# Establishment and application of a quick screening method for hypoglycemic compounds

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## 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).

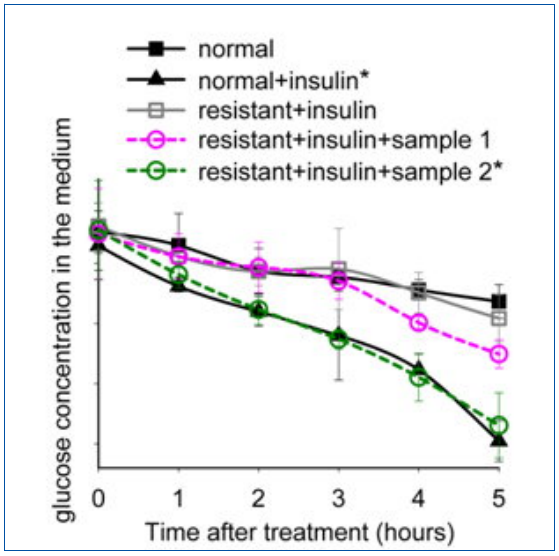


Figure 2. An example of results from the quick screening of hypoglycemic molecules. Normal represents normal cells without any treatment； normal+insulin is normal cells stimulated with insulin; resistant+insulin is insulin-resistant cells stimulated with insulin; resistant+insulin+sample 1 is insulin-resistant 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.