

# Single Nucleotide Polymorphisms in the Peroxisome Proliferator-Activated Receptor $\delta$ Gene Are Associated With Skeletal Muscle Glucose Uptake

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The peroxisome proliferator-activated receptors (PPARs) belong to a superfamily of nuclear receptors. It includes PPAR- $\delta$ , a key regulator of fatty acid oxidation and energy uncoupling, universally expressed in different tissues. The PPAR- $\delta$  gene (PPARD) maps to 6p21.2-p21.1 and has 11 exons and spans 35 kbp. We investigated the effects of single nucleotide polymorphisms (SNPs) of PPARD on whole-body, skeletal muscle, and subcutaneous adipose tissue glucose uptake in 129 healthy individuals using the hyperinsulinemic-euglycemic clamp technique combined with fluorine-18-labeled fluorodeoxyglucose ( $^{18}\text{F}$ ]FDG) and positron emission tomography (PET). Three of six SNPs of PPARD and their haplotypes were significantly associated with whole-body insulin sensitivity.  $^{18}\text{F}$ ]FDG-PET scanning indicated that SNPs of PPARD primarily affected insulin sensitivity by modifying glucose uptake in skeletal muscle but not in adipose tissue. Our results give evidence that SNPs of PPARD regulate insulin sensitivity particularly in skeletal muscle. *Diabetes* 54: 3587–3591, 2005

The peroxisome proliferator-activated receptors (PPARs) belong to a superfamily of nuclear receptors. Three different PPAR isoforms ( $\alpha$ ,  $\gamma$ , and  $\delta/\beta$ ) have been identified. PPAR- $\alpha$ , primarily involved in fatty acid oxidation, is mainly expressed in liver and to a lesser extent in heart and skeletal muscle. PPAR- $\gamma$  is expressed in white and brown adipose tissue and has a pivotal role in adipogenesis, lipid storage, and glucose homeostasis (1). PPAR- $\delta$  is expressed in a variety of tissues, with high levels in skeletal muscle, and it is a key regulator of fatty acid oxidation and energy uncoupling (2,3).

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$^{18}\text{F}$ ]FDG, fluorine-18-labeled fluorodeoxyglucose; PET, positron emission tomography; PPAR, peroxisome proliferator-activated receptor; PPARD, PPAR- $\delta$  gene; SNP, single nucleotide polymorphism; WBGU, whole-body glucose uptake.

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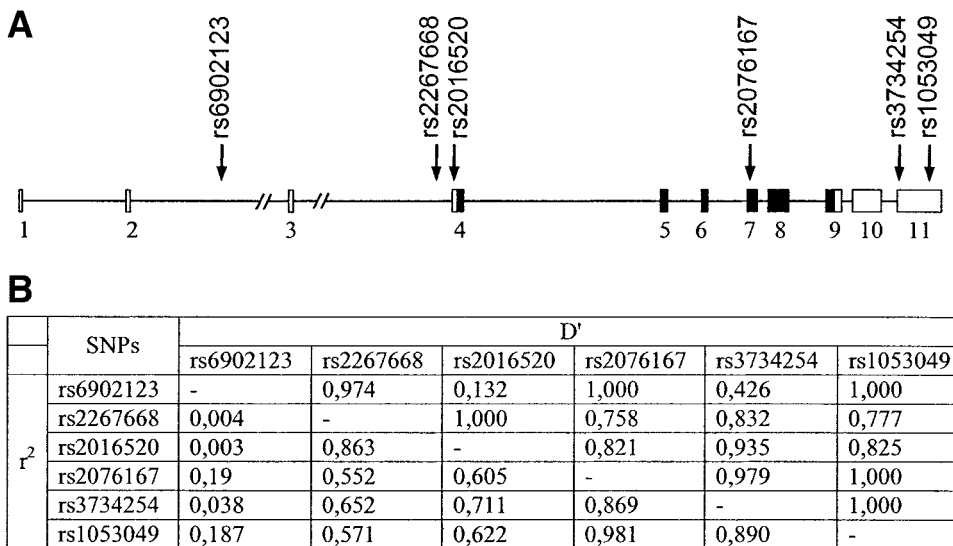
Fatty acids and their metabolites are natural ligands to PPARs, and they have been shown to activate PPAR- $\delta$  (4). Activation of PPAR- $\delta$  with a synthetic compound promotes reverse cholesterol transport by increasing the serum HDL level in mice (5) and monkeys (6). Furthermore, PPAR- $\delta$  lowers the levels of LDL and triglycerides (6). Activation of PPAR- $\delta$  also leads to increased expression of genes involved in fatty acid oxidation and energy uncoupling in skeletal muscle (7). Subsequent energy dissipation results in reduced adiposity, thereby protecting against diet-induced obesity and improving insulin sensitivity (2,3,7). Therefore, it is reasonable to assume that single nucleotide polymorphisms (SNPs) in the PPAR- $\delta$  gene (PPARD) may influence insulin sensitivity in the whole body and insulin sensitive tissues.

PPARD has been mapped to 6p21.2-p21.1 and has 11 exons and spans 35 kbp (8). Nine SNPs of PPARD have been reported (9), and these polymorphisms have been associated with fasting plasma glucose levels and BMI but not with the risk of type 2 diabetes or insulin resistance. In a previous study (9), insulin sensitivity was not measured in skeletal muscle or in adipose tissue. Therefore, we examined the impact of several SNPs of PPARD on whole-body, skeletal muscle, and subcutaneous adipose tissue glucose uptake in healthy individuals using the hyperinsulinemic-euglycemic clamp technique combined with fluorine-18-labeled fluorodeoxyglucose ( $^{18}\text{F}$ ]FDG) and positron emission tomography (PET).

## RESEARCH DESIGN AND METHODS

A total of 129 volunteers (109 men and 20 women, aged  $33 \pm 1$  year, and BMI  $27.3 \pm 0.5$  kg/m<sup>2</sup>), who had previously participated in PET studies at Turku PET Centre, Finland, were recruited in this study as previously described (10). They all were healthy, as judged by their medical history, physical examination, and laboratory tests, and were not taking any medication. Written informed consent was obtained after the nature, purpose, and potential risks of the study were explained. The commission on ethics of the municipal hospital district of southwest Finland approved the study protocol.

Studies were performed after an overnight fast, and subjects were lying in a supine position throughout the PET scanning. Two catheters were inserted: one in an antecubital vein for the infusion of glucose and insulin and the injection of  $^{15}\text{O}$ ]H<sub>2</sub>O and  $^{18}\text{F}$ ]FDG and the other for blood sampling either in the radial artery or in the antecubital vein of the opposite upper extremity, warmed with a heating pillow to arterialize the venous blood. At 0 min, an intravenous infusion of insulin ( $1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was started for 140  $\pm$  20 min. At 50  $\pm$  5 min,  $^{15}\text{O}$ ]H<sub>2</sub>O was intravenously injected, and a dynamic scan of both femoral regions for 6–15 min was performed in 65 subjects as previously described (11).  $^{18}\text{F}$ ]FDG was injected at 90  $\pm$  20 min, and a dynamic scan of the femoral region was performed for 20–30 min as previously described (12). Forty-two subjects performed intermittent isomet-



**FIG. 1. A:** Gene map shows SNPs genotyped in *PPARD* (NM\_006238). Coding exons are marked by black boxes and 5' and 3' untranslated regions by white boxes. Genotyped SNPs are shown with NCBI's dbSNP accession numbers. **B:** Linkage disequilibrium ( $D'$ ,  $r^2$ ) are shown among SNPs in *PPARD*.

ric exercise with one leg during the scan, but only the measurements of the noncontracting, i.e., control leg, were used. Whole-body insulin sensitivity was assessed using the hyperinsulinemic-euglycemic clamp technique (10). Image acquisition and processing have been previously described (10).

**Measurement of skeletal muscle and adipose tissue glucose uptake.** Femoral muscle and adipose tissue glucose uptake were calculated using the three-compartment model of [ $^{18}\text{F}$ ]FDG kinetics (13). Plasma and tissue time activity curves were graphically analyzed to quantitate the fractional phosphorylation rate ( $K_t$ ) for the tracer (14). The rates of glucose uptake were obtained by multiplying  $K_t$  by the plasma glucose concentration divided by a lumped constant, which accounts for the differences in transportation and phosphorylation of [ $^{18}\text{F}$ ]FDG and glucose. A lumped constant of 1.2 for skeletal muscle and 1.14 for adipose tissue were used as previously described (15,16).

**Measurement of femoral muscle blood flow and oxygen consumption.** The calculation of blood flow with [ $^{15}\text{O}$ ]H $_2$ O-PET is based on the Kety's (17) principle of inert gas exchange between blood and tissues. The autoradiographic method was employed to calculate the blood flow pixel by pixel by using the arterial input curve corrected for dispersion and delay (18).

**Measurement of whole-body glucose uptake.** The rates of whole-body glucose uptake (WBGU) were measured independently of the PET measurements with the hyperinsulinemic-euglycemic clamp technique. Euglycemia (plasma glucose  $\sim 5$  mmol/l) was maintained with a variable rate of 20% glucose infusion based on arterial plasma glucose measurements taken every 10 min. WBGU ( $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was calculated between 60 and 120 min of hyperinsulinemia.

**Biochemical analyses.** Plasma glucose was determined in duplicate by the glucose oxidase method and serum insulin concentration by immunoassay as previously described (10). Serum total and HDL cholesterol and triglycerides were assessed using standard enzymatic methods (Boehringer Mannheim, Mannheim, Germany). Serum LDL cholesterol was calculated using the Friedewald formula (19).

**Genotyping of the SNPs of PPARD.** DNA analyses were performed at the University of Kuopio, Finland. Genotyping was done using TaqMan Allelic Discrimination Assays (Applied Biosystems, Foster City, CA). Our genotyping success rate was 100%. The TaqMan genotyping reaction was amplified on a GeneAmp PCR System 2700 (50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min), and fluorescence was detected on an ABI Prism 7000 sequence detector (Applied Biosystems). Selection of the SNPs was based on the genotype data available from HapMap project's website (20). Genotype data from Utah residents with ancestry from northern and western Europe were examined. Linkage disequilibrium statistics were calculated and haplotype blocks were visualized by using the Haploview software (21). Six SNPs were chosen from different haplotype blocks to properly cover the genomic region of *PPARD*.

**Statistical methods.** All calculations were done with the SPSS/Win statistical program (version 11.5.1 for Windows; SPSS, Chicago, IL). All data are given as means  $\pm$  SD. Insulin and triglyceride concentrations were log transformed before statistical analyses to achieve a normal distribution. Adjustment for confounding factors was performed with ANCOVA when comparing the genotype groups.  $P$  value  $<0.05$  was considered statistically significant.

## RESULTS

We selected six SNPs of *PPARD* for genotyping: rs6902123, rs2267668, rs2016520, rs2076167, rs1053049, and rs3734254. The observed allele frequencies in all genotyped SNPs were in Hardy-Weinberg equilibrium. The minor allele frequencies were 0.03 for rs6902123, 0.13 for rs2267668, 0.14 for rs2016520, 0.14 for rs2076167, 0.14 for rs1053049, and 0.12 for rs3734254.

Location of SNPs for *PPARD* and their linkage disequilibrium are shown in Fig. 1. Table 1 shows clinical and metabolic parameters according to different SNPs. Subjects with the AG genotype of rs2267668 had lower blood pressure than subjects with the AA genotype ( $P = 0.044$ ). Subjects with the TT genotype of rs2076167 had higher total ( $P = 0.049$ ) and LDL ( $P = 0.047$ ) cholesterol than subjects with the TC genotype. Furthermore, subjects with the TT genotype of rs1053049 had higher total ( $P = 0.047$ ) and LDL ( $P = 0.050$ ) cholesterol than subjects with the TC genotype. After the adjustment for age and sex, these differences lost their statistical significance. None of the SNPs was significantly associated with BMI. The TC genotype of rs6902123 ( $P = 0.005$ ), the TC genotype of rs2076167 ( $P = 0.022$ ), and the TC genotype of rs1053049 ( $P = 0.029$ ) were significantly associated with higher rates of WBGU after the adjustment for age and sex. Similarly, these genotypes were significantly associated with higher skeletal muscle glucose uptake (rs6902123,  $P = 0.001$ ; rs2076167,  $P = 0.023$ ; and rs1053049,  $P = 0.028$ ). In contrast, none of the SNPs was associated with adipose tissue glucose uptake.

Based on six SNPs, seven haplotypes were found. However, four of them were very uncommon ( $n = 1-4$ ), and statistical analyses could not be performed. Therefore, we constructed haplotypes based on those three SNPs showing a significant association with the rates of WBGU and skeletal muscle glucose uptake (rs6902123, rs2076167, and rs1053049). Four haplogenotypes were found (hgt1: TTT/TTT,  $n = 97$ ; hgt2: TTC/TTT,  $n = 1$ ; hgt3: TCC/TTT,  $n = 23$ ; and hgt4: CCC/TTT,  $n = 8$ ). Subjects with the hgt1 had higher systolic blood pressure ( $131 \pm 15$  vs.  $125 \pm 13$  mmHg,  $P = 0.040$ ), total cholesterol ( $4.7 \pm 1.0$  vs.  $4.3 \pm 0.6$  mmol/l,  $P = 0.047$ ), and LDL cholesterol ( $2.9 \pm 0.9$  vs.  $2.5 \pm 0.6$  mmol/l,  $P = 0.050$ ) than carriers of other haplogenotypes.

Figure 2 shows the rates of WBGU and skeletal muscle and subcutaneous adipose tissue glucose uptake in carriers of hgt1, hgt3, and hgt4. The rates of WBGU differed among the haplogenotypes (unadjusted  $P = 0.012$ , adjusted for age and sex  $P = 0.012$ ), and they were lower among the carriers of hgt1 compared with subjects with hgt3 or hgt4 (unadjusted  $P = 0.016$ , adjusted for age and sex  $P = 0.031$ ). Similarly, skeletal muscle glucose uptake differed among the haplogenotypes (unadjusted  $P = 0.005$ , adjusted for age and sex  $P = 0.004$ ), and it was lower among carriers of hgt1 compared with subjects with hgt3 or hgt4 ( $P = 0.019$ , adjusted for age and sex  $P = 0.027$ ). Blood flow did not differ among the haplogenotypes. No differences among the haplogenotypes were found with respect to subcutaneous adipose tissue glucose uptake.

**DISCUSSION**

We investigated the relationship of SNPs in PPAR $\delta$  with whole-body, skeletal muscle, and adipose tissue glucose uptake. We demonstrated for the first time that SNPs in PPAR $\delta$  were significantly associated with whole-body insulin sensitivity as assessed by the euglycemic-hyperinsulinemic clamp. Furthermore, results of the [ $^{18}$ F]FDG-PET scanning indicated that SNPs of PPAR $\delta$  affect insulin sensitivity primarily by modifying skeletal muscle glucose uptake.

During hyperinsulinemia, the majority of glucose is utilized by skeletal muscle, and adipose tissue glucose uptake contributes only modestly to whole-body insulin sensitivity. An agonist inducing activation of PPAR- $\delta$  has been shown to promote lipid oxidation and energy uncoupling in skeletal muscle (3,6). It is possible that SNPs of PPAR $\delta$  alter gene transcription, affecting lipid oxidation, energy uncoupling, and insulin sensitivity in humans similarly to animal models (3,6,22,23). Further studies are needed to confirm that SNPs associated with glucose uptake in our study are functional. Also, in the adipose tissue the activation of PPAR- $\delta$  results in increased fatty acid oxidation and energy dissipation, thereby protecting against excess adiposity and diet-induced obesity (2). However, in our study SNPs of PPAR $\delta$  were associated with insulin sensitivity only in skeletal muscle but not in adipose tissue.

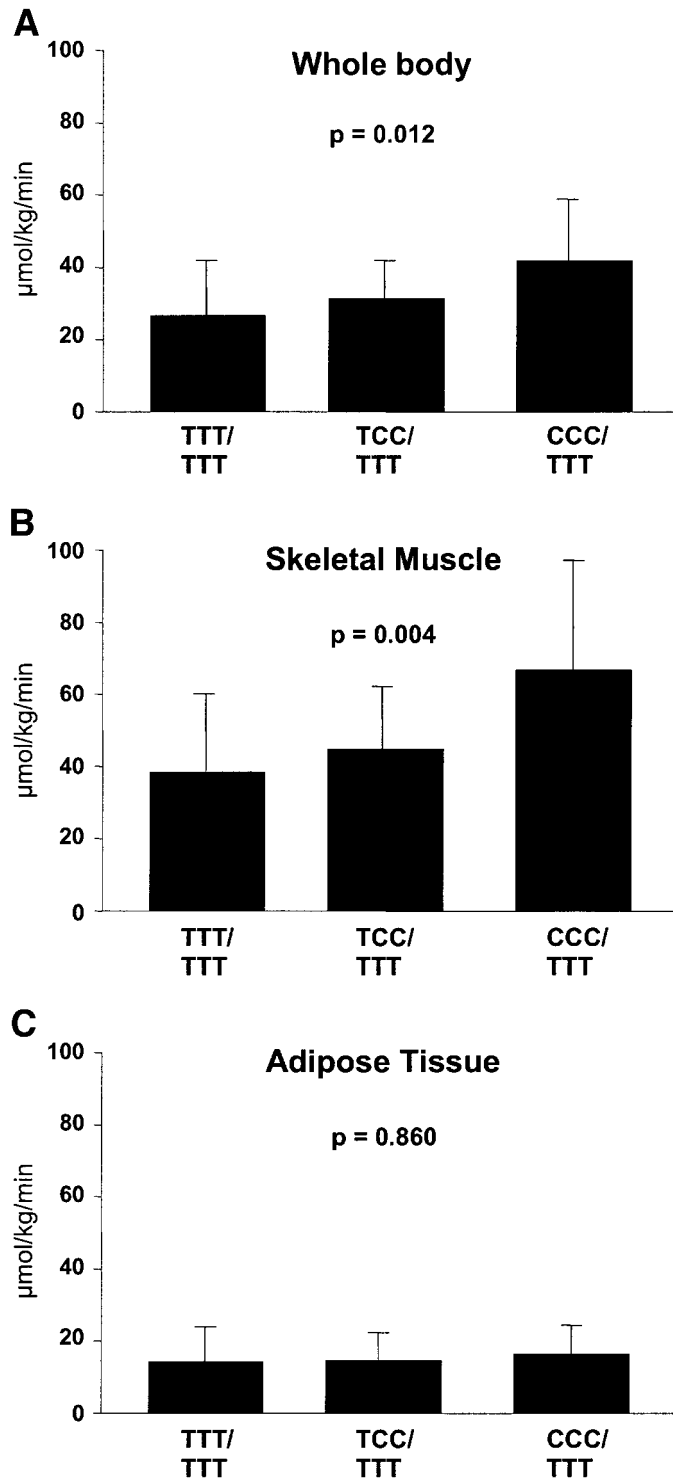
Only one previous study has evaluated the relationship of SNPs in PPAR $\delta$  with obesity and glucose metabolism. In the study by Shin et al. (9), SNPs of PPAR $\delta$  were associated with BMI and plasma glucose level but not with insulin resistance measured by the homeostasis model assessment index. In our study, subjects homozygous for hgt1 had somewhat higher BMI, suggesting the possible role of PPAR $\delta$  polymorphism in controlling obesity. In accordance with previous studies (6), we also found that SNPs of PPAR $\delta$  were associated with total and LDL cholesterol levels, although after the adjustment for age and sex these results lost their statistical significance.

In our study, the associations of three SNPs of PPAR $\delta$  and their haplogenotypes with WBGU ( $P = 0.005$ – $0.029$  for SNPs and  $P = 0.012$  for haplogenotype after the adjustment for age and sex) and skeletal muscle glucose uptake ( $P = 0.001$ – $0.028$  for SNPs and  $P = 0.004$  for haplogenotype after the adjustment for age and sex) were statistically significant. The possibility of type 1 error is possible because of multiple comparisons. However, even after the correction for multiple comparisons (six SNPs), the difference between the haplogenotypes was still statistically

**TABLE 1**  
The association of SNPs of PPAR $\delta$  on BMI, blood pressure, lipids, and the rates of WBGU, SMGU, and SFGU

SNP	rs6902123		rs2267668		rs2016520		rs2076167		rs1053049		rs3734254	
Genotype	TT	TC	AA	AG	TT	TC	TT	TC	TT	TC	TT	TC
<i>n</i>	121	8	99	30	98	31	98	31	97	32	101	28
Men/women	102/19	7/1	82/17	27/3	81/17	28/3	82/16	27/4	81/16	28/4	84/17	25/3
Age (years)	32.6 ± 10.4	32.5 ± 11.3	33.4 ± 10.9	30.0 ± 8.5	33.4 ± 10.9	30.1 ± 8.4	33.3 ± 10.7	30.4 ± 9.1	33.2 ± 10.7	31.0 ± 9.6	33.0 ± 10.6	31.1 ± 9.7
BMI (kg/m <sup>2</sup> )	27.3 ± 5.7	26.0 ± 4.6	27.7 ± 5.8	25.7 ± 5.0	27.7 ± 5.8	26.0 ± 5.0	27.7 ± 5.8	25.9 ± 5.1	27.7 ± 5.8	25.9 ± 5.0	27.6 ± 5.8	26.1 ± 5.0
Systolic blood pressure (mmHg)	130 ± 14	124 ± 16	131 ± 15	124 ± 14*	131 ± 15	125 ± 14	131 ± 15	125 ± 11	131 ± 15	125 ± 13	131 ± 15	127 ± 13
Diastolic blood pressure (mmHg)	82 ± 11	75 ± 12	82 ± 12	81 ± 10	82 ± 12	81 ± 10	83 ± 11	78 ± 11	82 ± 11	79 ± 11	82 ± 11	81 ± 11
Total cholesterol (mmol/l)	4.6 ± 1.0	4.2 ± 0.6	4.6 ± 1.0	4.4 ± 0.7	4.6 ± 1.0	4.4 ± 0.7	4.7 ± 1.0	4.2 ± 0.6	4.7 ± 1.0	4.3 ± 0.6	4.6 ± 1.0	4.3 ± 0.6
LDL cholesterol (mmol/l)	2.8 ± 0.9	2.4 ± 0.4	2.9 ± 0.9	2.6 ± 0.6	2.9 ± 0.9	2.6 ± 0.6	2.9 ± 0.9	2.5 ± 0.6	2.9 ± 0.9	2.5 ± 0.6	2.9 ± 0.9	2.6 ± 0.6
HDL cholesterol (mmol/l)	1.2 ± 0.3	1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.3	1.3 ± 0.4	1.2 ± 0.3	1.2 ± 0.4	1.3 ± 0.3	1.2 ± 0.4	1.3 ± 0.3	1.2 ± 0.4	1.3 ± 0.3
Triglycerides (mmol/l)	1.2 ± 0.8	1.3 ± 0.3	1.2 ± 0.7	1.2 ± 1.0	1.2 ± 0.7	1.2 ± 1.0	1.2 ± 0.9	1.0 ± 0.4	1.2 ± 0.9	1.0 ± 0.4	1.2 ± 0.8	1.0 ± 0.4
WBGU ( $\mu$ mol/kg/min)	27.6 ± 14.3	41.6 ± 16.7†	27.6 ± 15.3	31.3 ± 12.8	27.8 ± 15.4	31.4 ± 12.6	26.6 ± 14.7	34.4 ± 13.8‡	26.8 ± 14.7	33.7 ± 14.1§	27.6 ± 15.5	31.6 ± 11.6
SMGU ( $\mu$ mol/kg/min)	39.6 ± 23.3	66.5 ± 30.5¶	39.9 ± 25.2	45.7 ± 21.9	39.7 ± 25.3	46.2 ± 21.6	38.4 ± 24.2	50.4 ± 23.7	38.6 ± 23.8	50.0 ± 23.8#	40.0 ± 25.9	45.9 ± 18.0
SFGU ( $\mu$ mol/kg/min)	14.4 ± 8.5	16.4 ± 7.7	14.6 ± 8.8	14.2 ± 7.0	14.6 ± 8.9	14.4 ± 6.9	14.4 ± 8.8	15.1 ± 7.3	14.4 ± 8.8	14.9 ± 7.2	14.5 ± 8.9	14.7 ± 6.8

Data are means ± SD. \* $P = 0.044$ ; † $P = 0.005$ ; ‡ $P = 0.022$ ; § $P = 0.029$ ; ¶ $P = 0.001$ ; || $P = 0.023$ ; # $P = 0.028$ . All  $P$  values are adjusted for age and sex. SFGU, subcutaneous fat glucose uptake; SMGU, skeletal muscle glucose uptake.



**FIG. 2.** The rates of whole-body (A), skeletal muscle (B), and subcutaneous adipose tissue (C) glucose uptake ( $\mu\text{mol}/\text{kg}/\text{min}$ ) according to haplotypes of PPAR $\Delta$ . P values indicate the differences among the three haplotypes after the adjustment for age and sex.

significant ( $P < 0.008$ ) for skeletal muscle glucose uptake. The correction for multiple comparisons in this case is very conservative given the fact that several SNPs were in tight linkage disequilibrium. Furthermore, SNPs of PPAR $\Delta$  were more tightly linked with skeletal muscle glucose uptake than with the rates of WBGU, which is expected given the previous evidence that PPAR- $\delta$  agonists enhance

basal and insulin-stimulated glucose uptake in skeletal muscle (24).

In conclusion, our results give the first evidence that SNPs of PPAR $\Delta$  are regulating insulin sensitivity, particularly in skeletal muscle. Further studies should focus on SNPs of PPAR $\Delta$  as predictors for type 2 diabetes.

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