



## Proposal of Meal Tolerance Test (MTT) For Investigating Ability of Insulin Secretion for Small Carbohydrate Load

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### Abstract

Authors and collaborators have continued clinical practice and research on diabetes for long, and begun Low Carbohydrate Diet (LCD) at first in Japan. We have proposed super-, standard-, petite-LCD methods with 12%, 26%, 40% of carbohydrate, and developed medical and social LCD movement by Japanese LCD promotion association (JLCDPA). For research protocol, subjects were 10 healthy young medical staff. Two tests were 75gOGTT and meal tolerance test (MTT) of breakfast of super-LCD with 300kcal and 6g of carbohydrate. Blood glucose and immunoreactive insulin (IRI) were measured at 0 min and 30 min. Results of glucose and IRI in median value (0-30min) showed as follows: i) OGTT; 89.5 mg/dL to 130.5 mg/dL, 5.1  $\mu$ U/mL to 40.6  $\mu$ U/mL, ii) MTT; 93.5 mg/dL to 84.5 mg/dL, 4.9 $\mu$ U/mL to 10.6  $\mu$ U/mL (significant increase,  $p < 0.05$ ). The increments of IRI for GTT (carbo-75g) and MTT (carbo-6g) were analyzed. There was a significant correlation between increments of IRI in GTT and MTT ( $p < 0.05$ ). Blood glucose in MTT tended to decrease from 0 min to 30 min. These results suggested that insulin secretion would be sufficient and relatively excessive for 6g of carbohydrate amount.

### Keywords

Low Carbohydrate Diet (LCD), Japanese LCD Promotion Association (JLCDPA), Meal Tolerance Test (MTT), Super-LCD Glycemic Index (GI)

### Abbreviations

LCD: Low Carbohydrate Diet, JLCDPA: Japanese LCD Promotion Association, MTT: Meal Tolerance Test, IRI: Immunoreactive Insulin, OGTT: Oral Glucose Tolerance Test, GI: Glycemic Index

## Introduction

Diabetes mellitus has been widely prevalent in developed countries and also in developing countries worldwide [1]. It may bring various harmful influences from the medical, social, and economic points of view [2]. Diabetes has chronic complications including macrovascular and microvascular disorders, which are usually without pain, discomfort, or remarkable symptoms [3]. These pathophysiological problems come from an elevated level of blood glucose. That is why decreasing the level of the daily profile of blood glucose has been emphasized for long.

Regarding the therapeutic way for diabetic patients, nutritional therapy has been a fundamental method. In recent years, recommended diet therapy has seemed to be changing from calorie restriction (CR) to a low carbohydrate diet (LCD). Previously, LCD was initiated by Atkins and others in the medical and health care region [4]. Its evidence was clarified for some studies among LCD, CR, and a Mediterranean diet [5]. Successively, the definition of LCD has been proposed for nutritional research of LCD [6]. After that, the clinical benefit of LCD has been predominantly found in various reports worldwide [7,8].

On the other hand, the author's et al. in Japan has begun LCD at first in Japan [9]. After that, we have informed the efficacy of LCD with three patterns of LCDs, which are super-LCD, standard-LCD, petite-LCD with 12%, 26%, 40% of carbohydrate amount, respectively [10]. We have developed an LCD movement by the activities of the Japanese LCD promotion association (JLCDPA) [11]. Further, clinical research on LCD has been continued so far, such as blood glucose profiles on CR and LCD, elevated ketone bodies in the axis of newborn-pregnant mother-placenta-fetus, continuous glucose monitoring (CGM), meal tolerance test (MTT), and so on [12].

For the research of MTT in the meal habit of Japan, we usually have rice as the main food in our daily life [13]. Then, we have proposed a trial of MTT of carbohydrate using usual Japanese traditional breakfast [14]. Recently, several reports about diabetic treatment have been observed [15,16]. In this article, the diabetic topic concerning MTT and insulin secretion would be

described.

## Subjects and Methods

Subjects enrolled in the current study were healthy people. They are working as the medical staff including males 5 and female 5 with the age of 22-32 years old. They have been healthy with no remarkable diseased states before. They have the value of average body mass index (BMI) as  $20.7 \pm 1.4 \text{ kg/m}^2$  and their BMI was all within normal limits.

Methods have two kinds of tests for the investigation. In order to evaluate the changes in blood glucose and insulin value, we conducted a 75g oral glucose tolerance test (75gOGTT) and a meal tolerance test (MTT). The former is the usual test for evaluating the diabetic status, using the intake of 75g of carbohydrate (TRELAN-G 50<sup>R</sup>, exam No. 22000AMX00569, 300 kcal).

In contrast, the latter is one of the tests for evaluating the responses of blood glucose, using actual meal as breakfast. The meal of this protocol included several foods, which are tomato, broccoli, omelet with mayonnaise, and consommé soup. The detailed nutritional factors included 6g of carbohydrate, 24g of fat, and 13g of protein with 300 kcal as a total calorie.

The two tests were conducted for 10 people at intervals of one week, and each was assigned 5 OGTTs and 5 MTTs at random. In both of the tests, we measured the blood levels of glucose before, 30 min and 120 min, and the value of insulin (immunoreactive insulin IRI) for before and 30 min. From the obtained data mentioned above, the increment of IRI (0-30 min) was calculated as delta ( $\Delta$ ) of IRI. The correlation of both deltas of IRI between OGTT and MTT was investigated.

### Statistical Analysis:

In the current investigation, obtained data were shown by the box plot method including median, quartiles (25% and 75%), maximum and minimum. When we evaluate the significant difference for the correlation of the two factors, the presence of the significance was judged at the level of  $p < 0.05$ .

### Ethical Considerations:

The current study has been fundamentally conducted with ethical principles based on the Declaration of Helsinki. Furthermore, the additional comment was performed by the Ethical Guidelines for Research for Humans, with the Good Clinical Practice (GCP). There has been a consideration for the protection of human rights. In addition, furthermore, the “Ethical Guidelines for Epidemiology Research” was applied as the guideline. This concept was shown by the Ministry of Health, Labor and Welfare, and the Ministry of Education, Culture, Sports, Science, and Technology in Japan.

The author and colleagues have established an ethical committee in the hospital. The medical and ethical committee has expert professionals such as a physician, nurse, nutritionist, pharmacist, and that

of legal specialty. For the current study, our discussion has been conducted with appropriate situations and has decided to all of the agreements. In the light of the subjects, informed consents with agreements and also written documents were obtained from all of the subjects. This investigation has been registered by the National University Hospital Council of Japan (ID: #R000031211).

### Results

#### Response of Glucose:

Ten healthy subjects had two tests of OGTT and MTT. The median value of blood glucose was 89.5 mg/dL, 130.5 mg/dL, 76.5 mg/dL, in 0 min, 30 min and 120 min for GTT, respectively (**Fig-1**, left). Similarly, glucose in median was 93.5 mg/dL, 84.5 mg/dL, 90.0 mg/dL for MTT, respectively (**Fig-1**, right).

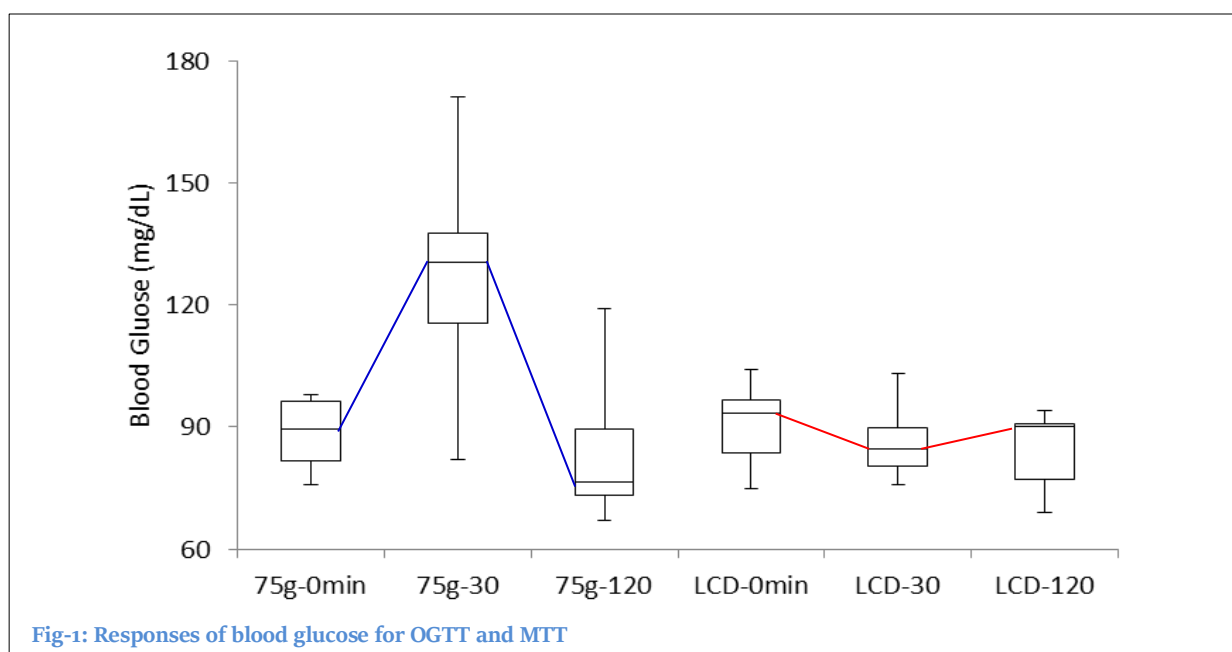


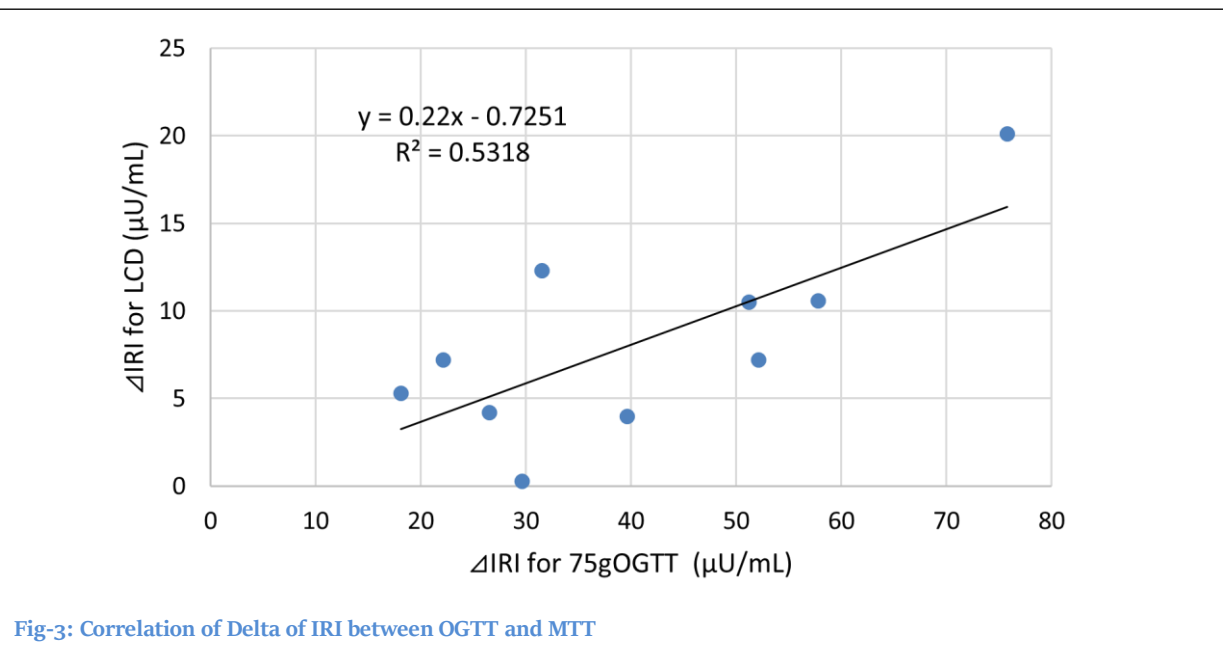
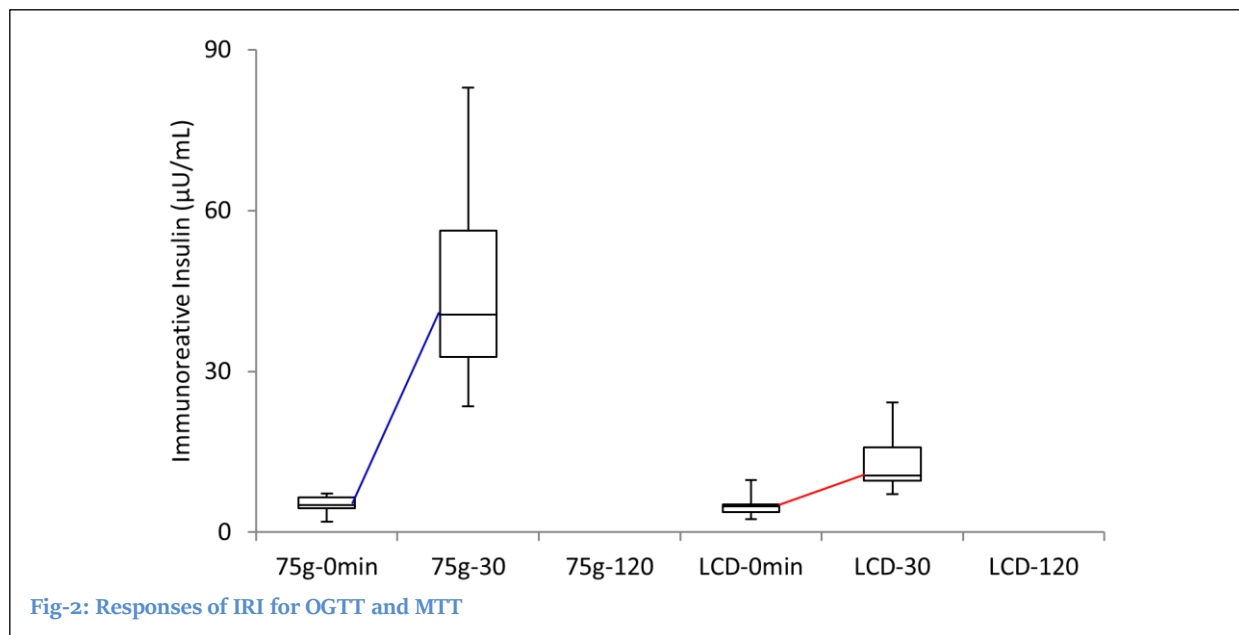
Fig-1: Responses of blood glucose for OGTT and MTT

#### Response of Insulin:

Ten healthy subjects had two tests of OGTT and MTT. The median value of immunoreactive insulin (IRI) was 5.1  $\mu$ U/mL and 40.6  $\mu$ U/mL in 0 min and 30 min for GTT, respectively (**Fig-2**, left). Similarly, IRI in the median was 4.9  $\mu$ U/mL and 10.6  $\mu$ U/mL in 0 min and 30 min for MTT, respectively (**Fig-2**, right). The latter MTT statistically showed that the increase in insulin concentration from 0 to 30 minutes was significantly increased ( $p < 0.05$ ).

#### Delta of Insulin:

For the stimuli of GTT and MTT, the increase of insulin secretion was found. The increment of IRI between 0 min and 30 min is called delta ( $\Delta$ ) of IRI. For 10 subjects, the delta of IRI values for GTT and MTT were calculated. The results were analyzed for the correlation between the delta of IRI in GTT and MTT. The results showed a significant correlation ( $p < 0.05$ ) between the delta of IRI in GTT and MTT (**Fig-3**).



## Discussion

This study describes the effects of carbohydrates on the MTT. This is related to the Glycemic Index / Glycemic Load (GI / GL) issues. GI / GL was proposed by Jenkins et al., in which there are different carbohydrates for influencing potential metabolic responses with different glycemic responses (GR) [17]. The lower GI and GL have been beneficial for decreased risk of diabetes and cardiovascular disease (CVD) [18]. Among them, 42 breakfast cereals were investigated. The factors included GI (in vivo versus predicted) and GL (g) for serving 30g (in vivo versus predicted). They showed that GI is  $68 \pm 9.2$ , GL (g) =  $14.7 \pm 3.1$  g/30g, Sucrose =  $20.1 \pm 8.5$ , Starch  $51.1$

$\pm 10.0$  g [18].

In our current study, the results showed that MTT had a lower blood glucose response than 75g OGTT (Fig-1). Concerning the amount of carbohydrate, MTT has 6g, while 75gOGTT has 75g. According to the official comment of the American Diabetes Association (ADA), the only carbohydrate directly affects blood glucose levels [19]. Consequently, the results of the MTT study seem to be reasonable. It should be noted that the absorption rate of carbohydrates varies due to several factors, such as dietary GI / GL of various food contents and the presence of liquid. Among them,

75gOGTT would be one of the most rapid absorption ways of many amounts of carbohydrate. The reason is that the TRELAN -G50<sup>R</sup> contains not plain water but carbonated water. Furthermore, the absorption speed of carbohydrate may be influenced by the actual meal situation containing proteins and lipids.

Regarding insulin response, both GTT and MTT showed a significant increase (**Fig-2**). Blood glucose in MTT (median value) tended to decrease from 0 minutes to 30 minutes. The reason seemed to be that insulin secretion from the pancreas was sufficient for 6 g of carbohydrate, and its amount may be relatively excessive. Consequently, there was relatively postprandial decreased blood glucose after 30 minutes.

There was a significant correlation between GTT and MTT concerning the increased degree of insulin (**Fig-3**). In other words, cases with larger insulin response to GTT showed better response to MTT with small doses of carbohydrate 6g [20]. This reason may be due to individual differences and also certain levels in the responsiveness. Several related factors may be included, such as pancreatic  $\beta$ -cell reactivity, the secretory ability of insulin, insulin resistance for target organ, absorption rate, body mass index (BMI), and others [21]. Further, we investigated the correlation between the delta of IRI and BMI, but it revealed no significant relationship.

As regards MMT, there were several reports for the clinical challenge of breakfast. A protocol was a test meal of breakfast including 500 kcal in energy and 55% of carbohydrates [22]. It has 69g of carbohydrate, and it brought the increased response of IRI and blood glucose. From the reported data, glucose and IRI increased from 167 to 230 mg/dL in glucose, and 12 to 37  $\mu$ U/ml in IRI during 0 to 30 min, respectively.

Authors had also reported MMT using the meal which contains 70g of carbohydrate [14] This protocol meal was from the standard CR meal where the Japan Diabetes Association (JDA) has recommended for long [23]. It contains rice, miso soup, egg, and others which have been a traditional Japanese meal for breakfast [24]. We investigated 48 diabetic patients categorized into 3 groups due to HbA1c values. Each group showed

the following result: HbA1c value was 6.0 %, 7.8%, 9.7% in HbA1c, glucose increased 117 to 150 mg/dL, 166 to 203 mg/dL, 218 to 299 mg/dL in median during 30 min, IRI increased 4.4 to 12.8 $\mu$ U/mL, 4.5 to 13.5 $\mu$ U/mL, 4.2 to 9.9 $\mu$ U/mL in IRI (0-30 min), respectively. Consequently, enough responses of glucose and IRI were found, suggesting the clinical usefulness of carbo-70 MTT [14].

Furthermore, there is another protocol formula of MTT for breakfast [25]. It contains standard three macronutrients, which are 450 kcal, protein 15%, fat 35%, and carbohydrate 50%. From this, 56g of carbohydrate is included in the formula. Before this study, a model for the international standard test meal was challenged for the investigation of postprandial hyperglycemia and hyperlipidemia [26].

Another study was described concerning the second meal phenomenon [27]. Two types of breakfast were tried. Carbo group had breakfast with PFC = 15: 20: 65, while the protein group had breakfast with PFC = 35: 20: 45. Both groups had same lunch, where the protein group showed lower glucose and higher IRI response [27]. These findings may be related with improved glucose tolerance because of GLP-1-induced insulin response after MTT [28].

From the result of MTT, the blood glucose level after 30 minutes tended to be lower than that at 0 minutes, which was median data. Observing the actual data in 7 out of 10 cases, the blood glucose levels at 30 minutes were lower than those at 0 minutes. As a matter of fact, the secretion of insulin from the pancreas has been proved, though the stimulated load was as small as 6 g of carbohydrate.

In this study, a large amount of insulin was secreted by GTT (carbo-75 g) and blood glucose was increased, while a small amount of insulin was secreted by MTT (carbo-6 g) and blood glucose was the same or slightly decreased at 30 min. Hyperinsulinemia in blood has been known to have some negative effects on health. They include the generation of active oxygen, increased risk of aging, arteriosclerosis, Alzheimer's disease, cancer, and others.

Recently, Dr. Igaki and collaborators, Kyoto University has shown the mechanism between cancer risk and metabolic disease [29]. They reported the research on *Drosophila* eye imaginal epithelium and oncogenic scribble (scrib) mutant cells. The results showed that hyperinsulinemia promotes epithelial tumorigenesis by the mechanism of abrogating cell competition [29]. Furthermore, one of the well-known oral hypoglycemic agent (OHA) metformin can repress scrib tumorigenesis under hyperinsulinemia.

This research has some limits. Although GTT and MTT were performed on the same healthy subject, the number of cases was small and the relationship between many related biomarkers has not been examined. If 10 subjects should be obese with very high BMI, it is expected that the results would be different from this time because of the presence of insulin resistance. The response of blood glucose and insulin can be obtained by small administration of 6g of carbohydrate. Various protocols can be tried with a different condition.

In summary, we examined the insulin secretory response of healthy subjects by MTT with 6 g of carbohydrate. As the case has a better insulin response to 75gOGTT, one has also a higher response to 6g of carbohydrate. Even with only 6 g, the insulin secretory response was sufficient and the blood glucose after 30 minutes showed a tendency to decrease. It is necessary to continue a detailed evaluation of these phenomena in the future.

### Conflict of Interest

All authors have read and approved the final version of the manuscript. The authors have no conflicts of interest to declare.

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