Brief Genetics Report

The Stimulation-Induced Increase in Skeletal Muscle Glycogen Synthase Content Is Impaired in Carriers of the Glycogen Synthase *Xba*I Gene Polymorphism

Josée St-Onge, Denis R. Joanisse, and Jean-Aimé Simoneau

Associations between glycogen synthase gene (*GYS1*) polymorphism and states of insulin resistance and type 2 diabetes have been reported. The purpose of this study was to establish if the *GYS1* genotype impacts on the content of glycogen synthase (GS) protein in muscle measured under basal and stimulated conditions. To examine this, *GYS1 Xba*I and Met416Val polymorphisms and thigh muscle GYS1 protein content were determined at rest, both before and after several weeks of neuromuscular electrical stimulation in carriers and noncarriers of the mutations. The allelic frequency was 0.086 for the *Xba*I mutation (A2) and 0.006 for the Met416Val in our cohort of French-Canadian subjects. When measured at rest, the GS protein content in muscle was similar among carriers and noncarriers of the *Xba*I variant. However, the stimulation-induced increase (23%) in the amount of GS muscle protein normally seen in wild-type individuals was impaired in those carrying the *Xba*I mutation. These data demonstrate that some individuals, because of their genetic background, are unable to stimulate the process of GS protein accumulation in skeletal muscle. These results could explain why some individuals appear to be genetically predisposed to developing skeletal muscle insulin resistance when exposed to unfavorable metabolic environments. *Diabetes* 50:195–198, 2001

Glycogen synthase (GS) is a key regulatory enzyme in glucose metabolism and plays a role in the perturbed insulin-stimulated glucose storage of skeletal muscle in obese nondiabetic and type 2 diabetic patients (1–3). Because states of altered insulin-stimulated glucose metabolism appear to be strongly genetically determined, the identification of mutations that might cause a defect in GS protein expression is a logical next step. Several polymorphic sites have been identified for the glycogen synthase gene (*GYS1*). Among the most frequently studied *GYS1* polymorphisms are the *Xba*I and Met416Val mutations located in intron 14 and exon 10, respectively (4,5). A number of studies have shown significant associations between these two *GYS1* gene markers and type 2 diabetes and hypertension (4,6–8), although this is not seen in all reports (5,9–12). To our knowledge, whether carriers or noncarriers of a specific *GYS1* polymorphism have different GS protein content in their skeletal muscle has only been verified by Groop et al. (4). The authors conclude that the skeletal muscle of individuals carrying the *Xba*I A1A1 wild-type alleles contains a similar amount of GS protein to that of individuals with the A1A2 alleles. However, this study included only type 2 diabetic patients, and it cannot be excluded that the similarity between the two groups was due to long-term and confounding skeletal muscle metabolic changes caused by the diabetic state. The current study was undertaken to explore whether healthy subjects possessing *GYS1* polymorphisms (i.e., X*ba*I or Met416Val) have a different GS protein content in their skeletal muscle compared with noncarriers under basal conditions or after a chronic increase in muscular contractile activity.

As recently described for other populations (Swedish [0%] and Danish [0.4%] [13]), the genotype frequency of the Met416Val mutation was relatively low (only 1.3%) in our cohort of French-Canadian subjects (116 men and 41 women) when compared with Finnish (17%) (5) or Japanese (~10%) (8) groups. Only two subjects in our cohort were heterozygous for this mutation (i.e., a carrier genotype frequency of 1.3%). Because of this low number of carrier subjects, no further investigation was pursued with this mutation. Interestingly, none of a cohort of 130 French individuals carried the Met416Val mutation (J.S.-O., J.-A.S., C. Bouchard, unpublished data). Given its low frequency in many different populations, it is unlikely that the Met416Val mutation contributes significantly to insulin resistance in the general population. In addition, a recent report showed no effect of Met416Val on GS activity (13), further casting doubt on its potential role in insulin resistance. However, the genotype frequency of the *Xba*I variant in French-Canadians (17.2%) was similar to that previously reported in other ethnic groups (4,7,9,11,12). Of the

From the Division of Kinesiology, Department of Social and Preventive Medicine, Faculty of Medicine, Laval University, Ste-Foy, Québec, Canada.

Address correspondence and reprint requests to Denis R. Joanisse, PhD, Division of Kinesiology, Department of Social and Preventive Medicine, Faculty of Medicine, Room 0223 PEPS, Laval University, Ste-Foy, Québec, Canada, G1K 7P4. Email: denis.joanisse@kin.msp.ulaval.ca.

Received for publication 13 April 2000 and accepted in revised form 8 September 2000.

J.-A.S. is deceased.

CK, creatine kinase; COX, cytochrome c oxidase; GS, glycogen synthase; NMES, neuromuscular electrical stimulation; PCR, polymerase chain reaction; TBST, Tris-buffered saline with Tween.

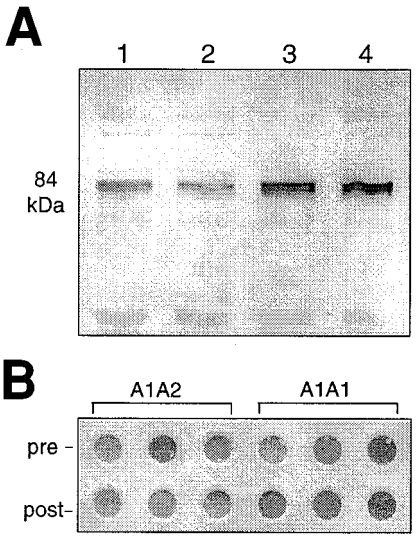

FIG. 1. GS protein content in human vastus lateralis. *A*: Representative Western blot (8 µg total protein per lane) showing GS content in sedentary individuals (*lanes 1* and *2*) and highly trained endurance athletes (*lanes 3* and *4*). *B*: Representative dot blot (5 µg total protein per well, samples run in triplicate) from a carrier and a noncarrier of the A2 allele before (pre) and after (post) NMES.

157 subjects, 27 were identified as carrying the A1A2 alleles, whereas all others were homozygous for the A1 allele.

To determine if the *Xba*I polymorphism influences GS protein content in skeletal muscle (measured by dot blot, Fig. 1), vastus lateralis muscle biopsies (14) were taken from 21 carriers and 21 noncarriers pair-matched for sex, age, BMI, and level of physical activity. As shown in Fig. 2, no difference was found under basal conditions in GS protein content measured in muscle from carriers of the *Xba*I variant allele (46.9 ± 12.2 U/g) and noncarriers (44.9 ± 10.2 U/g), which is in agreement with the work of Groop et al. (4).

The originality of our study lies in the fact that we verified if carriers and noncarriers of the *Xba*I polymorphism responded differently to a standardized environmental stimulus recognized to alter GS protein content in skeletal muscle. The knee extensor muscles of seven matched pairs of carriers and noncarriers were subjected to neuromuscular electrical stimulation lasting 6 weeks combined with vastus lateralis muscle biopsies taken before and after the protocol. Neuromuscular electrical stimulation (NMES) leads to significant alterations in skeletal muscle characteristics in humans (15). In addition to functional and contractile property changes, the content or activity of markers of different metabolic pathways, including GS activity, has been substantially modulated after as little as 4 weeks of chronic low-frequency NMES (16). Not only was the NMES-induced increase in skeletal muscle GS protein content recently confirmed in humans, the increase was shown to occur concomitantly with improved insulin-stimulated whole-body glucose uptake (17). However, the magnitude of the changes varied substantially among individuals.

As illustrated in Fig. 3, the key finding of the present study is that the significant stimulation-induced increase (23%) in skeletal muscle GS protein content seen in individuals having the *Xba*I A1A1 wild-type genotype was impaired in the carriers of the A2 variant allele (genotype effect and interaction $P < 0.05$). This result does not arise from global differences in the response of skeletal muscle from the two groups of subjects to NMES. This is evidenced by the similar behaviors of creatine kinase (CK) activity (unchanged) and cytochrome c oxidase (COX) activity (increased by 22%; NMES effect $P < 0.05$); both of these results are consistent with previous findings (15). Therefore, the difference measured in GS content after NMES appears to be genotype related.

Our results are particularly interesting in light of the recent report of Orho-Melander et al. (6), which showed increased susceptibility of *Xba*I polymorphism carriers to hypertension, insulin resistance, and earlier onset of type 2 diabetes. Our data provide clues to a mechanism that may explain their results. It is unlikely that the *Xba*I polymorphism, located in untranslated intron 14, causes any alteration in the activity of GS itself. Other than direct effects on mRNA stability, this genetic variant may be in linkage disequilibrium with other yet unidentified mutations within the coding or flanking regions of the *GYS1* gene. Our results suggest that whatever the regulatory mechanisms involved, the differences in GS content become manifest only under certain specific conditions. Thus, the present study supports the thrifty genotype hypothesis proposed by Neel (18), stipulating that some individuals have a survival advantage during periods of restrictive energy intake but, when exposed to periods of decreased energy expenditure and food abundance, favor fat deposition. Insulin resistance would be a major factor involved in the channeling of ingested fat towards tissues such as adipose tissue and skeletal muscle for storage rather than skeletal muscle for oxidation (19). Indeed, the results of our study show that individuals differ in their capacity to alter the content of an important metabolic marker of skeletal muscle insulin-stimulated glucose storage in response to a challenge (NMES) based on their different genetic background. If, in the nonresponder individuals, a similar lack of adjustment is taking place in response to environmental conditions, such as a positive energy balance or a diabetogenic diet, it follows that this could increase their likelihood to develop skeletal muscle insulin resistance and type 2 diabetes over time.

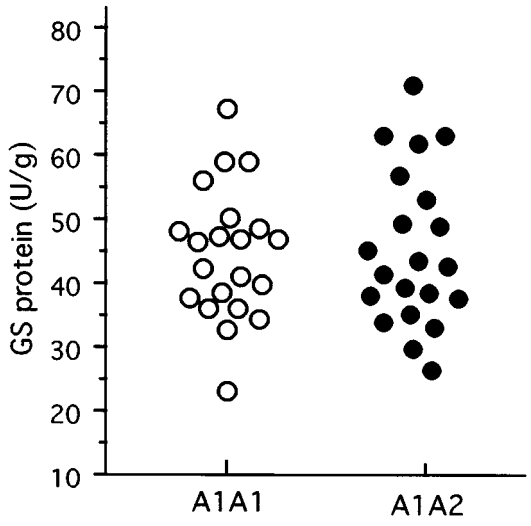

FIG. 2. Skeletal muscle GS protein content in carriers (●) (n = 21) and noncarriers (○) (n = 21) of the *Xba*I *GYS1* gene polymorphism measured under basal conditions.

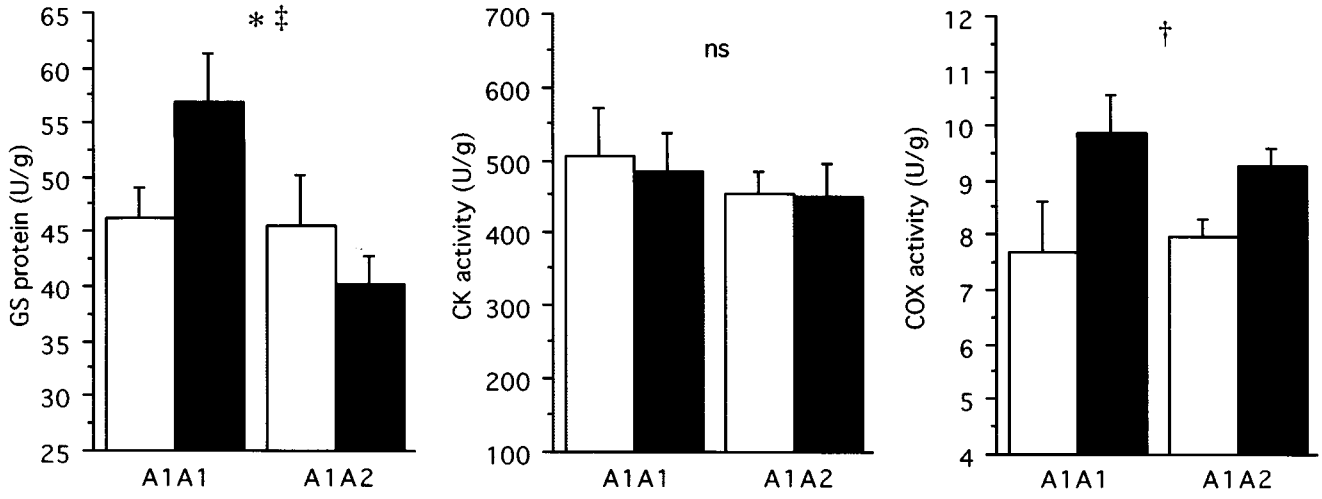

FIG. 3. Skeletal muscle GS protein content and CK and COX enzyme activities in carriers (n = 7) and noncarriers (n = 7) of the *Xba*I *GYS1* gene polymorphism measured before (□) and after (■) 6 weeks of low-frequency neuromuscular electrical stimulation. *Significant effect of genotype; †significant effect of NMES; ‡significant effect of genotype X NMES interaction. Effects are considered significant for $P < 0.05$.

In conclusion, the allelic frequency of the *GYS1 Xba*I variant observed in the present study was similar to previously reported results and significantly more frequent than the Met416Val mutation, which was relatively rare in our cohort of French-Canadian subjects. Confirming previous results, muscle biopsies taken under basal conditions from the carriers and noncarriers of the *Xba*I polymorphism contained similar GS protein content. However, the novel and important finding of the present study is that the stimulation-induced increase in skeletal muscle GS protein content normally seen in individuals having the wild-type genotype is completely impaired in those carrying the *Xba*I mutation. These data demonstrate that some individuals, because of their genetic background, are unable to stimulate the processes of GS protein accumulation in skeletal muscle. These results could explain why some individuals appear to be genetically predisposed to develop skeletal muscle insulin resistance when exposed to unfavorable metabolic environments.

RESEARCH DESIGN AND METHODS

Subjects. Blood samples were obtained from 157 (116 men and 41 women) unrelated healthy normal-weight Caucasians aged 18 to 35 years and living in the Quebec City area. Genomic DNA was extracted from frozen peripheral-blood leukocytes by standard methods. Among these participants only two subjects were heterozygotes for the Met416Val mutation and were not tested further. A subgroup of 21 subjects (14 men and 7 women) of the 27 identified as carrying the A1A2 alleles, as well as a subgroup of 21 noncarriers pair-matched (14 men and 7 women) based on age (25.7 ± 5.3 vs. 25.6 ± 4.4 years, mean ± SD), BMI (22.8 ± 2.4 vs. 23.1 ± 2.5 kg/m^2), and level of physical activity (6.7 ± 6.2 vs. 5.7 ± 5.0 h/week), agreed to undergo a vastus lateralis muscle biopsy (14). Of these subjects, 14 (7 carriers and 7 noncarriers pair-matched on the same criteria as above; 5 men and 2 women in each group) further agreed to participate in the neuromuscular stimulation protocol with vastus lateralis muscle biopsies taken before and after the protocol. The study was approved by the Medical Ethics Committee of Laval University and appropriate informed consent was obtained from all the subjects.

Determination of the *Xba*I and Met416Val polymorphisms. The *Xba*I polymorphism in intron 14 of the *GYS1* gene was genotyped using the previously described polymerase chain reaction (PCR) method (7). The A1 and A2 alleles were determined by the absence or recognition of the *Xba*I restriction site formed by the substitution of a cytosine for a thymine in the *GYS1* nucleotide sequence of intron 14. The Met416Val polymorphism was genotyped using the previously described PCR method (20). The wild-type and mutated alleles were determined by the presence or absence of the *Nla*III restriction site that occurs from a nucleotide substitution changing a methionine to a valine in the amino acid sequence of the GS protein.

NMES protocol. The procedures used were previously described (15). Briefly, knee extensor muscles of both thighs were subjected to 3 h of NMES per day, 6 days per week for 6 weeks. NMES was delivered at a low frequency (8 Hz) using a portable battery-powered stimulator (Respond II; EMPI) and 3-in diameter round adhesive electrodes (Pals Plus 9000; EMPI).

GS protein content. Muscle samples of the subjects, frozen in liquid nitrogen and kept at –70°C until used, were homogenized (1:40 wt:vol) in buffer (Na-K-PO_4 100 mmol/l, EDTA 2 mmol/l, pH 7.2). The total protein content of the muscle homogenates was determined using the Bio-Rad Protein Assay method. Quantification of the GS protein content was done with the use of an antibody provided by J. C. Lawrence at the University of Virginia School of Medicine, Charlottesville, VA. Mini-PROTEAN II and Trans-Blot Cell equipment (Bio-Rad) were used for sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blotting following the procedures recommended by the manufacturer. Of the total protein, 8 µg was electrophoretically separated (0.75-mm thick 10% SDS gels) and electrically transferred (60 min at 100 V) to polyvinylidene fluoride membranes (Immobilon; Millipore). After blocking in 5% powdered milk, membranes were incubated for 1 h with 0.05 µg/ml affinity-purified GS antibody, washed three times in Tris-buffered saline with 0.1% Tris-buffered saline with Tween-20 (TBST) solution, and incubated with rabbit anti-chicken antibodies conjugated to alkaline phosphatase (5,000-fold dilution). Membranes were washed again three times in TBST before antibody binding was detected using the alkaline phosphatase reaction. A single major band was found at ~84 kDa corresponding to the molecular weight of GS (Fig. 1*A*). Dot blots (Fig. 2*B*) (5 µg of total protein per well) were subsequently used to quantify protein levels, and these were made in triplicate for each sample. The reaction product of each blot was scanned and analyzed twice with the use of National Institutes of Health Image analysis software. A gradient concentration of human latissimus dorsi muscle proteins (from 2 to 18 µg) was deposited on each membrane and served as internal standard. The results were expressed as arbitrary units of protein per gram of wet weight muscle (U/g).

CK and COX enzyme activities. The activities of CK and COX from pre- and poststimulated muscles were spectrophotometrically determined by standard methods (21) and expressed in U (µmol substrate consumed/min)/g wet wt muscle.

Statistical analysis. Unpaired Student's t tests were used to test for significant differences among genotypes. Two-factor analyses of variance were used to test for differences in the stimulation-induced changes in muscle GS protein content and CK and COX enzyme activities between carriers and noncarriers of the *Xba*I gene polymorphism. Data are presented as means ± SD.

ACKNOWLEDGMENTS

This work was supported by the Fonds de la Recherche en Santé du Québec, the Natural Science and Engineering Research Council of Canada, and the Medical Research Council of Canada.

The cooperation of our research volunteers is gratefully acknowledged. The collaboration of Yves Gélinas, MSc; Rémy Thériault, PhD; and Germain Thériault, MD is also appreciated. We would like to give special thanks to Dr. David E. Kelley for his critical comments.

REFERENCES

1. Bogardus C, Lillioja S, Stone K, Mott D: Correlation between muscle glycogen synthase activity and in vivo insulin action in man. *J Clin Invest* 73:1185–1190, 1984
2. Damsbo P, Vaag A, Hother-Nielsen O, Beck-Nielsen H: Reduced glycogen synthase activity in skeletal muscle from obese patients with and without type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 34:239–245, 1991
3. Thorburn AW, Gumbiner B, Bulacan F, Brechtel G, Henry RR: Multiple defects in muscle glycogen synthase activity contribute to reduced glycogen synthesis in non-insulin dependent diabetes mellitus. *J Clin Invest* 87:489–495, 1991
4. Groop LC, Kankuri M, Schalin-Jantti C, Ekstrand A, Nikula-Ijas P, Widen E, Kuismanen E, Eriksson J, Franssila-Kallunki A, Saloranta C, Koskimies S: Association between polymorphism of the glycogen synthase gene and non-insulin-dependent diabetes mellitus. *N Engl J Med* 328:10–14, 1993
5. Rissanen J, Pihlajamaki J, Heikkinen S, Kekalainen P, Mykkanen L, Kuusisto J, Kolle A, Laakso M: New variants in the glycogen synthase gene (Gln71His, Met416Val) in patients with NIDDM from eastern Finland. *Diabetologia* 40:1313–1319, 1997
6. Orho-Melander M, Almgren P, Kanninen T, Forsblom C, Groop LC: A paired-sibling analysis of the XbaI polymorphism in the muscle glycogen synthase gene. *Diabetologia* 42:1138–1145, 1999
7. Schalin-Jantti C, Nikula-Ijas P, Huang X, Lehto M, Knudsen P, Syvanne M, Lehtovirta MT, Tikkanen T, Tikkanen I, Groop LC: Polymorphism of the glycogen synthase gene in hypertensive and normotensive subjects. *Hypertension* 27:67–71, 1996
8. Shimomura H, Sanke T, Ueda K, Hanabusa T, Sakagashira S, Nanjo K: A missense mutation of the muscle glycogen synthase gene (M416V) is associated with insulin resistance in the Japanese population. *Diabetologia* 40:947–952, 1997
9. Babadjanova G, Allolio B, Beuschlein F, Chuchalin A, Reincke M: Polymorphism of the glycogen synthase gene and non-insulin-dependent diabetes mellitus in the Russian population. *Metabolism* 46:121–122, 1997
10. Gharavi AG, Phillips RA, Finegood DT, Lipkowitz MS: Glycogen synthase polymorphism, insulin resistance and hypertension. *Blood Press* 5:86–90, 1996
11. Kadowaki T, Kadowaki H, Yazaki Y: Polymorphism of the glycogen synthase gene and non-insulin-dependent diabetes mellitus. *N Engl J Med* 328:1568–1569, 1993
12. Zouali H, Velho G, Froguel P: Polymorphism of the glycogen synthase gene and non-insulin-dependent diabetes mellitus. *N Engl J Med* 328:1568, 1993
13. Orho-Melander M, Shimomura H, Sanke T, Rasmussen SK, Nanjo K, Pedersen O, Groop LC: Expression of naturally occurring variants in the muscle glycogen synthase gene. *Diabetes* 48:918–920, 1999
14. Simoneau JA, Bouchard C: Human variation in skeletal muscle fiber-type proportion and enzyme activities. *Am J Physiol* 257:E567–E572, 1989
15. Theriault R, Theriault G, Simoneau JA: Human skeletal muscle adaptation in response to chronic low-frequency electrical stimulation. *J Appl Physiol* 77:1885–1889, 1994
16. Henriksson J, Chi MM, Hintz CS, Young DA, Kaiser KK, Salmons S, Lowry OH: Chronic stimulation of mammalian muscle: changes in enzymes of six metabolic pathways. *Am J Physiol* 251:C614–C632, 1986
17. Hjeltnes N, Galuska D, Bjornholm M, Aksnes AK, Lannem A, Zierath JR, Wallberg-Henriksson H: Exercise-induced overexpression of key regulatory proteins involved in glucose uptake and metabolism in tetraplegic persons: molecular mechanism for improved glucose homeostasis. *FASEB J* 12: 1701–1712, 1998
18. Neel JV: The thrifty genotype revisited. In *The Genetics of Diabetes Mellitus*, Kobberling J, Tattersall R, Eds. London, Academic Press, 1982, p. 283–293
19. Simoneau J-A, Kelley DE: Metabolic aspects of skeletal muscle in obesity. In *Progress in Obesity Research*. Vol. 8. Ailhaud G, Guy-Grand B, Eds. London, John Libbey & Company, 1999, p. 411–414
20. Orho M, Nikula-Ijas P, Schalin-Jantti C, Permutt MA, Groop LC: Isolation and characterization of the human muscle glycogen synthase gene. *Diabetes* 44:1099–1105, 1995
21. Howald H, Pette D, Simoneau JA, Uber A, Hoppeler H, Cerretelli P: Effect of chronic hypoxia on muscle enzyme activities. *Int J Sports Med* 11 (Suppl 1): S10–S14, 1990