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Original Article

The role of genetic variation in TCF7L2 and KCNJ11, dietary intake, and physical activity on fasting plasma glucagon-like peptide-1 in male adolescents

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Abstract

Background Transcription factor 7-like 2 (TCF7L2) and potassium voltage-gated channel subfamily j member 11 (KCNJ11) gene polymorphisms have been associated with type 2 diabetes mellitus (T2DM) via regulation of insulin production. Ingested nutrients induce glucagon-like peptide-1 (GLP-1), which in turn induces insulin secretion.

Objective To evaluate the relationship between TCF7L2 and KCNJ11 gene polymorphism, dietary intake, and physical activity on fasting plasma GLP-1 in normal male adolescents.

Methods This observational study with a cross-sectional design included 54 male adolescents selected from high schools in Yogyakarta, Indonesia. Interviews were done to collect data on energy intake and physical activity. The GLP-1 and insulin levels were measured from fasting blood plasma. The TCF7L2 and KCNJ11 gene polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results Fasting GLP-1 was positively correlated with energy intake ($r=0.276$; $P=0.047$), but not with physical activity ($r=0.011$; $P=0.936$). The GLP-1 concentration was not associated with TCF7L2 and KCNJ11 gene polymorphisms (all $P>0.05$). In subjects with an EE genotype (KCNJ11), GLP-1 was not correlated with insulin ($r=-0.036$; $P=0.435$). However, in subjects with an EK genotype (KCNJ11), GLP-1 was positively correlated with insulin ($r=0.394$; $P=0.026$).

Conclusion GLP-1 concentration is positively correlated with body weight. Among male adolescents with a genetic variation in KCNJ11 (EK genotype), there is a significant correlation between GLP-1 and insulin signalling. **[Paediatr Indones. 2017;57:239-45; doi: http://dx.doi.org/10.14238/pi57.5.2017.239-45]**.

Keywords: *KCNJ11; TCF7L2; GLP-1; diet; physical activity*

Type 2 diabetes mellitus (T2DM) is a disturbance in glucose metabolism as shown by high blood glucose, due to a disorder in insulin secretion or sensitivity.[1] In 2000, the World Health Organization reported that 171 million people worldwide suffered from T2DM.[2] And this disease was responsible for deaths of 1.5 million people in 2012.[3] Epidemiological studies have shown indicators of T2DM in adulthood can be detected from younger ages.[4,5] Insulin resistance and hyperglycemia are good indicators of early onset T2DM, and are prevalent in obese children and adolescents.[6,7]

Environmental and genetic factors have long been associated with insulin resistance in adolescents and the development of T2DM in adults. Dietary factors, such as high energy and fat intake as well as low dietary fiber intake, were shown to be related to increased risk of insulin resistance.[8–10] Obese

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nutritional status increased the risk of insulin resistance; and weight loss was associated with improvement of insulin sensitivity.[11–13]

In addition to environmental factors, several genetic variations that have been associated with increased risk of T2DM. In this study, we aimed to evaluate the role of genetic polymorphism in the TCF7L2 (transcription factor 7-like 2) and KCNJ11 (the potassium inwardly rectifying channel, subfamily J, member 11) genes. Both genes are important in glucose metabolism via regulation of insulin secretion. TCF7L2 gene is located on chromosome 10q25.3 and produces a high-mobility box-containing transcription factor.[14] The TCF7L2 has an important role in the signalling process of glucose metabolism, and variations in this gene have been associated with T2DM.[15] KCNJ11 is a member of the potassium channel gene that is located at 11p15.1 and responsible for production of Kir6.2. Kir6.2 is a component of the ATP-sensitive potassium channel (KATP) in pancreatic beta cells and important in regulation of insulin secretion.[16] Recently, a KCNJ11 gene polymorphism was reported to be associated with increased risk of T2DM.[17]

The TCF7L2 and KCNJ11 genes were associated with T2DM via regulation of insulin production. In pancreatic cells, insulin is released in response to several signals, including glucagon-like peptide-1 (GLP-1). GLP-1 is secreted by enteroendocrine cells (L cells) in the intestinal epithelium, in response to ingested nutrients.[18] GLP-1 induces postprandial insulin secretion and reduces post-prandial hyperglycemia. The effect of GLP-1 as a stimulator of glucose-dependent insulin release has been confirmed by many studies using different approaches.[19-21]

Although the TCF7L2 and KCNJ11 gene products induce insulin secretion from different pathways, they are connected to the regulation of GLP-1. TCF7L2, known as a transcription factor, has an ability to regulate production of GLP-1.[22,23] The KCNJ11 gene produces protein that stimulates insulin secretion via KATP channels. In an animal model, mutation of the KCNJ11 gene induced disturbances of insulin response to the GLP-1 signal.[24]

The role of TCF7L2 and KCNJ11 gene polymorphisms on regulation of GLP-1 production is an interesting connection that is not well understood. GLP-1 may be a good early indicator of T2DM, especially in younger aged children. To our knowledge, no studies to date have highlighted the connection between those genes and GLP-1 production in adolescents. Therefore, we aimed to evaluate the relationship between TCF7L2 and KCNJ11 on fasting plasma GLP-1 levels in adolescents. In addition to genetic factors, we also analyzed for a possible correlation between GLP-1 and lifestyle factors, such as dietary intake and physical activity in male adolescents.

Methods

This observational study with cross-sectional design included normal male adolescents from Yogyakarta, Indonesia. A total of 54 subjects aged 16-18 years were randomly selected from 10 high schools. Those with no medical problems were asked to participate in this study. Subjects were categorized as normal weight, overweight, or obese, based on WHO criteria.[25] This study was approved by the *Medical and Health Research Ethics Committee* (MHREC), Universitas Gadjah Mada Medical School.

Anthropometric measurements were done by trained personnel. Body weight was measured using A digital scale (0.1 kg precision) and height was measured using a microtoise (0.1 cm precision). Neck and waist circumferences were measured using non-stretchable plastic tape. Measurements were done twice, and the means of those measurements were used for further analysis. This study was part of an observational study on genetic, metabolic, and lifestyle aspects of metabolic syndrome in adolescents.[26]

Interviews were conducted to collect data on lifestyle, including dietary intake and physical activity. Dietary intake was measured using a validated, semi-quantitative food frequency questionnaire.[26] Physical activity was measured using an international physical activity questionnaire.[27] Blood collection was done in the morning after 10 hours of fasting. Blood specimens were placed in EDTA tubes and separated into plasma and buffy coat. Plasma was separated from whole blood, then stored at -80°C prior to use. GLP-1 was measured using an enzyme immunoassay (EIA) (*Sigma Aldrich*); insulin was measured using using an enzyme-linked immunoassay (ELISA) (DRG). The DNA sample was isolated from buffy coat using a

DNA isolation kit (Promega). KCNJ11 (Glu23Lys) and TCF7L2 (rs12255372) gene polymorphisms were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), with primers, and restriction enzymes, and conditions shown in **Table 1**.

Table 1. Primers and restriction enzymes used for PCR-RFLP

Genes	Primers (forward)	Primers (reverse)	Restriction enzymes	Incubation temperature & time
KCNJ11 (Glu23Lys)	5'-GACTCTGCAGT-GAGGCCCTA-3'	5'-ACGTTG-CAGTTGCCTTTCTT-3'	5U BAN II	60°C for 3 hours
TCF7L2 (rs12255372)	5'-CTG GAA ACT AAG GCG TGA GG -3'	5'- GGG TCG ATG TTG TTG AGC TT -3'	BseGI	65°C for 3 hours

Statistical analysis was done using *GraphPad Prism version 5.00 for Windows, GraphPad Software*, San Diego, California, USA. Data is presented as mean (standard deviation, SD). Independent T-test was used to compare the anthropometric measures, energy intake, physical activity, GLP-1 level, and insulin level between genotypes. Pearson's correlation test was used to evaluate for possible correlations between plasma GLP-1, insulin level, energy intake, and physical activity. Spearman's correlation test was used to analyze data that were not normally distributed. Results with $P<0.05$ were considered to be statistically significant.

Results

A total of 54 male adolescents were involved in this study. Characteristics of subjects are shown in **Table 2**. Nutritional status was based on BMI for age Z-score. The number of subjects with normal weight (-2<BMI<2), overweight (2<Z-score<3), and obese (Z-score > 3) were 43 (79.63%), 6 (11.11%), and 5 (9.26%), respectively.

The association between TCF7L2 and KCNJ11 gene polymorphisms and anthropometric measures, dietary intake, and endocrine signals are shown in **Table 3**. In TCF7L2 gene polymorphism, no subjects had the TT genotype, hence, the association analysis compared the dominant GG genotype and heterozygote GT genotype. TCF7L2 gene polymorphism was associated with higher body weight, taller height, and greater waist circumference ($P<0.05$). Dietary intake and mean physical activity were not significantly different between those with the GT and the GG genotypes of TCF7L2.

With regards to KCNJ11, all three genotypes were evaluated. We compared the dominant genotype (EE) group to genotypes with the K allele (EK + KK) group, but found no significant associations between KCNJ11 gene polymorphism and anthropometric measures, mean physical activity, or dietary intake.

The fasting plasma insulin and GLP-1 levels were measured in all subjects, and compared between KCNJ11 (EE and EK+KK) and TCF7L2 (GG and GT) genotypes. As shown in **Figure 1**, neither insulin nor GLP-1 significantly differed between KCNJ11 genotypes ($P=0.806$; $P=0.411$, respectively) nor between TCF7L2 genotypes ($P=0.455$; $P=0.531$, respectively).

Table 2. Characteristics of subjects

Characteristics	N=54
Mean age (SD), years	16.3 (0.6)
Mean anthropometric measures (SD)	
Body weight, kg	67.3 (18.7)
Height, cm	168.9 (6.6)
BMI, kg/m^2	23.6 (6.4)
BMI for age Z-score	0.4 (1.6)
Neck circumference, cm	35.4 (5.1)
Waist circumference, cm	80.7 (16.3)
Mean daily dietary intake (SD)	
Energy intake, kcal	3195 (1,097)
Protein intake, g	96.3 (37.2)
Fat intake, g	90.1 (39.8)
Carbohydrate intake, g	483.5 (164.9)
Fiber intake, g	8.9 (4.2)
Mean physical activity (SD), Mets-minute	2397 (1,346)
Mean fasting plasma insulin (SD), µIU/mL	14.8 (10.1)
Mean fasting plasma GLP-1 (SD), pg/mL	61.6 (27.8)

Table 3. The association of TCF7L2 and KCNJ11 gene polymorphisms with anthropometric measurements, dietary intake, and endocrine signals.

Measurements	TCF7L2			KCNJ11		
	GG (n=49)	GT (n=5)	P value	EE (n=23)	EK + KK (n=31)	P value
Mean age (SD), years	16.3 (0.6)	16.4 (0.5)	0.880	16.4 (0.5)	16.3 (0.7)	0.404
Mean anthropometric measures (SD)						
Body weight, kg	65.8 (18.0)	81.9 (20.9)	0.029	69.2 (20.8)	65.8 (17.1)	0.637
Height, cm	168.0 (6.0)	177.6 (6.9)	0.001	168.3 (6.2)	169.4 (7.0)	0.531
BMI	23.4 (6.4)	26.1 (7.1)	0.144	34.5 (7.3)	22.9 (5.7)	0.540
Z score BMI for age	0.3 (1.6)	1.1 (1.4)	0.175	0.6 (1.7)	0.3 (1.5)	0.529
Neck circumference, cm	35.2 (5.3)	36.8 (2.7)	0.071	35.2 (3.3)	35.5 (6.2)	0.751
Waist circumference, cm	78.9 (14.7)	98.4 (21.6)	0.016	81.6 (17.0)	80.1 (16.0)	0.840
Meand daily dietary intake (SD)						
Energy intake, kcal	3,144 (969)	3,697 (2,080)	0.287	3,135 (965)	3,240 (1,199)	0.731
Protein intake, gr	95.3 (34.9)	106.4 (60.0)	0.988	93.8 (35.9)	98.2 (38.7)	0.720
Fat intake, gr	88.5 (37.3)	106.4 (63.1)	0.777	85.6 (40.8)	93.5 (39.4)	0.474
Carbohydrate intake, gr	477.3 (149.6)	543.6 (294.2)	0.397	483.6 (145.1)	483.4 (180.5)	0.523
Fiber intake, gr	8.8 (4.1)	9.4 (5.6)	0.763	8.2 (3.0)	9.4 (4.9)	0.461
Mean physical activity, Mets-minute (SD)	2,372 (1,322)	2,640 (1,720)	0.612	2,630 (1,544)	2,224 (1,176)	0.463

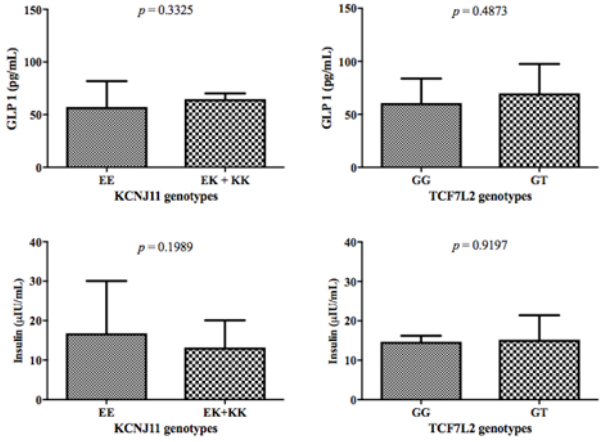

Figure 1. Fasting plasma GLP-1 and insulin levels in genotypes of KCNJ11 and TCF7L2

The possible correlation between insulin and GLP-1 was evaluated in all subjects as well, within the genotype groups. In all subjects, fasting GLP-1 was not associated with fasting insulin level (r=0.063; P=0.326). Subjects were divided by KCNJ11 genotypes, EE and EK. The correlation between insulin and GLP-1 in KK genotypes was not analyzed because the number of subjects is too low. As shown in **Figure 2**, in subjects with the EE genotype, GLP-1 was not correlated with insulin (r=-0.036; P=0.435). However, the EK genotype group had a significant correlation between GLP-1 and insulin (r=0.394; P=0.026). We did not analyze for a possible correlation within TCF7L2 genotypes

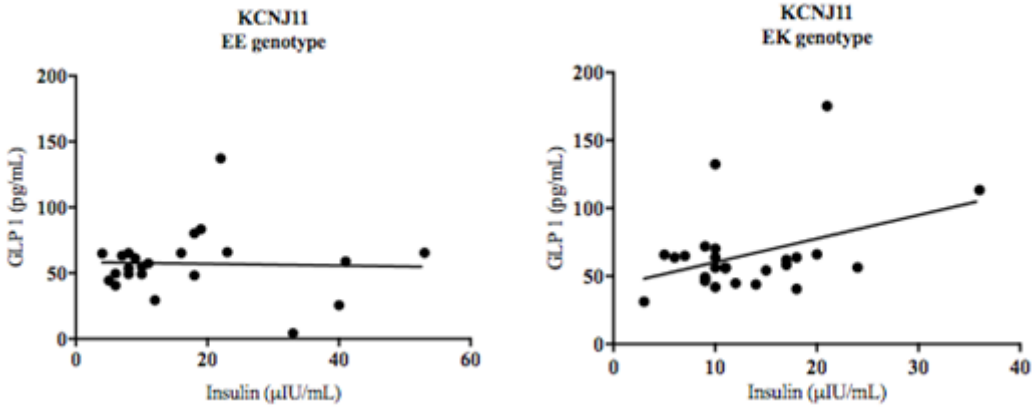

Figure 2. The correlation between fasting insulin and GLP-1 according to KCNJ11 genotypes. In the EE genotype, GLP-1 was not correlated with insulin (r=-0.036; P=0.435) but in the EK genotype, GLP-1 had a significant, positive correlation with insulin (r=0.394; P=0.026).

because of the small number of subjects in one of the genotype groups.

In order to evaluate lifestyle factors, we analyzed the relationship between energy intake, physical activity, and fasting plasma GLP-1. Total energy intake had a positive, significant association with GLP-1, after controlling for weight and age (r=0.276; P=0.047). However, there was no significant correlation between physical activity and GLP-1 (r=0.011; P=0.936) (**Figure 3**). Body weight was also significantly correlated with fasting GLP-1 level (age controlled, r=0.444; P=0.001).

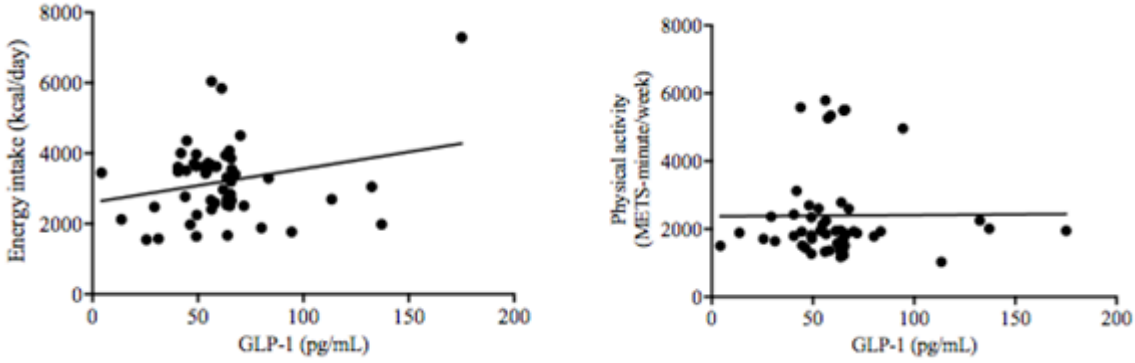

Figure 3. The correlation between fasting plasma GLP-1 on energy intake (r=0,276; P=0.047) and physical activity (r=0.011; P=0.936), by Pearson's correlation analysis (2-tailed, corrected for body weight and age).

Discussion

In this study, we evaluated factors for possible associations with GLP-1 production in male adolescents, including lifestyle and genetic factors. With regards to lifestyle, fasting GLP-1 production was positively correlated with total energy intake, but not with physical activity. With regards to genetics, fasting GLP-1 concentration was not associated with TCF7L2 or KCNJ11 gene polymorphisms. However, the correlation between fasting GLP-1 and insulin level was dependent on genetic background. In subjects with the EE genotype of KCNJ11, GLP-1 was not associated with insulin. However, those with the EK genotype of KCNJ11 had a positive correlation between GLP-1 and insulin.

Because of its insulinotropic effect, GLP-1 production has been associated with better glucose metabolism.[27] Postprandial GLP-1 secretion was reported to be lower in T2DM patients than those without T2DM, and administration of a GLP-1 analogue improved glucose control in T2DM patients.[28,29] Because GLP-1 induces insulin secretion, it has been argued that the increasing GLP-1 production might lead to hyperinsulinemia and insulin resistance.

Growing evidence has shown that GLP-1 production is an early indication of metabolic syndrome. Munoz *et al.* showed that obese individuals had higher fasting GLP-1 concentration and also disturbed diurnal GLP-1 variation.[30] Additionally, Yamaoka-Tojo *et al.* reported that a peripheral GLP-1 concentration was positively correlated with metabolic syndrome. They suggested that the conditions of overnutrition and obesity increased GLP-1 production and lead to GLP-1 dysfunction.[31]

Those ideas were supported in this study. We showed that body weight was positively correlated with fasting GLP-1. Additionally, energy intake was also correlated with fasting GLP-1, independent of body weight and age. This showed that habitual energy intake was correlated with fasting GLP-1 production our subjects. In all subjects, we showed that GLP-1 was not correlated with insulin concentration. However, after analyzing by genetic profile, we observed a positive correlation between GLP-1 and insulin level in subjects with the EK genotype of KCNJ11 gene. Interestingly, this correlation was not seen in the EE genotype of KCNJ11 gene. These results are evidence of the importance of KCNJ11 gene polymorphism on

the insulinotropic effect of GLP-1.

The interaction between KCNJ11 and GLP-1 action has been shown in an animal trial. Hugill *et al.* induced a KCNJ11 point mutation in mice that induced impaired glucose tolerance and defective insulin secretion. Additionally, they showed that mice with KCNJ11 gene mutation had an impaired response to GLP-1 and glucose-dependent insulinotropic peptide (GIP).[24] To date, the mechanism of how the genetic mutation in the KCNJ11 gene affects GLP-1 production or the insulin response to GLP-1 remains unknown. As such, this study is the first to assess genetic variation in KCNJ11 and the insulin response to GLP-1 production. While our study was observational, had a small number of subjects, and assessed blood specimens taken in a fasting state, our results provide a basis for further study on the physiological effect of KCNJ11 on GLP-1 and insulin regulation.

TCF7L2 and KCNJ11 genes are important in regulating insulin release and genetic variation of those genes were associated with T2DM15-17. In this study, we analyzed the association of those genetic polymorphisms with GLP-1 and insulin concentration in male adolescents. Analyzing the correlation between GLP-1 and insulin was necessary because suspect that GLP-1 and insulin can be used as an early indicator of metabolic disturbance in glucose metabolism. However, in this study we did not find a significant difference in GLP-1 and insulin level among those with TCF7L2 and KCNJ11 gene variations.

In this study, we also showed that TCF7L2 gene polymorphism was associated with higher body weight, taller height, and greater waist circumference. Subjects with T allele of those gene variation has more body weight and waist circumference than those without T allele. However, because the number of subjects in GT group was very low (only 5 subjects), it is very important to be careful when generate conclusion based on this finding.

In conclusion, GLP-1 and insulin have a significant association in male adolescents with the EK genotype of KCNJ11, perhaps shedding more light on the association between KCNJ11 gene variation and insulin resistance as well as T2DM. Additionally, GLP-1 is associated with nutritional status and energy intake. Because this study involved only a small number of subjects and exclusively male adolescents, we suggest further study on a broader population.

Acknowledgement

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Conflict of Interest

None declared.

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