PHYSICAL ACTIVITY IS STRONGLY INVERSELY RELATED TO POST-CHALLENGE PLASMA GLUCOSE AND GLYCEMIC SPIKES IN A RISK POPULATION FOR TYPE 2 DIABETES

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ABSTRACT

AIM: To examine the relationship between physical activity (PA) and various cardiometabolic risk factors during an oral glucose tolerance test (OGTT), including glycemic spikes (PGS) in individuals at risk for type 2 diabetes.

SUBJECTS AND METHODS: A total of 949 middle-aged subjects from the Risk factors in Impaired Glucose Tolerance for Atherosclerosis and Diabetes (RIAD) trial aged 40-70 years were included in the present cross-sectional analysis. Standard 75 g OGTT was performed and blood was collected every 30 min for 2 hours for measurements of plasma glucose (PG) and other cardiometabolic risk factors. PA was assessed using interviewer-administered questionnaire.

RESULTS: Post-challenge PGS and maximal PG (PGmax) during OGTT were significantly lower in individuals with high PA vs. individuals with low PA even after body mass index (BMI) adjustment (p = 0.026 and p = 0.035, respectively). In univariate analysis post-challenge PG 30, 60, 90, and 120 minutes, PGS and PGmax during OGTT were significantly inversely correlated to PA. This correlation was attenuated but remained significant after adjustment for BMI. Fasting PG and glycosylated hemoglobin were not correlated to PA. Significantly higher fasting and post-challenge insulin levels were found among subjects with low vs. medium (p < 0.05) and high PA (p < 0.05). Post-challenge C-peptide and proinsulin levels were significantly lower in participants with high vs. participants with low PA (p < 0.05 for all). The relationship between 2-h PG and PA was observed also in lean subjects and in subjects with normal fasting glucose. In multivariate analysis PA was a significant independent determinant of 2-h PG.

CONCLUSION: We found a strong inverse relationship between PA and various post-challenge cardiometabolic parameters during OGTT, including glycemic spikes, in a population at risk for diabetes. This relationship was only partially dependent on BMI.

Keywords: exercise, sedentary lifestyle, post-challenge plasma glucose, glycemic spikes, post-challenge insulin, body mass index

INTRODUCTION

Sedentary lifestyle is one of the strongest predictors of obesity and type 2 diabetes mellitus and is a major contributor to the current worldwide diabetes epidemic.¹-³ Increased physical activity has been shown to be associated with lower total and cardiovascular mortality as well as with reduced risk of type 2 diabetes and metabolic syndrome.⁴-⁷ Prospective studies in various ethnic groups have demonstrated that lifestyle intervention can prevent type 2 diabetes in subjects with impaired glucose tolerance (IGT).⁸-¹² Thus, the risk of diabetes was reduced by 58% in both the Finnish Diabetes Prevention study, and in the American Diabetes Prevention Program which was superior to the medical treatment with metformin.⁹,¹⁰ Furthermore, regular physical activity was shown to reduce the risk of developing type 2 diabetes independently of dietary changes and weight reduction.¹³

The pandemic of type 2 diabetes is among the...
greatest public health problems nowadays. Patients with type 2 diabetes have a 2-4 fold increased cardiovascular risk and premature mortality, and this risk starts to increase very early, parallel to the development of the disease.14-16 In the early phase of diabetes and in prediabetes, postprandial hyperglycemia and glycemic fluctuations seem to be more atherogenic than fasting hyperglycemia.17,18 Thus, in a population at risk for type 2 diabetes we showed that carotid intima-media thickness (IMT), a generally accepted marker of atherosclerosis, is more strongly associated with 2-h post-challenge glucose and glycemic spikes than with fasting glucose and glycosylated haemoglobin.18 Hence, it is of interest to investigate the determinants of non-diabetic postprandial hyperglycemia to successfully enable combating its progression to diabetes and macrovascular complications. Some studies have recently reported beneficial independent association of physical activity with 2-h postprandial glucose level in non-diabetic subjects.19-22 So far, there have been no data reported on the relationship between glycemic spikes as parameter of glucose fluctuations and physical activity.

AIM
To analyse this question a population predisposed to develop type 2 diabetes seems to be most appropriate. Therefore, the aim of the present study was to investigate the relationship of physical activity and various glycemic parameters within an OGTT, including glycemic spikes, in a population at risk for diabetes. Furthermore, we examined the post-challenge changes of specific insulin, C-peptide and proinsulin during OGTT in relation to physical activity, as well as the relationship between physical activity and post-challenge glucose in lean, overweight and obese subjects.

PATIENTS AND METHODS
PATIENTS AND STUDY DESIGN
Subjects were consecutive participants of the RIAD (Risk factors in IGT for Atherosclerosis and Diabetes) trial, details of which were previously published.23 In brief, 1139 subjects (aged 40-70 years) were included, who were at risk of developing type 2 diabetes, such as family history of type 2 diabetes, overweight or obesity and/or hyper/dyslipoproteinemia. Known diabetes, medication affecting glucose tolerance, liver and kidney diseases, functional thyroid gland disorders and acute infections were the exclusion criteria. In the current analysis we excluded participants without conducted oral glucose tolerance test and report the findings in the remaining 949 subjects.

The study participants underwent a standard examination according to a special protocol including: physical examination, resting ECG; questionnaire for lifestyle, own and family medical history; and examination of accepted risk factors for atherosclerosis. All subjects were asked to abstain from heavy exercise or sedentary behaviour as well as from food excess or fasting for three days prior to the test. The study protocol was approved by the Ethics Committee of the University of Dresden. The investigation conforms to the principles outlined in the Declaration of Helsinki and all subjects gave written informed consent prior to participation.

ANTHROPOMETRIC MEASUREMENT AND BLOOD PRESSURE ASSESSMENT
Weight and height were measured by standard techniques. Body mass index (BMI) was calculated as the body weight (kg) divided by the squared height (m²) of the subject. The waist circumference was measured using a plastic tape at the midpoint between the lower rib margin and the iliac crest and the hip circumference at the level of the trochanter. Blood pressure was examined in a sitting position after a rest of at least 5 minutes. Two consecutive measurements were performed within 3 minutes and the second one was taken into consideration.

PHYSICAL ACTIVITY ASSESSMENT
Physical activity was assessed using interviewer-administered questionnaire. Participants were asked to report the different kinds of activities they participated in, the average time spent on these activities per occasion and the total time per week spent on each activity during the past year. This information was used to calculate subjects’ weekly physical activity energy expenditure, expressed in metabolic equivalent-minutes per week (MET-min/week) using the compendium of physical activities.24 Based on the weekly volume of physical activity participants were classified into 3 physical activity categories in accordance with the U.S. Department for Health and Human Services “2008 Physical activity guidelines for Americans “low activity” < 500 MET-min/week, “medium activity” 500-1000 MET-min/week, and “high activity” > 1000 MET-min/week.25

BLOOD SAMPLE ANALYSIS
Venous blood was drawn after an overnight fast of at least 10 h. EDTA plasma and serum were separated...
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by centrifugation (4000 rpm for 8 minutes at 4 °C). Aliquots of plasma and serum were immediately frozen with liquid nitrogen and were stored at -80°C until analysis of insulin, proinsulin, C-peptide and other metabolic parameters. Plasma glucose, lipids and HbA1c were determined using fresh material. A standard OGTT was performed with 75 g glucose (Glucodex, Rougier Inc, Chambly, Quebec, Canada) and blood was collected for the measurement of plasma glucose every 30 min for 2 hours. Plasma glucose was measured by the hexokinase method (inter assay coefficient of variation (CV) = 1.5 %). The post-challenge glucose spikes (PGS) were defined as the difference between the maximal post-challenge glucose level during OGTT (PGmax), irrespective of the time after glucose challenge, and the level of fasting plasma glucose. Glycosylated hemoglobin (HbA1c) was examined by high performance liquid chromatography (HPLC) on a Diamat analyzer (Bio- Rad Laboratories, Munich, Germany).

Plasma triglycerides and total cholesterol were measured enzymatically on a Ciba Corning Express Plus analyzer using commercially available test kits (Boehringer, Mannheim, FRG). High-density lipoprotein (HDL) cholesterol was determined after precipitation with dextran sulfate on a Ciba Corning Express Plus analyzer (Boehringer, Mannheim, FRG). Low-density lipoprotein (LDL) cholesterol level was calculated using Friedewald equation.

Specific insulin and C-peptide were measured by enzyme immunoassays (Medgenix Diagnostics Fleurus, Belgium) in a fasting state as well as 30, 60, 90 and 120 minutes during the OGTT. Specific insulin (inter assay CV = 7.6 %) showed no cross-reactivity to human proinsulin. Proinsulin was analyzed by highly specific enzyme immunoassay (DGR Instruments, Marburg, FRG). The monoclonal antibody used in this assay recognizes a proinsulin specific epitope. It showed cross-reactivity with proinsulin 32-33 split of less than 1.5 % and none with human insulin and human C-peptide.

Statistical analyses

Data are presented as n, mean ± SEM, or percentage (%) as respectively indicated. One way ANOVA and chi-square tests were used for comparisons between groups of physical activity. Correlation analyses were performed using Pearson correlation coefficient. Two-h post-challenge plasma glucose during OGTT was assessed in tertiles of fasting plasma glucose and groups of physical activity, as well as among lean, overweight, and obese subjects and groups of physical activity. The difference in 2-hour plasma glucose in these tertiles was evaluated using one-way ANOVA. Multiple linear regression analysis was applied to identify the strongest determinants of 2-hour plasma glucose. Differences were considered significant at p < 0.05. All statistical analyses were performed using SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL).

Results

As shown in Table 1 the examined subjects were of middle age and had BMI in the overweight range. Subjects with medium physical activity (PA) level were slightly older than those with low activity level, however the difference was of borderline significance (p = 0.034). Individuals with high activity level had significantly lower BMI and waist circumference when compared to individuals classified as having medium and low activity level. Subjects with high level of physical activity exhibited also lower blood pressure, which was statistically significant vs. individuals with low PA for diastolic blood pressure. Besides, lower total cholesterol level was found among subjects with high PA vs. subjects with medium PA. No significant differences were observed with respect to the other lipid parameters, HbA1c, family history of diabetes and smoking habits between the PA categories.

In univariate correlation analysis post-challenge plasma glucose levels 30 min (r = -0.079, p = 0.027), 60 min (r = -0.082, p = 0.022), 90 min (r = -0.115, p = 0.001), and 120 min (r = -0.140, p < 0.001), as well as PGmax (r = -0.134, p = 0.001) and PGS (r = -0.121, p = 0.001) during OGTT were significantly inversely correlated to PA level. This correlation was attenuated but remained significant after adjustment for age, sex and BMI (p = 0.024, p = 0.05, p = 0.009, p = 0.017, p = 0.01, p = 0.03 for post-challenge plasma glucose at 30, 60, 90, 120 min, PGmax, and PGS during OGTT, respectively). Fasting plasma glucose and HbA1c were not found to be correlated to PA.

When PGmax during OGTT was compared in categories of PA (Table 2), a statistically significant difference was observed between subjects with high PA level and subjects with low PA level (p = 0.035). Similarly, PGS (Table 2) were found to be significantly lower in individuals classified as having high PA compared to those with low PA (p = 0.026). Both findings were confirmed after adjustment for age and sex; and were attenuated, but remained significant after an additional adjustment for BMI.
Fig. 1 presents the dynamic changes in plasma glucose (PG), specific insulin, C-peptide, and pro-insulin during OGTT in the categories of PA. With respect to PG (Fig. 1a) no differences were found at baseline (fasting plasma glucose); whereas at 30, 60 and 90 min during OGTT individuals with high PA level had significantly lower PG levels in comparison with individuals with low PA level and at 120 minutes to individuals with medium and low PA level.

Specific insulin concentrations (Fig. 1b) were lower in participants with medium and high PA when compared to participants with low PA at baseline (fasting state) and at 30 min during OGTT. At 60 min significant difference was found only between individuals with high and low activity level. Specific insulin was significantly lower among individuals with high activity compared to individuals with both medium and low PA level at 90 and 120 min during OGTT.

During the OGTT no differences were observed in fasting C-peptide levels between categories of PA (Fig. 1c), whereas at 30, 60, and 90 min subjects with high PA had significantly lower levels in comparison to subjects with low activity; and at 60 min subjects with medium activity had lower levels compared to subjects with low activity. Similarly to the findings for PG and specific insulin, C-peptide concentration 2 hours after OGTT was significantly lower in subjects with high vs. subjects with medium and low activity, respectively.

With respect to proinsulin (Fig. 1d) no significant differences between groups were found at baseline and at 30 min, whereas at 60, 90, and 120 min during OGTT post-challenge proinsulin levels were significantly lower among participants with high PA compared to those with low PA.

When 2-hour post-challenge PG level during OGTT was examined in tertiles of fasting PG and categories of PA (Fig. 2) a significant trend towards increase in 2-hour PG was observed parallel to the decrease of PA level in the first and the second, but not in the third tertile of fasting PG.

We further examined 2-hour PG during OGTT in lean, overweight, and obese subjects and categories of PA (Fig. 3). Among lean individuals 2-hour PG level increased significantly in trend parallel to the decrease in the level of PA. Among overweight subjects, although a comparable trend

| Table 1. Characteristics of the examined subjects (n = 949) in categories of physical activity |
|-----------------------------------------------|----------------|----------------|----------------|
| Characteristic                              | Low activity  | Medium activity | High activity  |
| (n = 119)                                    | (n = 509)      | (n = 321)       |
| Age (years)                                 | 53.2 ± 0.70    | 54.9 ± 0.35 *   | 54.5 ± 0.45    |
| Sex (male/female)                           | 54/65          | 244/265         | 162/159        |
| Body mass index (kg/m²)                     | 28.3 ± 0.49    | 27.4 ± 0.20     | 26.4 ± 0.20 *  |
| Waist circumference (cm)                    | 95.7 ± 1.26    | 94.4 ± 0.60     | 90.9 ± 0.65 *  |
| Systolic blood pressure (mmHg)              | 138.4 ± 1.92   | 135.6 ± 0.91    | 134.5 ± 1.17   |
| Diastolic blood pressure (mmHg)             | 85.7 ± 1.11    | 84.8 ± 0.48     | 83.21 ± 0.63 * |
| Total cholesterol (mmol/l)                  | 5.88 ± 0.11    | 5.91 ± 0.06     | 5.73 ± 0.06    |
| LDL cholesterol (mmol/l)                    | 3.55 ± 0.11    | 3.55 ± 0.05     | 3.50 ± 0.05    |
| HDL cholesterol (mmol/l)                    | 1.45 ± 0.05    | 1.43 ± 0.02     | 1.43 ± 0.03    |
| Triglycerides (mmol/l)                      | 1.94 ± 0.16    | 2.03 ± 0.11     | 1.75 ± 0.94    |
| HbA1c (%)                                   | 5.76 ± 0.06    | 5.69 ± 0.03     | 5.70 ± 0.05    |
| Family history of diabetes (%)              | 63.0           | 59.9           | 58.3           |
| Smoking history (%)                         | 11.8           | 13.0           | 15.0           |

Data are n, %, or mean ± SEM; * p < 0.05 vs. individuals with low activity; † p < 0.05 vs. individuals with medium activity.

| Table 2. Maximal plasma glucose (PGmax) and post-challenge glucose spikes (PGS) in mmol/l during OGTT in categories of physical activity |
|-----------------------------------------------|--------|--------|
| Categories of physical activity               | PGmax  | PGS    |
| Sedentary                                    | 11.40 ± 0.33 | 5.18 ± 0.25 |
| Moderately active                            | 11.04 ± 0.14 | 4.89 ± 0.11 |
| Active subjects                              | 10.66 ± 0.18* | 4.58 ± 0.14* |

Data are mean ± SEM; * p < 0.05 vs. sedentary subjects.
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Overweight individuals with high level of PA showed significantly lower 2-hour PG level in comparison with overweight individuals with medium level of PA. Similar results were found for the joint association of PA level and obesity status with PGmax and PG spikes (data not shown).

In a multiple regression analysis including age, sex, BMI, waist circumference, PA level, and family history of type 2 diabetes as covariates, we found that PA along with age and waist circumference was a significant independent determinant of 2-hour PG, whereas BMI, sex, and family history of type 2 diabetes were not (Table 3).

DISCUSSION

In the present analysis we found a strong inverse relationship between physical activity and various post-challenge glucose parameters during OGTT in a population at risk for type 2 diabetes. This relation was attenuated but remained significant after adjustment for age, sex and BMI. The novel finding of this study is the observed beneficial association between physical activity and post-challenge glycemic spikes that are believed to be atherogenic. It has been repeatedly shown that glycemic variability appears to be more harmful than sustained hyperglycemia for the development of diabetic complications, which is mediated by activating oxidative stress. Thus, the urinary excretion rate of 8-iso-PGF2α, a known marker of oxidative stress, was found to be strongly correlated with glycemic variability that was assessed by the mean amplitude of glycemic excursions, estimated by continuous glucose monitoring systems. Besides, acute glucose spikes were reported to activate the...
release of plasma 3-nitrotyrosine, another marker of oxidative stress, in healthy subjects and type 2 diabetic patients.27

Previously, several studies have reported an independent association between physical activity and 2-h post-challenge glucose.19-22 Thus, being physically active and total PA time were shown to be independently and negatively associated with 2-h PG in the large population-based cross-sectional AusDiab study among individuals without history of type 2 diabetes aged ≥ 25 years.19 Similarly, sedentary behaviour (indicated by television viewing time) was found to be positively associated with 2-h PG, but not fasting PG, independent of physical activity time and adiposity status.20 Higher levels of leisure time PA were reported to be associated with lower 2-h glucose, independent of the level of abdominal obesity, in a cross-sectional population-based random sample of 1812 middle-aged Finnish adults without a history of cardiovascular disease or diabetes.21 Similarly, free-living PA energy expenditure was demonstrated to be inversely associated with 2-h glucose during a standard 75-g OGTT independently of adiposity in adult Cameroonian without known diabetes.22 Furthermore, light-intensity PA was found to be independently associated

Table 3. Determinants of 2-h postchallenge plasma glucose

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>β</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.035</td>
<td>0.097</td>
<td>0.01 to 0.06</td>
<td>0.003</td>
</tr>
<tr>
<td>Physical activity</td>
<td>-0.360</td>
<td>-0.083</td>
<td>-0.63 to -0.09</td>
<td>0.010</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.039</td>
<td>0.180</td>
<td>0.01 to 0.07</td>
<td>0.010</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.062</td>
<td>0.099</td>
<td>-0.02 to 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td>0.275</td>
<td>0.049</td>
<td>-0.20 to 0.75</td>
<td>NS</td>
</tr>
<tr>
<td>Family history of diabetes</td>
<td>0.159</td>
<td>0.028</td>
<td>-0.20 to 0.52</td>
<td>NS</td>
</tr>
</tbody>
</table>

B - unstandardized regression coefficient; β – standardized regression coefficient.
with 2-h PG in a prospective observational study in non-diabetic subjects. Our study confirms the beneficial independent association between PA level and 2-h PG during OGTT in a Caucasian population at risk for type 2 diabetes. It also extends this finding to a variety of other post-challenge parameters during a standard OGTT – such as 30’, 60’ and 90’ post-challenge PG, maximal PG during OGTT and glycemic spikes – which, so far, has not been reported within one and the same population. Since post-challenge hyperglycemia is thought to be more atherogenic than fasting hyperglycemia in non-diabetic individuals and in the early phase of type 2 diabetes the positive effect of increased physical activity on postprandial glucose level could be useful to protect from development of macrovascular disease. Currently there is strong evidence that occupational and leisure-time PA is associated with reduced incidence of coronary artery disease (CAD) events. The data in the literature demonstrate a graded relationship of decreasing CAD rates with increasing PA levels. It goes beyond the scope of the present work to discuss the mechanisms involved in the vascular protective effect of PA, but the beneficial impact on post-challenge hyperglycemia could be of importance in this respect.

Consistent with previous reports, we did not find a significant correlation between fasting PG and physical activity. Fasting and postprandial hyperglycemia although interrelated have different pathophysiology. Postprandial hyperglycemia predominantly reflects skeletal muscle insulin resistance, whereas fasting hyperglycemia is characterized primarily by impaired hepatic insulin sensitivity. The beneficial impact of PA on postprandial but not fasting glucose could be explained by the fact that PA is directly associated with improved peripheral insulin sensitivity. We also did not demonstrate any difference in HbA1c level in the three groups of PA in our population at risk for type 2 diabetes but without known history of it. Studies on the effect of exercise in patients with type 2 diabetes reported a HbA1c reduction of 0.5 to 1 %, depending on the baseline HbA1c level.

We found higher fasting and post-challenge insulin levels in subjects with low PA in comparison with the individuals with respectively medium and high PA. Fasting hyperinsulinemia is a known characteristic feature of sedentary lifestyle reflecting lower insulin sensitivity. Some studies have suggested that compensatory hyperinsulinemia in insulin resistant subjects might result in potential increase in cardiovascular and cancer events. Thus, postprandial hyperinsulinemia was shown to be independently associated with CAD, irrespective of fasting glucose, postprandial glucose, and fasting insulin levels in non-diabetic subjects. Therefore, reducing postprandial insulin level by increased PA could contribute to prevention of atherosclerosis. Although it is not the purpose of the current paper to analyse insulin secretion in categories of physical activity, we would briefly mention that insulin concentrations alone without taking into account corresponding glucose values may be misleading with respect to insulin secretion capacity. However, with respect to proinsulin response to glucose load our data suggest that PA protects the B-cells from harmful effects of postprandial spikes and hyperglycemia.

Epidemiological and experimental studies have convincingly shown the protective role of PA in reducing the risk of developing type 2 diabetes. Furthermore, lifestyle intervention in subjects at risk for type 2 diabetes resulted in sustained lifestyle changes and reduced diabetes incidence, which remained after the individual lifestyle counseling had been stopped. Prospective studies have demonstrated that physically active lean subjects are at the lowest and inactive obese subjects – at the highest risk to develop type 2 diabetes, however less is known about the protective role of PA in lean individuals. Currently, it is strongly emphasized on the importance of being physically active for hyperglycaemic and obese subjects as a preventive measure against development of type 2 diabetes and cardiovascular disease. The present analysis shows that the linear relationship between 2-h post-challenge glucose and physical activity was also observed in lean subjects and in subjects with fasting PG below 5.6 mmol/l. This supports the notion that regular PA should be recommended by health professionals on a population level, including normoglycemic and lean individuals. This is consistent with a report of the Women’s Health Study on the joint effect of PA and BMI on coronary heart disease, reinforcing the importance of both being lean and physically active.

CONCLUSIONS

We found a strong inverse relationship between physical activity level and various post-challenge glucose parameters during OGTT, including glycemic spikes, in a population at risk for type 2 diabetes.
This relation was only partially dependent on BMI, since it was attenuated but remained significant after BMI was taken into account.

REFERENCES


зависимость между стоимостьями сахара в крови на 30, 60, 90 и 120 мин. после ОГТТ, между гликемическими пиками и максимальной стоимостью глюкозы. Наблюдаемая зависимость оказалась слегка измененной, но осталась значимой после коррекции влияния ИМТ. Корреляция между уровнями сахара в крови натощак, уровнями гликированных гемоглобинов и ФА не установлена. Достоверно более высокие уровни сахара в крови и инсулина после ОГТТ наблюдались среди лиц с низкой ФА по сравнению с лицами с умеренной (p < 0.05) и высокой ФА (p < 0.05). Уровни С-пептида и проинсулина более низки среди лиц с высокой ФА по сравнению с лицами с низкой ФА. Наблюдаемая разница статистически достоверна (p < 0.05). Взаимосвязь между сахаром в крови 2 ч. после ОГТТ и уровнями ФА наблюдалась и среди лиц с нормальной массой тела и среди лиц с нормальными исходными стоимостью сахара в крови натощак. В мультивариантном анализе установлено, что ФА представляет собой независимый фактор, определяющий уровень глюкозы 2 ч. после ОГТТ.

Заключение: Настоящее исследование установило сильную обратно пропорциональную зависимость между уровнями ФА, между множеством кардио-метаболических факторов риска после проведения ОГТТ, включая и гликемические пике среди лиц с повышенным риском развития сахарного диабета типа 2. Наблюдаемая зависимость была только частично определена ИМТ.