Elevations in blood glucose before and after the appearance of islet autoantibodies in children

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Supplemental material

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Supplemental Table 1. Visit age and mean non-fasting pre- and post-prandial blood glucose values

| Visit  | N children with glucose measurement | Age at visit; Median (Min – Max) (years) | Mean pre-prandial glucose concentration (mg/dl (mmol/L)) | Mean 30 min. post prandial glucose concentration (mg/dl (mmol/L)) | Mean 60 min. post prandial glucose concentration (mg/dl (mmol/L)) |
|--------|-------------------------------------|-------------------------------------------|------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| Visit 1| 1,038                               | 0.51 (0.33 – 0.59)                        | 92.6 (5.14)                                                | 105.0 (5.83)                                                  | 100.8 (5.60)                                                  |
| Visit 2| 1,014                               | 0.67 (0.48 – 0.80)                        | 90.6 (5.03)                                                | 104.8 (5.82)                                                  | 99.0 (5.50)                                                   |
| Visit 3| 1,003                               | 0.83 (0.64 – 0.99)                        | 89.1 (4.95)                                                | 103.0 (5.72)                                                  | 98.4 (5.47)                                                   |
| Visit 4| 911                                 | 1.16 (0.94 – 1.34)                        | 88.7 (4.93)                                                | 99.6 (5.53)                                                   | 96.9 (5.38)                                                   |
| Visit 5| 775                                 | 1.50 (1.39 – 1.69)                        | 88.1 (4.89)                                                | -                                                            | -                                                            |
| Visit 6| 590                                 | 1.99 (1.75 – 2.21)                        | 89.4 (4.97)                                                | -                                                            | -                                                            |
| Visit 7| 354                                 | 2.49 (2.41 – 2.73)                        | 90.4 (5.02)                                                | -                                                            | -                                                            |
| Visit 8| 194                                 | 3.00 (2.94 – 3.17)                        | 91.4 (5.08)                                                | -                                                            | -                                                            |
| Visit 9| 73                                  | 3.46 (3.39 – 3.60)                        | 92.6 (5.14)                                                | -                                                            | -                                                            |
Supplemental Table 2. SNPs and their respective weights used to calculate the glucose GRS

| SNP          | Risk allele | Nearest gene | Allele Weight |
|--------------|-------------|--------------|---------------|
| Rs340874     | C           | PROXI        | 0.439         |
| Rs11071657   | A           | FAM148B      | 0.270         |
| Rs11558471   | A           | SLC30A8      | 0.911         |
| Rs4506565    | T           | TCF7L2       | 0.885         |
| Rs560887     | C           | G6PC2        | 2.532         |
| Rs10830963   | G           | MTNR1B       | 2.262         |
| Rs4607517    | A           | GCK          | 2.093         |
| Rs2191349    | T           | DGKB/TMEM195 | 1.013         |
| Rs780094     | C           | GCKR         | 0.979         |
| Rs11708067   | A           | ADCY5        | 0.911         |
| Rs7944584    | A           | MADD         | 0.709         |
| Rs10885122   | G           | ADRA2A       | 0.743         |
| Rs174550     | T           | FADS1        | 0.574         |
| Rs11605924   | A           | CRY2         | 0.506         |
| Rs11920090   | T           | SLC2A2       | 0.675         |
| Rs7034200    | A           | GLIS3        | 0.608         |

SNP, single nucleotide polymorphism; GRS, genetic risk score
Table 3. Multivariate linear regression analysis (site-harmonized glucose measurements, adjusted for visit age)

| Variable               | Visit 1 – Visit 4 (age 4 months – 1.35 years) | Visit 5 – Visit 7 (age 1.4 years – 2.7 years) |
|------------------------|----------------------------------------------|---------------------------------------------|
|                        | N observations | N children | Estimate [95% CI] | P-value | N observations | N children | Estimate [95% CI] | P-value |
| **Gender**             |                |            |                  |         |                |            |                  |         |
| Female                 | 1,419          | 373        | Reference        | -       | 619            | 285        | Not included     | -       |
| Male                   | 1,416          | 378        | 1.2 [0.3; 2.1]   | 0.0099  | 563            | 274        |                  |         |
| **BMI**                | 2,835          | 751        | 0.4 [0.1; 0.7]   | 0.0029  | 1,182          | 559        | Not included     | -       |
| **Glucose GRS**        | 2,835          | 751        | 0.2 [0.1; 0.4]   | 0.0016  | 1,182          | 559        | 0.4 [0.2; 0.7]   | 0.0011  |
| **Any islet autoantibodies** |                |            |                  |         |                |            |                  |         |
| No                     | 2,662          | 706        | Not included     | -       | 1,182          | 559        |                  |         |
| Yes                    | 173            | 45         |                  |         | 77             | 38         | 4.30 [1.2; 7.2]  | 0.006   |
| **INS genotype**       | 2,835          | 751        |                  |         | 1,182          | 559        | Not included     | -       |
| AA                     | 1,603          | 425        | -1.4 [-2.3; -0.5]| 0.0017  | 652            | 317        |                  |         |
| other                  | 1,232          | 326        | Reference        |         | 530            | 242        |                  |         |

BMI Body Mass Index, GRS Genetic Risk Score, INS Insulin
Supplemental Table 4. Multivariate linear regression analyses (site-harmonized glucose measurements, adjusted for visit age) of post prandial glucose values at 30 min after food intake

| Variable                          | N observations | N children | Estimate (95% CI) | P-value |
|-----------------------------------|----------------|------------|-------------------|---------|
| Sex                               | 2,544          | 747        |                   |         |
| Female                            | 1,249          | 370        | Reference         | –       |
| Male                              | 1,295          | 377        | 2.4 (1.1; 3.6)    | 0.0002  |
| **Glucose GRS**                   | 2,544          | 747        | 0.3 (0.1; 0.5)    | 0.003   |
| Any islet autoantibodies          | 2,544          | 747        |                   |         |
| No                                | 2,429          | 715        | Reference         |         |
| Yes                               | 115            | 32         | 5.4 (2.4; 8.4)    | 0.0004  |

CI, confidence interval; GRS, genetic risk score;
Supplemental Table 5. Univariate linear regression analyses (site-harmonized pre-prandial glucose measurements, adjusted for visit age): sensitivity analysis excluding data from UK and Sweden.

| Variable                                      | Visit 1 – Visit 4 | Visit 5 – Visit 9* |
|-----------------------------------------------|-------------------|-------------------|
|                                               | N observations    | N children | Estimate (95% CI) | P-value | N observations | N children | Estimate (95% CI) | P-value |
| **Sex**                                       |                   |            |                  |         |                |            |                  |         |
| Female                                        | 3,124             | 826       | Reference        | –       | 1,609          | 620        | Reference         | –       |
| Male                                          | 1,566             | 412       | 1.4 (0.7; 2.1)   | <0.0001 | 762            | 306        | 1.1 (–0.2; 2.4)   | 0.085   |
| **First degree relative with T1D**            |                   |            |                  |         |                |            |                  |         |
| No                                            | 1,348             | 355       | Reference        | –       | 612            | 258        | Reference         | –       |
| Mother                                        | 753               | 199       | 0.7 (–0.2; 1.6)  | 0.14    | 427            | 156        | 0.9 (–0.7; 2.5)   | 0.27    |
| Other                                         | 1,023             | 272       | 1.0 (0.2; 1.9)   | 0.012   | 570            | 206        | –0.8 (–2.3; 0.7)  | 0.28    |
| **BMI**                                       | 3,121             | 826       | 0.6 (0.4; 0.8)   | <0.0001 | 1,605          | 620        | 0.8 (0.3; 1.2)    | 0.00051 |
| **Glucose GRS**                               | 2,233             | 588       | 0.2 (0.1; 0.3)   | 0.0040  | 1,069          | 433        | 0.4 (0.1; 0.6)    | 0.0066  |
| **Any islet autoantibodies**                  | 3,124             | 826       | Reference        | –       | 1,360          | 620        | Reference         | –       |
| No                                            | 2,882             | 763       | Reference        | –       | 1,235          | 566        | Reference         | –       |
| Yes                                           | 242               | 63        | –0.1 (–1.4; 1.3) | 0.91    | 125            | 54         | 2.2 (–0.1; 4.5)   | 0.059   |
| **Multiple islet autoantibodies**             | 3,124             | 826       | Reference        | –       | 1,360          | 620        | Reference         | –       |
| No                                            | 2,952             | 781       | Reference        | –       | 1,269          | 583        | Reference         | –       |
| Yes                                           | 172               | 45        | 0.1 (–1.5; 1.6)  | 0.93    | 91             | 37         | 3.0 (0.3; 5.7)    | 0.028   |
| **INS genotype**                              | 3,124             | 826       | –1.1 (–1.8; –0.4)| 0.0024  | 1,609          | 796        | 0.2 (–1.1; 1.5)   | 0.77    |
| AA                                            | 1,756             | 463       | Reference        | –       | 892            | 352        | Reference         | –       |
| other                                         | 1,368             | 363       | Reference        | –       | 717            | 268        | Reference         | –       |

CI, confidence interval; T1D, type 1 diabetes; BMI, body mass index; GRS, genetic risk score; INS, Insulin gene
Supplemental Table 6. Univariate linear regression analyses (site-harmonized glucose measurements, adjusted for visit age): sensitivity analysis using pre-prandial glucose values at visit 1 only

| Variable                                | N observations | Estimate (95% CI) | P-value |
|------------------------------------------|----------------|-------------------|---------|
| **Visit 1 (Age 4 – 7 months)**           |                |                   |         |
| **Sex**                                  | 1,038          |                   |         |
| Female                                   | 511            | Reference         | –       |
| Male                                     | 527            | 1.8 (0.4; 3.2)    | 0.013   |
| **First degree relative with T1D**       | 1,038          |                   |         |
| No                                       | 489            | Reference         | –       |
| Mother                                   | 231            | −0.73 (−2.6; 1.1) | 0.44    |
| Other                                    | 318            | 0.05 (−1.6; 1.7)  | 0.94    |
| **BMI**                                  | 1,038          | 1.1 (0.7; 1.5)    | <0.0001 |
| **Glucose GRS**                          | 743            | 0.2 (−0.1; 0.5)   | 0.17    |
| **Any islet autoantibodies**             | 1,038          |                   |         |
| No                                       | 966            | Reference         |         |
| Yes                                      | 72             | −0.2 (−3.1; 2.6)  | 0.88    |
| **Multiple islet autoantibodies**        | 1,038          |                   |         |
| No                                       | 985            | Reference         |         |
| Yes                                      | 53             | 0.5 (−2.7; 3.8)   | 0.76    |
| **INS genotype**                         | 1,038          |                   |         |
| AA                                       | 580            | −2.3 (−3.8; −0.9) | 0.0016  |
| other                                    | 458            | Reference         |         |

CI, confidence interval; T1D, type 1 diabetes; BMI, body mass index; GRS, genetic risk score; INS, Insulin gene
**Supplemental Table 7:** List of SNPs used to determine study eligibility and risk score calculation

| SNP        | Gene, Allele, or Haplotype                      | Score weight for genotype or per allele |
|------------|-----------------------------------------------|----------------------------------------|
| rs17426593 | HLA DR4-DQ8/DR4-DQ8                            | 3.15                                   |
| rs2187668  |                                               | 3.98                                   |
| rs7454108  | HLA DR3/DR4-DQ8                               |                                        |
| rs3129889  | HLA DRB1*1501                                 | Exclusion criteria for first degree relatives |
| rs1794265  | HLA DQB1*0503                                 | Exclusion criteria for first degree relatives |
| **HLA class I** |                                     |                                        |
| rs1264813  | HLA A 24                                      | 0.43                                   |
| rs2395029  | HLA B 5701                                    | 0.92                                   |
| **Non-HLA SNPs** |                                   |                                        |
| rs2476601  | PTPN22                                        | 0.76                                   |
| rs2816316  | RGS1                                          | 0.16                                   |
| rs3024505  | IL10                                          | 0.22                                   |
| rs1990760  | IFIH1                                         | 0.16                                   |
| rs3087243  | CTLA4                                         | 0.16                                   |
| rs10517086 | C4orf52                                       | 0.19                                   |
| rs2069763  | IL2                                           | 0.11                                   |
| rs6897932  | IL7R                                          | 0.19                                   |
| rs3757247  | BACH2                                         | 0.19                                   |
| rs9388489  | C6orf173                                      | 0.14                                   |
| rs6920220  | TNFAIP3                                       | 0.15                                   |
| rs1738074  | TAGAP                                         | 0.05                                   |
| rs7804356  | SCAP2                                         | 0.15                                   |
| rs4948088  | COBL                                          | 0.17                                   |
| rs7020673  | GLIS3                                         | 0.23                                   |
| rs12722495 | IL2RA                                         | 0.47                                   |
| rs947474   | PRKCA                                         | 0.15                                   |
| rs10509540 | RNLS/C10orf59                                 | 0.25                                   |
| rs689      | INS                                           | 0.65                                   |
| rs4763879  | CD69                                          | 0.06                                   |
| rs2292239  | ERBB3                                         | 0.36                                   |
| rs3184504  | SH2B3                                         | 0.24                                   |
| rs1465788  | ZFP36L1                                       | 0.13                                   |
| rs17574546 | RASGRP1                                       | 0.13                                   |
The risk score is calculated by multiplying the number of risk alleles (i.e., 0, 1 or 2 for each single SNP) with the weight assigned to each SNP and then summing up the weighted contributions of all SNPs plus an additive constant of 3.15 for infants who have the HLA DR4-DQ8/DR4-DQ8 genotype or 3.98 for infants who have the HLA DR3/DR4-DQ8 genotype. As an example, the risk score for a child with HLA DR4-DQ8/DR4-DQ8, homozygous for the risk allele of rs1264813 (weight 0.43), heterozygous for the risk allele of rs2395029 (weight 0.92), homozygous for the non-risk allele of rs2476601 (weight 0.76) and for all other SNPs in the genetic risk score is calculated as follows:

Risk score = 3.15 + (2 * 0.43) + (1 * 0.92) + (0 * 0.76) + 0 = 4.93
Supplemental Figure 1. Histogram showing the distribution of the glucose genetic risk score (count of measurements for each unit of score).
Supplemental Figure 2. Linear regression of pre-prandial glucose values by visit age in the infancy period (visits 1–4; 4 months–1.35 years of age) according to the child’s first-degree relative status: none (blue), mother (green), and other (red).
Supplemental Figure 3. Linear regression of post-prandial glucose values (60 min after food intake) by visit age in the infancy period (visits 1–4; 4 months–1.35 years of age) for islet autoantibody-positive (blue) and islet autoantibody-negative (red) children.
Supplemental Figure 4. Schematic of the method used to calculate the differences in glucose values in autoantibody positive children (blue points) from the linear regression curves of glucose values in autoantibody negative children (red)
Supplemental Acknowledgements

**GPPAD Coordinating Center:** Melanie Gündert, Florian Haupt, Stefanie Arnolds, Miriam Bißbort, Karina Blasius, Nadine Friedl, Cigdem Sanverdi, Gertrud Göppel, Martin Heigermoser, Bianca Höfelschweiger, Manja Jolink, Krisztian Kisfügedi, Nadine Klein, Ramona Lickert, Claudia Matzke, Kim Muñoz Alvarez, Rebecca Niewöhner, Marlon Scholz, Katharina Schütte-Borkovec, Franziska Voß, Andreas Weiβ, José Maria Zapardiel Gonzalo, Sarah Schmidt, Philipp Sifft, Heidi Kapfelsberger, Merve Vurucu, Katharina Sarcletti, Melanie Sporreiter, Stefanie Jacobsen, and Ivo Zeller.

**Belgium Clinical Center:** Kristina Casteels, Charlien Jannsen, Anre Rochtus, An Jacobs, Hilde Morobé, Jasmin Paulus, Brontë Vrancken, Natalie Van den Driessche, Renka Van Heyste, Janne Houben, Leyla Smets, and Veerle Vanhuyse.

**Germany, Dresden Clinical Center:** Ezio Bonifacio, Reinhard Berner, Sari Arabi, Ruth Blechschmidt, Sevina Dietz, Franziska Ehrlich, Gita Gemulla, Zahra Gholizadeh, Sophie Heinke, Raphael Hoffmann, Angela Hommel, Franziska Andrea Lange, Anja Loff, Robert Morgenstern, Anett Piller, Marc Weigelt, Marie Zielmann, and Nicole Zubizarreta.

**Germany, Hanover Clinical Center:** Olga Kordonouri, Thomas Danne, Laura Galuschka, Ute Holtkamp, Nils Janzen, Carolin Kruse, Sarah Landsberg, Karin Lange, Erika Marquardt, Felix Reschke, Frank Roloff, Kerstin Semler, Thekla von dem Berge, and Jantje Weiskorn.

**Germany, Munich Clinical Center:** Anette G. Ziegler, Peter Achenbach, Melanie Bunk, Simone Färber-Meisterjahn, Willi Grätz, Ines Greif, Melanie Herbst, Anna Hofelich, Melina Kaiser, Heidi Kaltenecker, Esra Karapinar, Annika Kölln, Benjamin Marcus, Annette Munzinger, Jasmin Ohli, Claudia Ramminger, Franziska Reinmüller, Veronika Vollmuth, Tiziana Welzhofer, and Christiane Winkler.
Poland Clinical Center: Agnieszka Szypowska, Mariusz Ołtarzewski, Sylwia Dybkowska, Katarzyna Dżygało, Lidia Groele, Katarzyna Kajak, Dorota Owczarek, Katarzyna Piechowiak, Katarzyna Popko, Agnieszka Skrobot, Rafał Szpakowski, Anna Taczanowska, Beata Zduńczyk, and Anna Zych.

Sweden Clinical Center: Helena Elding Larsson, Markus Lundgren, Åke Lernmark, Daniel Agardh, Hanna Samuelsson, Sofie Alstrom Mortin, Carin Andrén Aronsson, Rasmus Bennet, Charlotte Brundin, Susanne Dahlberg, Lina Fransson, Ida Jönsson, Sara Maroufkhani, Zeliha Mestan, Caroline Nilsson, Anita Ramelius, Evelyn Tekum Amboh, Carina Törn, and Ulrika Ulvenhag.

UK, Oxford Clinical Center: Matthew Snape, John A Todd, Genevieve Haddock, Owen Bendor-Samuel, James Bland, Edward Choi, Rachel Craik, Kimberly Davis, Sophia Hawkins, Arancha de la Horra, Yama Farooq, Clare Scudder, Ian Smith, Fenella Roseman, Hannah Robinson, Nazia Taj, Manu Vatish, Louise Willis, Conor Whelan, and Tabitha Wishlade.

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