HbA1c not-under-control group N	Mean	SD	t-value
Vigorous day	93	.19	4.48	117	26.34	3.44	1.07
Vigorous time	93	12.90	.07	117	.915	.07	-1.96*
Moderate day	93	3.42	15.76	117	140.93	20.10	-2.02*
Moderate time	93	200.90	259.10	117	78.52	12.97	.46
Walking day	93	3.61	3.16	117	155.88	53.27	-4.77**
Walking time	93	132.74	167.50	117	177.72	42.15	-1.83

Table 2 Physical activities of participants in HbA1c under-control group and not-under-control group

Among the HbA1c under-control group, 13.58% subjects reported that they did not have regular exercise habits, compared with 19.39% in not-under-control group. While among those who have regular exercise habits, 64.19% of the subjects in well-controlled group and 57.14% in the not-under-control group indicated that "walking" as their main physical activity. According to the results of self-reported IPAQ, subjects between these two groups spent 5.79 ± 1.86 times / 215.87 ± 166.72 minutes, and 5.41 ± 2.12 times / 150.71 ± 71.55 minutes on walking per week, respectively. The average duration of each walking was 37 minutes in the HbA1c under-control group while 28 minutes in the HbA1c not-under-control group. There was a negative and significant correlation between walking minute/week and HbA1c (r=-.22, p<0.05) , results were shown as Figure 1.

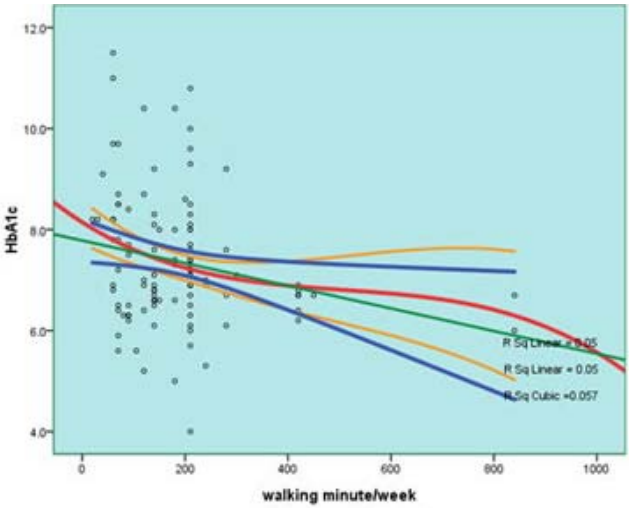

Figure 1. The relationship between HbA1c and walking minute/week

In addition, the results suggest that there was an inverse-J shape between blood glucose/HbA1c and total physical activity, which indicated the moderate/vigorous lifestyle might play an important role in the treatment of type 2 diabetes.

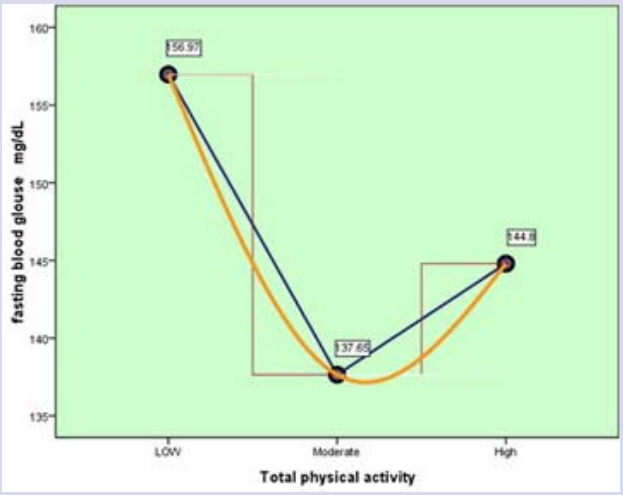

Figure 2. The relationship between fasting blood glucose and total physical activity

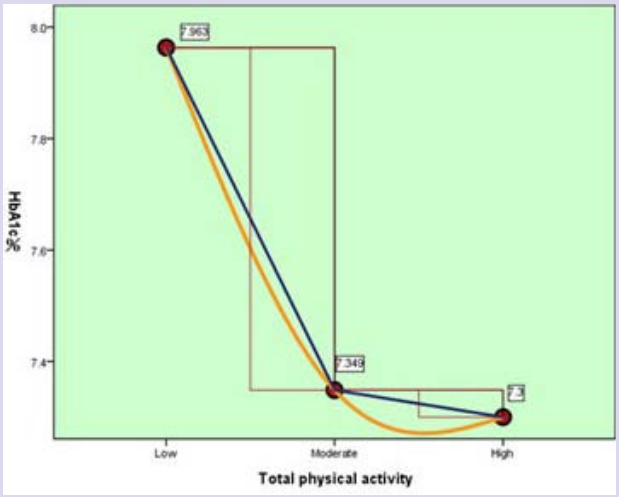

Figure 3. The relationship between HbA1c and total physical activity

Conclusions

The aim of this study is to assess the efficacy of physical activity in controlling blood glucose and glycated haemoglobin (HbA1c) among type 2 diabetes population. A total of 210 subjects were recruited and were measured their physical activity by IPAQ in 2009. Regular physical activity in diabetes care is known to induce an immediate decreasing of blood glucose levels, increased insulin sensitivity, and a decline of glycated haemoglobin. The results from this study show that low-intensity physical activity, such as walking, was related a significant acute reduction of blood glucose levels and glycated haemoglobin in type 2 diabetes patients.

Our findings are also consistent with those of 2 previous studies conducted among diabetes populations. The results indicated that patients in the HbA1c under-control group spent 216 minute/week on walking comparing to 150 minute/week in the HbA1c not-under-control group. This allows for a more common use of this preventive measure in this group of patients. Our study has demonstrated the difference of lifestyle in control blood glucose in patients with type 2 diabetes, but further studies will be needed to document long-term influence. Demonstrating the immediate effects on blood glucose in connection with a bout of low-intensity exercise can be useful in patient education and may help diabetes patients acquire a more physically active way of life. An over-30 min walk dairy can be used to demonstrate that diabetes patients can induce lowered glucose levels and get one more handle on their disease.

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Address:No.1,Shueh Fu Road, Neipu, PingTung 912,TAIWAN
Website:http://www.npust.edu.tw
Phone:08-7703202
GPN:2009802044