Original Article

Genetic and Nongenetic Regulation of *CAPN10* mRNA Expression in Skeletal Muscle

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The gene encoding calpain-10 (*CAPN10*) has been identified as a candidate gene for type 2 diabetes. Our aim was to study the impact of genetic (heritability and polymorphisms) and nongenetic (insulin, free fatty acids, and age) factors on *CAPN10* mRNA expression in skeletal muscle using two different study designs. Muscle biopsies were obtained before and after hyperinsulinemic-euglycemic clamps from 166 young and elderly monozygotic and dizygotic twins as well as from 15 subjects with normal (NGT) or impaired glucose tolerance (IGT) exposed to an Intralipid infusion. We found hereditary effects on both basal and insulin-exposed *CAPN10* mRNA expression. Carriers of the type 2 diabetes–associated single nucleotide polymorphism (SNP)-43 G/G genotype had reduced *CAPN10* mRNA levels compared with subjects carrying the SNP-43 A-allele. Age had no significant influence on *CAPN10* mRNA levels. Insulin had no significant effect on *CAPN10* mRNA levels, neither in the twins nor in the basal state of the Intralipid study. However, after a 24-h infusion of Intralipid, we noted a significant increase in *CAPN10* mRNA in response to insulin in subjects with NGT but not in subjects with IGT. In conclusion, we provide evidence that mRNA expression of *CAPN10* in skeletal muscle is under genetic control. Glucose-tolerant but not glucose-intolerant individuals upregulate their *CAPN10* mRNA levels in response to prolonged exposure to fat. *Diabetes* 54: 3015–3020, 2005

Type 2 diabetes is a complex trait with both environmental and hereditary factors contributing to the overall pathogenesis (1,2). The gene encoding calpain-10 (*CAPN10*), located on chromosome 2q37, has been identified as a candidate gene for type 2 diabetes (3). Calpain-10 is a member of the calpain family of nonlysosomal cysteine proteases that catalyzes the cleavage of specific substrates involved in a number of cellular functions. The exact functions of calpain-10 remain to be determined, but it is expressed in many tissues involved in glucose homeostasis such as skeletal muscle, liver, pancreas, and adipose tissue. Interestingly, recent results suggest that calpain-10 may be involved in GLUT4 translocation to the cell membrane in adipocytes (4) and regulation of pancreatic insulin secretion (5,6) as well as pancreatic β-cell apoptosis (7). Allele-specific variability in expression of a number of genes, including *CAPN10*, has been observed (8). In Pima Indians, the diabetes-associated intronic single nucleotide polymorphism (SNP)-43 G/G genotype was associated with reduced muscle *CAPN10* mRNA expression and impaired insulin-stimulated glucose metabolism, mainly impaired glucose oxidation (9). In addition, the SNP-43 G-allele has been associated with both elevated plasma free fatty acid (FFA) (10) and triglyceride levels (11). Elevated plasma FFA levels are common in obesity and type 2 diabetes and may directly contribute to the development of insulin resistance (12).

Our aim was to study the effect of genetic and nongenetic factors on *CAPN10* mRNA expression in skeletal muscle. To accomplish this, we quantified *CAPN10* mRNA levels in muscle biopsies obtained before and after hyperinsulinemic-euglycemic clamps in young and elderly monozygotic and dizygotic twins as well as in subjects with normal (NGT) or impaired glucose tolerance (IGT) after exposure to fat infusion. We used a generalized estimating equations (GEEs) model to test the influence of different factors on *CAPN10* mRNA expression and the impact of *CAPN10* SNP-43 and mRNA expression on glucose and lipid metabolism.

RESEARCH DESIGN AND METHODS

Twin study protocol. Subjects were identified through The Danish Twin Register and selected as previously described (13,14). A total of 98 young (aged 25–32 years) and elderly (aged 58–66 years) twin pairs were included in the clinical examination (13,14). We were able to obtain both blood samples and skeletal muscle biopsies from 83 of the twin pairs (28 younger monozygotic, 20 younger dizygotic, 15 elderly monozygotic, and 20 elderly dizygotic; Table 1). Among the elderly twins, 77% had NGT, 19% had IGT, and 4% had previously unknown type 2 diabetes. Of the young twins, 98% had NGT and 2% had IGT. Zygosity was determined by polymorphic genetic markers.

Clinical examination. Subjects underwent 2 days of clinical examinations separated by 1–2 weeks. Day 1 included a standard 75-g oral glucose tolerance test and anthropometric measures (i.e., BMI, waist-to-hip ratio, and a dual-energy X-ray absorptiometry scanning to determine body composition), as previously described (13). On day 2, subjects underwent a 2-h hyperinsulinemic-euglycemic clamp preceded by a 30-min intravenous glucose tolerance test performed as previously described (13). After the intravenous glucose

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FFA, free fatty acid; GEE, generalized estimating equation; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; NOGM, nonoxidative glucose metabolism; SNP, single nucleotide polymorphism.

TABLE 1
Clinical characteristics of twins participating in the study

	Young twins	Elderly twins	*P*
n (male/female, MZ/DZ)	96 (56/40, 56/40)	70 (28/42, 30/40)	0.02, 0.03
Age (years)	28 ± 2	62 ± 2	<0.0001
BMI (kg/m^2)	24.1 ± 3.2	26.5 ± 4.7	0.0001
Waist-to-hip ratio	0.84 ± 0.09	0.88 ± 0.10	0.008
Percentage body fat (%)	22.0 ± 6.8	28.9 ± 9.7	<0.0001
Fat mass (kg)	16.3 ± 6.2	21.5 ± 9.0	<0.0001
Fasting plasma glucose (mmol/l)	5.4 ± 0.3	5.9 ± 0.8	<0.0001
Fasting plasma insulin (μU/ml)	5.8 ± 2.7	5.9 ± 3.5	0.5

Data are means ± SD. DZ, dizygotic twins; MZ, monozygotic twins.

tolerance test, a primed-continuous insulin infusion (40 mU · m^{-2} · min^{-1}) was initiated and continued for 2 h. A steady state was defined as the last 30 min of the 2-h clamp period. A variable infusion of glucose (180 g/l) maintained euglycemia during insulin infusion, with monitoring of plasma glucose concentration every 5–10 min during the basal and clamp periods using an automated glucose oxidation method (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA). Indirect calorimetry was performed using a computerized flow-through canopy gas analyzer system (Deltarac; Datex, Helsinki, Finland).

Plasma insulin concentrations were analyzed as previously described (13). Insulin-stimulated glucose uptake was defined as the glucose infusion rate during steady state. The glucose uptake, glucose oxidation, and fat oxidation were expressed per kilogram lean body mass as determined by dual-energy X-ray absorptiometry scan. Nonoxidative glucose metabolism (NOGM) was calculated as glucose uptake minus glucose oxidation, as determined by indirect calorimetry.

Intralipid study protocol. Seven unrelated male subjects with IGT and a first-degree family history of type 2 diabetes and eight male control subjects matched for age and BMI with NGT and no family history of type 2 diabetes participated in the study (Table 2). IGT was diagnosed according to World Health Organization criteria.

Experimental protocol. All subjects participated at three separate occasions in a hyperinsulinemic-euglycemic clamp (performed as described for the twin study) with prior infusion of Intralipid for 0, 2, or 24 h as previously described in detail (15). Skeletal muscle biopsies were obtained before and at the end of the clamps.

Intralipid is a fat emulsion consisting of 12% palmitic acid (C16:0), 4% stearic acid (C18:0), 21% oleic acid (C18:1 n-9), 53% linoleic acid (C18:2 n-6), 7% α-linolenic acid (C18:3 n-3), and 3% other acids. To achieve a physiologic 10–30% elevation of the fasting plasma FFA concentration, 20% Intralipid was infused at a rate of 40 ml · kg body wt^{-1} · h^{-1} through a polyethylene catheter inserted into an antecubital vein. During the baseline experiment (protocol 1), saline was infused for 2 h before the study start and continued throughout the study period. During protocol 2, the Intralipid infusion was given for 2 h before the study start and continued throughout the study period. During protocol 3, the subjects were admitted to the hospital, and the Intralipid infusion was initiated 24 h before the study start and continued throughout the study period.

Plasma insulin concentrations were determined using the 1234 AutoDELFIA immunoassay system (Wallac Oy, Turku, Finland). Plasma FFAs were measured using an enzymatic colometric method (Wako, Richmond, VA).

All subjects

Muscle biopsy. Muscle biopsies were obtained from the vastus lateralis muscle under local anesthesia in subjects participating in both protocols using a modified Bergström's needle (including suction) before and after the hyperinsulinemic-euglycemic clamps. Biopsies were immediately frozen in liquid nitrogen and stored at −80°C for later analysis. Both studies were approved by the regional ethics committees.

Measurement of CAPN10 mRNA using real-time RT-PCR. In the twin study, extraction of total RNA from the muscle biopsies was performed with the TRI reagent (Sigma-Aldrich, St. Louis, MO) and in the Intralipid study with the guadinium thiocyanate method (16). cDNA was synthesized using Superscript II RNase H$^-$ Reverse Transcriptase (Life Technologies, Gaithersburg, MD) and random hexamer primers (Life Technologies). Real-time PCR in the twin study was performed using the ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA) and in the Intralipid study using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems) according to the manufacturer's instructions. Primers and probe for *CAPN10* mRNA quantification in the twin study were ordered as a ready-to-use mix of primers and an FAM-labeled probe (Hs 00225048_m1; Applied Biosystems) and in the Intralipid study designed using Primer Express Software (Applied Biosystems), and the sequences were *CAPN10* forward: 5′-CAT TCA CAG CCA GGA GAT GCT-3′, *CAPN10* reverse: 5′-CTG TTA GGT TTT CAT CAC TGC CAT-3′, and *CAPN10* probe: 5′(TET)-CCAGTT CCT CCA AGA GGT CTC CGT-(Tamra)3′ (TAG, Copenhagen, Denmark). The CAPN10 primers and probes recognize seven of the eight isoforms of calpain-10, all except calpain-10g. In both studies, *Cyclophilin A* was used as an endogenous control to standardize the amount of cDNA added to the reactions using a ready-to-use mix of primers and a VIC-labeled probe (Applied Biosystems). All samples were run in duplicate, and data were calculated using the standard curve method and expressed as a ratio to the *Cyclophilin A* reference.

Genotyping. DNA was extracted from blood using a conventional method (17). The *CAPN10* SNP-43 was genotyped using allelic discrimination in the ABI PRISM 7900 Sequence Detection System (Applied Biosystems). Primers and probes were designed using Assay by Design (Applied Biosystems).

Statistical methods. Monozygotic twins have identical genotypes, and any differences are theoretically due to environmental factors. Dizygotic twins, however, share on average 50% of their genes. The extents to which monozygotic twins are more alike than dizygotic twins are therefore presumed to reflect a genetic influence on the phenotype in question. Genetic modeling to estimate the degree of genetic versus environmental influence on *CAPN10* mRNA expression was conducted separately in the two age-groups using standard Mx scripts. Standard univariate twin modeling based on linear structural equations was used in the study (18). The applied model is based upon the assumption that phenotypic variation can be decomposed into additive genetic, genetic dominance, or shared environmental and unique

TABLE 2
Clinical and metabolic characteristics of men with NGT and IGT in the Intralipid study

	NGT	IGT	*P*
n	8	7	
Age (years)	53 ± 6	57 ± 4	0.2
BMI (kg/m^2)	32.7 ± 3.9	32.0 ± 3.1	0.8
Fasting plasma glucose (mmol/l)	6.0 ± 0.2	6.5 ± 0.6	0.2
Fasting plasma insulin (μU/ml)	9.0 ± 4.1	14.9 ± 8.4	0.1
2-h glucose (mmol/l)	5.6 ± 0.8	9.0 ± 0.9	0.001
Glucose uptake during clamp (mg · kg lean body mass^{-1} · min^{-1})	8.8 ± 2.2	5.1 ± 1.3	0.008
FFAs (mmol/l)	0.44 ± 0.11	0.40 ± 0.16	0.4

Data are means ± SD. 2-h glucose is plasma glucose 2 h after an oral glucose load (75 g glucose).

environmental effects. Additive genetic effects result from single gene effects added over multiple loci, whereas dominant genetic factors refer to genetic interaction within the same locus. Common environment refers to environmental factors shared by twins reared in the same family, and unique environment represents the environmental experiences that are unique for the individual twin. The fit for each model was assessed by maximum likelihood methods and resulted in a χ^2 goodness-of-fit index and probability value, which tested the agreement between the observed and the predicted statistics. With a low χ^2 and a high P value, there is no significant difference between the observed and expected models, and data fit the model. When selecting between nonnested models, the models with the lowest Akaikes Information Criterion were preferred. Akaikes Information Criterion is an alternative fit index and another way of expressing the fit. The smaller Akaikes Information Criterion, the better when distinguishing between different models.

GEE incorporating both family identifier and twin status were used to fit general linear models for the twin data using the approach of Zeger and Liang (19). GEE analyses have been applied on this particular twin material once before (20). We modeled the within-twin pair association as a correlation where we allowed the correlation to be different for the monozygotic and dizygotic twins. We reached the final models using backward selection regression.

Data are presented as means ± SD (for clinical variables) or means ± SE (for *CAPN10* mRNA expression). The χ^2 test was used to identify significant departures from the Hardy-Weinberg equilibrium, using only one random twin from each pair. Comparisons of *CAPN10* mRNA expression between different groups in the Intralipid study were performed using the nonparametric Wilcoxon or Mann-Whitney statistics and for the twin material using nonparametric statistics (P values) and a GEE analysis where adjustments for age, sex, BMI, and intra–twin-pair relationship were made (P_a values). Statistical operations were performed using the Number Cruncher Statistical Software (NCSS, Kaysville, UT) and Stata (StataCorp LP, College Station, TX). All tests applied were two tailed, and $P < 0.05$ was considered significant.

RESULTS

Twin study

CAPN10 mRNA expression in skeletal muscle: effect of age, insulin, heritability, and variation at SNP-43. The original study population consisted of 98 twin pairs and has previously been described (13,14). We were able to obtain both blood samples and skeletal muscle biopsies from 83 of the twin pairs (Table 1).

There was no significant difference in *CAPN10* mRNA levels between young and elderly twins neither in the basal state (0.39 ± 0.04 vs. 0.36 ± 0.04, $P = 0.1$, $P_a = 0.5$) nor after the 2-h hyperinsulinemic-euglycemic clamp (0.38 ± 0.03 vs. 0.35 ± 0.04, $P = 0.1$, $P_a = 0.7$). The insulin clamp had no significant effect on *CAPN10* mRNA levels neither in all individuals (from 0.38 ± 0.03 to 0.37 ± 0.02, $P = 0.3$) nor in the young (from 0.39 ± 0.04 to 0.38 ± 0.03, $P = 0.8$) or elderly twins (from 0.36 ± 0.04 to 0.36 ± 0.04, $P = 0.2$).

Biometric models were calculated for basal and postclamp *CAPN10* mRNA expression to estimate the degree of genetic versus environmental influence (online appendix 1 [available at http://diabetes.diabetesjournals.org]). In the elderly twins, there was a major genetic component influencing both basal (a^2 [additive genetic] = 0.84, e^2 [unique environment] = 0.16) and postclamp *CAPN10* mRNA levels ($a^2 = 0.57$, $e^2 = 0.43$). In the younger twins, basal *CAPN10* mRNA levels fitted a model with both a genetic and a slightly higher environmental component ($a^2 = 0.43$, $e^2 = 0.57$), while postclamp *CAPN10* mRNA levels fitted a model pointing toward a major genetic component ($a^2 = 0.66$, $e^2 = 0.34$). Genotype frequencies for the *CAPN10* SNP-43 were in Hardy-Weinberg equilibrium (G/G = 0.48, G/A = 0.45, and A/A = 0.07, $P > 0.05$ in an analysis including only one random twin from each pair). Subjects with the *CAPN10* SNP-43 G/G genotype exhibited decreased *CAPN10* mRNA levels both before (0.33 ± 0.03 [$n = 79$] vs. 0.43 ± 0.04 [$n = 86$], $P = 0.03$) and after insulin clamp (0.30 ± 0.03 [$n = 77$] vs. 0.44 ± 0.04 [$n = 83$], $P = 0.0004$) compared with individuals carrying the SNP-43 A-allele (Fig. 1). The difference observed after insulin clamp ($P_a = 0.01$) but not the difference observed at the basal state ($P_a = 0.1$) was still significant after adjustments for age, sex, BMI, and intra–twin-pair correlations.

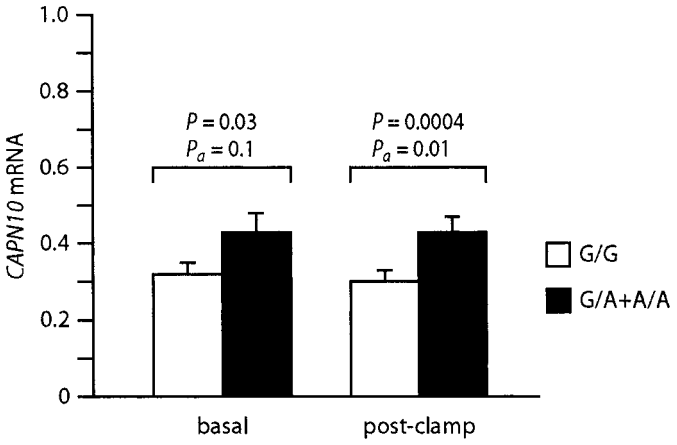

FIG. 1. Association between *CAPN10* mRNA expression in skeletal muscle and SNP-43 genotype. Carriers of the SNP-43 G/G genotype ($n = 79$ before and $n = 77$ after insulin clamp) had reduced *CAPN10* mRNA levels compared with carriers of the A-allele ($n = 86$ before and $n = 83$ after insulin clamp). mRNA levels were quantified with real-time PCR and normalized to the level of endogenous *Cyclophilin A*. Results are expressed as the means ± SE. Both Mann-Whitney statistics (P values) and a GEE analysis adjusting for age, BMI, sex, and intra–twin-pair relationship (P_a values) are used to calculate statistical differences between groups.

GEE modeling. GEE modeling was used to test whether any of the following parameters influence the basal and insulin-stimulated *CAPN10* mRNA levels in skeletal muscle: *CAPN10* SNP-43 genotype (G/G [0] or G/A and A/A [1]), zygosity (monozygotic [0] or dizygotic [1]), birth weight (continuous [g]), age (young [0] or elderly [1]), sex (men [0] or women [1]), percentage body fat (continuous [%]), total body aerobic capacity ($V\text{o}_{2\text{max}}$) (continuous [ml · kg^{-1} · min^{-1}]), and the interactions between sex and percentage body fat as well as SNP-43 and sex, age, birth weight, percentage body fat, and $V\text{o}_{2\text{max}}$, respectively. The final models were reached using backward selection regression (online appendix 2). Basal *CAPN10* mRNA expression was positively related to the interaction between SNP-43 genotype and $V\text{o}_{2\text{max}}$ (regression coefficient [R] = 0.003, $P = 0.04$). Insulin-stimulated *CAPN10* mRNA levels were influenced by sex ($R = 0.36$, $P = 0.01$), the interaction between SNP-43 and birth weight ($R = 0.00005$, $P = 0.002$), and the interaction between sex and percentage body fat ($R = -0.01$, $P = 0.02$).

Since calpain-10 has been suggested to be involved in GLUT4 translocation (4), and *CAPN10* SNP-43 associated with impaired insulin-stimulated glucose metabolism (8), the GEE model was also used to test whether basal *CAPN10* mRNA expression along with any of the following parameters influence metabolic turnover rates: *CAPN10* SNP-43 genotype (G/G [0]) or G/A and A/A ([1]), zygosity (monozygotic [0]) or dizygotic ([1]), birth weight (continuous [g]), age (young [0] or elderly [1]), sex (men [0] or women [1]), percentage body fat (continuous [%]), $V\text{o}_{2\text{max}}$ (continuous [ml · kg^{-1} · min^{-1}]), and the interactions between sex and percentage body fat, *CAPN10* mRNA and sex, age, birth weight, percentage body fat, and $V\text{o}_{2\text{max}}$, respectively, and SNP-43 and sex, age, birth weight, percentage body fat, and $V\text{o}_{2\text{max}}$, respectively. The final mod-

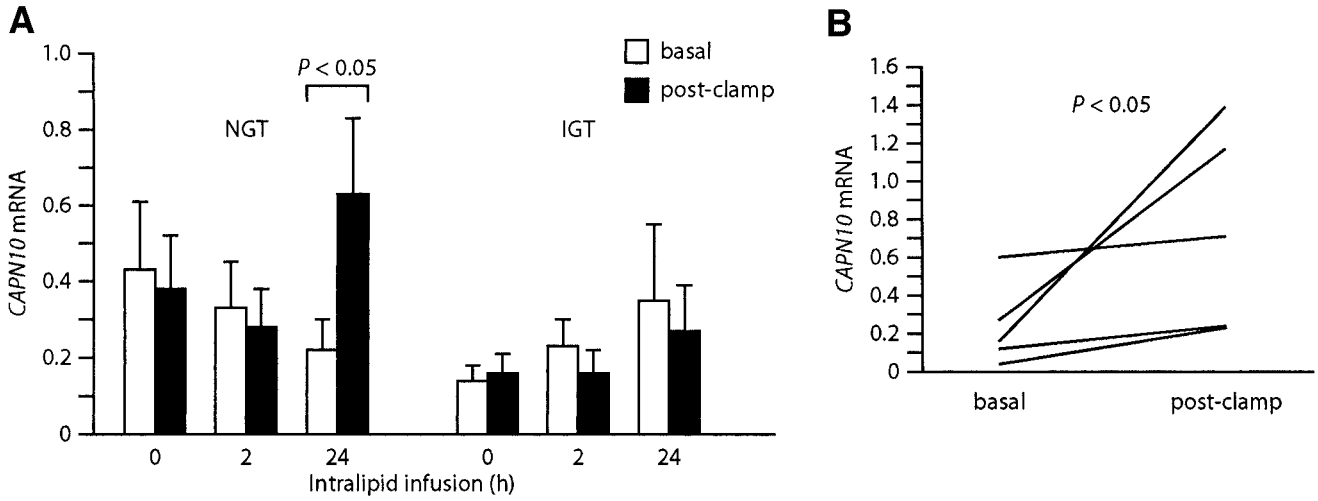

FIG. 2. ***A*: *CAPN10* mRNA expression in skeletal muscle obtained before and after hyperinsulinemic-euglycemic clamps with prior infusion of lipids (Intralipid) for 0, 2, and 24 h from subjects with NGT and IGT. Results are expressed as the means ± SE. *B*: *CAPN10* mRNA expression in skeletal muscle obtained before and after hyperinsulinemic-euglycemic clamps with prior infusion of Intralipid for 24 h from subjects with NGT. The individual expression levels are shown. The 24-h infusion of Intralipid resulted in a significant increase in *CAPN10* mRNA expression in response to insulin in subjects with NGT ($P = 0.04$). mRNA levels were quantified with real-time PCR and normalized to the level of endogenous *Cyclophilin A*.**

els were reached using backward selection regression (online appendix 3). Insulin-stimulated glucose uptake was positively related to Vo_{2max} ($R = 4.0$, $P < 0.001$) and the interaction between SNP-43 genotype and birth weight ($R = 0.03$, $P = 0.01$) and negatively related to the interaction between SNP-43 genotype and percentage body fat ($R = -2.8$, $P = 0.02$). Glucose oxidation was influenced by age ($R = -21.8$, $P < 0.001$) and birth weight ($R = 0.01$, $P = 0.01$). Fat oxidation was influenced by age ($R = 6.6$, $P = 0.001$) and sex ($R = -4.7$, $P = 0.02$) and NOGM by Vo_{2max} ($R = 3.9$, $P < 0.001$). We were unable to detect an association between *CAPN10* mRNA expression and metabolic turnover rates by the GEE methodology.

By ANOVA, young carriers of different *CAPN10* SNP-43 genotypes differed significantly concerning glucose uptake during clamp (G/G = 11.5 ± 3.2 [$n = 43$], G/A = 11.4 ± 3.4 [$n = 44$], and A/A = 14.2 ± 2.3 mg · kg lean body mass^{-1} · min^{-1} [$n = 9$], $P = 0.03$) and NOGM (G/G = 6.8 ± 2.8 [$n = 43$], G/A = 6.7 ± 2.8 [$n = 44$], and A/A = 9.9 ± 2.4 mg · kg lean body mass^{-1} · min^{-1} [$n = 9$], $P = 0.01$), although the significances disappeared when adjustments for age, sex, BMI, and intratwin correlation were made ($P_a = 0.16$ and $P_a = 0.05$, respectively). No significant discrepancies between different *CAPN10* SNP-43 carriers and other parameters including BMI, waist-to-hip ratio, birth weight, fasting plasma glucose, fasting plasma insulin, fat percentage, fat mass, lean body mass, glucose oxidation, or fat oxidation during clamp or Vo_{2max} were observed in young or elderly twins (data not shown).

Intralipid study

***CAPN10* mRNA expression in skeletal muscle: effect of Intralipid.** The clinical and metabolic characteristics of the subjects are shown in Table 2 and have been previously described in detail (15). There were no significant differences between NGT and IGT subjects in preclamp *CAPN10* mRNA levels at baseline (0.43 ± 0.18 [$n = 8$] vs. 0.14 ± 0.04 [$n = 6$], $P = 0.3$) or after 2 (0.33 ± 0.12 [$n = 8$] vs. 0.23 ± 0.07 [$n = 7$], $P = 0.7$) or 24 h of Intralipid infusion (0.22 ± 0.08 [$n = 6$] vs. 0.35 ± 0.20 [$n = 7$], $P = 0.6$), nor were there any significant differences between NGT and IGT subjects in postclamp *CAPN10* mRNA levels at baseline (0.38 ± 0.13 [$n = 8$] vs. 0.16 ± 0.05 [$n = 7$], $P = 0.1$) or after the 2-h (0.28 ± 0.10 [$n = 7$] vs. 0.16 ± 0.06 [$n = 6$], $P = 0.3$) or 24-h lipid infusion (0.63 ± 0.23 [$n = 6$] vs. 0.27 ± 0.12 [$n = 7$], $P = 0.2$) (Fig. 2*A*).

As observed in the twin study, insulin had no significant effect on *CAPN10* mRNA levels neither in the NGT (from 0.43 ± 0.18 to 0.38 ± 0.16 [$n = 8$], $P = 0.6$) nor in the IGT group (from 0.14 ± 0.04 to 0.11 ± 0.02 [$n = 6$], $P = 0.6$) at baseline or after 2 h of Intralipid infusion (NGT subjects from 0.37 ± 0.12 to 0.28 ± 0.11 [$n = 6$], $P = 0.6$ and IGT subjects from 0.25 ± 0.08 to 0.16 ± 0.06 [$n = 6$], $P = 0.2$). However, after the 24-h Intralipid infusion, a significant increase in *CAPN10* mRNA was observed in response to insulin in the NGT group (from 0.24 ± 0.09 to 0.75 ± 0.22 [$n = 5$], $P = 0.04$) but not in the IGT group (from 0.35 ± 0.20 to 0.27 ± 0.12 [$n = 7$], $P = 0.9$) (Fig. 2*B*).

DISCUSSION

The aim of this study was to investigate the effect of different genetic factors, including heritability estimates and genetic variation on one side versus nongenetic factors including age, insulin, and plasma FFA levels on the other, on *CAPN10* mRNA expression in skeletal muscle.

The inability of insulin to regulate the *CAPN10* mRNA expression, found in both our study populations at baseline, confirms previous data on calpain-10 protein levels (4). However, after a prolonged low-grade infusion of lipids, subjects with NGT were able to upregulate their *CAPN10* mRNA levels in response to insulin. Little is known about how muscle protects itself from occasional fat exposure after, e.g., a fat-rich meal. Several studies have shown that elevated FFA levels play a key role in the development of skeletal muscle insulin resistance (12). An increased supply of FFA substrates results in a metabolic switch from oxidizing glucose to oxidizing FFAs (21) but also in increased reesterification of FFAs to triglycerides. Of note, increased intramyocellular triglyceride concentrations have been associated with skeletal muscle insulin resistance (22–24). Elevated FFA levels and intracellular lipids appear to inhibit insulin signaling, leading to a

reduction in insulin-stimulated muscle glucose transport that may be mediated by a decrease in GLUT4 translocation (12). Since calpain-10 has been suggested to be involved in GLUT4 translocation (4), the increase in *CAPN10* mRNA levels in response to Intralipid and insulin seen in the NGT subjects could be a way to protect against insulin resistance secondary to elevated FFAs. Recently, calpain-10 has also been shown to participate in a novel apoptosis pathway in pancreatic β-cells (7). This pathway is initiated by the fatty acid palmitate. It is possible that the increase in *CAPN10* mRNA is part of an ordered pathway including apoptosis as opposed to an unordered lysis of cells that otherwise would take place. This reasoning is hypothetical, since this apoptosis pathway has not been described in muscle cells but certainly seems worthwhile pursuing.

It is well known that there is an age-related impairment of glucose tolerance (25–27). It is characterized by both alterations in glucose-induced insulin release from pancreatic β-cells and resistance to insulin-mediated glucose disposal primarily in skeletal muscle. To test whether reduced *CAPN10* expression in skeletal muscle could be one of the mechanisms responsible for the decreased glucose tolerance in older subjects, we compared *CAPN10* mRNA levels in young and elderly twins. We have previously shown this to be the case concerning peroxisome proliferator–activated receptor γ coactivator 1α and 1β in this study population (20). However, we found no significant difference in *CAPN10* mRNA expression between the two age-groups.

Twin studies have been used extensively in medical research to determine the potential role of genes versus environment in the etiology of human disease. The heritability data from this investigation suggest that a large amount of the variability in *CAPN10* mRNA expression is due to genetic factors. Of the genetic variants in *CAPN10*, the G-allele of SNP-43 seems to show the strongest association with type 2 diabetes in the original study sample (3) as well as in the Botnia population (10). We have recently found that the SNP-43 G-allele is associated with both higher fasting FFA and triglyceride levels compared with the A-allele (10,11). In line with a study in Pima Indians (9) and a study where we investigated *CAPN10* mRNA levels in adipose tissue (11), we observed decreased *CAPN10* mRNA levels in subjects with the G/G genotype compared with carriers of the A-allele in the twin study, although after adjusting for age, BMI, sex, and intra–twin-pair relationship the difference was only significant for the expression after insulin clamp. The population we used for the Intralipid study was too small to allow conclusions as to genotype-specific phenotypic associations.

Using the GEE methodology, we found further support for SNP-43 being involved in the regulation of *CAPN10* mRNA expression in skeletal muscle. The interaction between SNP-43 and $V_{O_{2max}}$, a measure of the aerobic capacity, significantly influenced the basal *CAPN10* mRNA levels, while sex and the interactions between sex and percentage body fat as well as SNP-43 and birth weight significantly influenced the insulin-stimulated *CAPN10* mRNA levels in the twin study population. In other words, carriers of the SNP-43 A-allele will increase the basal *CAPN10* mRNA levels more compared with subjects with the SNP-43 G/G genotype in response to an improvement in $V_{O_{2max}}$.

In a GEE analysis performed to identify factors influencing insulin-stimulated glucose uptake, we found that $V_{O_{2max}}$ and the interactions between SNP-43 genotype and birth weight correlated positively and the interaction between SNP-43 genotype and percentage body fat correlated negatively to this variable. Using the same analysis method, $V_{O_{2max}}$ was the only factor that significantly influenced NOGM (positively). In an ANOVA, young carriers of different *CAPN10* SNP-43 genotypes diverged in glucose uptake during clamp and NOGM, although the significances were attenuated after adjustments for age, sex, BMI, and intra–twin-pair relationship. It is noteworthy that the mRNA expression levels of *CAPN10* did not significantly contribute to explain the variation in these metabolic parameters in the twin clamp study. The Pima Indian study suggested that the decreased rates of glucose turnover observed in subjects carrying the SNP-43 G/G genotype resulted from decreased rates of glucose oxidation and that there was a positive correlation between *CAPN10* mRNA levels in skeletal muscle and glucose oxidation (9). We were not able to find any associations between this measurement and *CAPN10* mRNA expression or SNP-43 genotype in the present investigation. This may be explained by different experimental design or different epistatic interactions operating in American Pima Indians and Scandinavian Caucasians. The Pima Indians have the world's highest reported incidence and prevalence of type 2 diabetes (30). It should be noted that results from e.g., association studies on genetic variation in *CAPN10* and type 2 diabetes vary between different ethical populations (31). The discordance may also be due to the fact that the younger subjects in the twin study and the subjects in the Pima Indian study have a similar average age, although the Scandinavian subjects are leaner.

In conclusion, this study shows that *CAPN10* mRNA expression in skeletal muscle is a heritable trait and may at least partly be explained by the SNP-43. *CAPN10* mRNA expression is not regulated by insulin alone, but after a long-term infusion of FFAs, subjects with NGT were able to upregulate their *CAPN10* mRNA levels in response to a hyperinsulinemic-euglycemic clamp, possibly as a way to escape insulin resistance.

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