Lifestyle-related diseases develop through the accumulation of undesirable lifestyle habits both prior to the onset of disease as well as during normal healthy life. Accordingly, early detection of, and intervention in, metabolic disorders is desirable, but is hampered by the lack of an established evaluation index for young individuals. The purpose of this study was to investigate the utility of a biomarker of health in young female subjects. The subjects were young healthy Japanese females in whom energy expenditure was measured for a period of 210 min after a test meal. In addition, Δplasma glucose and Δserum insulin were calculated from the fasting and 30 min values. Δplasma glucose and Δserum insulin levels varied widely compared to fasting levels. Both the area under the curve of carbohydrate oxidation rate and serum free fatty acid levels were higher in individuals in the high Δplasma glucose group. Moreover, Δplasma glucose was higher in individuals in the high Δserum insulin group than in the low Δserum insulin group. We conclude that nutritional balanced liquid loading test using Δplasma glucose and Δserum insulin as the evaluation index is useful for the detection of primary metabolic disorders in young females.

Key Words: lifestyle-related diseases, primary metabolic disorders, early detection, postprandial metabolic response, mixed meal

The incidence of metabolic syndrome has been increasing with the increasing prevalence of lifestyle behaviors such as overfeeding and/or immobilization. Individuals with metabolic syndrome are at increased risk for developing lifestyle-related diseases such as type 2 diabetes, hypertension, dyslipidemia and cardiovascular disease, as well as increased mortality from cardiovascular disease. Lifestyle-related diseases develop through the accumulation of undesirable lifestyle habits both prior to the onset of disease as well as during normal healthy life. Recent reports have described metabolically obese, normal weight (MONW) individuals, who have normal body weight but exhibit a cluster of obesity-related characteristics, including excess visceral fat, insulin resistance and hyperinsulinemia. These observations in apparently healthy young individuals emphasize the importance of the early detection of, and early intervention in, metabolic disorders, a goal which is hampered by the lack of an established evaluation index in young individuals.

Postprandial hyperglycemia, which is characteristic of prediabetic patients, is a reflection of reduced insulin sensitivity and secretory capacity. Hyperglycemia leads to increased secretion of late-phase insulin, thereby promoting the accumulation of fat and the development of insulin resistance. Epidemiological and interventional studies have shown that postprandial hyperglycemia is an independent risk factor for cardiovascular disease and, furthermore, oxidative stress induced by postprandial hyperglycemia impairs vascular endothelial function and plays a role in atherosclerosis. As the foundation for a variety of lifestyle-related diseases postprandial hyperglycemia is considered useful as an index for the early detection of metabolic disorders. Currently, diagnosis of impaired glucose tolerance is based upon the 2-h plasma glucose level in a 75 g oral glucose tolerance test (OGTT), a time point at which the plasma glucose of most healthy young subjects has returned to normal levels. Since compensatory metabolic responses are thought to occur soon after eating, we hypothesized that the metabolic status of young subjects could be ascertained by evaluating metabolic changes taking place soon after eating. It has been reported in middle aged subjects, that performing OGTT and examining insulin concentration 30 min after OGTT is likely to be a strong contributor to a diagnosis of impaired glucose tolerance. A 75 g OGTT often elicits false reactive hypoglycemia with adverse epigastric symptoms and, moreover, high dose monosaccharide loading does not reflect the normal daily blood glucose excursion and insulin response. In addition, plasma glucose levels in healthy subjects do not cause a dose-dependent increase in glucose above a certain value. The possibility exists therefore that the administration of high doses of monosaccharide to young subjects would not provide for the detection of subtle metabolic changes.

Insow (Meiji Co., Ltd., Tokyo, Japan) is a nutrient balanced liquid formula that contains isomaltulose, a sucrose isomer found in honey. Isomaltulose has been shown to be metabolized by isomaltulose and, in the intestine, is less rapidly, although completely, cleaved than sucrose. In our previous study, ingestion of this formula suppressed postprandial hyperglycemia and excessive insulin secretion in rats and humans. Since analyzing the postprandial metabolic responses after Insow loading may allow us to detect primary metabolic changes in young subjects, here we investigated its use a biomarker to indicate the degree of health in young subjects.

Materials and Methods

Subjects. 128 young females were recruited as subjects in this study, of which 26 with missing data were excluded from analysis, giving a final sample of 102 females. Written informed consent was obtained from all subjects, and the study was ap-
proved by the Ethics Committee of the University of Shizuoka. The clinical and biological characteristics of the subjects are shown in Table 1. The mean values ± SD of age and body mass index (BMI) were 21.4 ± 1.3 years and 19.9 ± 2.1 kg/m², respectively. Glucose metabolism of all subjects was confirmed as normal based on fasting plasma glucose (FPG), fasting serum insulin (FIRI) and HbA1c levels of 91.8 ± 4.5 mg/dl, 5.7 ± 2.3 μU/ml and 5.2 ± 0.2 %, respectively. Hepatic and renal function were normal in all subjects.

**Test meal.** The test meal was Inslow the main source of carbohydrate in which is isomaltulose, which is absorbed at a slower rate than sucrose. The protein, fat and carbohydrate concentrations in Inslow are 20.0%, 29.7%, and 50.3%, respectively.

**Study protocol.** Test days were selected to fall outside the menstrual periods of the subjects. All the subjects were asked to avoid heavy exercise and intake of alcohol 24 h before the day of the study. The subjects were instructed to eat the same prescribed dinner (2,717 kJ; protein: 13.3%, fat: 12.4%, carbohydrate: 74.3%) at 1,830 h before their test day, after which their food and drink intake was limited to water.

All subjects rested for 20 min prior to measurement of resting energy expenditure over 30 min before fasting blood samples were collected (baseline). Subjects consumed Inslow (250 ml; 1,046 kJ) within 10 min of collection of the baseline blood sample after which postprandial energy metabolism was measured for 210 min while the subject was at rest. Gas samples were taken over a 15 min period every 30 min (15 min interval) during the 210 min. Peripheral blood samples were collected 30 min after consumption of Inslow.

**Energy metabolism measurements.** Oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were measured using an automatic, computerized indirect calorimeter (Aero monitor AE 300, Minato Medical Science, Tokyo, Japan) during which continuous ventilatory volumes (VO₂ and VCO₂) were displayed on a computer screen at 15-s intervals, and mean min-by-min values were recorded. Respiratory quotient (RQ) was determined from O₂ and VCO₂. Data of VO₂ and VCO₂ were obtained by averaging the stable 10 min period in 15 min. Energy expenditure (EE) and carbohydrate oxidation rates (Cox) and fat oxidation rates (Fox) were calculated using the tables described by Lusk. The incremental area under the curve (AUC) for Cox and Fox were calculated for the 210 min period following ingestion of test meal.

**Blood analysis methods and anthropometric measurements.** Plasma and serum samples were separated and stored at −80°C until analysis. Fasting blood samples were used for analysis of plasma glucose, serum insulin and biochemical examination, and 30 min blood samples were used for analysis of plasma glucose and serum insulin (SRL Inc, Tokyo, Japan). Anthropometric measurements were determined using a bioelectrical impedance analysis method (TANITA-RBF-215; TANITA Corporation, Tokyo, Japan). ΔPG (plasma glucose) and ΔIRI (serum insulin) were calculated from the fasting and 30 min values (ΔPG; PG 30 min – FPG, ΔIRI; 30 min IRI – FIRI).

**Statistical analysis.** Data are reported as mean ± SD. Correlation between ΔPG or ΔIRI and anthropometric and metabolic traits were assessed with Pearson’s correlation coefficient and multiple regression analysis. ΔPG and ΔIRI were divided into quartiles, the difference in continuous variables was analyzed using one-way analysis of variance (ANOVA). The Tukey post hoc test was used to determine the source of significant variance among the groups. All statistical analyses were performed using the Statistical Package of Social Science (SPSS for Windows, ver. 19.0, SPSS, Chicago, IL).

**Results**

**Individual differences of plasma glucose (PG) and serum insulin (IRI) levels.** The distribution of PG and serum IRI levels in fasting and postprandial 30 min are shown in Fig. 1. Postprandial PG and IRI were distributed over a wide range compared to the fasting values (FPG: 83.0–104.0 mg/dl; FIRI: 1.3–13.9 μU/ml; PG at 30 min: 63.0–139.0 mg/dl; IRI 30 min: 3.7–106.0 μU/ml). Similar to PG and IRI at 30 min, ΔPG and ΔIRI were distributed over a wide range.

**Table 1. Characteristics of the study subjects**

| Characteristic          | Mean ± SD   |
|-------------------------|-------------|
| Age (year)              | 21.4 ± 1.3  |
| Height (cm)             | 159.2 ± 5.6 |
| Weight (kg)             | 50.6 ± 6.0  |
| BMI (kg/m²)             | 19.9 ± 2.1  |
| Percentage body fat (%) | 23.2 ± 4.2  |
| PG (mg/dl)              | 91.8 ± 4.5  |
| IRI (μU/ml)             | 5.7 ± 2.3   |
| HbA1c (NGSP) (%)        | 5.2 ± 0.2   |
| Total protein (g/dl)    | 7.0 ± 0.4   |
| Albumin (g/dl)          | 4.4 ± 0.2   |
| Total cholesterol (mg/dl)| 167.1 ± 23.3 |
| HDL cholesterol (mg/dl) | 62.8 ± 13.1 |
| Triglyceride (mg/dl)    | 65.0 ± 22.6 |
| FFA (mEq/l)             | 0.38 ± 0.14 |

Data are means ± SD. BMI, body mass index; PG, plasma glucose; IRI, serum insulin; HDL, high density lipoprotein; FFA, free fatty acid.
Association of ΔPG and ΔIRI levels with anthropometric, energy metabolism and blood biomarkers. ΔPG levels were positively correlated with fasting Fox, Cox AUC, ΔIRI and free fatty acid (FFA) levels, and negatively correlated with fasting Cox and resting RQ (Table 2A). ΔIRI levels were positively correlated with body fat percentage, BMI and ΔPG levels were and negatively correlated with fasting PG levels (Table 2B).

To evaluate the association of ΔPG and ΔIRI levels with anthropometric, energy metabolism and and blood biomarkers, we used multiple linear regression analysis. FFA, AIIR, Cox AUC and fasting IRI levels were significant and independent factors [standardized partial regression coefficients = 0.308, 0.277, 0.215 and -0.206, respectively; multiple correlation coefficient adjusted for degrees of freedom = 0.235 (Table 3A) contributing to the variance in elevated ΔIRI levels.

ΔPG and ΔIRI levels were divided into quartiles (ΔPG; quartile 1 = –21.0–4.3 mg/dl, quartile 2 = 4.4–11.0 mg/dl, quartile 3 = 11.1–17.8 mg/dl, quartile 4 = 17.9–45.0 mg/dl) (ΔIRI; quartile 1 = –21.0–4.3 mg/dl, quartile 2 = 4.4–11.0 mg/dl, quartile 3 = 11.1–17.8 mg/dl, quartile 4 = 17.9–45.0 mg/dl), which were distributed over a wide range. It has been reported that insulin concentrations 30 min after a 75 g OGTT correlate with plasma glucose levels at 2 h after the same test, as well as with impaired glucose tolerance and insulin secretion during the early postprandial phase. In this study, although subjects with high ΔIRI had normal BMIs, there is a risk of their developing obesity in the future. East Asians including Japanese have a high risk of developing lifestyle-related diseases such as diabetes even if they are lean. In this study, even though there was no difference in BMI, differences were observed in ΔPG and ΔIRI, so we cannot predict the metabolic disorders by using BMI as a biomarker. These results suggest that evaluation of postprandial PG and IRI 30 min after loading is useful for early detection of metabolic disorders.

When we examined the relationship between ΔPG level and FFA, AIIR, Cox AUC and fasting IRI, we found that FFA was significantly higher in the high ΔPG group (quartile 4) than in the low ΔPG group (quartile 1). An elevated plasma FFA concentration results in an increase in intracellular fatty acyl-CoA and

Table 2. Correlation between ΔPG or ΔIRI and anthropometric, metabolic traits

| (A) | ΔPG | Pearson’s product-moment correlation coefficient |
| --- | --- | --- |
| Percentage body fat | -0.013 |
| BMI | -0.051 |
| Fasting Fox | 0.307** |
| Fox AUC | -0.029 |
| Fasting Cox | -0.260** |
| Cox AUC | 0.305** |
| ΔRQ | 0.164 |
| Fasting IRI | -0.155 |
| ΔIRI | 0.206* |
| Triglyceride | 0.068 |
| FFA | 0.358** |

| (B) | ΔIRI | Pearson’s product-moment correlation coefficient |
| --- | --- | --- |
| Percentage body fat | 0.262** |
| BMI | 0.278** |
| Fasting Fox | 0.033 |
| Fox AUC | -0.005 |
| Fasting Cox | 0.093 |
| Cox AUC | 0.102 |
| ΔRQ | 0.173 |
| Resting RQ | -0.003 |
| 30-min RQ | -0.003 |
| ΔRQ | 0.148 |
| Fasting PG | -0.023** |
| ΔPG | 0.206* |
| Triglyceride | 0.150 |
| FFA | 0.091 |

Table 3. Association between ΔPG or ΔIRI and anthropometric and metabolic traits

| (A) | ΔPG | β | p value | R² |
| --- | --- | --- | --- | --- |
| FFA | 0.308 | 0.001 | 0.326 |
| ΔIRI | 0.277 | 0.003 | 0.291 |
| Cox AUC | 0.215 | 0.018 | 0.236 |
| Fasting IRI | -0.206 | 0.027 | -0.221 |

| (B) | ΔIRI | β | p value | R² |
| --- | --- | --- | --- | --- |
| BMI | 0.290 | 0.003 | 0.296 |
| ΔPG | 0.221 | 0.020 | 0.230 |

Data were used for multiple linear regression analysis. PG, plasma glucose; IRI, serum insulin; FFA, free fatty acid; Cox, carbohydrate oxidation; AUC, area under the curve; BMI, body mass index.

Discussion

In the present study we investigated whether the metabolic status of young females can be measured based on metabolic changes taking place soon after food loading. While the fasting laboratory data of subjects were normal, PG and IRI values at 30 min after loading were distributed over a wide range. It has been reported that insulin concentrations 30 min after a 75 g OGTT correlate with plasma glucose levels at 2 h after the same test, as well as with impaired glucose tolerance and insulin secretion during the early postprandial phase. It is possible that elevated ΔPG and ΔIRI levels in our study may reflect the onset of impaired glucose tolerance. Another study has reported that insulin concentrations 30 min after a 75 g OGTT strongly correlate with changes in adult body weight and waist circumference measured over a 6 year period. In this study, although subjects with high ΔIRI had normal BMIs, there is a risk of their developing obesity in the future. East Asians including Japanese have a high risk of developing lifestyle-related diseases such as diabetes even if they are lean. In this study, even though there was no difference in BMI, differences were observed in ΔPG and ΔIRI, so we cannot predict the metabolic disorders by using BMI as a biomarker. These results suggest that evaluation of postprandial PG and IRI 30 min after loading is useful for early detection of metabolic disorders.
Fig. 2. Association of ΔPG with anthropometric and metabolic traits. BMI (A), Cox AUC (B), fasting IRI (C), FFA (D), ΔIRI (E). 1st quartiles; −21.0–4.3 (mg/dl), 2nd quartiles; 4.4–11.0 (mg/dl), 3rd quartiles; 11.1–17.8 (mg/dl), 4th quartiles; 17.9–45.0 (mg/dl). The differences among the four groups were assessed by one-way ANOVA. *p<0.05. PG, plasma glucose; BMI, body mass index; Cox AUC, area under the curve for carbohydrate oxidation rates; FFA, free fatty acid; IRI, serum insulin.

Fig. 3. Association of ΔIRI with anthropometric and metabolic traits. BMI (A), Cox AUC (B), fasting IRI (C), FFA (D), ΔPG (E). 1st quartiles; 1.6–25.0 (μU/ml), 2nd quartiles; 25.1–39.1 (μU/ml), 3rd quartiles; 39.2–45.8 (μU/ml), 4th quartiles; 45.9–98.2 (μU/ml). The differences among the four groups were assessed by one-way ANOVA. *p<0.05. IRI, serum insulin; BMI, body mass index; Cox AUC, area under the curve for carbohydrate oxidation rates; FFA, free fatty acid; PG, plasma glucose.
diacyl glycerol concentrations, which result in activation of protein kinase C (PKC)-theta and increased insulin receptor substrate-1 (IRS-1) serine phosphorylation. This in turn leads to decreased IRS-1 tyrosine phosphorylation, decreased activation of IRS-1-associated phosphatidylinositol 3-kinase activity and decreased insulin-stimulated glucose transport activity. A previous study in non-obese subjects has demonstrated that subjects with insulin-resistance have higher FFA levels than insulin-sensitive subjects. In the present study, we observed a trend towards increased ARI levels with increasing ΔPG. Based on these data, we speculate that subjects with high ΔPG level may exhibit mild insulin resistance induced by increased serum FFA concentration. The mean of the Cox AUC, which reflects the rate of postprandial carbohydrate oxidation, was significantly higher in the high ΔPG group than in low ΔPG group. While Cox during insulin infusion has been shown to be higher in individuals with normal glucose tolerance compared to those with impaired glucose tolerance (IGT) and type 2 diabetes, elevated glucose and insulin concentrations correlate with increased postprandial Cox in healthy men. We suggest therefore that enhanced insulin secretion and carbohydrate oxidation during the course of the deterioration in glucose tolerance represent compensatory reactions to postprandial hyperglycemia.

ΔPG was significantly higher in the high ARI group than in the low ARI group, suggesting that excessive insulin secretion was induced by increasing postprandial PG levels. Postprandial hyperglycemia is not only characteristic of the early stage of diabetes, but is also an independent risk factor for cardiovascular disease. Elevated glucose levels stimulate reactive oxygen species production through PKC-dependent activation of NAD(P)H oxidase in both vascular smooth muscle cells and endothelial cells, leading to the acceleration of atherosclerosis. In addition to IGT subjects the high insulin response group was characterized by higher BMI, subcutaneous fat area, uric acid levels and HOMA-beta than the low insulin response group. These facts suggest the importance of the early diagnosis of postprandial hyperglycemia and hyperinsulinemia. Our study suggests the possibility that elevated ΔPG and ARI levels represent a compensatory metabolic response to maintain homeostasis in healthy female subjects. Although lifestyle intervention programs are reportedly beneficial in preventing diabetes in subjects with IGT, improvement of lifestyle may also be indicated for healthy subjects.

While the OGTT is currently used to identify glucose intolerance, false reactive hypoglycemia has often been associated with adverse epigastric symptoms, including discomfort, anxiety and lethargy, and does not reflect daily glucose excursions and insulin response. Accordingly, mixed meals containing protein and fat in addition to carbohydrate, have been recently developed for the OGTT. Compared to a liquid test meal such as that used in this study, a solid test meal complicates the standardization of the conditions of ingestion, including the number of mastications and the time required to eat. Moreover, since the main carbohydrate in our test meal is isomaltulose, which is digested and absorbed more slowly than glucose and sucrose, this meal does not induce rapid increases in plasma glucose and insulin levels and their associated side effects.

In summary, nutritional balanced liquid loading test using ΔPG and ARI as the evaluation index is useful for the detection of primary metabolic disorders in young subjects. However, metabolic syndrome is a complex disease such as environmental and genetics, so it is necessary to further analysis of the genetic evaluation. And we must follow up to reveal whether subjects with high ΔPG and ARI will develop metabolic syndrome.

Acknowledgments

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology in Japan (for MS, HA).

Abbreviations

AUC area under the curve
BMI body mass index
Cox carbohydrate oxidation rates
EE energy expenditure
FFA free fatty acid
FIRI fasting serum insulin
Fox fat oxidation rates
FPG fasting plasma glucose
IGT impaired glucose tolerance
IRI serum insulin
IRS-1 insulin receptor substrate-1
OGTT oral glucose tolerance test
PG plasma glucose
PKC protein kinase C
RQ respiratory quotient
VO2 oxygen consumption
VCO2 carbon dioxide production

Conflict of Interest

No potential conflicts of interest were disclosed.

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