Glucose metabolism in discordant monozygotic twins for cardiorespiratory fitness

Metabolismo de glicose em gêmeos monozigóticos discordantes para aptidão cardiorrespiratória

Metabolismo de glucosa en gemelos monocigóticos discordantes para aptitud cardiorrespiratoria

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ABSTRACT

Objective: To determine if glucose and insulin concentrations are regulated by cardiorespiratory fitness (VO_{2max}) regardless of their genetic effects.

Methods: This cross-sectional study enrolled 38 pairs of young monozygotic twins (11 to 18 years-old). All subjects underwent a progressive maximal exercise test on a treadmill to determine the VO_{2max} with gas exchange analysis (MedGraphics VO2000® - Medical Graphics Corp., St. Paul, MN). Blood samples were drawn after fasting to determine glucose and insulin levels. Monozygosity was confirmed by genotyping 15 informative genetic markers. Nine pairs had at least $10mL.kg^{-1}.min^{-1}$ difference in VO_{2max} and were divided into the more and less active group, according to their VO_{2max}. Mean differences between more and less active groups were evaluated by Wilcoxon's test for paired data.

Results: On average, twins from the more active group presented a 17% ($13.5\pm3.7mL.kg^{-1}.min^{-1}$) higher VO_{2max} compared to their less active siblings. No significant differences were observed between the groups for fasting insulin (36.5±34.6 *versus* 25.3±13.7mg/dL; $p<0.813$). However, the more active twins had lower fasting glucose than the less active ones (82.9±7.3 *versus* 86.7±7.6mg/dL; $p<0.010$).

Conclusions: In this case-control study (discordant monozygotic twins), the less active co-twins were characterized by higher fasting plasma glucose levels. This implies that poor cardiorespiratory fitness can be associated with defective glucose metabolism regardless of genetic factors.

Key-words: monozygotic; twin study; physical fitness; case-control studies.

RESUMO

Objetivo: Verificar se as concentrações de glicose e insulina em jejum são reguladas pela aptidão cardiorrespiratória ($VO_{2máx}$), independentemente dos efeitos genéticos.

Métodos: Dados de 38 pares de gêmeos monozigóticos (11 a 18 anos) foram analisados transversalmente. Os participantes foram submetidos a um teste de esforço máximo com ergoespirometria aberta (MedGraphics VO2000® – Medical Graphics Corp., St. Paul, MN) e à coleta de sangue para estimar a concentração de glicose e insulina em jejum.

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A zigosidade foi determinada por intermédio da investigação de concordância dos gêmeos em relação a 15 marcadores genéticos polimórficos. Nove pares demonstraram diferença média intrapar para o consumo máximo de oxigênio ≥10mL.kg^{-1}.min^{-1} e foram divididos em dois grupos, de alta e baixa aptidão. Os grupos foram comparados a partir do teste pareado de Wilcoxon, tendo em vista a assimetria dos dados.

Resultados: Em média, os gêmeos do grupo de alta aptidão apresentaram consumo máximo de oxigênio 17% superior (13,5±3,7mL.kg^{-1}.min^{-1}) a seus irmãos menos aptos. Não houve diferença entre os grupos para as concentrações de insulina (36,5±34,6 *versus* 25,3±13,7mg/dL; p<0,813), porém, os gêmeos mais aptos demonstraram menor concentração de glicose do que seus contrapares menos aptos (82,9±7,3 *versus* 86,7±7,6mg/dL; p<0,010).

Conclusões: Neste estudo, caracterizado como caso-controle (gêmeos monozigóticos discordantes), o irmão com menor aptidão cardiorrespiratória apresentou maior concentração de glicose em jejum, sugerindo que a baixa aptidão cardiorrespiratória está associada a distúrbios no metabolismo de glicose.

Palavras-chave: monozigóticos; estudo em gêmeos; aptidão física; estudos de casos e controles.

RESUMEN

Objetivo: Verificar si las concentraciones de glucosa e insulina en ayuno son reguladas por la aptitud cardiorrespiratoria (VO2máx), independiente de los efectos genéticos.

Métodos: Datos de 38 pares de gemelos monocigóticos (11 a 18 años) fueron analizados transversalmente. Los participantes fueron sometidos a una prueba de esfuerzo máximo con ergoespirometría abierta (MedGraphics VO2000® Medical Graphics Corp., St. Paul, MN) y a la recolección de sangre para estimar la concentración de glucosa e insulina en ayuno. La cigosidad fue determinada por medio de la investigación de concordancia de los gemelos respecto a 15 marcadores genéticos polimórficos. Nueve pares demostraron diferencia mediana intrapares para el consumo máximo de oxígeno ≥10mL.kg^{-1}.min^{-1} y fueron divididos en dos grupos, de alta y baja aptitud. Los grupos fueron comparados a partir de la prueba pareada de Wilcoxon, teniendo en vista la asimetría de los datos.

Resultados: En promedio, los gemelos del grupo de alta aptitud presentaron consumo máximo de oxígeno el 17% superior (13,5±3,7mL.kg^{-1}.min^{-1}) a sus hermanos menos aptos. No hubo diferencia entre los grupos para las concentraciones de insulina (36,5±34,6 versus 25,3±13,7mg/dL; p<0,813), pero los gemelos más aptos demostraron menor concentración de glucosa que sus contrapares menos aptos (82,9±7,3 versus 86,7±7,6mg/dL; p<0,010, respectivamente).

Conclusiones: En este estudio, caracterizado como caso-control (gemelos monocigóticos discordantes), el hermano con menor aptitud cardiorrespiratoria presentó mayor concentración de glucosa en ayuno, sugiriendo que la baja aptitud cardiorrespiratoria está asociada a disturbios en el metabolismo de glucosa.

Palabras clave: monocigóticos; estudio en gemelos; aptitud física; estudio control de casos.

Introduction

Non-insulin-dependent diabetes mellitus, or type 2 diabetes (DM2), is characterized by a high blood glucose concentration[1]. Its prevalence has been increasing rapidly in several countries, regardless of their level of development, and this increase was observed in both sexes, all ages, all ethnic groups, and all education levels[2,3]. Although this disease affects predominantly adults and older individuals, it is increasingly more common among children and youngsters[4,5].

Despite the fact that DM2 is considered a multifactorial disease, evidence points out to defects in glucose metabolism, more specifically in the secretion of insulin by beta-cells, to reduced action of insulin on tissues (sensitivity), or to both[6,7]. Among the risk factors associated with insulin resistance, glucose intolerance and DM2, one of the most important is poor cardiorespiratory fitness[8,9].

Maximal oxygen uptake (VO_{2max}), an indicator de cardiorespiratory fitness, can be seen as an objective parameter of physical fitness status and/or practice of daily physical activity[9]. Regular physical activities and high VO_{2max} are considered determinant behavioral or environmental factors in the prevention and treatment of DM2 due to their influence in glucose metabolism[10,11]. However, the association between VO_{2max}, glucose tolerance and sensitivity to insulin is established without taking into account the genetic influences inherent to each individual. A strategy to evaluate the impact of VO_{2max} on glucose metabolism, regardless of genetic factors, is investigating monozygotic (MZ) twins discordant for cardiorespiratory fitness. This method is known as case-control model and provides a great opportunity to determine if a feature (phenotype) acts on other features, regardless of genetics[12-14]. Thus, this study with MZ twins aimed to determine if glucose and insulin concentrations are regulated by VO_{2max}, without regards of genetic effects.

Method

By means of a population survey conducted in 2008, 98 pairs of same-sex twins (11 to 18 years-old), born between 1990 and 1997 (53 female pairs and 45 male pairs), were identified in the municipality of Rio Claro, state of São Paulo, Brazil. All state and private schools of the municipality were visited. Of the 98 pairs identified, in 31 of them one or both twins refused to participate in the study and 13 were not located. A total of 54 healthy pairs (35 female pairs and 19 male pairs), who were not being treated with drugs at the time of assessments, accepted to take part in the study. A paternity test revealed that the sample comprised 38 pairs of MZ twins and 16 pairs of dizygotic (DZ) twins. In view of the design of this study, only MZ twins were included in the research, i.e., 38 pairs.

MZ twins and their parents and/or guardians were previously informed about the experimental procedures used and gave written informed consent. The intervention protocols were approved by the Research Ethics Committee of Universidade Estadual Paulista (Unesp) under protocol no. 5.093 and comply with the regulations of Resolution 196/96 of the National Health Council of the Brazilian Ministry of Health on research involving human beings.

One week after cardiorespiratory capacity (VO_{2max}) was assessed, the twins came to the clinical analysis laboratory at 7:30 a.m., after overnight fasting (between 10 and 12 hours), accompanied by their guardians. After remaining at rest for 30 minutes, the twins had blood samples taken from the antecubital vein, using the vacuum blood collection system (Vacutainer™ Becton Dickinson Company, Plymouth, United Kingdom). Aliquots were dispensed into 4-mL tubes with anticoagulant (fluoride associated with ethylenediaminetetraacetic acid (EDTA) 1mg/mL blood and 1mg/mL EDTA) and into 3.5-mL tubes with heparin for analysis purposes. Plasma was used to determine fasting glucose and insulin. Nearly 150µL of the blood collected were directly pipetted onto QIAcard® (Qiagen, Valencia, USA) for further DNA analysis.

Twins were assigned as MZ or DZ by genotyping genetic markers (DNA), such as minisatellite loci, also known by the acronym STR (*short tandem repeat*). Sixteen autosomal STRs (CSF1PO, D2S1338, D3S1358, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, D5S51, FGA, TH01, TPOX, vWA and amelogenin *locus*) from the DNA samples were analyzed by PCR amplification, using the Identifiler kit, according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA)

Anthropometric measures of body mass (BM) and height were obtained for the calculation of body mass index (BMI) in kg/m^2. In order to determine VO_{2max}, a physical stress test (PST) was performed on an ATL Super® treadmill (Inbrasport, Porto Alegre, RS, Brazil), with incline set to 1%, in the morning and the afternoon, from 9:00 a.m. to 11:30 a.m. and from 2:00 to 6:00 p.m., in a room with the temperature maintained between 20 and 25ºC. After a period of five minutes in which subjects became familiar with the ergometer at different speeds (4 to 7km/h), they remained at rest on the treadmill for five minutes in standing position.

Then the test started with a protocol consisting of an initial speed of 4km/h, with progressive increments of 1km/h per minute in workload. Verbal encouragement was given in an attempt to achieve maximal physical exertion. Besides volitional exhaustion, respiratory exchange rate (RER) higher than 1.15 and rating of perceived exertion of 20 (220-age) were also used as indicators for terminating the test. Minute volume (VE), VO_2 and carbon dioxide production (VCO_2) were continuously recorded (metabolic analyzer MedGraphics VO2000® Medical Graphics Corp., St. Paul, MN) at rest and during PST. The equipment was calibrated previously to the development of the research and at the beginning of each PST. VO_{2max} was collected breath by breath, and the value adopted to analyze the data was recorded as the mean oxygen uptake in the last 30 seconds before PST was terminated.

Continuous Measurement of Pulmonary Gas Exchange

In order to apply the model of studies with MZ twins (case-control), the absolute intrapair difference in VO_{2max} was established as 10mL.kg^{-1}.min^{-1} in order to determine which twin pairs were discordant for cardiorespiratory fitness. Analyzes were performed using a statistical package (SPSS, version 13.0), with significance level set at $p<0.05$. Initially, the Shapiro-Wilk test was used to analyze data distribution. The Wilcoxon's test was applied to assess the presence of differences between discordant twins (higher and lower VO_{2max}) regarding anthropometric and biochemical variables.

Results

Of the 38 pairs of MZ twins included in the research, only nine had an intrapair difference in VO_{2max} equal to or higher than 10mL.kg^{-1}.min^{-1} (discordant). Discordant twins, four male pairs and five female pairs, with a mean age of 13.9±2.2 years, were stratified into two subgroups, one comprising the twin with

Table 1 - Anthropometric characteristics of nine pairs of monozygotic twins discordant for maximal oxygen uptake†

	Higher maximal VO_2 (9)	Lower maximal VO_2 (9)	*p*-value
Maximal VO_2 ($mL.kg^{-1}.min^{-1}$)	45.9±10.0	32.4±10.6	0.008
BM (kg)	46.4±9.0	46.2±8.7	0.953
Height (cm)	155.7±11.5	156.4±11.0	0.343
BMI (kg/m^2)	18.9±1.4	18.7±1.5	0.594
Age (years)	13.9±2.2		
Sex (♂/♀)	(4/5)		

Maximal VO_2: maximal oxygen uptake; BM: body mass; BMI: body mass index; †: intrapair difference for maximal VO_2 equal to or higher than $10mL.kg^{-1}.min^{-1}$; data expressed as mean and standard deviation (±).

Table 2 - Fasting insulin and glucose concentrations of nine pairs of monozygotic twins discordant for maximal oxygen uptake

Variables	Higher maximal VO_2*	Lower maximal VO_2	*p*-value
FI (mg/dL)	36.5±34.6	25.3±13.7	0.813
FG (mg/dL)	82.9±7.3	86.7±7.6*	0.010

*intrapair difference for maximum oxygen uptake equal to or higher than $10mL.kg^{-1}.min^{-1}$; FI: fasting insulin; FG: fasting glucose.

higher VO_{2max} and the other comprising the twin with lower VO_{2max} (Table 1). It bears stressing that the relative difference within the pairs of discordant twins (with higher and lower VO_{2max}) ranged between 16.9 and 42.1% (mean of 30.6±9.5%), while for the concordant twins the range was from 0.3 to 33% (mean of 10.6±8.2%). In turn, the absolute mean of these differences was $13.5±3.7mL.kg^{-1}.min^{-1}$ for discordant pairs and $3.6±2.3mL.kg^{-1}.min^{-1}$ for concordant pairs (data not shown).

Discordant twins had mean values of 45.9±10.0 versus $32.4±10.6mL.kg^{-1}.min^{-1}$ for the sibling twin with higher and lower VO_{2max}, respectively ($p<0.01$), but showed no differences in anthropometric variables (Table 1). The difference in VO_{2max} between co-twins with higher and lower VO_{2max} prevailed even when the pairs were separated by sex, i.e., females (40.6±6.3 versus 27.6±7.2) and males (52.4±10.5 versus 38.4±12.0) – data not shown.

Table 2 provides information on variables related to glucose metabolism. It was observed that among the pairs of discordant MZ twins the concentration of fasting glucose was significantly lower in the co-twin with higher VO_{2max}. Therefore, there was no difference in fasting insulin.

Discussion

The hypothesis of this study was that the discordance in VO_{2max} (cardiorespiratory fitness) $\geq 10mL.kg^{-1}.min^{-1}$ between identical twins would be able to influence glucose metabolism, regardless of genetic effects. No differences were observed in BM, height or BMI between co-twins with higher and lower VO_{2max}. However, a significant difference in fasting glucose concentration was identified among discordant twins. In other words, co-twins with higher VO_{2max} had a lower glucose concentration when compared to their co-twins with lower VO_{2max} (82.9±7.3 versus 86.7±7.6mg/dL, respectively). Considering that the paired subjects were genetically identical, this result indicates that cardiorespiratory fitness can modulate plasma glucose concentration regardless of genetic factors, but there is no such evidence for fasting insulin.

Information from studies that used traditional methods of basic and applied research (without controlling for genetic effects), in addition to the knowledge gained from studies with MZ twins, provide grounds for the discussion of the results found in the present study. In this sense, the investigation of a sample of young MZ twins (mean of 25 years) discordant for obesity revealed associations between obesity and alterations in lipid metabolism(15), endothelial dysfunctions(16) and disorders in amino acid metabolism(17), independent of genetics. Physical activity assessed by a questionnaire in a sample of middle-aged female MZ twins revealed that the most physically active women had lower total-body and central adiposity than their more obese co-twins(13). Twins discordant for visceral fat (14 women and 9 men; 33 to 59 years) showed significant alterations in insulin sensitivity and glucose tolerance(18). The same sample demonstrated that plasma leptin levels were increased in obese twins, regardless of genetic effects(19).

Despite the significant number of studies analyzing the effects of obesity on metabolic risk factors independent of genetics, the use of a model of MZ twins discordant for exercise or cardiorespiratory fitness is still rare. The analysis of

35 pairs of MZ twins (10 female and 25 male with 40.5±6.8 years) discordant for vigorous exercise (the more active twins run an average of 63.0±20.4 km/wk whereas the less active twins had an average of 7km/week) revealed that an active behavior may reduce genetic influences on BMI[20]. In another study, an investigation of 14 pairs of MZ twins discordant for obesity (24 to 27 years) evidenced a significant association, with a defective expression of the genes that encode components of mitochondrial oxidative phosphorylation in adipose tissue among the less active co-twins[14]. The effects of physical activity and cardiorespiratory fitness on free fatty acid (FFA) uptake in skeletal muscle, the myocardium and liver were also analyzed, regardless of genetics, in nine pairs of MZ twins (25.9±1.7 years). The results revealed that hepatic FFA uptake was significantly lower (33%) in more active co-twins compared to their less active counterparts[21].

The present study, which analyzed the effects independent of genetics on glucose metabolism in MZ twins aged between 11 and 18 years, showed lower fasting glucose concentrations in MZ co-twins with higher cardiorespiratory fitness (VO_{2max}). However, the twins did not differ for anthropometric variables and plasma insulin concentrations, despite the discordance in VO_{2max}. It is interesting to point out that the similarity observed among MZ twins does not necessarily mean that the phenotype (feature) is genetic in its origin. This sample comprised young twins (11 to 18 years) who live together and therefore share the same environment. The similarity in this case could result from genetic and environmental sharing, while the discordance, in turn, should indicate that the disease/dysfunction is due at least partly to non-genetic factors[22]. An important difference between the present study and those that used the same model (case-control) is the fact that the latter investigated samples of older MZ twins (above 24 years of age) who did not live in the same household.

In addition to evidence that VO_{2max} can reduce genetic influences on BMI (obesity indicator)[20], influence the expression of genes of mitochondrial oxidative phosphorylation in adipose tissue[14] and reduce hepatic FFA uptake[21], it is suggested that a higher cardiorespiratory fitness is able to exert primary effects independent of genetic factors on metabolic diseases and disorders such as DM2. There is evidence that abnormalities in the rates of glucose use in children with first-degree relatives with DM2 (predisposed to DM2) can be improved by the increase in cardiorespiratory fitness[23].

However, it is important to highlight and justify some limitations of this study. The first one concerns the establishment of the discordance based on cardiorespiratory fitness. In this sense, since cardiorespiratory fitness (grounded on family aggregation and twin studies) shows significant genetic effects (50 to 67%)[24,25], the present results should be interpreted with caution. One of the assumptions of twin studies is that a phenotype carries a higher genetic effect when it shows low variance among MZ twins. In classical studies with MZ twins, the discordance is defined according to criteria well-founded in the literature and the phenotype is usually an acquired condition[26]. However, even in studies in which the variance allows to establish discordance between identical twins (e.g., one twin is diabetic/obese/hypertensive/sedentary and the other is not), it is still in question which genetic or environmental factor could have most significantly influenced such discordance[27,28].

Additionally, it was observed that the behavior of cardiorespiratory fitness changes throughout life and receives the influence of different factors, such as: age, sex and physical activity level. It bears stressing also that, besides the improvement in VO_{2max} provided by physical activity, the period corresponding to childhood and adolescence is marked by the progressive growth and development of components of the cardiorespiratory system, which determine VO_{2max} (lungs, heart and muscles)[29]. Thus, a possible discordance in VO_{2max} at this age among genetically identical individuals would be probably due to environmental effects (physical activity and/or physical exercise). In other words, the determinants that distinguish the aerobic fitness of MZ twins could be attributed to the environment, because in this model the alterations observed in VO_{2max} in function of the very growth and development of different systems would be under genetic control. Considering the increase in VO_{2max} induced by growth, at this stage it is less likely to reduce (lose) aerobic capacity than to increase it. Therefore, disregarding errors from the subject, the rater and the measuring equipment, it is possible that co-twins from the discordant pair who showed higher VO_{2max} in this study are really more active than their twin sibling.

Finally, regarding the limitations of this study, it is interesting to address the cutoff value of $10mL.kg^{-1}.min^{-1}$ adopted to establish the discordance between MZ twins. In a longitudinal study with MZ and DZ twins discordant for cardiorespiratory fitness (26.4±4.9 versus $32.5±5.5mL.kg^{-1}.min^{-1}$), the researchers used a lower cutoff value ($\approx 6mL.kg^{-1}.min^{-1}$) and emphasized that intrapair differences were enough to identify metabolic disorders[30]. In another research, the investigators adopted a cutoff value of 9% to determine discordance (mean of 18±10%) in VO_{2max} among MZ twins. The more and the less active twins showed values of 50.9±5.1 versus $43.4±6.7mL.\ kg^{-1}\ min^{-1}$

respectively[21]. It bears stressing that in the present study the relative difference between twins with higher and lower VO_{2max} (discordant) ranged from 16.9 to 42.1%, with mean of 30.6±9.5% (data not shown). Furthermore, the absolute mean of these differences was 13.5±3.7mL.kg^{-1}.min^{-1} for discordant pairs (Table 1) and nearly 7.5mL.kg^{-1}.min^{-1} for the study by Hannukainen *et al*[21].

Considering thus the limitations of the study and the results found, it is possible to suggest that the case-control model supports the hypothesis that cardiorespiratory fitness can modulate glucose concentration in childhood and adolescence, regardless of genetic factors. This finding reveals the importance of cardiorespiratory fitness in the prevention of metabolic disorders associated with glucose tolerance and confirms what experimental studies that did not control for genetic factors have been emphatically reporting, i.e., that a lower cardiorespiratory fitness (at all ages) is associated with disorders in glucose metabolism, among other issues.

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