No effect of the Trp64Arg β_3 -adrenoceptor variant on in vivo lipolysis in subcutaneous adipose tissue

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Summary A Trp64Arg variant in the human β_3 -adrenoceptor is associated with earlier onset of non-insulin-dependent diabetes mellitus and obesity in several populations. The present study investigated in vivo lipolysis in individuals homozygous for the 'variant' allele coding for arginine (**Arg**) in position 64 of the β_3 -adrenoceptor or homozygous for the 'wild type' tryptophan (**Trp**) allele. Subjects were 25 healthy, non-diabetic Pima Indians, 8 **Arg** (2 males, 6 females; aged 34 ± 9 years, BMI 36.2 ± 7.7 kg/m², 43 ± 11 % body fat [mean $\pm$ SD]), and 17 **Trp** (9 males, 8 females; aged 30 ± 5 years, BMI 30.4 ± 6.1 kg/m², 39 ± 9 % body fat). After an overnight fast, a microdialysis probe was inserted in the subcutaneous adipose tissue and perfused with Ringer's solution. Dialysate was collected in 10-min fractions during a 30-min baseline and during 40 min with isoproterenol, a non-selective β -adrenergic agonist, added to the perfusate (1 μ mol/l). Changes in rate of lipolysis

were assessed as changes in dialysate glycerol concentration. The relative changes in dialysate glycerol concentrations in response to isoproterenol, expressed as percent over baseline, were similar in the two groups (i.e. 63 ± 30 and 74 ± 28 % in the **Arg** and **Trp** subjects, respectively). The results were also similar in the two groups after adjustment for sex and percentage of body fat. No differential effect of isoproterenol on blood flow was demonstrated between the two groups (assessed by the ethanol dilution technique). These results are consistent with in vitro studies showing no functional effect of the β_3 -adrenoceptor variant, and/or indicate that the β_3 -adrenoceptor is not very important for subcutaneous adipose tissue lipolysis. [Diabetologia (1997) 40: 838–842]

Keywords Microdialysis, obesity, NIDDM, beta-adrenergic receptor subtypes, Pima Indians.

The recently described variant of the human β_3 -adrenoceptor (Trp64Arg) is associated with earlier onset of non-insulin-dependent diabetes mellitus (NIDDM) [1, 2], abdominal obesity [2, 3], insulin resistance [2, 4], propensity for weight gain [5, 6], difficulty in losing weight [3], low metabolic rate [3, 7], and high BMI [4, 8, 9]. The mechanism(s) underlying

these associations are not known, but differences in lipolytic activity have been suggested. Stimulation of the abdominal subcutaneous adipocytes in vivo with a β_3 -adrenoceptor specific agonist increases lipolysis [10].

The present study was designed to investigate whether in vivo lipolysis in subcutaneous adipose tissue (assessed using microdialysis) was influenced by the variant of the β_3 -adrenoceptor. This study was conducted in Pima Indians, a population known for its propensity to obesity and having the highest reported allele frequency of the β_3 -adrenoceptor variant (31 % [1]). In contrast with other studies, we exclusively studied subjects homozygous for either the arginine allele or for the 'wild type' tryptophan allele. There was no difference in in vivo isoproterenol-stimulated

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Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; Arg, arginine; Trp, tryptophan.

Table 1. Characteristics of subjects studied

	Arg64 homozygotes 'Variant'	Trp64 homozygotes 'Wild type'	Group effect
Male/female	2/6	9/8	
Age (years)	34 $\pm$ 9	30 $\pm$ 5	NS
Weight (kg)	98 $\pm$ 21	83 $\pm$ 18	NS
Height (cm)	165 $\pm$ 5	166 $\pm$ 7	NS
BMI (kg/m ²)	36.2 $\pm$ 7.7	30.4 $\pm$ 6.1	NS
Body fat (%)	43 $\pm$ 11	39 $\pm$ 9	NS
Waist/thigh ratio	1.66 $\pm$ 0.24	1.61 $\pm$ 0.12	NS
Fasting plasma insulin (pmol/l)	165 (12–318)	81 (12–216)	NS
Fasting plasma glucose (mmol/l)	4.5 $\pm$ 0.6	4.2 $\pm$ 1.1	NS
Fasting plasma glycerol (mmol/l)	1.04 (0.55–16.4)	0.88 (0.55–7.52)	NS

Data are mean $\pm$ SD or (range)

lipolysis between the two groups, consistent with the in vitro studies showing no functional effect of the variant [11, 12]

Subjects and methods

Subjects. We studied 25 Pima Indians (Table 1). The subjects were recruited based on their previously established β_3 -adrenoceptor genotype [1], being either homozygous for the variant arginine allele (**Arg**) or homozygous for the 'wild type' tryptophan allele (**Trp**). All subjects were healthy as determined by medical history, physical examination, and routine blood and urine tests, and none took medications or smoked. All subjects were non-diabetic as determined by an oral glucose test (75 g) according to World Health Organization (WHO) criteria [13]. The study was approved by the National Institute of Diabetes and Digestive and Kidney Diseases Institutional Review Board and the Tribal Council of the Gila River Indian Community, and the subjects gave written, informed consent. The subjects resided in our metabolic research unit and were given a weight maintenance diet (20% protein, 30% fat, 50% carbohydrate) for at least 3 days prior to microdialysis. Body composition was determined by total body dual-energy X-ray absorptiometry (DPX-1; Lunar Radiation Corporation, Madison, Wis., USA) [14].

Microdialysis. The microdialysis procedure has been described in detail elsewhere [15]. The probe used had a polyurethane, double-lumen 30 mm $\times$ 1.2 mm shaft (CMA/20; CMA/Microdialysis, Stockholm, Sweden), the distal 10 mm of which was covered with a semipermeable membrane (cut-off 20 kDa). The in vitro recovery for glycerol at 5 μ l/min for this probe is 26 $\pm$ 1% (M. Boschmann, unpublished observations). At 07.00 hours, after an overnight fast, with the subject in the supine position, the probe was inserted into the subcutaneous adipose tissue of the abdomen, close to the umbilicus. No local anaesthesia was used. The probe was perfused with Ringer's solution (Abbott Industries, North Chicago, Ill., USA) by a precision pump (CMA/102; CMA/Microdialysis) at a rate of 5 μ l/min. A semipermeable membrane at the tip of the probe allows for substances in the extracellular fluid to enter the probe and be retrieved in the dialysate, and for a drug to be added to the perfusate in quantities having only local effects. The dialysate was collected in 10-min fractions. The first 30 min of dialysate was discarded. Then, baseline samples were collected for 30 min before adding isoproterenol HCl 2 mg/dl (Abbott) to the Ringer's solution to achieve a final concentration of 1 μ mol/l. Samples were collected for another 40 min during

isoproterenol stimulation. Throughout the experiment, ethanol was added to the perfusate at a concentration of 50 mmol/l for measurement of local blood flow. The samples were fractionated into two portions to be analysed for glycerol (to measure lipolysis) and ethanol. The fractions were stored until analysis at -70 and $+5^\circ\text{C}$, respectively.

Analyses. Dialysate glycerol was determined by a luminometric assay [16, 17]. Baseline dialysate glycerol concentration was calculated as the mean value of the three 10-min fractions. Isoproterenol-stimulated dialysate glycerol concentration was calculated as the mean value of the three 10-min fractions collected from 10–40 min. Effect of isoproterenol on dialysate glycerol concentration was expressed as percent increase in dialysate glycerol concentration over baseline. Dialysate ethanol was determined using a standard enzymatic assay [18]. Local blood flow was expressed as the ratio of ethanol concentration in the outgoing dialysate to ethanol concentration in the incoming dialysate, the ethanol outflow/inflow ratio [19]. To estimate the reproducibility of the microdialysis method, baseline glycerol concentration was measured in 45 individuals using two probes inserted simultaneously approximately 10 cm apart in the same fat depot. For the two determinations in the same volunteer the coefficient of variation was 27% (S. Snitker, unpublished observations).

Statistical analysis. Statistical analyses were performed using the SAS 6.08 statistical programs (SAS Institute, Cary, N.C., USA). Group comparisons were made by two-tailed *t*-test and multiple linear regression when adjusting for covariates. Results are given as mean $\pm$ SD or as mean (range) when indicated.

Results

During baseline, the dialysate glycerol concentration was similar in the two groups (38 $\pm$ 15 [**Arg**], 32 $\pm$ 12 μ mol/l [**Trp**]; $p = 0.37$). During isoproterenol stimulation, dialysate glycerol concentration was significantly different from baseline in both groups (60 $\pm$ 22 [**Arg**], 54 $\pm$ 18 μ mol/l [**Trp**]). The relative change in dialysate glycerol concentration in response to local isoproterenol infusion, expressed as percent increase over baseline is shown in Figure 1. The mean of the individual relative increase was similar in the two groups (63 $\pm$ 30 [**Arg**], 74 $\pm$ 28% [**Trp**]; $p = 0.40$). Ethanol outflow/inflow ratio was similar in the two

Fig 1. Relative change in dialysate glycerol concentration in response to local isoproterenol infusion. The bars represent the means; symbols represent individual values. The difference between the means of the two groups was not significant

groups during baseline (0.81 ± 0.10 [Arg], 0.90 ± 0.22 [Trp]; $p = 0.28$) and during isoproterenol infusion (0.78 ± 0.11 [Arg], 0.87 ± 0.16 [Trp]; $p = 0.14$). Ethanol outflow/inflow ratio during isoproterenol infusion did not differ significantly from baseline in either group. After adjustment for percentage body fat and for sex, the change in dialysate glycerol concentration was still similar in the two groups (63 ± 33 [Arg], 73 ± 37 % [Trp]; $p = 0.51$). Sex was not a significant independent determinant of change in dialysate glycerol.

Discussion

The Trp64Arg allele of the β_3 -adrenoceptor results in the replacement of tryptophan with arginine in position 64 in the first intracellular loop of the receptor. The frequency of the Trp64Arg allele varies in different populations (e.g. 0.31 in Pima Indians vs 0.08 in United States Caucasians). The Arg64 allele has been associated with an earlier onset of NIDDM [1, 2], abdominal obesity [2, 3], insulin resistance [2, 4], propensity for weight gain [5, 6], difficulty in losing weight [3], low metabolic rate [3, 7], and high BMI [4, 8, 9]. Knowing that the β_3 -adrenoceptor is expressed mainly in subcutaneous and visceral adipose tissue depots [20], and that the β_3 -adrenoceptor is involved in the lipolytic response to catecholamines, we hypothesized that the above associations may be the result of impaired lipolytic activity in individuals carrying the Arg64 allele. In contrast to the hypothesis, the in vivo lipolytic response to isoproterenol in abdominal subcutaneous adipose tissue was similar in two groups of Pima Indian subjects, one group homozygous for the arginine allele, the other homozygous for the tryptophan allele.

Since it is not known which proportion of adrenergically stimulated lipolysis in subcutaneous adipose

tissue is mediated through the β_3 -adrenoceptors, a non-selective β -adrenergic agonist, isoproterenol, was used to stimulate lipolysis, mimicking the physiological situation in which endogenous catecholamines activate all three β -adrenoceptor subtypes. Stimulated lipolysis was measured as the increase in glycerol concentration in the microdialysate, a direct reflection of the concentration in the extracellular fluid of the adipose tissue. Isoproterenol was applied locally by adding it to the microdialysis perfusate. The increase in dialysate glycerol following isoproterenol administration was similar in the two groups, also after correction for changes in local blood flow and adjustment for percentage of body fat and for sex. These negative results could have three possible explanations: first, the Trp64Arg variant may not have any functional significance, as suggested by in vitro studies [11, 12]. Second, it is possible that the β_3 -adrenoceptor may play a negligible role for lipolysis in subcutaneous fat tissue, compared to β_1 - and β_2 -receptors. Studies suggest that the β_3 -adrenoceptor is preferentially expressed in visceral fat [20], and it is conceivable that the variant may have functional consequences only in visceral fat, and if so, possibly on thermogenesis rather than on lipolysis. Third, the Trp64Arg variant may cause a subtle functional defect not detectable with the present design. It is possible that stimulation with a β_3 -adrenergic agonist could have produced findings other than those obtained with isoproterenol. However, such findings would not necessarily be relevant to normal physiology.

In the present study, the two groups differed with regard to sex distribution. However, our analyses did not show a significant independent effect of sex on the lipolytic response to local isoproterenol infusion. Thus, it is unlikely that the difference in sex distribution influenced the results. Microdialysis with the membrane size and perfusion rate used in the present study does not result in a complete equilibrium for glycerol between the extracellular fluid and the dialysate. *Recovery*, i.e. the relationship between glycerol concentration in the dialysate and that in the extracellular fluid, may therefore have varied from one subject to another. We did not determine recovery for each subject. However, because of the number of subjects studied and the similarity in body composition between the two groups, we believe that individual differences in glycerol recovery did not mask differences between the groups. Changes in local blood flow were assessed with the ethanol dilution method. As the ethanol outflow/inflow ratio did not change significantly in response to isoproterenol, we were unable to demonstrate a change in local blood flow. This is in contrast with other investigators who have found a significant decrease in the ethanol outflow/inflow ratio – indicating an increase in local blood flow – during local infusion of isoproterenol $1 \mu\text{mol/l}$ [21]. This discrepancy is probably due to the perfusion

rate being higher than in the referenced study (5 vs 2.5 $\mu\text{l}/\text{min}$) – resulting in a lower sensitivity of the ethanol dilution method [22] – and the membrane being shorter (10 vs 20 mm), resulting in a lower delivery of isoproterenol to the tissue.

The literature is divided on whether the Arg64 allele has any functional significance. A multitude of studies report an association between the Trp64Arg allele and parameters related to NIDDM, obesity, or metabolic rate in several ethnic groups [1–9], but the findings have been inconsistent and the statistical associations rather weak. As recently reviewed [23], a number of reports refute that the Arg64 allele is associated with adverse metabolic conditions. The Trp64Arg variant was not associated with morbid obesity, weight gain, or response to weight-loss therapy in Finns [24]. When expressed in Chinese hamster ovary cells by Candelore et al. [11], the arginine β_3 -adrenoceptor type was functionally and pharmacologically similar to the tryptophan type. In a study by Li et al. [12] of isolated subcutaneous and visceral white fat cells from homozygotes for the Trp64 allele and from heterozygotes (no homozygotes for the Trp64Arg allele were studied), no differences were found in lipolysis. In a study by Tataranni et al. [25] in Pima Indians, the Trp64Arg allele, even in its homozygous form, was not associated with fasting plasma non-esterified fatty acid concentration or rate of appearance, nor with basal lipid oxidation rate. It is therefore conceivable that the apparent effect of the Arg64 allele on metabolism may be due to linkage disequilibrium with a nearby gene having an effect on metabolism.

In the present study, lipolysis was only assessed under baseline conditions and during stimulation with one concentration of isoproterenol, thus not allowing us to produce a dose/response curve. However, since the two types of β_3 -adrenoceptors have been carefully characterized in vitro (where they appear identical) [11], the present study – the first in vivo pharmacologic study of the Trp64Arg polymorphism – should be seen as further evidence that the Trp64Arg polymorphism has very little, if any, physiological significance. The first 10-min fraction of dialysate collected during the isoproterenol stimulation was disregarded, as a steady-state dialysate glycerol concentration was not reached until after 10 min. Based on the published observations of others, we assume that the concentration of isoproterenol used gave maximal or near-maximal rates of lipolysis [21]. In studies in which the same probes and the same fat region were used, exercise at 40 % of $\dot{V}O_{2\text{max}}$ resulted in a 38 % increase in dialysate glycerol concentration (S. Snitker, unpublished observations).

In conclusion, we could not demonstrate an effect of the Trp64Arg variant on lipolytic response to local non-selective β -adrenergic stimulation by isoproterenol in subcutaneous adipose tissue, as determined in

vivo by microdialysis. Other mechanisms may be responsible for the reported associations between the Trp64Arg variant and several aspects of obesity and NIDDM.

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