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11β-Hydroxysteroid dehydrogenase Type 1: genetic polymorphisms are associated with Type 2 diabetes in Pima Indians independently of obesity and expression in adipocyte and muscle

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Abstract

Aims/hypothesis. The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) modulates tissue-specific glucocorticoid concentrations by generating active cortisol. We have shown that adipose tissue 11β-HSD1 mRNA levels were associated with adiposity and insulinaemia. Here we conducted further expression and genetic association studies in Pima Indians.
Methods. The 11β-HSD1 mRNA concentrations were measured in abdominal subcutaneous adipocytes (n=61) and skeletal muscle tissues (n=64). Single nucleotide polymorphisms in the *HSD11B1* gene were genotyped in a larger group of full-blooded Pima Indians.
Results. Two representative SNPs (SNP1, n=706; SNP5, n=839) were associated with Type 2 diabetes mellitus (p=0.01), although neither SNP was associated with obesity. Among subjects with normal glucose tolerance, SNP1 (n=127) and SNP5 (n=159) were associated with insulin-mediated glucose uptake rates (p=0.03 and p=0.04), and SNP1 was further associated with fasting, 30-min, and 2-h plasma insulin concentrations (p=0.002, p=0.002 and p=0.03). Adipocyte 11β-HSD1 mRNA concentrations were correlated positively with adiposity and insulinaemia, and were additionally negatively correlated with insulin-mediated glucose uptake rates; nevertheless, the adipocyte 11β-HSD1 expression did not correlate with genotypes of the donors. The muscle 11β-HSD1 mRNA concentrations did not correlate with any anthropometric or metabolic variables.
Conclusions/interpretation. We confirmed that adipocyte 11β-HSD1 mRNA concentrations were associated with adiposity, and showed that genetic variations in the *HSD11B1* gene were associated with Type 2 diabetes mellitus, plasma insulin concentrations and insulin action, independent of obesity. The variable adipose expression might not be a primary consequence of these *HSD11B1* SNPs. Therefore, it is possible that the *HSD11B1* gene is under tissue-specific regulation, and has tissue-specific consequences.

Keywords Adipocyte · Genetic polymorphisms · Insulinaemia · 11β-Hydroxysteroid dehydrogenase type 1 · Obesity · Type 2 diabetes mellitus

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Abbreviations: 11β-HSD1, 11β-Hydroxysteroid dehydrogenase type 1 · SNPs, single nucleotide polymorphisms

Introduction

The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inactive cortisone to active cortisol and is expressed in many tissues including liver, adipose tissue, muscle, pancreas, gonads and brain [1, 2, 3, 4]. Changes in local concentrations of glucocorticoids due to 11β-HSD1 activity have been implicated in obesity and its metabolic consequences including insulin resistance and Type 2 diabetes mellitus [5].

Adipose tissue seems to be an important primary site for 11β-HSD1 action in obesity. The adipose 11β-

HSD1 mRNA and/or activity is positively correlated with obesity in at least four different populations [6, 7, 8, 9, 10, 11], though not all [12]. Transgenic mice overexpressing 11β-HSD1 in adipose tissue develop hyperphagia, visceral obesity, hyperglycaemia, hyperinsulinaemia, glucose intolerance, insulin resistance and hyperlipidaemia [13]. Consistent with these studies, we recently showed that increased 11β-HSD1 activity and mRNA levels in whole subcutaneous adipose tissue were associated with adiposity and hyperinsulinaemia in 19 Pima Indian subjects and 12 Caucasians [9].

Although many studies have focused on the role of 11β-HSD1 in the expansion of adipose tissue, modulation of 11β-HSD1 activity has an effect on multiple target tissues which may promote insulin resistance independently of obesity. For example, in non-obese glucose-intolerant patients, adipose 11β-HSD1 activity is not increased and hepatic 11β-HSD1 activity is maintained [14] compared with the down-regulation of hepatic 11β-HSD1 which occurs in obesity [15]. Inhibition of 11β-HSD1 with oral carbenoxolone improves hepatic insulin sensitivity [16] and has a greater effect in non-obese glucose-intolerant patients than healthy control subjects [17]. The expression of 11β-HSD1 in myoblast cultures stimulated with glucocorticoids was negatively correlated with insulin sensitivity [18]. The 11β-HSD1 knockout mice develop resistance to diet-induced hyperglycaemia through attenuation of hepatic gluconeogenic enzymes [19] and increased hepatic insulin sensitivity [20]. Notably, inhibition of 11β-HSD1 activity in the pancreas causes beta cells to secrete more insulin [21].

Here we expanded the expression study in isolated abdominal subcutaneous adipocytes of a larger group of Pima Indians, since an in situ hybridisation study showed that obese individuals had increased 11β-HSD1 mRNA expression in the adipocyte compartment of subcutaneous adipose tissue [8]. To better understand the role of 11β-HSD1 in the development of obesity and insulin resistance, we also measured the 11β-HSD1 mRNA concentrations in skeletal muscle tissues of a separate group of Pima Indians who had undergone detailed metabolic characterisation. Furthermore, we analysed the *HSD11B1* gene to identify single nucleotide polymorphisms that might associate with the metabolic phenotypes.

Materials and methods

Subjects. All subjects provided written informed consent prior to participation. All studies were approved by the Tribal Council of the Gila River Indian Community and the Institutional Review Board of the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK). Subcutaneous abdominal fat biopsies were obtained from 61 non-diabetic Pima or Tohono O'Odham Indians. Eight of these subjects overlapped with our previous study [9]. Muscle biopsies were obtained from 64 non-diabetic Pima or Tohono O'Odham subjects, 17 of whom also underwent the adipose tissue biopsy. All subjects with muscle biopsies and a subset of subjects with fat biopsies (n=30) underwent euglycaemic–hyperinsulinaemic clamp as described below. The anthropometric and metabolic variables of the subjects are described in Table 1. All subjects undergoing biopsies were in good health as determined by their medical history, physical examination and routine blood and urine tests, and none were taking medication at the time of the study. Genotyping and association studies were done in ~800 full-blooded Pima Indians who participated in our ongoing longitudinal study of the aetiology of Type 2 diabetes mellitus among the Gila River Indian Community in Arizona [22], which included a subset of ~150 subjects who underwent euglycaemic–hyperinsulinaemic clamp. Genotype data were available in all 61 subjects of the adipocyte expression study, and in 40 out of the 64 subjects of the muscle expression study. Diabetic status was determined according to the criteria of the World Health Organisation [23].

Table 1. Clinical characteristics of subjects for the adipose and muscle tissue biopsies

Characteristic	All subjects for adipose biopsy (n=61) Mean ± SD	Subset of subjects for adipose study with clamp measurements (n=30) Mean ± SD	Subjects for muscle biopsy (n=64) Mean ± SD
Sex (male/female)	37/24	18/12	37/27
Age (years)	30±7	31±7	30±7
Weight (kg)	104±37	92±25	99±28
Height (cm)	168±9	166±9	167±8
BMI (kg/m^2)	37±12	33±8	35±9
Body fat (%)	33±8	32±7	33±7
Waist (cm)	113±23	105±18	108±20
Fasting plasma glucose (mmol/l)	5.0±0.5	4.9±0.5	4.9±0.5
Plasma glucose at 2 h (mmol/l)	6.6±1.8	6.8±1.8	6.8±1.8
Fasting plasma insulin (μU/ml)	44±17	42±15	42±17
Plasma insulin at 2 h (μU/ml)	171±93[a]	183±98	148±2[b]
Glucose disposal rate at physiological insulin clamp (mg·min^{-1}·kg^{-1} EMBS)		2.6±0.7	2.5±1.4
Glucose disposal rate at maximally stimulating insulin clamp (mg·min^{-1}·kg^{-1} EMBS)		8.7±2.7	8.9±2.6

EMBS, estimated metabolic body size; [a] n=59; [b] n=63

Metabolic measurements. Volunteers were admitted to the Clinical Research Unit where they consumed a weight-maintaining diet (containing 50% of calories as carbohydrates, 30% as fat and 20% as protein) for 2 to 3 days before clinical testing. Body composition was measured by dual energy X-ray absorptiometry (DXA) using a total body scanner (DPX-L, Lunar Radiation, Madison, Wis., USA) as described previously [24]. Waist circumference was measured at the umbilicus in the supine position. All subjects with muscle biopsies and a subset of subjects with fat biopsies (n=30) underwent euglycaemic–hyperinsulinaemic clamp as described previously [25]. Briefly, after an overnight fast, a primed continuous intravenous insulin infusion was administered for 100 min at a constant rate of 40 $mU \cdot m^{-2}$ body surface $area \cdot min^{-1}$ (low-dose insulin; M-low), followed by a second insulin infusion for 100 min at 400 $mU \cdot m^{-2}$ min^{-1} (high-dose insulin; M-high). Steady-state plasma insulin concentrations of 155±26 μU/ml and 2481± 320 μU/ml respectively were achieved. Plasma glucose concentrations were maintained at ~5.5 mmol/l with a variable infusion of a 20% glucose solution. Rates of insulin-stimulated glucose disposal at low-dose and high-dose insulin concentrations were calculated for the last 40 min of each insulin infusion. The rate of endogenous glucose production was measured before insulin infusion (BSGO) and during the last 40 min of the low-dose insulin infusion (ENDM), using tritiated glucose and calculated from the Steele equation [26]. All measurements derived from the glucose clamp were normalised to estimated metabolic body size (EMBS, which equals fat-free mass +17.7 kg).

Fat biopsy and isolation of adipocyte RNA. After an overnight fast, subjects underwent subcutaneous abdominal fat needle biopsies under local anaesthesia with 1% lidocaine. The adipose biopsy was placed on a sterile nylon mesh and rinsed with sterile 0.9% NaCl solution. The tissue was then cleaned of visible connective tissue and blood vessels in Hank's Buffered Saline Solution (HBBS) supplemented with 5.5 mmol/l glucose. For adipocyte isolation, adipose tissue was digested in HBBS buffer containing 5.5 mmol/l glucose, 5% fatty acid free BSA (Introgen/Serologicals, Norcross, Ga., USA) and 3.3 mg/ml type I collagenase (Worthington Biochemical, Lakewood, N.J., USA) for 30 min in a water bath at 37 °C. The digestion mixture was passed through a sterile 230 mm stainless steel tissue sieve (Thermo EC, Holbrook, N.Y., USA) and the adipocytes were allowed to float by buoyancy. The supernatant containing adipocytes was collected. Adipocyte RNA was extracted using RNeasy Mini Kit from Qiagen (Valencia, Calif., USA). During the extraction, RNA was treated with DNAse using RNAse-free DNAse Set (Qiagen) per manufacturer's instruction.

Muscle biopsy and RNA extraction. Percutaneous needle biopsies were done on the vastus lateralis muscle under local anaesthesia with 1% lidocaine after a 12-h overnight fast and the biopsy specimens were immediately frozen in liquid nitrogen as described previously [27]. Total RNA was isolated from the frozen tissues homogenised in TRIzol Reagent (Life Technologies, Gaithersburg, Mass., USA) and further purified using RNeasy Mini Kit and RNAse-free DNAse Set (Qiagen) as described above.

cDNA synthesis. The quality of the RNA was assessed by agarose gel electrophoresis. One microgram of RNA from each sample was used to prepare oligo dT-primed cDNA using the Advantage RT for PCR kit (Clontech, Palo Alto, Calif., USA) following the manufacturer's recommendation and the cDNA was stored at –70 °C. Successful cDNA synthesis was verified by PCR amplification of β2-microglobulin transcript using forward primer 5′-TGTCTTTCAGCAAGGACTGGTC-3′ and reverse primer 5′-TGATGCTGCTTACATGTCTCGAT-3′.

Quantitative real-time PCR. The 11β-HSD1 transcript was quantified with Real Time PCR primer-probe sets using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, Calif., USA). The sequences for the 11β-HSD1 primers and Taqman probe were designed using Primer Express 1.5 software (Applied Biosystems). Primers were synthesised by Integrated DNA Technologies, (Coralville, Ia., USA). The probe sequence was 5′-6FAM CTTGGCCTCATAGACACAGAAACAGCCA BHQ1-3′ (Biosearch Technologies, Novato, Calif., USA). The sequence of the 11β-HSD1 forward primer was 5′-GGAATATTCAGTGTCCAGGGTCAA-3′ and the reverse primer was 5′-TGATCTCCAGGGCACATTCCT-3′. The human TATA box binding protein (TBP) gene transcript with optimised primers and probe (Pre-developed Taqman Assay Reagents Endogenous Control Human TBP kit, Applied Biosystems) was used as the normalisation gene. Real-time PCR was done by using Universal Taqman mix in the ABI 7700 sequence detector (Applied Biosystems). The final concentrations of primers and probe were 0.9 mmol/l and 0.25 mmol/l respectively. A standard curve for each primer-probe set was generated by serial dilution of a randomly chosen cDNA sample done in triplicate. Each sample was run in duplicate and the mean values of the duplicates were used to calculate transcript level. Real-time PCR was carried out as recommended by the manufacturer as follows: 50 °C, 2 min; 95 °C, 10 min; 95 °C, 15 s and 60 °C, 1 min for 40 cycles.

Identification and genotyping of polymorphisms. Genomic DNA samples from 20 non-diabetic unrelated Pima Indian subjects were sequenced across a ~4.0 kb region upstream of the transcription initiation site, the six exons, exon-intron junctions and the 5′ and 3′ UTR of *HSD11B1* gene. Samples were amplified in a GeneAmp PCR system 9700 as follows: 94 °C, 4 min; 94 °C, 15 s; 60 °C, 45 s and 72 °C, 1 min for 35 cycles; 72 °C, 5 min. Big Dye Terminator (Applied Biosystems) was used to do cycle sequencing and the samples were analysed on an automated capillary sequencer (model 3700, Applied Biosystems). Genotyping was done by the allelic discrimination TaqMan Assay by design (Applied Biosystems) for SNP5 and by direct sequencing using Big Dye Terminator, as described above, for SNP1 in full-blooded Pima Indian subjects. The allelic discrimination assay PCR was done as follows: 50 °C, 2 min; 95 °C, 10 min; 92 °C, 15 s and 60 °C, 1 min for 40 cycles in the 7700 Sequence detector (Applied Biosystems).

Statistical analysis. Statistical analyses were done by using the procedures of the Statistical Analysis System software (SAS Institute, Cary, N.C., USA). 11β-HSD1 mRNA levels were normalised to TBP by taking the residual values of 11β-HSD1 after linear regression to TBP transcript level. For the metabolic association analyses, plasma insulin concentrations and rates of glucose disappearance during the low-dose insulin infusion were logarithmically transformed to approximate normal distributions. General linear regression models were used to assess the relationships between mRNA concentrations or genotypes and measures of obesity and insulin action. For continuous variables, the generalised estimating equation (GEE) procedure was used to adjust for the appropriate covariates. The association analyses between adipocyte mRNA levels and metabolic variables were not adjusted for nuclear family membership since there were only five first-degree relatives in this dataset. However, the genotyped dataset had first, second or more degree relatives and all the association analyses between

genotypes and metabolic measures were adjusted for nuclear family membership. The Student's *t* test was done to evaluate genotype associations with expression levels.

The association analyses of genotypes with diabetes or obesity were done in full-blooded Pima Indians. The odds ratio was used to determine the strength of the association between the prevalence of the at-risk genotype and affection status. Associations were calculated under three different models: dominant, recessive, and additive. Linkage disequilibrium (LD) between SNPs was calculated with the Estimating Haplotypes program. The degree of LD was quantified by D′, which represents the extent of LD between alleles, expressed as a proportion of the maximum possible. The amount of shared information was expressed in r^2.

A p value of less than 0.05 was considered significant.

Results

The adipocyte 11β-HSD1 mRNA concentrations correlated positively with measures of obesity (% body fat and waist circumference) as well as fasting and 2-h plasma insulin concentrations during the OGTT (Table 2). In a subset of subjects who agreed to undergo a euglycaemic–hyperinsulinaemic clamp (n=30; Table 1), the adipocyte 11β-HSD1 mRNA concentrations also correlated negatively with glucose disposal rates at physiological and maximally stimulating insulin concentrations (Table 2). These correlations remained significant after adjusting for age and sex (Table 2).

Sequencing of the *HSD11B1* gene (~4.0 kb region upstream of the transcription initiation site, the six exons, exon-intron junctions and the 5′ and 3′ UTR) in DNA from 20 Pima Indians identified six variants (Table 3). Genotyping of these SNPs was carried out in 100 subjects to assess the extent of linkage disequilibrium. SNPs 1 and 2 were in 100% linkage disequilibrium with each other, and SNP4, SNP5 and SNP6 were in 100% linkage disequilibrium among themselves. SNP3 was very rare with a minor allele frequency of 1.7%, which we did not genotype further. Therefore SNP1 and SNP5 were selected as representatives of the two common genotypic groups, and were further genotyped for association studies in ~800 full-blooded Pima Indians. Analysis of this larger number of genotypes showed that, despite differences in frequencies, the common alleles at SNP1 (*G*, 0.86) and SNP5 (*G*, 0.54) were in very high linkage disequilibrium (D′=0.98). The amount of shared information was relatively low (r^2=0.21).

In the genotyped full-blooded Pima Indians, both SNP1 (n=706) and SNP5 (n=839) were associated with Type 2 diabetes mellitus under both additive (p=0.01 and 0.02 respectively) and recessive (p=0.01 for both SNPs) analytical models (Table 4). Among the subjects with normal glucose tolerance who had additionally undergone a euglycaemic–hyperinsulinaemic clamp, SNP1 (n=127) and SNP5 (n=159) were also associated with glucose uptake rates at maximally stimulating insulin concentrations (p=0.03 and p=0.04 respectively, after adjusting for age, sex, % fat and nuclear family membership; Table 5). SNP1 was additionally associated with fasting, 30 min and 2-h plasma insulin concentrations in response to a 75-g OGTT, (p=0.002, p=0.002 and p=0.03 respectively, after adjusting for age, sex, % fat and nuclear family membership). This SNP remained significantly associated with fasting insulin (p=0.02) and early insulin secretion (plasma insulin at 30-min; p=0.003) after adjusting for age, sex, % fat, 30-min glucose and glucose disposal rate at high-dose insulin concentrations and nuclear family membership. No significant associations were found between SNP1 or SNP5 and age, % fat, fasting, 30-min and 2-h glucose concentrations, glucose disposal rate at physiological insulin concentrations, and measures of hepatic insulin sensitivity (data not shown).

Although SNP5 was not significantly associated with plasma insulin concentrations in response to an OGTT, it showed the same trend as observed with SNP1 (Fig. 1). For example, subjects homozygous for the rare allele (*TT*) at SNP5 had increased mean plasma insulin concentrations at 30 min, despite having similar glucose concentrations. These subjects, homozygous for the *T* allele at SNP5, also had a significant decrease in glucose disposal, compared with subjects homozygous for the common allele (*GG*).

Both SNP1 (p=0.3, n=613) and SNP5 (p=0.8, n=728) were not associated with BMI. In addition, these SNPs were not associated with 11β-HSD1 mRNA concentrations in the abdominal subcutaneous adipocyte biopsies of 61 Pima Indians; for SNP1 (*GG* vs *GA*; No *AA*s available) p=0.32, and for SNP5 (*GG*+*GT* vs *TT*) p=0.89.

Table 2. Pearson correlations of the normalised *HSD11B1* expression in adipocytes with in vivo metabolic variables

Metabolic variables	r	p value	r^a	p value[a]	n
% Body fat	0.39	0.002	0.60	<0.0001	61
Waist circumference	0.77	<0.0001	0.75	<0.0001	61
Fasting plasma insulin	0.68	<0.0001	0.66	<0.0001	61
2-h plasma insulin	0.53	<0.0001	0.55	<0.0001	59
Glucose disposal rate at physiological insulin clamp	–0.60	0.0004	–0.62	0.0003	30
Glucose disposal rate maximally stimulating insulin clamp	–0.42	0.02	–0.36	0.047	30

[a] Adjusted for age and sex

Table 3. Characteristics of the six single nucleotide polymorphisms in the *HSD11B1* gene in Pima Indians

	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6
Alleles	*G/A*	*C/T*	*G/T*	*AAA/AA*	*G/T*	*A/G*
Allele frequency	*A*=0.14	*T*=0.14	*T*=0.02	*AA*=0.46	*T*=0.46	*G*=0.46
Position[a]	5′ upstream (–2940)	5′ upstream (–2679)	5′ upstream (–2431)	Intron 3 (+53)	Intron 3 (+94)	3′ UTR (+234)
Nucleotide position (Accession number) rs number	3334051 (NT_021877) rs 846910	3334312 (NT_021877) rs 3334312	3334561 (NT_021877) N/A	4436 (AH010971) N/A	4478 (AH010971) rs 12086634	3366897 (NT_021877) rs 6752

[a] The positions of the SNPs in the 5′ upstream sequence (relative to the transcription initiation start site), in intron 3 and the 3′UTR of *HSD11B1* gene, variant 1 are shown in parentheses. N/A, not applicable

Fig. 1. Metabolic responses in subjects based on their genotype for SNP1. Glucose (**a**) and insulin (**b**) curves are in response to a 75 g OGTT. The glucose disposal rates (**c**) were measured during maximally stimulating insulin infusion (2481±320 µU/ml). Given the low frequency of homozygotes for the rare allele for SNP1 (*AA* genotype), for statistical purposes these subjects were combined with the heterozygous subjects (*GA*) and this group was compared to the homozygotes for the common allele (*GG*). *GG*, circles; *AA*+*GA*, squares

Since SNP1 and SNP5 were associated with glucose disposal rate, which is primarily due to glucose uptake in the muscle, we investigated whether this association was attributable to differential 11β-HSD1 expression levels in skeletal muscle. There was no association between skeletal muscle 11β-HSD1 mRNA concentration and fasting plasma insulin (r=–0.05; p=0.68) or plasma insulin at 2 h (r=0.04; p=0.77) dur-

Table 4. Genotypic distribution for SNP1 and SNP5 based on diabetic status

	Status	Genotype			*A* allele Dominant Model (*AA+GA* vs *GG*)		Additive Model (per number of *A* alleles)	
		GG	*GA*	*AA*	OR (95% CI)	*p* value	OR (95% CI)	*p* value
SNP1	Non-diabetic	226 (81.6)	48 (17.3)	3 (1.1)	1.72 (1.14–2.56)	**0.01**	1.64 (1.11–2.38)	**0.01**
	Diabetic	310 (72.3)	109 (25.4)	10 (2.3)				
	Status	Genotype			*T* allele Recessive Model (*TT* vs *GG+GT*)		Additive Model (per number of *T* alleles)	
		GG	*GT*	*TT*	OR (95% CI)	*p* value	OR (95% CI)	*p* value
SNP5	Non-diabetic	121 (36.8)	166 (50.5)	42 (12.8)	1.79 (1.14–2.8)	**0.01**	1.34 (1.05–1.72)	**0.02**
	Diabetic	158 (31)	238 (46.7)	114 (22.4)				

Number of subjects with each genotype and their percent of total in parentheses are given. Odds ratios (with 95% CI in parentheses) and significant *p* values from analyses under dominant, recessive, and additive models are presented. Data were adjusted for age, sex, birth date, and nuclear family membership

Table 5. Association of SNP1 and SNP5 with insulin sensitivity in Pima Indians with normal glucose tolerance

	SNP1[a]				SNP5[b]		
	GG (100)	*GA+AA* (27)	*p* value[c]	*p*value[d]	*GT+GG* (135)	*TT* (24)	*p* value[c] Recessive
Fasting plasma insulin (μU/ml)	35±2	46±5	**0.002**	**0.02**	37±2	47±5	0.22
Plasma insulin at 30 min (μU/ml)	244±16	324±31	**0.002**	**0.003**	252±14	320±44	0.26
Plasma insulin at 2 h (μU/ml)	139±10	187±24	**0.03**	0.06	148±10	182±26	0.91
Glucose disposal rate at physiological insulin during clamp ($mg \cdot kg^{-1}$ EMBS$\cdot min^{-1}$)	2.8±0.1	2.6±0.2	0.23		2.8±0.1	2.5±0.2	0.42
Glucose disposal rate at maximally stimulating insulin during clamp ($mg \cdot kg^{-1}$ EMBS$\cdot min^{-1}$)	9.3±0.2	8.4±0.4	**0.03**		9.4±0.2	8.3±0.4	**0.04**

[a] Due to the low frequency of SNP1, for statistical analyses, the homozygotes for the rare allele (*AA*) were combined with the heterozygotes (*GA*), and this combined genotypic group was then compared to the more common homozygous (*GG*). [b] For SNP5, data is analysed under a recessive model where the common (*GG*) subjects are combined with the heterozygotes (*GT*). [c] Adjusted for age, sex, percent body fat and nuclear family membership; [d] adjusted for age, sex, percent body fat, glucose, glucose disposal rate at high-dose insulin and nuclear family membership (when *p* values[c] were significant). The *p* values <0.05 are shown in bold

ing OGTT and glucose disposal rates at physiological insulin concentration (r=–0.06; *p*=0.65) or high insulin dose (r=0.04; *p*=0.78) during euglycaemic–hyperinsulinaemic clamp in a separate group of 64 Pima Indians (as described in Materials and methods). In addition, SNP1 (*GG* vs *GA*; No *AA*s, *p*=0.8) and SNP5 (*GG + GT* vs *TT* *p*=0.08) did not show any associations with the muscle 11β-HSD1 expression levels.

Discussion

In this study, we identified single nucleotide polymorphisms (SNPs) in the 5′ upstream and intronic regions of the *HSD11B1* gene in Pima Indians that were associated with Type 2 diabetes mellitus and insulin resistance, but not with obesity. The lack of association with BMI was also observed in a study that genotyped two microsatellite markers within the *HSD11B1* locus in two other populations [28]. SNP4 and 5 polymorphisms were identified in 8 individuals, but no correlation with obesity was found [29]. We also showed that increased 11β-HSD1 mRNA concentrations in isolated abdominal subcutaneous adipocytes correlated with increased obesity and plasma insulin concentrations as well as decreased insulin action as determined by glucose disposal rates. In contrast, there was no direct correlation between skeletal muscle 11β-HSD1 mRNA concentrations and insulin action. The results indicate that 11β-HSD1 is likely to have a complex role in determining adiposity and insulin action, which may be due to tissue-specific regulation of this enzyme.

SNP1, which is in 100% linkage disequilibrium with SNP2, was associated with Type 2 diabetes mellitus, fasting, 30-min and 2-h plasma insulin concentrations in response to an OGTT, and glucose disposal rate in response to a high-dose insulin clamp. Since both SNP1 and SNP2 are located in the 5′ upstream region of the *HSD11B1* gene, if either SNP is a functional variant, it would alter the mRNA concentration of the gene. A report has shown that two other 5′ upstream region SNPs of the *HSD11B1* gene (not present in the individuals we sequenced) are associated with reduced transcription [30]. Functional studies to investigate the potential effects of SNPs 1 and 2 on promoter activity are warranted. SNP5, which is in 100% linkage disequilibrium with SNP4 and SNP6, was similarly associated with Type 2 diabetes mellitus and glucose disposal in response to a high-dose insulin clamp. SNP4 and SNP5 are both intronic, and SNP6 is in the 3′UTR. Both SNPs 4 and 5 act as intronic enhancers that modulate 11β-HSD1 expression and activity [31]. Nevertheless, given the lack of association between SNP1 or SNP5 and obesity, there was no association between these SNPs and adipocyte 11β-HSD1 mRNA concentrations in the 61 subjects that we studied.

Although not tested in this study, the genetic variants in *HSD11B1* gene may affect 11β-HSD1 expression in tissues other than adipose or muscle. Since there is a notable association between SNP1 in the *HSD11B1* 5′ upstream region with early (30 min) insulin secretion, it is possible that either this SNP1 or SNP2, which is in 100% LD with SNP1 and also in the 5′ upstream region of the gene, modulates 11β-HSD1 expression in pancreatic beta cells. It has recently been reported that the 11β-HSD1 expression and activity were increased in islets of diabetic Zucker Diabetic Fatty rats [32]. This enzyme is also expressed in human pancreatic islets [21], and carbenoxolone, an inhibitor of 11β-HSD1 activity, can reverse inhibition of insulin secretion in pancreatic beta cells from ob/ob mice by glucocorticoids [21]. Another possibility of genetic variants in the *HSD11B1* gene affecting the enzyme expression in the liver seems unlikely, because we did not observe any associations between the SNPs in *HSD11B1* gene and measures of hepatic insulin sensitivity.

Screening the 5′ upstream sequences with MatInspector showed a heat shock factor transcription binding site in the SNP1 region. SNP2 is located in a stretch of nucleotides (TATTA(T/C)TTT TTA) that shares homology with the consensus MEF2 (myocyte enhancer factor 2) binding sequence $CTA(A/T)_4$ TA(G/A) [33] and also the consensus insulin promoter factor 1 (IPF1) sequence C/TTAATG on the complementary strand [34]. MEF2 isoforms are expressed in muscle and non-muscle cells in a cell-type-specific manner [35, 36]. The levels of MEF2 factors and DNA binding activity are decreased in skeletal muscle [36] and adipose tissue of STZ-induced insulin-deficient diabetic mice [37]. The IPF1 (PDX1/Pancreatic duodenal homeobox 1) is primarily expressed in beta cells and is required for pancreatic development and maintenance of beta cell function [38]. It is a key transcription regulator of many genes including insulin [39] and amylin [34]. Direct functional studies of these putative consensus binding sites in the 5′ upstream region of the *HSD11B1* gene are required to elucidate their role, if any, in regulating tissue-specific *HSD11B1* transcription.

Our results suggest that 11β-HSD1 is likely to have a complex role in determining adiposity and insulin action, which may be due to tissue-specific regulation of this enzyme. We propose that 11β-HSD1 activity in humans contributes to alterations in early insulin secretion, insulin resistance, and ultimately to increased risk of Type 2 diabetes mellitus through both an adiposity-associated mechanism (i.e. increased adipose mass results in increased adipose-specific 11β-HSD1 expression and pleotropic secondary effects cause insulin resistance) and an adiposity-independent mechanism. However, future studies in the Pima Indians, as well as replication of our findings in other metabolically characterised populations are necessary to substantiate these hypotheses.

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