Evaluation of Fasting State-/Oral Glucose Tolerance Test-Derived Measures of Insulin Release for the Detection of Genetically Impaired β-Cell Function

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Abstract

**Background:** To date, fasting state- and different oral glucose tolerance test (OGTT)-derived measures are used to estimate insulin release with reasonable effort in large human cohorts required, e.g., for genetic studies. Here, we evaluated twelve common (or recently introduced) fasting state-/OGTT-derived indices for their suitability to detect genetically determined β-cell dysfunction.

**Methodology/Principal Findings:** A cohort of 1364 White European individuals at increased risk for type 2 diabetes was characterized by OGTT with glucose, insulin, and C-peptide measurements and genotyped for single nucleotide polymorphisms (SNPs) known to affect glucose- and incretin-stimulated insulin secretion. One fasting state- and eleven OGTT-derived indices were calculated and statistically evaluated. After adjustment for confounding variables, all tested SNPs were significantly associated with at least two insulin secretion measures (p<0.05). The indices were ranked according to their associations’ statistical power, and the ranks an index obtained for its associations with all the tested SNPs (or a subset) were summed up resulting in a final ranking. This approach revealed area under the curve (AUC)\text{In}ulin(0-30)/AUC\text{Glucose}(0-30) as the best-ranked index to detect SNP-dependent differences in insulin release. Moreover, AUC\text{In}ulin(0-30)/AUC\text{Glucose}(0-30) corrected insulin response (CIR), AUC\text{C-Peptide}(0-30)/AUC\text{Glucose}(0-30), AUC\text{C-Peptide}(0-120)/AUC\text{Glucose}(0-120) two different formulas for the incremental insulin response from 0–30 min, i.e., the insulinogenic indices (IGI)2 and IGI1, and insulin 30 min were significantly higher-ranked than homeostasis model assessment of β-cell function (HOMA-B; p<0.05). AUC\text{C-Peptide}(0-120)/AUC\text{Glucose}(0-120) was best-ranked for the detection of SNPs involved in incretin-stimulated insulin secretion. In all analyses, HOMA-β displayed the highest rank sums and, thus, scored last.

**Conclusions/Significance:** With AUC\text{In}ulin(0-30)/AUC\text{Glucose}(0-30), CIR, AUC\text{C-Peptide}(0-30)/AUC\text{Glucose}(0-30), AUC\text{C-Peptide}(0-120)/AUC\text{Glucose}(0-120), IGI2, IGI1, and insulin 30 min, dynamic measures of insulin secretion based on early insulin and C-peptide responses to oral glucose represent measures which are more appropriate to assess genetically determined β-cell dysfunction than fasting measures, i.e., HOMA-B. Genes predominantly influencing the incretin axis may possibly be best detected by AUC\text{C-Peptide}(0-120)/AUC\text{Glucose}(0-120).

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Introduction

Recently, genome-wide association (GWA) scans in tens of thousands of human cases and controls using high-density single nucleotide polymorphism (SNP) arrays and subsequent meta-analyses of these data provided important new insights into the genetic architecture of complex diseases [1]. In the course of these studies, a series of nearly 20 novel type 2 diabetes risk loci were identified. In smaller but extensively and thoroughly phenotyped cohorts, many of the diabetogenic alleles were shown to affect β-cell function [2]. Despite this recent scientific progress, a shortcoming of the genetic findings up to now is that the sum of all reported common GWA-derived risk alleles only marginally improves the prediction of future type 2 diabetes, when combined with established clinical parameters, and only explains about 6% of the heritability of the disease [3]. Thus, it is anticipated that
many more loci remain to be discovered that act in an additive or even synergistic manner to increase the type 2 diabetes risk. Amongst others, the following strategies are currently discussed to find them: (i) use of SNP arrays of higher density, (ii) assessment of rare variants, and (iii) realization of GWA analyses using quantitative traits known to be crucially involved in the pathogenesis of type 2 diabetes, such as insulin secretion and insulin sensitivity [2,3].

One possibility to identify more loci affecting β-cell function is to determine insulin release in cohorts large enough to allow reliable genetic analyses. To estimate insulin release in such cohorts with reasonable effort, i.e., at low expenses in time and costs, fasting state- and several different oral glucose tolerance test (OGTT)-derived indices calculated from plasma insulin, C-peptide, and glucose concentrations are available [4–10]. However, which of these indices are best-suited to detect genetically determined β-cell dysfunction is unknown. Therefore, we evaluated, in this study, fasting state- (homeostasis model assessment of β-cell function [HOMA-B]) and OGTT-derived indices (insulin and C-peptide concentrations at 30 min of OGTT, insulinogenic indices [IGIs], area under the curve [AUC]_{Insulin(0-30)}/AUC_{Glucose(0-30)}, AUC_{C-Peptide(0-30)/AUC_{Glucose(0-30)}, AUC_{Insulin(0-120)/AUC_{Glucose(0-120)}, AUC_{C-Peptide(0-120)/AUC_{Glucose(0-120)}, oral disposition index [DI oral]}, corrected insulin response [CIR], and first-phase insulin secretion) for their suitability to detect altered insulin release due to confirmed type 2 diabetes risk SNPs convincingly described to affect specific aspects of β-cell function, such as glucose-stimulated insulin secretion (GSIS), incretin-stimulated insulin secretion (ISIS), or incretin release. For this investigation, we included the type 2 diabetes risk loci/SNPs MTNR1B rs10830963, HHEX rs7923837, CDKAL1 rs7754840, TCF7L2 rs7903146, WFS1 rs10010131, and KCNV2 rs151290.

Materials and Methods

Ethics statement

From all participants, informed written consent to the study was obtained, and the Ethics Committee of the Medical Faculty of the University of Tübingen approved the study protocol.

Subjects

A cohort of 1364 White individuals was recruited from the ongoing Tübingen family study for type 2 diabetes (TUF) that currently encompasses ~2000 participants at increased risk for type 2 diabetes (non-diabetic individuals from Southern Germany with family history of type 2 diabetes or diagnosis of impaired fasting glycaemia) [11]. More than 99.5% of the TUF participants are of European ancestry. All participants underwent the standard procedures of the study protocol including medical history and physical examination, assessment of smoking status and alcohol consumption habits, routine blood tests, and an OGTT. Selection of the present study cohort was based on the absence of newly diagnosed diabetes and the availability of complete sets of clinical and genotype data. Moreover, the participants were not taking any medication known to affect glucose tolerance or insulin secretion. The subject characteristics are given in Table 1. From this cohort, a subset of 274 individuals additionally underwent a frequently sampled intravenous glucose tolerance test (IVGTT).

OGTT and IVGTT

A standard 75-g OGTT was performed after a ten-hour overnight fast, and venous blood samples were drawn at time-points zero, 30, 60, 90, and 120 min for the determination of plasma glucose, insulin, and C-peptide concentrations. In those individuals who agreed to undergo the IVGTT, baseline samples (~10, -,5, and 0 min) were collected before a glucose dose of 0.3 g/kg body weight was given. Blood samples for the measurement of plasma glucose and insulin were obtained at 2, 4, 6, 8, 10, 20, 30, 40, 50, and 60 min.

Laboratory measurements

Plasma glucose was determined using a bedside glucose analyzer (glucose oxidase method, Yellow Springs Instruments, Yellow Springs, OH, USA). Plasma insulin and C-peptide concentrations were measured by commercial chemiluminescence assays for ADVIA Centaur (Siemens Medical Solutions, Fernwald, Germany) according to the manufacturer’s instructions.

Selection of loci/SNPs

From each confirmed type 2 diabetes risk locus previously reported to affect specific aspects of β-cell function, we selected one representative SNP based on the availability of genotype data and on the robustness of the SNP’s β-cell effect in our cohort. As loci/SNPs associated with GSIS, we selected MTNR1B rs10830963 [12,13], HHEX rs7923837 [14,15], and CDKAL1 rs7754840 [16,17]. As loci/SNPs predominantly associated with

| Parameter | Count or Mean ± SD |
|-----------|--------------------|
| Women/men (N) | 661/331 | 83/54 | 95/33 |
| Age (yrs) | 37±12 | 43±13 | 41±13 |
| BMI (kg/m²) | 27.2±6.9 | 31.7±10.2 | 30.1±7.3 |
| Fasting glucose (mmol/l) | 4.90±0.39 | 5.84±0.25 | 5.10±0.30 |
| Glucose 120 min (mmol/l) | 5.61±1.13 | 6.22±0.99 | 8.71±0.76 |
| Fasting insulin (pmol/l) | 53.3±43.1 | 80.5±73.0 | 72.7±52.7 |
| Fasting C-peptide (pmol/l) | 578±252 | 773±389 | 673±296 |
| HOMA-B (U/mmol* | 134±115 | 116±106 | 153±104 |
| Insulin 30 min (pmol/l)* | 465±375 | 507±369 | 530±397 |
| C-Peptide 30 min (pmol/l)* | 1992±841 | 2182±1008 | 2040±910 |
| GI_ (×10⁻⁶)* | 164±229 | 148±131 | 123±93 |
| GI_ (×10⁻⁶)* | 51.5±41.6 | 46.7±36.0 | 50.5±38.4 |
| DI oral (mmol⁻¹)* | 3.67±5.86 | 2.19±1.88 | 1.90±1.19 |
| CIR (l/mmol⁻¹ ×10⁻⁶)* | 1642±1450 | 1141±943 | 1204±866 |
| First-phase insulin secretion (pmol/l)* | 1234±784 | 1247±893 | 1280±865 |
| AUC_{Insulin(0-30)/AUC_{Glucose(0-30)} (×10⁻⁶)*} | 40.0±30.3 | 39.3±28.6 | 45±29.8 |
| AUC_{Insulin(0-120)/AUC_{Glucose(0-120)} (×10⁻⁶)*} | 58.6±42.4 | 63.9±41.3 | 66.9±50.1 |
| AUC_{C-Peptide(0-30)/AUC_{Glucose(0-30)} (×10⁻⁶)*} | 201±77 | 198±85 | 192±75 |
| AUC_{C-Peptide(0-120)/AUC_{Glucose(0-120)} (×10⁻⁶)*} | 324±105 | 322±116 | 289±100 |

*Seventeen subjects with calculated negative values in one or more of the twelve insulin secretion indices tested were excluded (N=1347). AUC – area under the curve; BMI – body mass index; CIR – cleared insulin response; DI – disposition index; HOMA-B – homeostasis model assessment of beta-cell function; IFG – impaired fasting glycaemia; IG – insulinogenic index; IGT – impaired glucose tolerance; NGT – normal glucose tolerance; OGTT – oral glucose tolerance test.

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ISI/secretin release, we selected TCF7L2 rs7903146 [18;19, WFSI rs10010131 [20], and KCNJ11 rs151290 [21]. All SNPs were genotyped in the whole cohort in the course of earlier studies [12;15;17;18;20;21] using TaqMan assays (Applied Biosystems, Foster City, CA, USA) and passed the quality controls. Details on this as well as on minor allele frequencies, genotyping success rates, and Hardy-Weinberg equilibrium are reported in the aforementioned references.

Calculations

Insulin secretion derived from the fasting state was calculated as HOMA-B: 20.05 × ln(I0) / (G0 - 3.5) with I0 = fasting insulin in μU/ml and G0 = fasting glucose in mmol/l [5]. All other insulin secretion indices were derived from the OGTT with insulin and C-peptide concentrations given in pmol/l, and glucose concentration given in mmol/l. AUCs of insulin, C-peptide, and glucose concentrations during the entire 120 min of the OGTT were calculated according to the trapezoid method as: 0.5 × [(I0 + I10) + (c120) + (G30 - 3.89)] [4]. First-phase insulin secretion was calculated as: [I0 – I0] / (G0 – G0) [6]. Insulin sensitivity derived from the OGTT was estimated as proposed by Matsuda and DeFronzo [22]: 10000 / [(G0 × I0) × Gmean]1.5. Fasting insulin clearance was calculated as CP0 / I0 with CP0 = fasting C-peptide, insulin clearance during the OGTT was calculated as AUC C-Peptide / AUC Insulin [3]. Acute insulin response (AIR) derived from the IVGTT was used as gold standard for the assessment of insulin secretion and calculated as: 0.5 × [(I0 + I10) + (I30 + I138.7) + (I88.5 + I138.7)] / [I0 + I10 + I30 + I138.7 + I88.5 + I138.7 + I00].

Statistical analyses

Prior to analysis, all continuous data were log-transformed in order to approximate normal distribution. Multiple linear regression analysis was performed using the least-squares method. In the regression models, the insulin secretion parameter was chosen as dependent variable, the SNP genotype (additive inheritance model) as independent variable, and gender, BMI, and OGTT-derived insulin sensitivity as confounding variables. In addition, the SNP genotype (additive inheritance model) was tested as dependent variable and the insulin secretion parameter as independent variable with inclusion of the aforementioned confounders in the models. Since the critical confounding variables age, BMI, and OGTT-derived insulin sensitivity did not achieve normal distribution even after applying the ladder of powers (probably due to the inclusion/exclusion criteria of our study), we additionally performed linear regression models including these parameters as nominal variables after stratification into quartiles. A p-value ≤ 0.05 was considered statistically significant. Multiple linear regression analyses, post hoc power calculations [statistical power (1-β) and least significant number (lsn; i.e., the sample size expected to be needed to achieve statistical significance) to detect the effect size given by the default settings (square root of the sum of squares for the hypothesis divided by N)], and Wilcoxon rank sum tests were performed using the statistical software package JMP 4.0 (SAS Institute, Cary, NC, USA).

Results

Seventeen subjects with calculated negative values in single insulin secretion measures were excluded from all analyses resulting in a final cohort of 1347 individuals. Since the phenotype (insulin release) is determined by the genotype, we started our analyses using the insulin secretion index as dependent variable and the SNP genotype (additive inheritance model) as independent variable. As expected, all tested loci/SNPs were significantly associated with at least two of the indices after adjustment for the confounding variables gender, age, BMI, and OGTT-derived insulin sensitivity (p ≤ 0.05, Table 2; additional statistical data given in Table S1). Most secretion indices identified three, four, or five of the six tested loci/SNPs to be significantly associated with insulin release, whereas HOMA-B detected MTNR1B rs1030963 only. Inclusion of the confounding parameters age, BMI, and OGTT-derived insulin sensitivity as nominal variables (after stratification into quartiles) in the linear regression models resulted in very similar statistical data (Table S2). After adjustment for gender, age, and BMI, none of the SNPs showed significant association with insulin clearance either in the fasting state (p > 0.1) or during the OGTT (p > 0.06).

To evaluate which indices are most appropriate to detect genetically determined differences in insulin release, we first calculated the post hoc least significant numbers for all associations and converted them into ranks with indices that displayed the lowest least significant number being the best-ranked (Table 2). Then, we summed up the ranks of each insulin secretion index obtained for all the tested SNPs and ranked the indices according to their rank sums (Table 3). Using this approach, AUC Insulin(0-30) / AUC Glucose(0-30) was identified as the best-ranked index (Table 3). Moreover, AUC Insulin(0-30) / AUC Glucose(0-30), CIR, AUC C-Peptide(0-30) / AUC Glucose(0-30), AUC C-Peptide(0-120) / AUC Glucose(0-120), IG1, IG1, and insulin 30 min, but not C-peptide 30 min, first-phase insulin secretion, AUC Insulin(0-120) / AUC Glucose(0-120), or DI oral, were significantly higher-ranked than HOMA-B (p < 0.05; Table 3). To avoid over-adjustment of DI oral, a secretion parameter already normalised for a rough estimate of insulin sensitivity (i.e., fasting insulin), this parameter was also tested in the absence of additional adjustment for OGTT-derived insulin sensitivity. This analysis resulted in a somewhat higher rank sum (57) that, however, had no impact on this index’ overall rank (rank 11). When summing up the ranks of the indices obtained for the three loci/SNPs affecting GSIS, i.e., MTNR1B rs1030963, HHEX rs7923837, and CDKAL1 rs7754840, AUC Insulin(0-30) / AUC Glucose(0-30) again turned out to be the highest-ranked index (Table 3). Notably, when summing up the ranks obtained for the three loci/SNPs predominantly affecting GSIS, i.e., TCF7L2 rs7903146, WFSI rs10010131, and KCNJ11 rs151290, AUC C-Peptide(0-120) / AUC Glucose(0-120) was the best-ranked index (Table 3). In the GSIS and ISIS subgroups, statistical analysis of the rankings was inappropriate due to the small sample sizes. In all rankings, HOMA-B displayed the highest rank sums and, thus, represented the lowest-ranked index. Assessing the SNP genotype (additive inheritance model) as dependent variable and the insulin secretion parameter as independent variable with inclusion of the aforementioned confounders in the multiple regression models yielded very similar rankings (Tables S3 and S4).

Interestingly, the indices that performed best in all these analyses, i.e., AUC Insulin(0-30) / AUC Glucose(0-30) and CIR, also revealed the best correlations with IVGTT-derived AIR (both r = 0.76), and HOMA-B, the lowest ranked index, showed the weakest correlation with AIR (r = 0.64, N = 274; Table 4).

Discussion

In this study, we intended to identify, among twelve fasting state- and common (or recently introduced) OGTT-derived
Table 2. Statistical data of the SNPs’ associations with indices of insulin release and ranking of the indices according to their lsn.

| Parameter                        | MTNR1B rs10830963 | HHEX rs7923837 | CDKAL1 rs7754840 | TCF7L2 rs7903146 | WFS1 rs10010131 | KCNQ1 rs151290 |
|----------------------------------|------------------|---------------|-----------------|-----------------|----------------|----------------|
|                                  | p                | 1-β           | lsn (rank)      | p               | 1-β           | lsn (rank)     |
| HOMA-B                           | <0.0001          | 0.99          | 345 (10)        | 0.9             | 0.07          | 34337 (12)     |
| Insulin 30 min                   | <0.0001          | 1.00          | 196 (5)         | 0.0010          | 0.92          | 587 (11)       |
| C-Peptide 30 min                 | <0.0001          | 1.00          | 241 (8)         | 0.0005          | 0.95          | 536 (9)        |
| IG₁                              | <0.0001          | 1.00          | 201 (6)         | 0.0002          | 0.96          | 483 (6)        |
| IG₂                              | <0.0001          | 1.00          | 164 (2)         | 0.0005          | 0.95          | 527 (8)        |
| DI oral                          | <0.0001          | 1.00          | 288 (9)         | 0.0008          | 0.93          | 568 (10)       |
| CIR                              | <0.0001          | 1.00          | 164 (2)         | 0.0001          | 0.98          | 447 (3)        |
| AUCInsulin0-30/AUCGlucose0-30    | <0.0001          | 1.00          | 153 (1)         | 0.0001          | 0.97          | 456 (4)        |
| AUCInsulin0-120/AUCGlucose0-120  | 0.0002           | 0.97          | 478 (12)        | <0.0001         | 0.99          | 356 (2)        |
| AUCC-Peptide0-30/AUCGlucose0-30  | <0.0001          | 1.00          | 180 (4)         | 0.0003          | 0.96          | 500 (7)        |
| AUCC-Peptide0-120/AUCGlucose0-120| 0.0001           | 0.97          | 453 (11)        | <0.0001         | 1.00          | 338 (1)        |

Seventeen subjects with calculated negative values were excluded (N = 1347). Prior to multiple linear regression analysis, all continuous variables were log-e-transformed to approximate normal distribution. In the multiple linear regression models, the insulin secretion parameter was chosen as dependent variable, the SNP genotype (additive inheritance model) as independent variable and gender, age, BMI, and OGTT-derived insulin sensitivity as confounding variables. AUC – area under the curve; BMI – body mass index; CIR – cleared insulin response; DI – disposition index; HOMA-B – homeostasis model assessment of beta-cell function; IG₁ – insulinogenic index; lsn – least significant number (sample size expected to be needed to achieve statistical significance); SNP – single nucleotide polymorphism.

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measures feasible for genetic studies in large cohorts, the indices best-suited to detect genetically determined alterations of insulin release. Since the suitability of the indices for detection of altered β-cell function may depend on the SNPs’ pathomechanisms, we additionally analysed the SNPs affecting GSIS separately from those affecting the incretin axis (ISIS or incretin release). It was not

The primary aim of this study to evaluate the performance of the fasting state- and OGTT-derived estimates of insulin secretion by comparing them with gold standard measures derived from laborious and expensive methods, such as IVGTT or hyperglycemic clamp.

Using summation of the ranks derived from post hoc least significant numbers, we show here that AUCC = area under the curve; CIR – cleared insulin response; DI – disposition index; GSIS – glucose-stimulated insulin secretion; HOMA-B – homeostasis model assessment of β-cell function; IGI – insulinogenic index; ISIS – incretin-stimulated insulin secretion/incretin production; lsn – least significant number; SNP – single nucleotide polymorphism.

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Table 3. Ranking of the indices of insulin release according to their rank sums.

| Overall ranking (all SNPs tested) | Ranking for detection of GSIS (MTNR1B, HHEX, and CDKAL1 SNPs) | Ranking for detection of ISIS (TCF7L2, WFS1, and KCNQ1 SNPs) |
|-----------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| Rank Parameter                    | Rank sum (from lsn) Rank                                    | Rank Parameter                    | Rank sum (from lsn) Rank                                    |
|-----------------------------------|---------------------------------------------------------------|-----------------------------------|---------------------------------------------------------------|
| 1 AUCC (0-30)/AUC (0-30)          | 24* 1                                                         | 1 AUCC (0-30)/AUC (0-30)          | 7 1                                                         |
| 2 CIR                             | 28* 2                                                         | CIR                               | 10 2                                                         |
| 3 AUCC (0-30)/AUC (0-30)          | 29* 3                                                         | IG2                               | 13 3                                                         |
| 4 AUCC (0-120)/AUC (0-120)        | 29* 4                                                         | First-phase insulin secretion     | 16 4                                                         |
| 5 IG1                             | 33* 5                                                         | Insulin 30 min                    | 17 AUCC (0-30)/AUC (0-30) 17                               |
| 6 IG1                             | 36* 6                                                         | C-Peptide 30 min                  | 19 6                                                         |
| 7 C-Peptide 30 min                | 39 7                                                         | CIR                               | 20 7                                                         |
| 8 Insulin 30 min                  | 42* 8                                                         | AUCC (0-120)/AUC (0-120)          | 23 8                                                         |
| 9 First-phase insulin secretion   | 42 9                                                         | IGI2                              | 24 9                                                         |
| 10 AUCC (0-120)/AUC (0-120)       | 46 10                                                        | Insulin 30 min                    | 25 10                                                        |
| 11 DI oral                        | 55                                                           | C-Peptide 30 min                  | 25 11                                                        |
| 12 HOMA-β                         | 64 12                                                        | HOMA-B                            | 34 HOMA-B                                                    |

*Significantly different from HOMA-B (p < 0.05; Wilcoxon rank sum test). AUC – area under the curve; CIR – cleared insulin response; DI – disposition index; GSIS – glucose-stimulated insulin secretion; HOMA-B – homeostasis model assessment of β-cell function; IGI – insulinogenic index; ISIS – incretin-stimulated insulin secretion/incretin production; lsn – least significant number; SNP – single nucleotide polymorphism.

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Table 4. Association of the fasting- and OGTT-derived indices of insulin release with IVGTT-derived AIR (N = 274).

| AIR  | Parameter       | Rank sum | R  | P  |
|------|-----------------|----------|----|----|
| AUCC (0-30)/AUC (0-30) | 0.76     | <0.0001 |
| CIR  | 0.76            | <0.0001  |
| IG2  | 0.75            | <0.0001  |
| First-phase insulin secretion | 0.74     | <0.0001 |
| IGI1 | 0.72            | <0.0001  |
| AUCC (0-30)/AUC (0-30) | 0.72     | <0.0001 |
| Insulin 30 min | 0.71 | <0.0001 |
| DI oral | 0.70 | <0.0001 |
| AUCC (0-120)/AUC (0-120) | 0.70     | <0.0001 |
| C-Peptide 30 min | 0.68 | <0.0001 |
| AUCC (0-120)/AUC (0-120) | 0.67     | <0.0001 |
| HOMA-β | 0.64 | <0.0001 |

Prior to multiple linear regression analysis, all continuous variables were log-transformed to approximate normal distribution. In the multiple linear regression models, AIR was chosen as dependent variable, the fasting-OGTT-derived insulin secretion index as independent variable and gender, age, BMI, and OGTT-derived insulin sensitivity as confounding variables. AIR – acute insulin response; AUC – area under the curve; CIR – cleared insulin response; DI – disposition index; HOMA-B – homeostasis model assessment of β-cell function; IGI – insulinogenic index; IVGTT – intravenous glucose tolerance test; OGTT – oral glucose tolerance test. doi:10.1371/journal.pone.0014194.t004
derived AIR. In these latter analyses with IVGTT-derived AIR as gold standard, HOMA-B again revealed the weakest correlation and, thus, was confirmed to be less useful for the detection of impaired β-cell function.

A limitation of our study is that the results were generated in a single study population and, thus, clearly need replication in other comparably genotyped and phenotyped cohorts of similar or larger sample size. Furthermore, the ranking of the insulin secretion indices may also depend on the ethnicity. Since our study cohort was nearly exclusively comprised of White European subjects, similar analyses in other ethnicities would be interesting.

Finally, we conclude that, according to our data, AUCCGlucose(0-30)/AUCGlucose(0-30)-along with CIR, AUCC-Peptide(0-30)/AUCGlucose(0-30), AUCC-Peptide(0-120)/AUCGlucose(0-120), IGI2, IGI1, and insulin 30 min, b-determined AUCGlucose. These findings, if replicated in comparably sized and multiple linear regression models, the insulin secretion parameter calculated negative values were excluded (N = 1347). In the quartiles. Given are the p-value, estimate and the standard deviation of the minor allele’s effect. Seventeen subjects with calculated negative values were excluded (N = 1347). Prior to multiple linear regression analysis, all continuous variables were loge-transformed to approximate normal distribution. In the multiple linear regression models, the SNP genotype (additive inheritance model) was chosen as dependent variable, the insulin secretion parameter as independent variable and gender, age, BMI, and OGTT-derived insulin sensitivity as confounding variables. AUC - area under the curve; BMI - body mass index; CIR - cleared insulin response; DI - disposition index; HOMA-B - homeostasis model assessment of beta-cell function; IGI - insulinogenic index; SD - standard deviation; SNP - single nucleotide polymorphism. Found at: doi:10.1371/journal.pone.0014194.s002 (0.07 MB DOC)

Table S3 Statistical data of the SNPs’ associations with indices of insulin release using the genotype as dependent variable. Seventeen subjects with calculated negative values were excluded (N = 1347). Prior to multiple linear regression analysis, all continuous variables were loge-transformed to approximate normal distribution. In the multiple linear regression models, the SNP genotype (additive inheritance model) was chosen as dependent variable, the insulin secretion parameter as independent variable and gender, age, BMI, and OGTT-derived insulin sensitivity as confounding variables. AUC - area under the curve; BMI - body mass index; CIR - cleared insulin response; DI - disposition index; HOMA-B - homeostasis model assessment of beta-cell function; IGI - insulinogenic index; SD - standard deviation; SNP - single nucleotide polymorphism. Found at: doi:10.1371/journal.pone.0014194.s003 (0.08 MB DOC)

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Author Contributions
Conceived and designed the experiments: NS HUH AF. Performed the experiments: SAHS MH CK MG KK FM. Analyzed the data: SAHS HS. Conceived and designed the experiments: NS HUH AF. Performed the experiments: SAHS MH CK MG KK FM. Analyzed the data: SAHS HS. Contributed to discussion: SAHS HS FM NS HUH AF. Reviewed/edited manuscript: NS HUH AF.

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