Diabetologia (2002) 45: 276–282

Diabetologia

Rapid communication

Polymorphism in the *Calpain 10* gene influences glucose metabolism in human fat cells

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Abstract

Aims/hypothesis. A common *G* to *A* polymorphism (*UCSNP-43*) in the *Calpain 10* gene was recently found to be associated with Type II (non-insulin-dependent) diabetes mellitus and variations in post-absorptive and insulin stimulated glucose metabolism in vivo. We aimed to study the influence of *Calpain 10* polymorphism on insulin action in fat cells.
Methods. Calpain 10 polymorphism (*UCSNP-19*, *-43* or *-63*) were set in relation to lipolysis and lipogenesis in isolated subcutaneous adipocytes of 46 apparently healthy non-obese subjects.
Results. For *UCSNP-43* the *G/G* genotype had two-fold higher basal and insulin stimulated rates as compared with *AA/AG* genotypes. However, there was no genotype effect on basal or insulin inhibited lipolysis rates in fat cells. The protein amount of GLUT 4 in adipocytes was not influenced by the polymorphism. Fat cells expressed mRNA for the *Calpain 10* gene at a relatively high concentration, about 4 amol/μg RNA, which is similar to that of uncoupling protein-2. Neither a *UCSNP-19* nor a *UCSNP-63* polymorphism in the *Calpain 10* gene was found to be associated with basal or insulin-induced adipocyte lipolysis and lipogenesis. None of the polymorphisms influenced body mass index or fasting plasma concentrations of insulin and glucose in 693 non-obese healthy subjects.
Conclusions/interpretation. The *Calpain 10* gene could be involved in the regulation of glucose metabolism but not lipolysis in human fat cells, although it does not involve adipocyte GLUT-4 protein content. It is possible that the *Calpain 10* gene predisposes to diabetes by influencing the glucose metabolism. [Diabetologia (2002) 45: 276–282]

Keywords Adipose tissue, GLUT-4, insulin, lipogenesis, lipolysis, mRNA, polymerase chain reaction.

The genetic and environmental factors that contribute to the development of Type II (non-insulin dependent) diabetes mellitus are not well defined but resistance of insulin-mediated glucose transport into peripheral tissues (mainly muscle and adipose tissue) is an important factor [1]. The insulin signalling pathways to its different molecular targets in fat cells and muscle have been partly identified [2, 3]. It appears that the initial pathways, ie. binding to the receptor, activation of insulin receptor substrates and activation of phosphadityl inositol 3 kinase (PI-3-kinase) are similar; signals to various metabolic events after that such as activation of the major glucose carrier protein GLUT-4 and inhibition of lipolysis could differ [4].

Recent studies show that Type II diabetes is associated with a common *G* to *A* polymorphism (*UCSNP-43*) within the *Calpain 10* gene in different cohorts [5]. Although this finding suggests a new pathway for development of Type II diabetes, the biological function of the *Calpain 10* gene is not known [6]. However, the Calpain 10 gene polymorphism, i. e. homozygocity for *G* at *UCSNP-43*, has been associated with reduced post-absorptive and insulin-stimulat-

Received: 2 July 2001 and in revised form: 28 September 2001

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Table 1. Genotype distribution of different polymorphisms in *Calpain 10* gene in a large non-obese cohort

Genotype	Men	Women
UCSNP-19		
Del/Del	36	94
Del/Ins	124	311
Ins/Ins	73	253
UCSNP-43		
AA	18	49
GA	107	268
GG	108	341
UCSNP-63		
GG	187	565
TG	92	45
TT	1	1

Values for gender distribution of each polymorphism were compared by chi-square (χ^2) analysis. No significant effect of gender was observed

ed glucose turnover in obese, non-diabetic Pima Indians [7]. This suggests that the *Calpain 10* gene is involved in the regulation of glucose transport. We tested this hypothesis using isolated human fat cells. These cells are interesting as a model for insulin action in human beings because, they are relatively easily available and also because insulin has several well-defined metabolic actions in these cells (such as stimulation of glucose metabolism and inhibition of lipolysis). In an ongoing project aiming to characterize insulin action in isolated human subcutaneous fat cells, we genotyped 46 healthy, non-obese subjects for three known polymorphisms in the *Calpain 10* gene, *UCSNP-19*, *-43* and *–63*. Genotype data were set in relation to insulin action on lipolysis and lipogenesis in fat cells. In addition, expression of Calpain 10 mRNA and GLUT-4 protein were investigated in the adipocytes and an examination of the clinical effects of the polymorphisms were investigated in a large non-obese cohort.

Subjects and methods

Subjects. The first study comprised 46 consecutively recruited non-obese subjects of both sexes (9 males/37 females) who participated in a project to investigate insulin action in fat cells. Of these, 37 were healthy volunteers recruited by local advertisement whereas the remaining were referred to our ambulatory surgical dayward for benign, elective surgery (uncomplicated gallstones or inguinal hernia) and were otherwise healthy. No subject was on regular medication, including anticonception drugs for women. Age ranged from 21 to 62 years. Five of the females were menopausal. BMI ranged from 20 to 29 kg/m^2. Obesity was defined as BMI of more than 30 kg/m^2 according to the World Health Organisation criteria. The healthy volunteers and the subjects undergoing surgery came to the hospital in the morning at 07:30 h after an overnight fast. A venous blood sample was taken at fast for determination of plasma glucose by the hospital's routine chemistry laboratory and fasting plasma insulin by a radioimmunoassay kit (Pharmacia-Amersham, Uppsala, Sweden). Thereafter, the healthy volunteers underwent a fat biopsy under local anaesthesia [8]. The day surgery patients were given general anaesthesia as described [8)] and a subcutaneous fat biopsy was obtained from the abdominal skin incision at the start of operation. Up to this point only saline was administered intravenously. We have previously shown that adipocyte metabolic regulation is the same in fat cells obtained by local or general anaesthesia given in this way [8].

In a second study, subcutaneous adipose tissue was obtained from subjects undergoing elective abdominal surgery because of various non-malignant disorders at the start of operation. This tissue was only used to study the mRNA expression of the genes for *Calpain 10*, *UCP-2* and *18S*.

A third study was carried out to assess the frequency distribution of the different polymorphisms in the *Calpain 10* gene (Table 1). For this purpose venous blood was obtained from a heterogeneous group of 891 non-obese subjects (658 women, 233 men). The non-obese subjects included for investigation on insulin action described above were also included in this cohort. Among all these subjects 693 (524 women, 169 men) were classified as healthy according to the same criteria as for those undergoing fat biopsy. This group was subjected to clinical analysis (Table 2). The remaining 198 subjects were heterogeneous (diabetes, hypertension, dyslipidemia, polycystic ovary syndrome) and therefore not considered for further analysis.

Adipocyte lipolysis and lipogenesis. The amount of subcutaneous adipose tissue obtained by biopsy in local anaesthesia or

Table 2. Clinical data with *Calpain 10* genotypes in 693 healthy non-obese subjects

Calpain 10 Genotypes	Age (years)	BMI (kg/m^2)	Insulin (mU/l)	Glucose (mmol/l)
UCSNP-19				
Del/Del	42 ± 1	23.4 ± 0.3	6.4 ± 0.6	4.8 ± 0.1
Del/Ins	41 ± 1	23.8 ± 0.1	6.7 ± 0.2	4.9 ± 0.1
Ins/Ins	42 ± 1	23.8 ± 0.2	6.7 ± 0.3	4.8 ± 0.1
UCSNP-43				
AA	40 ± 2	24.1 ± 0.4	6.8 ± 0.4	5.0 ± 0.1
GA	42 ± 1	23.7 ± 0.2	6.4 ± 0.2	4.9 ± 0.1
GG	42 ± 1	23.1 ± 0.1	6.8 ± 0.3	4.9 ± 0.1
UCSNP-63				
GG	41 ± 1	23.8 ± 0.1	6.7 ± 0.2	4.9 ± 0.1
GT	42 ± 1	23.4 ± 0.2	6.4 ± 0.4	5.0 ± 0.1

Values are means ± SEM. They were compared by analysis of variance. No significant differences were observed. T63T subjects were too few to analyse. Insulin and glucose are measures in fasting venous plasma

from elective surgery at the day ward was 0.5–1 g. Whenever possible about 300 mg of tissue was frozen at –70 °C for subsequent protein determination (see below). The remaining tissue was immediately used for metabolic investigations as described [9]. In brief, isolated fat cells were prepared and fat cell size as well as the number of fat cell incubated were determined. In one set of experiments lipolysis was investigated. Dilute suspensions of fat cells were incubated in duplicate in a buffer (pH 7.4) containing albumin (20 mg/ml), glucose (1 mg/ml) and adenosine deaminase (1 U/ml) (to remove tracer amounts of adenosine which might influence the antilipolytic effect of insulin), and 8-bromo cyclic AMP (1 mmol/l) (phosphodiesterase sensitive cyclic AMP analogue that stimulates lipolysis) with or without increasing concentrations of human crystalline insulin (10^{-15}-10^{-7} mol/l). Incubations were carried out in duplicate for 2 h at 37 °C with air as the gas phase. At the end of incubation an aliquot of the medium was removed for glycerol determination (lipolysis index). In another set of experiments, the uptake of radiolabelled glucose into total lipids of fat cells (lipogenesis) at a very low total glucose concentration, where glucose transport is a rate-limiting step, was determined [10]. Fat cells were incubated (2 % vol/vol) in a medium (pH 7.4.) containing albumin (20 mg/ml), glucose (final concentration 2/ml) and 3-^{3}H-glucose ($5 \cdot 10^6$ cpm/ml) in the absence or presence of increasing concentrations of crystalline human insulin (10^{-15} – 10^{-6} mol/l) for 2 h in duplicate at 37 °C with air as the gas phase. Incubation was terminated by the addition of sulphuric acid. A scintillation fluid was added and incubation tubes were subjected to radioactive counting. Radioactive uptake into total lipids of fat cells is proportional to glucose transport [10]. This method, which is easy and less tissue consuming, gives the same information about basal and insulin-stimulated glucose transport as the tissue consuming "golden standard" 3-O-methyl-glucose method (10).

From the concentration-response experiments insulin action was evaluated in two ways. Firstly, the curves were linearised by log-logit transformation and the concentration yielding half maximal effect was estimated. From this value pD_2 was determined, which is the negative logarithm value of the molar half-maximal effective hormone concentration. Secondly, insulin action on lipolysis and lipogenesis at maximum effective concentration was determined (insulin responsiveness). Insulin acts according to the spare-receptor hypothesis in human fat cells, which means that only a fraction of the receptors has to be occupied by the hormone to obtain a full metabolic response [11]. Because of this, changes in pD_2 reflect changes in early events in insulin receptor signal transduction whereas changes in insulin responsiveness reflect more distal events as well [12]. In all subjects, for both lipolysis and lipogenesis, a maximum insulin effect was reached so it was always possible to calculate pD_2 and insulin responsiveness. Values for lipolysis and lipogenesis were related to the number of fat cells incubated.

Protein isolation and Western blot analysis. Frozen subcutaneous adipose tissue, approximately 300 mg, was crushed and lysed in protein lysis buffer (1 % Triton-X100, TRIS-HCL pH 7,6, 150 mmol/l NaCl) supplemented with protease inhibitors (1 mmol/l PMSF and Complete, Boehringer Mannheim, Germany), and homogenized using a microtome. The homogenate was centrifuged at 14000 rpm for 30 min and the infranatant was removed and saved, All steps were carried out at 4 °C to minimize the risk of protein degradation. The protein content in each sample was determined using a kit of reagents from Pierce, Rockford, Ill., USA. One-hundred micrograms of total protein was then loaded on polyacrylamide gels and separated by standard SDS-polyacrylamide gel electrophoresis. To control for differences in gel migration, exposure time, antibody incubation etc, samples from *G/G* and *G/A* subjects were run on the same gels and transferred to the same PVDF membranes (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom). Blots were blocked for 1 h in room temperature in TRIS-Buffered-Saline with 0.1 %. Tween-20 and 5 % nonfat dried milk. This was followed by an overnight incubation at + 4 % C in the presence of a polyclonal rabbit antibody directed against human GLUT-4 (Biogenesis, New Fields, Poole, UK). Secondary anti-rabbit antibodies (1:4000) conjugated to horseradish peroxidase were from Sigma, (St Louis, Mo., USA). Antigen-antibody complexes were detected by chemiluminescence using a kit of reagents from Pierce (Supersignal, Rockford, Rockford, Ill., USA) and blots were exposed to high-performance chemiluminescence film (Amersham, Little Chalfont, UK). Films were scanned and the optical density of each specific band analysed using the program Image (NIH, Bethesda, Maryland, Md., USA) and expressed as $OD \cdot mm^2 \cdot 100$ μg of total protein.

The determination of Calpain 10 mRNA. In order to make a semi-quantitative measurement, adipose tissue expression of calpain10 mRNA was related to the amount of UCP2 mRNA. For both UCP2 and calpain10, 0.5 μg RNA was used for cDNA synthesis with specific anti-sense primers, as described [7, 14]. Using these primers, the sum of the eight isoforms of Calpain 10 mRNA previously described [7], were identified as a single PCR product. The identity of this product was determined by direct sequencing (Cybergene, Stockholm, Sweden). Thereafter, Calpain10 and UCP2 cDNA were amplified simultaneously in the same PCR-experiment using specific forward and anti-sense primers. The primers used for UCP2 were forward primer 5'-ATGGACGCCTACAGAACCAT and reverse primer 5'-CTGGGCCACCATCTTTATCA. To control for different annealing temperature of the UCP2 and Calpain10 primers, multiple PCR experiments were done with a temperature gradient ranging from 48 °C to 60 °C. The optical density of the agarose gel bands for Calpain10 and UCP2 was determined using a TINA 2.09G software (Raytest, Straubenhardt, Germany). After correcting for size differences of the PCR products, the ratio between the optical density for calpain10 (121 bp) and for UCP2 (290 bp) was calculated. The absolute level of UCP2 mRNA was also determined using quantitative RT-competitive PCR, as described [14].

Genotyping. DNA was prepared from venous blood. The *Calpain 19* (*Ins/Del*) variant was genotyped by PCR using forward primer 5'-GTT TGG TTC TCT TCA GCG TGG AG-3' and reverse primer 5'-CAT GAA CCC TGG CAG GGT CTA AG-3'. The *Calpain 43* (*G/A*) polymorphism was genotyped using forward primer 5'-GCT GGC TGG TGA CAT CAG TGC and reverse primer 5'-ACC AAG TCA AGG CTT AGC CTC ACC TTC ATA-3' and subsequent digestion with enzyme NdeI. The *Calpain 63* (*G/T*) variant was genotyped using forward primer 5'-AAG GGG GGC CAG GGC CTG ACG GGG GTG GCG-3' and reverse primer 5'-AGC ACT CCC AGC TCC TCA TC-3', digestion using enzyme HhAI. Previously genotyped DNA [5] was used to confirm the accuracy of determining the three polymorphisms.

Statistics. Values are means ± SEM. Student's unpaired *t* test, analysis of variance, analysis of co-variance (using sex and age as co-factors), linear regression analysis and chi-square χ^2 analysis were carried out. A p value of 0.05 or less was considered to be statistically significant.

Table 3. Findings in fat cells with different genotypes and alleles of the *UCSNP-43* polymorphism in the *Calpain 10* gene

	AA	*AG*	AA/AG	GG	P (AA/AG vs GG)	P (AA/AG vs GG)
Fat cell volume, pl	501 ± 169	531 ± 4	525 ± 44	481 ± 34	0.71	0.39
Lipolysis rate (pmol · 10 cells · 2 h)						
8 bcAMP	22 ± 4	20 ± 2	20 ± 2	21 ± 2	0.84	0.99
+ Insulin	9 ± 3	8 ± 1	8 ± 1	8 ± 1	0.80	0.92
Lipogenesis rate (amol · 10 cells · 2 h)						
Basal	1.9 ± 0.7	1.6 ± 0.3	1.6 ± 0.2	3.2 ± 0.4	0.016	0.019
+ Insulin	3.6 ± 1.7	3.5 ± 0.5	3.5 ± 0.5	7.8 ± 1.1	0.009	0.026
pD_2 lipolysis	14.8 ± 0.2	14.6 ± 0.1	14.6 ± 0.1	14.4 ± 0.2	0.64	0.33
pD_2 lipogenesis	13.7 ± 0.3	13.6 ± 0.3	13.8 ± 0.2	13.7 ± 0.3	0.34	0.78

Values are means ± SEM. They were compared by analysis of variance and gender and age were included as co-variables in the *AA/AG* vs *GG* comparison. + Insulin = value at maximum effective insulin concentration. pD_2 = negative logarithm of half maximum effective insulin concentration (mol/l)

Table 4. Findings with lipogenesis in different subgroups as regards polymorphism in the *Calpain 10* gene

	UCSNP-43 genotypes		
	AA/AG	*GG*	*p*
Women			
n	22		
Basal	1.9 ± 0.3	3.2 ± 0.5	0.04
Insulin	4.4 ± 0.6	8.0 ± 1.2	0.03
Local anaesthesia			
n	17	20	
Basal	1.7 ± 0.3	3.1 ± 0.5	0.03
Insulin	3.9 ± 0. 6	8.0 ± 1.3	0.01
Ins-Ins and *G-G* in *UCSNP-19* and *–63*, respectively			
n	11	8	
Basal	1.7 ± 0.3	3.1 ± 0.4	0.007
Insulin	3.2 ± 0.5	9.7 ± 2.1	0.004

Values were compared by Student's *t* test. See legend to Table 1 for further details

Results

Genotype frequency. As a result of genotyping of the polymorphisms at *UCSNP-19*, *–43* and *–63* in the *Calpain 10* gene in the whole material show, the *Ins19Del* and *G43A* polymorphisms were common and the allele frequency ranged from 30 to 70% (Table 1). The *G63T* polymorphism was less common, the uncommon allele occurred among 8% of the subjects. All genotypes were in Hardy-Weinberg equilibrium and there was no significant difference in genotype distribution between genders. It should be noted that the sum of alleles in Table 1 was not equal for all *UCSNPs*. This is due to the occasional occurrence of typing error.

Lipolysis, lipogenesis and clinical data. The phenotypic effects of the three polymorphisms in the *Calpain 10* gene were investigated. As regards clinical data in the 694 healthy non-obese subjects, none of the *UCSNP –19, –43* and *–63* polymorphisms was associated with age, BMI, fasting plasma glucose or fasting plasma insulin (Table 2). As regards adipocyte data (in the 46 subjects investigated) neither *Ins19Del* nor *G63T* had any significant effect on fat-cell volume, lipolysis and lipogenesis (values not shown). However, some marked phenotypic effects of the *A43G* polymorphism were observed (Table 3). Thus, *GG* subjects had about twice as much basal and maximum insulin-stimulated lipogenesis compared with AA subjects; heterozygotes did not differ from *AA* homozygotes. These differences remained when *AA* and *AG* subjects were combined. In addition, *GG* subjects had significantly increased insulin-stimulated lipogenesis even when the basal value was substracted (values not shown). However, there was no genotype effect on pD_2 for lipogenesis. With regard to lipolysis, no effect of the *A43G* polymorphism on control or insulin-inhibited lipolysis rates was observed. This was also true for pD_2 for insulin inhibition of lipolysis. For illustrative purpose the mean concentration-response curves for lipolysis and lipogenesis are given in Figure 1. For *AA/AG* subjects the lipolysis curves were completely superimposed over that of *GG* subjects but for lipogenesis the two curves were different. *GG* subjects were much more responsive to insulin than *AA/AG* subjects. There was no effect of the *A43G* polymorphism on BMI, plasma glucose, plasma insulin and fat-cell volume. A minor but not significant difference in age and gene distribution was observed. However, the genotypic effect on basal and insulin-stimulated lipogenesis persisted after statistical correction for gender and age.

Because the material was somewhat heterogeneous, subgroup analysis of the *UCSNP-43* polymorphism was done (Table 4). In women the differences in basal and maximum insulin stimulated lipogenesis remained significant for the *A43G* polymorphism. Thus basal and insulin-stimulated lipogenesis were about twofold higher in *GG* compared with *AA/GG* women. The number of men was too small for a separate statistical analysis. We also investigated separately those subjects who were healthy volunteers (having fat removed by biopsy under local anaesthe-

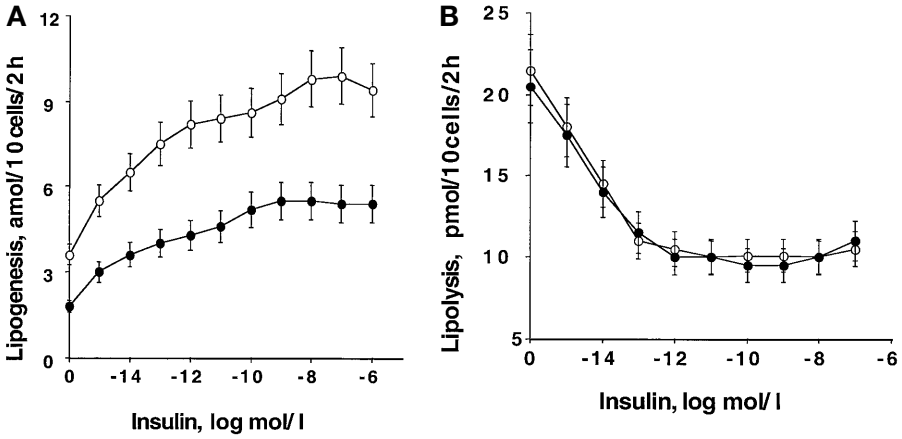

Fig. 1 (A, B). Effect of a *UCSNP-43* polymorphism in the *Calpain 10* gene (*A* to *G*) on lipolysis and glucose transport in human subcutaneous fat cells. Cells were incubated in the absence or presence of insulin at increasing concentrations. The right-hand graph shows data with lipolysis, expressed as glycerol release. The left-hand graph shows data with lipogenesis expressed as glucose incorporation into lipids. Mean concentration-response curves (± SEM) for *AA/AG* and *GG* subjects are depicted

Fig. 2. Effect of the *UCSNP-43 Calpain 10* polymorphism (*A* to *G*) on the protein content of GLUT-4 in adipose tissue of women. GLUT-4 was determined by Western blot analysis. Individual data are depicted. *A* = *AG*, *G* = *GG*

sia). In this group too, basal and insulin-stimulated lipogenesis were twofold higher in *GG* subjects. Finally, we considered the influence of the *Ins19Del* and *G63T* polymorphisms on our findings of the *G43A* polymorphism. Of nine theoretical haplotypes of *UCSNP-19* and *–63*, the only common was *Ins19Ins-G63G* occurring in 19 subjects. Data for the subjects carrying the specific *UCSNP-19/-63* homozygous haplotype are shown in Table 3. Even with this particular background of *Calpain 10* gene structure, the effect of the *A43G* polymorphism on lipogenesis remained. In other words, basal and maximum insulin-stimulated lipogenesis were about twofold higher in the *G43G* subjects carrying *Ins19Ins* in combination with *G63G*.

Because a few elderly post-menopausal women were included, we investigated the relation between age and adipose glucose transport in women. Neither basal nor insulin responsiveness of lipogenesis was influenced by age in an important way (adjusted $r^2 = 0.01$ for basal and 0.04 for responsiveness).

GLUT-4 protein expression. The results of lipogenesis indicate that subjects with the *G/G* allele have a more efficient glucose transport system. This could be due to an increase in the protein concentration of the major glucose transport protein, GLUT-4. We investigated the GLUT-4 protein content in *G/G* and *G/A* subjects (Fig. 2). We only had tissue left from a limited subset of patients due to the relatively large amounts of tissue needed for lipolysis and lipogenesis determination. Ten *G/G* and seven *G/A* women subjects (randomly selected) were therefore included and total cytosolic protein was isolated. Western blot analysis was done with a commercially available antibody directed against human GLUT-4. Protein from all 17 subjects was used in the same Western blot. Densitometric scanning showed almost identical mean values in *G/G* and *G/A* subjects (1717 ± 137 and 1698 ± 225 OD · mm^2 · 100 µg total protein, respectively).

Calpain10 mRNA. Calpain 10 mRNA was expressed in both adipose tissue and cells (Fig. 3). Sequencing of the PCR-product also revealed that it corresponded to the expected sequence of Calpain 10 mRNA. Result of the semi-quantitative measurement of calpain10 is shown in Figure 4. For both Calpain 10 and UCP2, the highest intensity of the PCR-products was found at temperatures 52 to 58 °C. From these four PCR-experiments, the mean ratio between the optical density of the Calpain10 and UCP2 bands was determined and found to be 1.7. The absolute amount of UCP2 mRNA, as calculated from RT-competitive-PCR, was 2.4 amol/µgRNA, in turn resulting in a level of Calpain10 mRNA of 4.4 amol/µg RNA.

Discussion

This study presents evidence for an association between the *Calpain 10* gene and adipocyte glucose metabolism. Lipogenesis was measured at a low glucose concentration when transport and not further metabolism is rate limiting for adipocyte utilization of glucose. It is therefore probable that the lipogenesis data above all represents insulin action on glucose

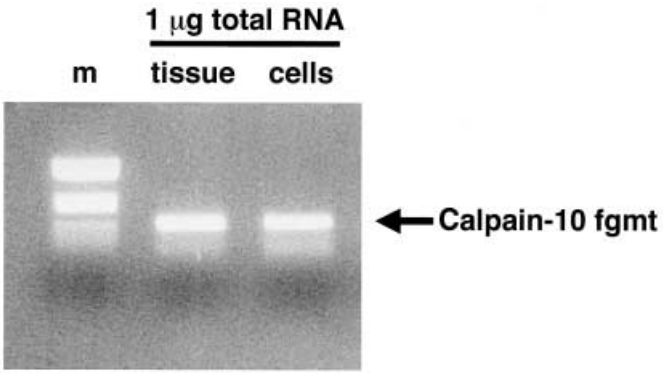

Fig. 3. Calpain 10 mRNA expression determined by PCR. Results with pieces of adipose tissue and isolated fat cells are depicted

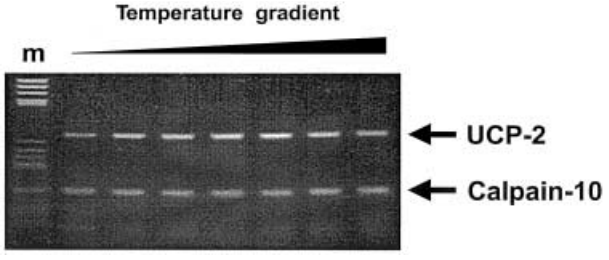

Fig. 4. Agarose gel used for semi-quantitative determination of Calpain10 mRNA level in adipose tissue. The ratio between the optical density for Calpain10 and UCP2 gel bands was determined

transport rather than the further metabolism of glucose. Three recently described polymorphisms in the gene were investigated. In accordance with previous observations [5, 7] two were common and one was relatively rare. Only the common *A43G* polymorphism had phenotypic effects, which were apparently independent of the genetic variance at *UCSNP-19* and *–63*. Interestingly, it is this polymorphism that is associated with Type II diabetes [5].

We observed that *A43G* was strongly associated with variations in basal and insulin-stimulated adipocyte lipogenesis. Basal and maximum insulin-stimulated lipogenesis was twofold enhanced in *G* homozygotes compared with subjects carrying the A-allele. There was no difference between **A** heterozygotes and **A** homozygotes. However, insulin sensitivity (ie. pD_2) of lipogenesis was not influenced by the polymorphism. Furthermore, no measure of adipocyte lipolysis was influenced by *Calpain 10* gene polymorphisms. Taken together, these data suggest that the genetic variance is selectively linked to insulin action on glucose metabolism. This indicates that the effect resides at some distal step(s) in insulin action, below PI3 K, where the signal transduction step to lipolysis and glucose metabolism diverge in fat cells [4]. It is probable that *Calpain 10* has direct effects in human fat cells because the gene was relatively highly expressed in these cells as evidenced by RT-PCR experiments. In adipose tissue, total Calpain 10 mRNA concentration was about 4 amol/μg RNA. This concentration of mRNA expression is about the same as that of *UCP-2*. However, we do not know how the *Calpain 10* gene might influence glucose metabolism but a number of regulatory steps in insulin signalling to GLUT-4 or to other glucose transporters in fat cells could be involved as well as direct effects on the cellular trafficking of glucose transport proteins [15]. It should be noted, though, that the *Calpain-10* gene probably does not regulate the total amount of GLUT-4 in fat cells because there was no effect of genetic variance on adipocyte expression of the GLUT-4 protein. On the other hand, it must be considered that the *G43A* polymorphism in the *Calpain 10* gene may be in linkage disequilibrium with variations in another gene nearby on chromosome 2.

The study group was somewhat heterogeneous but we do not believe a selection bias has influenced the results. The same type of data was generated in various subgroup analyses. In another study basal (ie. post absorptive) and insulin-stimulated peripheral glucose uptake were lower in G43G subjects [7]. The differences could be explained by the fact that we made a direct study of glucose metabolism in fat cells or that different study groups were included: we investigated lean Scandinavian subjects whereas the former study was done on obese Pima Indians. Obesity might interact with the effect of the polymorphism on glucose metabolism. Such genotype-phenotype interactions are not present in the non-obese state. Another possibility is that variations in glucose metabolism of fat cells have unique effects on whole body glucose homeostasis. Recently, if was observed that selective adipose tissue reduction of GLUT-4 impairs insulin action in muscle and liver [16]. The *Calpain 10* gene might have specific effects on glucose metabolism in the target tissues for insulin so that actions in fat and muscle influence glucose homeostasis in different ways. Furthermore, the *Calpain 10* gene is expressed in several mRNA isoforms in human tissues [7, 17]. It is possible that this results in the formation of different Calpain 10 peptides in liver, muscle and fat cells which, in turn, have tissue-specific actions. With our PCR method it was just possible to detect the total amount of Calpain 10 mRNA. Because we could not study adipocyte gene expression in the same subjects as we studied adipocyte metabolism, no attempts were made to measure isoforms of Calpain 10 mRNA. However, when previous (7) and present data are considered together it is tempting to speculate that the *Calpain 10* gene is involved in the regulation of insulin-stimulated glucose metabolism but this effect could vary from tissue to tissue. The fact that BMI, fasting plasma insulin and glucose were not influenced by the polymorphism indicates that the *Calpain 10* has separate effects in different

insulin target cells. However, it should be noted as regards clinical data that we analysed healthy non-obese subjects. Different findings could be at hand in obese or non-healthy subjects.

In summary, a common *UCSNP-43* polymorphism in the *Calpain 10* gene (*A* to *G*) which previously has been found to associate with Type II diabetes was found to be linked to basal and insulin-stimulated lipogenesis but not to lipolysis in human fat cells. The findings suggest that the *Calpain 10* gene might be in control of glucose metabolism, at least in fat cells and that the diabetic effect of this gene is related to interactions with glucose metabolism.

Acknowledgements. The excellent technical assistance of B-M. Leijonhufvud, K. Hertel, K. Wåhlin, E. Sjölin and E. Dungner is greatly appreciated. This study was supported by grants from Swedish Medical Research Council, Swedish Diabetes Association, Novo Nordic Foundation, Wallenberg Foundation, Juvenile Diabetes Foundation, Söderberg Foundation, Swedish Society of Medicine, Thuring Foundation and Swedish Heart and Lung Foundation.

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