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The effects of a *HTR2B* stop codon and testosterone on energy metabolism and beta cell function among antisocial Finnish males

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ABSTRACT

Herein, we examined insulin resistance (IR), insulin sensitivity (IS), beta cell activity, and glucose metabolism in subjects with antisocial personality disorder (ASPD), and whether the serotonin 2B (5-HT2B) receptor and testosterone have a role in energy metabolism. A cohort of subjects belonging to a founder population that included 98 ASPD males, aged 25–30, was divided into groups based on the presence of a heterozygous 5-HT2B receptor loss-of-function gene mutation (*HTR2B* Q20*; n = 9) or not (n = 89). Serum glucose and insulin levels were measured in a 5 h oral glucose tolerance test (75 g) and indices describing IR, IS, and beta cell activity were calculated. Body mass index (BMI) was also determined. Concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid were measured in cerebrospinal fluid, and testosterone levels from serum. An IR-like state comprising high IR, low IS, and high beta cell activity indices was observed among ASPD subjects without the *HTR2B* Q20* allele. By contrast, being an ASPD *HTR2B* Q20* carrier appeared to be preventive of these pathophysiologies. The *HTR2B* Q20* allele and testosterone predicted lower BMI independently, but an interaction between *HTR2B* Q20* and testosterone lead to increased insulin sensitivity among *HTR2B* Q20* carriers with low testosterone levels. The *HTR2B* Q20* allele also predicted reduced beta cell activity and enhanced glucose metabolism. Reduced 5-HT2B receptor function at low or normal testosterone levels may be protective of obesity. Results were observed among Finnish males having an antisocial personality disorder, which limits the generality.

1. Introduction

Comorbidities of psychiatric and metabolic disorders are frequent. For example, more than 25% of individuals diagnosed with diabetes also suffer from depression (Lustman and Clouse, 2005). The combined impact of metabolic and psychiatric disorders decreases daily executive performance and quality of life, causes severe organ complications, and increases mortality, which support a rationale to further examine metabolic pathophysiologies associated with distinct psychiatric disorders. ASPD is a psychiatric disorder, with a prevalence of 1% (Lenzenweger et al., 2007), which

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is linked to an inherently impulsive lifestyle that puts ASPD patients at risk for metabolic disorders because of challenges in maintaining a healthy diet and physical exercise. A Finnish violent offender population saturated with ASPD individuals has been shown to exhibit elevated basal insulin levels (Ojala et al., 2015).

In addition to the clinical hypothesis that patients with ASPD are at an increased risk for insulin-related pathophysiologies, we also hypothesized that the serotonergic pathway could alter insulin secretion and glucose homeostasis. Indeed, preliminary evidence that was mostly obtained from animal study settings suggest that serotonin (5-HT) is involved in the control of islet function and links to insulin resistance (IR) (Bennet et al., 2015; Saunders et al., 2014). Moreover, some studies imply that functional silencing of the serotonin 2B (5-HT2B) receptor may have protective effects on risk for IR and type 2 diabetes (T2D) because the function of the receptor seems to alter islet function and glucose homeostasis (Bennet et al., 2016; Kim et al., 2010, 2015; Tikkanen et al., 2016; Yamada et al., 1998). However, the role of the G_q-coupled serotonin 5-HT2B receptor in human somatic health is poorly characterized, but in psychiatric research studies the 5-HT2B receptor has been recently shown to exert the following tangible effects on human behavior and psychiatric symptoms: increased impulsive behavior, alcohol-related problem-behavior, emotional dysregulation, mood disorders, and anxiety (Bevilacqua et al., 2010; Tikkanen et al., 2015).

To examine the effects of the serotonergic pathway, we studied heterozygous carriers of a 5-HT2B receptor gene mutation (*HTR2B* Q20*) detected in a Finnish young founder population (Bevilacqua et al., 2010). *HTR2B* Q20* is a point-mutation of the 5-HT2B receptor gene, which is located at 2q36-q37. The mutation results in interrupted expression of the 5-HT2B receptor in lymphoblastoid cells, which results in a 50% reduction of the expression of the receptor protein in heterozygous individuals (Bevilacqua et al., 2010). The prevalence of the hereditary *HTR2B* Q20* is relatively high (2.2%) in the Finnish general population. Testosterone was included in analyzes because testosterone levels has been suggested to be elevated in *HTR2B* knockout mice and heterozygous *HTR2B* Q20* carriers in comparison to carriers of the wild type allele (Bevilacqua et al., 2010). Also, reduced testosterone levels have been associated with IR, T2D and obesity (Haffner et al., 1994; Jones, 2010).

2. Material and methods

2.1. Subjects and subgroups

Subjects were obtained from a genotyped cohort that included Finnish alcoholic violent offenders, their relatives, along with healthy controls who were recruited by newspaper advertisements; the cohort included a total of 875 subjects. This cohort was collected to detect biological risk factors for psychiatric disorders and impulsive behaviors. All subjects diagnosed with ASPD that had participated in an oral glucose tolerance test (OGTT) were included without pre-selection, resulting in a total of 98 subjects, all males, who were separated for comparison into the following two groups: heterozygote *HTR2B* Q20* allele carriers ($n = 9$) and subjects homozygous for the wild-type *HTR2B* Q20 allele ($n = 89$). The genotyping procedure has been previously described in detail by Bevilacqua et al. (2010). Genetic and molecular analyzes were performed at the Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, NIH (Bethesda, MD, USA).

2.2. Laboratory tests

Serum levels of glucose, insulin, and testosterone, and gamma-glutamyl transferase were measured after fasting overnight. Insulin was analyzed using a radioimmunoassay method and quantified in antibody-coated test tubes (Count-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA). Between-assay variation for insulin was 4.6% at 215 pmol/l. All samples were assayed in duplicate. When results of duplicate measurements showed discrepancies of more than 5%, samples were reanalyzed (Virkkunen et al., 1994). For clinical convenience, insulin levels are reported as mU/L. None of the participants had previously used antidepressants or antipsychotics within the two weeks prior to the laboratory tests; lack of drug use status was verified using urine tests. All subjects ate the same food and were not allowed to exercise 24-h prior to measurements. OGTT was administered after 12- to 16-h fasting overnight. At 8 a.m., participants drank a solution containing 75 g glucose (Leiras, Turku, Finland), which was ingested as quickly as possible. From an antecubital vein, nine blood samples (15 mL each) were collected during the 5 h test. For the first 2 h of the test, subjects rested in bed. Thereafter, they were allowed to move about the ward, but resting was encouraged. Blood glucose concentrations were determined enzymatically. Blood samples were stored in tubes that contained sodium fluoride.

Levels of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) were measured from cerebrospinal fluid (CSF) that was obtained by lumbar puncture. A total of 12 mL CSF was collected and 5-HIAA concentrations were analyzed by liquid chromatography and electrochemical detection methods. Prior to puncture between 8 and 9 a.m. the subjects had only been allowed to drink water after 8 p.m. the night before. Moreover, they had eaten the same food and were not allowed to exercise 24 h prior the puncture. No subjects had used a psychoactive medication, such as selective serotonin reuptake inhibitors, within the previous two weeks, which was confirmed by urine tests.

2.3. Measurements of glucose levels, insulin resistance, insulin sensitivity, and beta cell activity

WHO, (2006) definitions for normal and pathophysiological glucose levels were used. Normal fasting glucose levels were defined as 3.9–6.0 mmol/L, impaired fasting glucose tolerance (IFG) was defined as 6.1–6.9 mmol/L, and diabetes was defined as $\geq$7.0 mmol/L. The following glucose reference values at 120 min measurements in the OGTT were used to define impaired glucose tolerance (IGT) and diabetes, respectively: $\geq$7.8 and $\leq$11.0 mmol/L and $\geq$11.1. Additionally, IR, IS, and beta cell activity were calculated using the homeostasis model assessment calculator (Levy et al., 1998) (https://www.dtu.ox.ac.uk/homacalculator, accessed 02.03.16), which utilizes fasting glucose and fasting insulin values. Normal reference values for HOMA2 IR, IS, and beta cell activity are 1.0, 100%, and 100%, respectively. Furthermore, IR and IS were assessed based on the whole body insulin sensitivity index (WBISI) (Matsuda and DeFronzo, 1999) calculated from measurements obtained from the OGTT utilizing glucose and insulin values at baseline, and at 30, 60, 90, and 120 min (http://mmatsuda.diabetes-smc.jp/MIndex.html, accessed 02.04.16). A WBISI value of $\leq$2.5 denoted pathological whole body insulin resistance.

2.4. Area under the curve (AUC)

AUC values were calculated using the trapezoidal method (Purves, 1992) representing the “average curve” because it has been suggested that subtle biological variance might not be detected when presenting the “curve of averages” alone (Allison et al., 1995; Matthews et al., 1990). When the golden standard 2 h AUC was used for calculations of the AUC value, it was subtracted from the baseline value for each subject. Then, mean values were compared between groups.

2.5. Fat percentage

Fat percentages were measured using a four-terminal portable impedance analyzer (BIA 101, RJL Systems, Detroit MI, USA), which is a bioelectrical impedance technique that measures total body water, fat-free body mass, and, consequently, fat percentage. Measurements were performed as described previously by Lukaski et al. (1985).

2.6. Psychiatric disorders

All subjects were examined based on the DSM-III-R semi-structured interview to detect psychiatric disorders (American Psychiatric Association, 1987; Spitzer et al., 1988). Experienced psychiatrists conducted the interviews and two research psychiatrists rated the interview data blindly under the supervision of a senior research psychiatrist. Inter-rater reliability was high, and the senior psychiatrist resolved any discrepancies.

2.7. Statistical analyzes

A normal distribution of variables was tested using the Kolmogorov–Smirnov test. Asymmetric variable variances of mean values were tested using the Mann–Whitney *U* test. Comparisons of mean values for normally distributed variables were performed using two-tailed independent samples *t*-test letting the Levene's test of equality of variances determine the appropriate p-value. Multiple linear regression analyses were conducted to detect predictors of the main metabolic dependents. The SPSS 22.0 software package (SPSS Inc., Chicago, IL, USA) was used for all calculations with a significance level set at the 95% CI.

2.8. Ethics

Written informed consent was obtained from each participant. The study protocol was approved by the Institutional Review Boards of the Department of Psychiatry at the University of Helsinki and the Helsinki University Central Hospital.

3. Results

3.1. Measurements of glucose, insulin, IR, IS, beta cell activity, AUCs, and testosterone

No subjects exhibited pathological fasting or OGTT glucose values (i.e., impaired fasting glucose, IGT, or diabetes). However, ASPD subjects without *HTR2B* Q20* had increased IR, decreased IS, and high beta cell activity values; a significant difference in AUCs was observed between the groups. Table 1 and Fig. 1 display the most notable differences between groups. Testosterone levels were equal between groups.

3.2. Effects of metabolic predictors and age on BMI, IR, IS and beta cell activity

The multiple regression analyses were adjusted for age, BMI (not in the model where BMI was entered as the dependent) and gamma-glutamyl transferase. They detected both main effects and an interaction, which are presented in Table 2 and Fig. 2. The main results are discussed in the Discussion section, but it is notable that also age and BMI seemed to predict outcomes in a consistent manner.

3.3. Psychiatric disorders

All subjects fulfilled the criteria for ASPD. Borderline personality disorder was somewhat frequent in both groups, as it was present in 33% of *HTR2B* Q20* carriers and 43% of subjects without the mutation. All subjects without *HTR2B* Q20* fulfilled the criteria for a diagnosis of alcohol dependence, whereas alcohol dependence criteria were fulfilled by 89% of subjects among *HTR2B* Q20* carriers as one subject received a diagnosis of alcohol abuse.

4. Discussion

Our main finding in the larger cohort of ASPD subjects without *HTR2B* Q20* was that they exhibited an IR-like state characterized by high IR, low IS, and high beta cell activity indices, even though they were non-obese and normoglycemic. IGT was not observed during the OGTT. Thus, it is notable that the glucose levels at the 2 h measurements in the OGTT among ASPD subjects without *HTR2B* Q20* were not sufficiently aberrant to claim clinically diagnostic IR or T2D. However, the combination of an IR-like state and normoglycemia that we observed might be interpreted as a constellation of elevated insulin secretion relative to normoglycemia, which has recently been reported to have a distinct genetic background in patients with diabetes (Dimas et al., 2014). On the other hand, the IR-like findings may be a regular milestone on the risk path to develop T2D. The ASPD subjects without *HTR2B* Q20* were non-obese and had a low fat percentage, which also could have a distinct genetic basis. Approximately 100 BMI-associated single nucleotide polymorphisms have been identified and determined to explain a small amount of BMI variation (2.7%) (Konttinen et al., 2015). By contrast, genome-wide estimates have suggested that common genetic variation accounts for 20–30% of the variance in BMI (Llewellyn et al., 2013; Locke et al., 2015). We may speculate that having a higher amount of brown adipose tissue (BAT) allows glucose to be actively cleared from circulation, making it a protective physiological feature that guards against weight gain (Hanssen et al., 2015; Harms and Seale, 2013), but we did not measure BAT. Peripheral 5-HT synthesis has been recently shown to regulate BAT thermogenesis along with associated energy expenditure (Crane et al., 2015), which could partly explain the normal BMI among ASPD subjects without the *HTR2B* Q20* allele as ASPD has been associated with aberrant 5-HT metabolite levels in the CSF (Virkkunen et al., 1994). Although the mechanisms are unclear, the normoglycemia observed could also be explained by that ASPD subjects have been found to have a tendency towards functionally low glucose levels and exhibit low glycogen formation (Virkkunen et al., 2007, 2009).

Being a *HTR2B* Q20* carrier appeared to be protective against the IR, IS, and beta cell activity pathophysiologies. The glucose metabolism was enhanced and insulin secretion were also lower among *HTR2B* Q20* carriers. These findings are in accord with earlier reports of the role of the 5-HT2B receptor in regulating insulin secretion and glucose homeostasis, which mainly analyzed knockout animals (Bennet et al., 2016; Kim et al., 2010, 2015; Yamada et al., 1998) but suggest that similar effects may also appear in humans. It would be unusual that one gene could exhibit such a large effect on the phenotype. However, in this present study, such a scenario could be possible because it included heterozygous *HTR2B* Q20* carriers with a 50% reduction in 5-HT2B receptor abundance and all subjects belonged to a young founder population, which has been estimated to increase the power to detect the effects of alleles that are present at low-frequencies (0.5–5%) on complex disorders (Lim et al., 2014). Our observation that the levels of the 5-HT metabolite 5-HIAA in the CSF showed no difference between groups also suggests that the effects that we

Table 1
Mean values of serum glucose and insulin obtained from a 5 h oral glucose tolerance test between carriers of a loss-of-function serotonin 2B receptor gene mutation (*HTR2B* Q20*) and subjects without the mutation (*HTR2B* Q20) in a cohort of males diagnosed with antisocial personality disorder. Mean values of insulin resistance, insulin sensitivity, beta cell indices, and areas under the curve (AUCs) are also displayed, as well as age, body mass index (BMI), fat percentages, gamma-glutamyl transferase, testosterone, and the serotonin metabolite 5-hydroxyindoleacetic acid concentrations in cerebrospinal fluid (CSF 5-HIAA).

		HTR2B Q20* (n = 9)	HTR2B Q20 (n = 89)	t or Z-score (df)	p
Age (years)		30.4 (±9.1)	25.3 (±8.6)	1697 (97)	0.093
BMI (kg/m2)		22.4 (±1.5)	25.2 (±3.8)	−2682 (98)	0.007
Fat percentage		17.6 (±4.7)	18.8 (±5.6)	−0.759 (97)	0.514
Testosterone, nmol/L		22.1 (±8.6)	19.5 (±6.6)	0.884 (96)	0.400
CSF 5-HIAA, pg/mL		55.4 (±24.4)	65.1 (±24.5)	−1505 (49)	0.121
Gamma-glutamyl transferase, U/L		28.4 (±21.2)	68.1 (±110)	−2.873 (95)	0.006
Fasting glucose, mmol/L		4.1 (±0.6)	4.1 (±0.5)	−0.191 (94)	0.849
Glucose at 15 min, mmol/L		5.3 (±0.5)	5.5 (±1.1)	−0.504 (94)	0.616
Glucose at 30 min, mmol/L		5.9 (±0.9)	6.8 (±4.5)	−1691 (94)	0.094
Glucose at 60 min, mmol/L		5.3 (±1.2)	6.9 (±2.0)	−2393 (94)	0.019
Glucose at 90 min, mmol/L		4.6 (±0.8)	6.0 (±1.7)	−2460 (94)	0.014
Glucose at 120 min, mmol/L		4.3 (±1.1)	5.4 (±1.2)	−2359 (94)	0.020
Glucose at 180 min, mmol/L		3.9 (±0.9)	3.9 (±1.0)	−0.151 (94)	0.880
Glucose at 240 min, mmol/L		3.4 (±0.5)	3.5 (±0.7)	−0.437 (94)	0.663
Glucose at 300 min, mmol/L		4.0 (±0.5)	4.2 (±3.5)	−0.161 (94)	0.872
Glucose AUC_{0-120} min, mmol-min/L		66 (±81)	204 (±139)	−2987 (94)	0.003
Fasting insulin, mU/L		8.6 (±3.0)	11.9 (±7.6)	−1292 (93)	0.199
Insulin at 15 min, mU/L		32 (±22)	39 (±34)	−0.657 (93)	0.513
Insulin at 30 min, mU/L		53 (±36)	67 (±46)	−0.894 (93)	0.374
Insulin at 60 min, mU/L		58 (±26)	82 (±61)	−2111 (93)	0.038
Insulin at 90 min, mU/L		42 (±16)	66 (±53)	−3126 (93)	0.004
Insulin at 120 min, mU/L		39 (±17)	61(±51)	−2650 (93)	0.013
Insulin at 180 min, mU/L		21 (±13)	28 (±25)	−0.902 (93)	0.370
Insulin at 240 min, mU/L		10.0 (±3.5)	15.0 (±13.7)	−2839 (93)	0.007
Insulin at 300 min, mU/L		7.9 (±1.3)	10.7 (±7.0)	−3170 (93)	0.002
Insulin AUC_{0-120} min, mU-min/L		2060 (±815)	3202 (±2391)	−3041(93)	0.005
HOMA2 indices[a]	Insulin resistance (1.0 = normal)	1.0 (±0.2)	1.4 (±0.9)	−3484 (93)	0.001
	Insulin sensitivity (100% = normal)	104 (±25)	88 (±37)	1309 (93)	0.194
	Beta cell activity (100% = normal)	129 (±34)	191 (±87)	−2090 (93)	0.039
Matsuda indices[b]	Insulin resistance (aberrant ≥ 2.5)	1.6 (0.6)	2.4 (1.8)	−2.910 (93)	0.008
	Insulin Sensitivity (normal ≥ 2.5)[c]	7.8 (±4.0)	5.7 (±2.9)	1990 (93)	0.049

df = degree of freedom values obtained from two-tailed independent samples Students *t*-tests for normally distributed variables. Numbers in parentheses in the Z-score column indicate the n-number available for the Mann–Whitney *U* test used to analyze variables that deviated from a normal distribution (BMI, glucose at 90 min, and glucose $AUC_{0-120min}$). The significance level was set at the 95% CI.

[a] HOMA2 = Homeostasis Model Assessment, based on fasting levels of glucose and insulin.

[b] Matsuda and DeFronzo (1999), based on glucose and insulin levels obtained from OGTT.

[c] Whole Body Insulin Sensitivity (WBISI).

observed may have been caused by altered function of the 5-HT2B receptor rather than effects resulting from variance in the physiological supply of serotonin. Additionally, the experiments performed by Bennet et al. (2016) suggest that the effects of the 5-HT2B receptor on insulin secretion involve both exocytotic and intracellular mechanisms. Also, they provided evidence that the 5-HT2B receptor alters Ca^{2+} influx and the rate of mitochondrial oxidative consumption. Such profound biological variation may indicate that effects on phenotype could be detected.

Additional underlying causes other than a direct effect of *HTR2B* Q20* on islet function and glucose homeostasis are currently speculative, although several likely possibilities exist. For example, our findings could be partially explained by gene–gene interactions. The 5-HT1D receptor (formerly known as 5-HTR1D$_\alpha$) gene, *HTR1D*, is of particular interest because a polymorphism has been detected in the coding region of this gene (Ozaki et al., 1995) and its expression level has been potentially linked to defective insulin secretion in human T2D (Bennet et al., 2015). The 5-HT1B receptor (formerly known as 5-HT1D$_\beta$) is also of interest because the protein is 64% identical and nearly pharmacologically indistinguishable from the 5-HT1D receptor protein (Lappalainen et al., 1995). Furthermore, a highly bioactive intracellular proteolytic enzyme (26 S proteasome non-ATP regulatory subunit 1) gene (*PSMD1*) is located at the same locus (chr2:231,056,-864–231,172,827; GRCh38/hg38) as *HTR2B*, completely overlapping it, but on the opposite strand (*PSMD1* is on + strand, whereas *HTR2B* is on -). The minor allele of rs79874540 A(T) enables the stop codon composition of *HTR2B* Q20*, whereas in *PSMD1*, rs79874540 is an intronic variant and does not have a known effect on the stability of the protein. However, one of the gene transcripts (ENST00000431051[+]) is a target of nonsense-mediated RNA decay (NMD), whereby the alternate allele might play a role. Although unlikely, it is possible that *PSMD1*-dependent functions are also aberrant among *HTR2B* Q20* carriers. *PSMD1* has been shown to alter the risk for some CNS and cardiac diseases, such as Parkinson's disease and cardiomyopathies (Chung et al., 2001; Predmore et al., 2010), but it has not yet been established to have a role in either islet function or glucose homeostasis. Moreover, complex neuronal interactions are likely to occur in the process that regulates the secretion of islet hormones. Some evidence suggests that both 5-HT and dopamine can alter IR in metabolic disorders (Konner and Bruning, 2012). More specifically, preliminary evidence suggests that high dopaminergic activity in the pancreas can inhibit glucose-stimulated insulin secretion (GSIS) and alter beta cell Ca^{2+} influx via dopamine receptor D3 (Ustione and Piston, 2012).

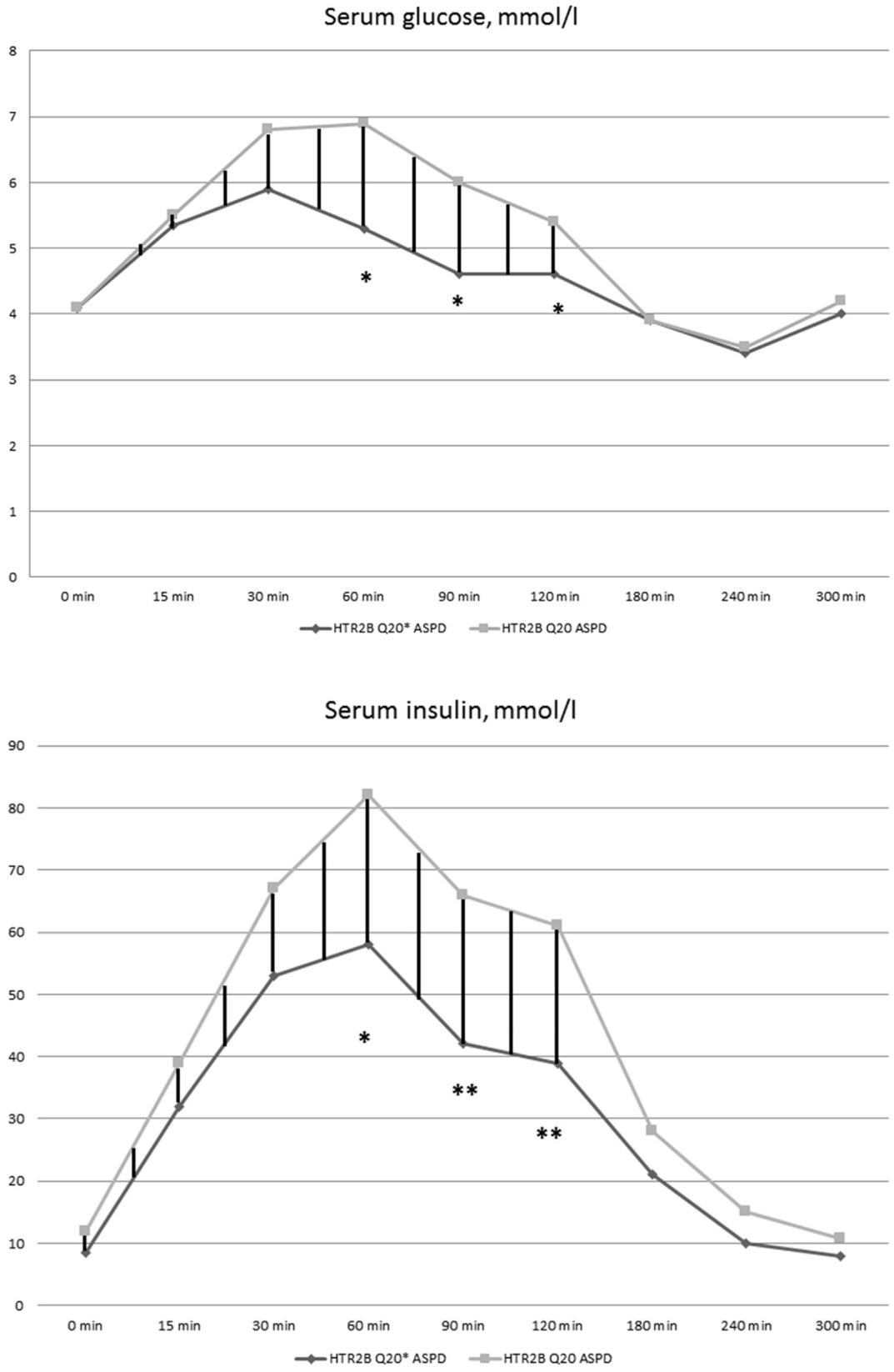

Fig. 1. Serum glucose and insulin mean value curves obtained from an oral glucose tolerance test among carriers of a loss-of-function 5-HT2B receptor gene mutation (*HTR2B* Q20*) versus subjects without the mutation (*HTR2B* Q20) in a sample comprising males diagnosed with antisocial personality disorder (ASPD). Functions show the "curve of averages", and the vertically striped areas show the significant differences ($p < 0.01$) in the area under the curve ($AUC_{0-120min}$) calculated using the trapezoidal method to describe the "average curve". * = $p < 0.05$, ** = $p < 0.01$.

Table 2
The effects of a *HTR2B* stop codon (*HTR2B* Q20*) and testosterone on metabolic parameters in a cohort of males diagnosed with antisocial personality disorder. GGT = Gamma-glutamyl transferase. R^2 = R squared explanatory power of the model. Results that are printed in bold font are significant at the 95% CI level.

Predictor	β (SE)	*t*	*p*	R^2	Predictor	β (SE)	*t*	*p*	R^2
Body mass index (BMI)				**0.12**	**Beta cell activity (HOMA2)**				**0.10**
HTR2B* Q20	**−2.94 (1.40)**	**−1.93**	**0.038**		***HTR2B* Q20***	**−65.7 (31.2)**	**−2.11**	**0.032**	
Testosterone	**−0.15 (0.06)**	**−2.66**	**0.009**		Testosterone	0.29 (1.40)	0.21	0.837	
HTR2B Q20* × testosterone	0.34 (0.32)	1.08	0.288		*HTR2B* Q20* × testosterone	−0.21 (7.35)	−0.03	0.978	
Age	−0.01 (0.04)	−0.03	0.975		BMI	2.74 (2.51)	1.09	0.279	
GGT	0.00 (0.01)	0.06	0.953		**Age**	**2.20 (0.99)**	**2.23**	**0.028**	
					GGT	0.04 (7.43)	0.01	0.996	
Insulin resistance (HOMA2)				**0.10**	**Insulin resistance (Matsuda)**				**0.10**
HTR2B Q20*	−0.25 (0.33)	−0.70	0.488		*HTR2B* Q20*	−0.46 (0.63)	−0.73	0.470	
Testosterone	−0.00 (0.01)	−0.69	0.493		Testosterone	−0.02 (0.03)	−0.80	0.425	
HTR2B Q20* × testosterone	0.02 (0.08)	0.32	0.748		*HTR2B* Q20* × testosterone	0.19 (0.25)	0.77	0.444	
BMI	**0.06 (0.03)**	**2.17**	**0.003**		BMI	0.09 (0.05)	1.75	0.084	
Age	0.00 (0.01)	0.90	0.893		Age	−0.02 (0.02)	−0.98	0.331	
GGT	0.00 (0.00)	0.32	0.748		GGT	0.00 (0.00)	0.89	0.378	
Insulin sensitivity (HOMA2)				**0.12**	**Insulin sensitivity (Matsuda)**				**0.19**
HTR2B Q20*	9.01 (13.1)	0.69	0.493		*HTR2B* Q20*	1.82 (1.08)	1.69	0.095	
Testosterone	0.76 (0.59)	1.30	0.199		Testosterone	0.04 (0.05)	0.80	0.428	
HTR2B Q20* × testosterone	−2.14 (3.10)	−0.70	0.492		***HTR2B* Q20* × testosterone**	**−1.10 (0.44)**	**−2.47**	**0.016**	
BMI	**−2.16 (1.06)**	**−2.04**	**0.045**		BMI	−0.16 (0.09)	−1.81	0.075	
Age	0.35 (0.42)	0.83	0.407		Age	0.06 (0.03)	1.78	0.079	
GGT	−0.04 (0.03)	−1.17	0.246		GGT	−0.00 (0.00)	−1.32	0.191	
Glucose $AUC_{0-120min}$				**0.25**	**Insulin $AUC_{0-120min}$**				**0.10**
HTR2B* Q20	**−152 (50)**	**−3.10**	**0.003**		*HTR2B* Q20*	−706 (872)	−0.81	0.420	
Testosterone	−2.18 (2.24)	−0.98	0.332		Testosterone	−10.6 (37.2)	-0.028	0.777	
HTR2B Q20* × testosterone	2.73 (11.8)	0.23	0.817		*HTR2B* Q20* × testosterone	−2.03 (196)	−0.01	0.992	
BMI	−0.34 (3.95)	−0.09	0.932		**BMI**	**216 (66.9)**	**3.25**	**0.002**	
Age	**3.67 (1.59)**	**2.33**	**0.022**		Age	35.5 (26.3)	1.35	0.181	
GGT	1.23 (2.0)	0.65	0.490		GGT	1.20 (2.15)	0.56	0.579	

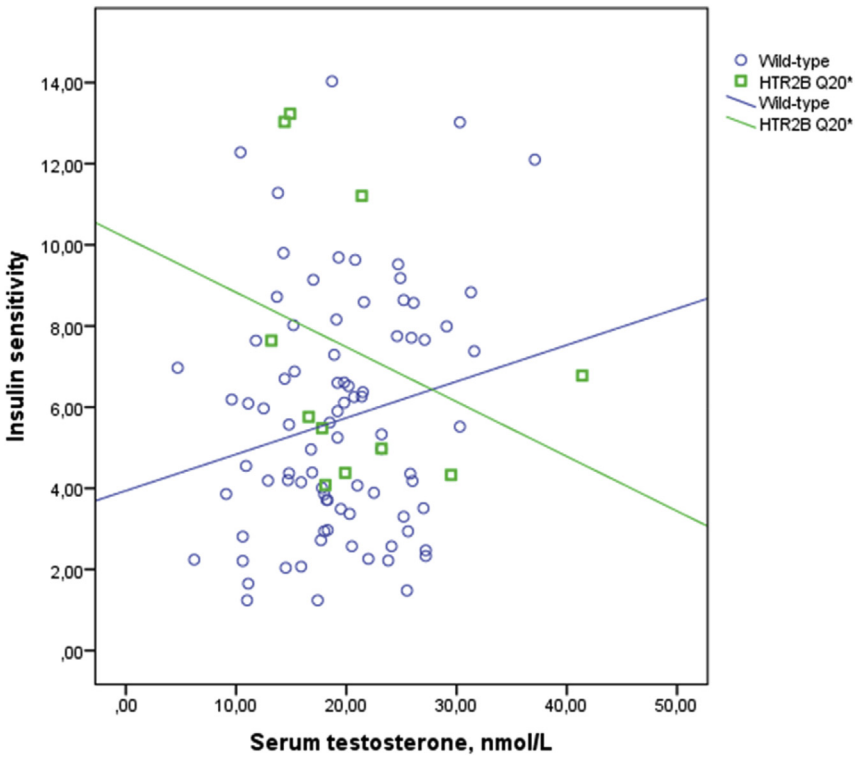

Fig. 2. The divergent regression lines show the joint effect of a *HTR2B* stop codon (*HTR2B* Q20*) and testosterone on insulin sensitivity as measured by the Whole Body Sensitivity Index (WBISI; ≤2.5 indicates pathological whole body insulin resistance) in a sample comprising males diagnosed with antisocial personality disorder. The data of the interaction term (*HTR2B* Q20* × testosterone) is β (SE) = −1.10 (0.44), $t = -2.47$, $p = 0.016$, $R^2 = 0.19$. Wild-type refers to subjects without the *HTR2B* Q20* allele.

Results extracted from the regression analyzes partly explain the mean value differences found in energy metabolism parameters between the comparison groups. The *HTR2B* Q20* allele predicted lower BMI, reduced beta cell activity, and reduced glucose excursion. However, testosterone also predicted low BMI, independently from the effect of the *HTR2B* Q20* allele. This main effect of testosterone on BMI is in line with earlier research (Haffner et al., 1994; Jones, 2010). Interestingly, an interaction occurred between *HTR2B* Q20* and testosterone to predict insulin sensitivity in a model that explained 19% of the variance ($\beta = -1.10$, SE = 0.44, $t = -2.47$, $p = 0.016$, $R^2 = 0.19$). This interaction suggests that the effect of testosterone is reversed among *HTR2B* Q20* carriers; they have an improved insulin sensitivity in the presence of low testosterone levels whereas subjects without this 5-HTR2B mutation have reduced insulin sensitivity in the same low testosterone environment. This effect may be driven by that *HTR2B* Q20* predicted reduced glucose excursion and reduced beta cell activity as the WBISI measures variation in both glucose and insulin levels after oral glucose challenge. Moreover, this interaction represent a potential causal mechanism for that *HTR2B* Q20* predicted reduced BMI in the model that accounted for the effect of testosterone. An interaction between the functioning of the 5-HT2B receptor and testosterone has not been reported in earlier research. The main effect of the *HTR2B* Q20* allele on BMI in humans is also a preliminary finding. However, it seems comprehensive as the serotonergic pathway has been linked to IR in animal settings (Bennet et al., 2015, 2016; Kim et al., 2015; Saunders et al., 2014). Also, high-rate catabolism of monoamines, including 5-HT, has been associated with low BMI in humans (Ducci et al., 2006). In theory, the interaction between *HTR2B* Q20* and testosterone would put *HTR2B* Q20* carriers with high testosterone levels at risk for reduced insulin sensitivity as compared to carriers of the wild-type allele. The regression lines crossed approximately at the testosterone level 27 nmol/L in our sample, which left 10% of the total sample above this pivotal point. Although our results are preliminary and correlative, the results suggest that the effect of testosterone on energy metabolism may partly be dependent of serotonergic mechanisms similar to the relationship between serotonin and insulin-related metabolism (Bennet et al., 2016; Tikkanen et al., 2016). Our results may describe a biochemical physiologic mechanism as the sample was homogeneous and likely exhibited only small variance in behavior. However, to understand the meaning and mechanisms of the relationships between energy metabolism, serotonergic pathway, and testosterone, GSIS laboratory settings should be conducted where the 5-HTR2B could be silenced and the testosterone environment could be altered simultaneously.

Alcohol consumption should be considered in our interpretation of the results because it has been reported to be high in a Finnish violent offender population with a high prevalence of ASPD (Tikkanen et al., 2009). Persistently abundant alcohol consumption is associated with an increased risk of chronic pancreatitis (Ammann, 2001), which can lead to irreversible islet destruction and, consequently, decreased insulin secretion. In this context, insulin responses to oral glucose load are severely impaired and result in overt hyperglycemia during OGTT—a pattern that we did not observe in any of our subjects. By contrast, modest, but regular alcohol consumption has been shown to be a protective factor against T2D (Carlsson et al., 2003). We included gamma-glutamyl transferase levels in the regression analyzes to decrease the possible bias of variance in alcohol consumption. However, all subjects had homogeneously a diagnosis of an alcohol use disorder, which is typical conjunct to ASPD. On the other hand, subjects had abstained for a four to eight week period prior to the tests because they were subjected to a court-ordered mandatory inpatient forensic mental status examination.

The small number of *HTR2B* Q20* subjects limits the power of our statistical analyzes. In addition to type I errors, type II errors may have occurred as the multiple linear regression analyzes are robust. Moreover, comparisons of results from human heterozygote studies with those of homozygous knockout animals should be cautious. However, the 50% reduction of 5-HTR2B receptor protein levels in the *HTR2B* Q20* subjects should lend support to hypothesize that comparisons to animal knockout studies could be made. We did not analyze environmental factors, which increases the risk of obtaining spurious results. E.g., dietary habits were not controlled for, but *HTR2B* Q20* has been linked to impulsive behaviors (Bevilacqua et al., 2010; Tikkanen et al., 2015), which would likely bias results in the opposite direction assuming that impulsive behavior decreases dietary control. A strength of our study was that the cohort was homogenous regarding ASPD diagnosis.

In future studies, the putative role of the 5-HT2B receptor in IR and T2D should be examined in different patient cohorts and risk groups. The importance of examining the effects of 5-HT receptors in diverse populations is emphasized by the finding that the effects of 5-HT pathways on insulin secretion appear to differ in non-diabetics and diabetics for unknown reasons. Evidence for this was provided by Bennet et al. (2015) who showed that (i) *HTR1D* and *HTR2A* are overexpressed in the islets of diabetic, but not non-diabetic donors, (ii) 5-HT1D receptor inhibits, whereas 5-HT2A receptor potentiates GSIS in non-diabetic, but not in diabetic islets, and (iii) diabetic islets lose sensitivity to the inhibitory effects of 5-HT on GSIS.

Together, our findings suggest that ASPD may be a risk group for insulin-related metabolic pathophysiology, which could be important to account for in clinical practice as IR, IS and beta cell activity indices has been shown to predict obesity, T2D, and cardiovascular diseases (Nolan and Faerch, 2012). Being an ASPD *HTR2B* Q20* carrier appeared to normalize insulin-related pathophysiologies, which implies that silencing the 5-HT2B receptor may have protective effects on metabolic disorders. Results were observed among Finnish males having an antisocial personality disorder, which limits the generality.

Authors' contributions

M.V contributed to the design of the study. R.T and M.V contributed to the structuring of the data and planning the focus of analysis. R.T had full access to all data and takes responsibility for data analysis. R.T, M.V, and T.S contributed to the disposition of the manuscript. M.K contributed with sampling and laboratory analyses. All authors contributed i) to the drafting of the manuscript, ii) intellectual assessment of the work, and iii) read the manuscript and approved the contributions.

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Conflicts of interest

All authors claim no conflict of interest.

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