



# Investigating optimal $\beta$ -cell-preserving treatment in latent autoimmune diabetes in adults: Results from a 21-month randomized trial

Ingrid K. Hals PhD<sup>1,2</sup> | Hanne Fiskvik Fleiner PhD<sup>3</sup> | Nina Reimers MD<sup>1</sup> |  
Marianne C. Astor MD<sup>4</sup> | Karin Filipsson MD<sup>5</sup> | Zuheng Ma PhD<sup>6</sup> |  
Valdemar Grill MD<sup>2</sup> | Anneli Björklund MD<sup>6</sup>

<sup>1</sup>Department of Endocrinology, St Olavs Hospital, Trondheim University Hospital, Trondheim, Norway

<sup>2</sup>Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

<sup>3</sup>Department of Clinical Pharmacology, St Olavs Hospital, Trondheim University Hospital, Trondheim, Norway

<sup>4</sup>Department of Medicine, Haukeland University Hospital, Bergen, Norway

<sup>5</sup>Department of Clinical Sciences, Lund University, Malmö, Sweden

<sup>6</sup>Department of Molecular Medicine and Surgery, Endocrine and Diabetes Unit, Karolinska University Hospital, Stockholm, Sweden

## Correspondence

Ingrid K Hals, PhD, Norwegian University of Science and Technology (NTNU), Department of Clinical and Molecular Medicine, Gastroenter 3rd Floor, Prinsesse Kristinas Gt 1, 7030 Trondheim, Norway.  
Email: ingrid.hals@ntnu.no

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## Abstract

**Aims:** To compare outcomes of glucagon-stimulated C-peptide tests (GSCTs) in people with latent autoimmune diabetes in adults (LADA) after a 21-month intervention with either insulin or the dipeptidyl peptidase-4 inhibitor sitagliptin.

**Research design and methods:** We included 64 glutamic acid decarboxylase (GAD) antibody-positive individuals, who were diagnosed with diabetes <3 years before the study, aged 30 to 70 years, and without clinical need for insulin treatment. We stratified participants by age and body mass index (BMI) and evaluated  $\beta$ -cell function by GSCT after a 48-hour temporary withdrawal of study medication.

**Results:** Age at randomization (mean 53 years), BMI (mean 27 kg/m<sup>2</sup>) and metabolic markers were similar between treatment arms. Glycated haemoglobin concentrations during intervention did not differ between arms. Fasting C-peptide concentrations after the intervention were similar, as were stimulated C-peptide levels (0.82  $\pm$  0.63 nmol/L after insulin, 0.82  $\pm$  0.46 nmol/L after sitagliptin; nonsignificant). Autoimmunity in the study population (estimated from GAD antibody titres and positivity/no positivity for zinc transporter 8 and islet antigen 2 antibodies) affected the evolution of the GSCT results significantly, which deteriorated in participants with high but not in those with low autoimmunity. Adjustment using analysis of covariance for the degree of autoimmunity did not alter the findings of no difference between treatment arms.

**Conclusions:**  $\beta$ -cell function after intervention was similar in patients with insulin- and sitagliptin-treated LADA, regardless of the strength of autoimmunity. Further, participants with low levels of GAD antibodies did not experience progressive deterioration of  $\beta$ -cell function over a 21-month period. Taken together, these findings could be useful for clinicians' choices of treatment in people with LADA.

Valdemar Grill and Anneli Björklund contributed equally to this study.

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**KEYWORDS**

clinical trial, insulin therapy, LADA, latent autoimmune diabetes in adults, randomized trial, sitagliptin,  $\beta$ -cell function

## 1 | INTRODUCTION

Latent autoimmune diabetes in adults (LADA) is usually diagnosed according to the following criteria: onset of diabetes above the age of 30 years; presence of  $\beta$ -cell directed antibodies, and mostly glutamic acid decarboxylase (GAD) antibodies; and no clinical need for insulin during the first 6 months after the diagnosis of diabetes. Using these criteria, LADA is a common form of diabetes, at least in populations in Europe.<sup>1</sup> People with LADA make up one-tenth of the total population with diabetes in many countries, and the condition may be more common than insulin-requiring type 1 diabetes<sup>2</sup>; treatment of LADA is therefore an important clinical issue.

Deficiency of  $\beta$  cells progresses faster in many people with LADA than in those with type 2 diabetes, presumably because of ongoing autoimmune assault in LADA.<sup>3</sup> The deficiency leads to insulin dependence, which occurs on average earlier in LADA than in type 2 diabetes.<sup>4</sup>  $\beta$ -cell deficiency to the point of insulin dependence is associated with poor metabolic control and diabetic complications,<sup>5–7</sup> which may be worse in LADA than in type 2 diabetes.<sup>8</sup> There is thus an urgent need for a therapy that retards  $\beta$ -cell demise; however, randomized studies on treatment for LADA are scarce and provide insufficient evidence with which to decide on the optimal treatment.<sup>9,10</sup>

In particular, there is ongoing debate regarding whether to treat LADA similarly to type 2 diabetes, with per-oral agents or with insulin (ie, before insulin is clinically needed). Evidence favouring early insulin treatment comes to some extent from pre-clinical data,<sup>11</sup> but mainly from a Japanese randomized study which found that insulin treatment retarded the progression of  $\beta$ -cell insufficiency as compared to anti-diabetic sulphonylureas (SUs).<sup>12</sup> That study has been criticized, however, for the use of an SU as a comparator, since SU has been shown in the long term to hasten  $\beta$ -cell deficiency in type 2 diabetes<sup>13</sup> and to exert  $\beta$ -cell toxicity in vitro.<sup>14</sup> A Cochrane review<sup>9</sup> concluded that randomized treatment studies without SUs in LADA are needed, and especially studies rigorously designed to assess a possible benefit of early insulin treatment.<sup>9</sup>

Against this background we designed a randomized study comparing the impact of early insulin treatment with a dipeptidyl peptidase-4 (DPP-4) inhibitor, sitagliptin, which prolongs the effect of the endogenous incretin hormones glucagon-like peptide-1 and gastric inhibitory polypeptide, thereby stimulating insulin secretion. Sitagliptin was chosen as comparator because it is a currently favoured drug for the treatment of type 2 diabetes, and, in contrast to SUs, has no recognized deteriorating effects on  $\beta$  cells in type 2 diabetes and has even been reported to exert beneficial effects in people with LADA.<sup>15</sup>

In Norway and Sweden, LADA populations are mostly overweight or obese, with ensuing insulin resistance. We therefore designed arms of the study to be add-ons to treatment with metformin, which is an insulin-sensitizer. We restricted recruitment to people who had neither near-optimal glucose control nor very poor glucose control, necessitating more intense pharmacological treatment. In this way we sought to avoid either over- or undertreating participants according to commonly accepted goals of treatment. After randomization we aimed for the metabolic control to be similar between treatment arms, thereby minimizing any influence of “glucotoxicity” on measures of  $\beta$ -cell function.

## 2 | RESEARCH DESIGN AND METHODS

### 2.1 | Inclusion and exclusion criteria

Men and women, aged 30 to 75 years, positive for GAD antibodies with <3 years of known diabetes, without pharmacological treatment for diabetes (except metformin) and with no clinical need for insulin treatment were eligible for the study. Glycated haemoglobin (HbA1c) concentration had to be at least 10% above the upper limit of normal (ULN) before treatment, or 5% above the ULN when on treatment with metformin, but not exceeding 60% above the ULN at the time of randomization. Fasting levels of C-peptide had to be  $\geq 0.3$  nmol/L.

Exclusion criteria were kidney failure (creatinine  $>150$   $\mu\text{mol/L}$ ), proliferative retinopathy with or without sequelae, myocardial infarction (within the last 6 months), unstable angina pectoris and other serious chronic diseases (such as glucocorticoid-treated asthma). We also excluded fertile women who planned to become pregnant during the study period.

### 2.2 | Recruitment and randomization

Many people were recruited after contact with their general practitioner upon receiving information of GAD antibody positivity. The treating doctor would first ask a potential participant whether he or she would accept being approached by study personnel for information on the study and possible participation. Other participants were recruited through screening of GAD antibodies (performed by the principal investigators) in health centres or through referral from general practitioners to hospital clinics in the study. The recruitment period took place between 2010 and 2016.

Recruited participants initiated metformin tablets, if they were not already receiving metformin. The dosage was increased during 1 to 2 months of a 3-month run-in period, aiming at 2 g/d. Participants who could not tolerate this dosage were treated continuously with a

lower dosage. Participants to be randomized after the run-in period were examined by a doctor and a nurse. The participants reported to a study centre in the morning after an overnight fast. Anthropometric measurements were carried out. Blood pressure was measured, and blood samples taken according to the study protocol. Participants were then randomized, non-blinded, into two arms of add-on medications to metformin, using a centralized randomization database at St Olavs University Hospital (Trondheim, Norway). One arm received add-on injections of insulin (insulin arm), the other the comparator sitagliptin (sitagliptin arm). Participant were stratified by age ( $\leq 53$  years or  $> 53$  years) and body mass index (BMI;  $\leq 26$  kg/m<sup>2</sup> or  $> 26$  kg/m<sup>2</sup>).

### 2.3 | Interventions

The interventions (insulin or sitagliptin) lasted 21 months. In Norway, study visits took place at Trondheim (St Olavs Hospital), at Namsos (Namsos Hospital) and at Bergen (Haukeland, Bergen University Hospital) and, in Sweden, at Stockholm (Karolinska University Hospital) and at Malmö (Skåne University Hospital).

Insulin (Insulatard<sup>®</sup>, a NPH-based insulin, Novo Nordisk, Copenhagen, Denmark) was injected subcutaneously at bedtime. Sitagliptin tablets (Januvia<sup>®</sup>, Merck, Sharp and Dome, Haarlem, the Netherlands) were given orally 100 mg/d. Treatments were adjusted by algorithms based on HbA1c and fasting glucose. If necessary, meal-time insulin was added in the insulin arm. In the sitagliptin arm, repaglinid (a stimulator of insulin release via mechanisms other than DPP-4 inhibitors) could be added as a "rescue" drug.

The primary endpoint in the trial was  $\beta$ -cell function, evaluated using glucagon-stimulated C-peptide tests (GSCTs) performed at baseline (ie, at randomization) and after 3, 9 and 21 months. Secondary outcomes were effects on insulin, proinsulin and proinsulin to C-peptide ratios. Definitions of the C-peptide variables are given in Table 1. Other design features are summarized in Table S1 in File S1.

### 2.4 | Measurements

An enzyme-linked immunosorbent assay (ELISA) was used to measure GAD antibodies (positive test if  $> 5$  UI/mL, measurement range

1-250 UI/mL) in Trondheim, Stockholm and Malmö, immunoprecipitation using translation labelled <sup>3</sup>H-GAD65 (positive test if  $\geq 0.08$  antibody index) was used in Oslo, and radioimmunoassay (RIA) for the determination of GAD antibodies in serum (positive test if  $> 0.9$  U/mL and measurement range of 0.1-300 U/mL) was used in Bergen. Antibodies against zinc transporter 8 (ZnT8) and islet antigen 2 (IA-2) were measured by ELISA (RSR Limited, Pontprennau, UK; range 10-2000 U/mL and 7.5-4000 U/mL, respectively). The thresholds used for positivity were 30 U/mL and 15 U/mL, respectively.

Blood pressure and body weight were measured at each study visit. Blood samples were collected in the overnight fasted state after a 48-hour withdrawal of study medicines (but not of metformin). HbA1c and fasting blood glucose were measured at each visit. A GSCT was performed by collecting blood samples before and 6 minutes after an intravenous injection of 0.5 mg glucagon. Secured serum samples were kept at  $-80^{\circ}\text{C}$ , pending measurements of antibodies, C-peptide, insulin and proinsulin. Levels of hormones were analysed by RIA (Millipore, Billerica, Massachusetts). According to the manufacturer, intra-assay variations (coefficient of variation [CV]) were within 1.5% to 6.9% for all three RIA kits.

Updated homeostatic model assessment (HOMA2) was used to measure insulin resistance (IR) based on levels of fasting C-peptide and fasting glucose. Measurements were made according to the calculator available at <https://www.dtu.ox.ac.uk/homacalculator/index.php>.

### 2.5 | Statistics

Analyses were performed using IBM SPSS Statistics 24. Results are presented as values at each time point and as changes from baseline. Significance testing was restricted to changes from baseline to the end of intervention (ie, at 21 months). Mann-Whitney *U* tests were used to assess GSCT results and other differences between treatment arms. We did not correct for multiple testing. Analysis of covariance was used to test for possible interactions between categories of autoimmunity and treatment (insulin or sitagliptin) on GSCT as well as for differences in GSCTs among the categories of autoimmunity, defined (arbitrarily) as follows: **low**: low levels of GAD antibodies and no positivity for other antibodies (ZnT8 and/or IA-2 antibodies); **middle**: low levels of GAD antibodies and positivity for other antibodies, or high levels of GAD antibodies and no positivity for other antibodies; and **high**: high levels of GAD antibodies and positivity for other antibodies. Low/high levels of GAD antibodies were defined as levels below/above medians of positive samples recorded at each measuring laboratory. Normality tests were performed by inspection of Q-Q plots and histograms of standardized residuals. Assessment of differences in GSCTs among the three categories of autoimmunity was repeated in a Kruskal-Wallis test.

### 2.6 | Power calculation

The calculation of power (made before the present study) was based on a study in people with type 2 diabetes.<sup>16</sup> The intra-individual CV

**TABLE 1** Definitions of C-peptide variables

Variable	Definition
Fasting C-peptide	Levels (nmol/L) after overnight fasting. Following a 48-hour withdrawal of study medicine at 3-, 9- and 21-month visits.
Stimulated C-peptide	Levels (nmol/L) at 6 minutes after injection of glucagon
Incremental C-peptide	Difference (in nmol/L) between fasting and stimulated levels of C-peptide
Proinsulin to C-peptide ratio	Ratio (in percent) between levels of stimulated proinsulin (pmol/L) and stimulated C-peptide (pmol/L)

between C-peptide glucagon tests was then 24.5%. Based on this CV, 52 participants would be needed to detect a 20% difference between treatments with a certainty of 80% at a *P* value of <.05.

## 2.7 | Study ethics and registration

The trial was registered at ClinicalTrials.gov (identifier: NCT01140438) and was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and approved by ethics committees in Norway and Sweden. Informed consent was obtained from all participants.

## 3 | RESULTS

### 3.1 | Characteristics of the study population

A total of 64 participants were randomized. In Norway, 25 were followed up in Trondheim, one in Namsos and five in Bergen. In Sweden, 32 were followed up in Stockholm and one in Malmö. The mean BMI at baseline was in the overweight category and metabolic control (HbA1c) was intermediate (as dictated by the study protocol; Table 2). Other baseline characteristics are given in Figure 1 (antibody

**TABLE 2** Characteristics of the study population

	Baseline values at the time of randomization		
	Total	Insulin arm	Sitagliptin arm
Women/men	29/35	15/17	14/18
Study medication	64	32	32
Age at randomization, years, median (IQR)	53 (45-60)	53 (46-58)	53 (43-61)
Age at randomization, range, years	31-70	33-69	31-70
Age at diabetes diagnosis, years, median (IQR)	53 (44-58)	53 (45-57)	52 (42-60)
Time, diagnosis to randomization, months, median (IQR)	9 (5-17)	9 (4-16)	11 (5-20)
BMI, kg/m <sup>2</sup> , mean ± SD	26.8 ± 5.1	26.9 ± 5.4	26.8 ± 4.8
BMI, kg/m <sup>2</sup> , range	18-45	18-45	18-36
Fasting C-peptide, nmol/L, mean ± SD	0.6 ± 0.4	0.7 ± 0.4	0.6 ± 0.3
Hb1Ac, mmol/mol, mean ± SD	51 ± 8.0	51 ± 7.0	51 ± 9.0
Systolic blood pressure, mmHg, mean ± SD	132 ± 14	133 ± 16	131 ± 12
Diastolic blood pressure, mmHg, mean ± SD	79 ± 10	80 ± 10	79 ± 7
Autoimmunity category, low/middle/high	16/27/21	9/14/9	7/13/12

Abbreviations: BMI, body mass index; HbA1c, glycated haemoglobin; IQR, interquartile range (25th and 75th percentile).

positivity) and in Table S2 in File S1 (clinical data) and Table S3 in File S1 (metformin dosage).

### 3.2 | Dropouts and data management

The accumulated dropout was 9.4%. Details are given in the Supporting Information. Results of GSCTs from the dropouts at 3 to 9 months were carried forward according to the principle of intention-to-treat. Significance testing without these data carried forward did not change the study results.

### 3.3 | Study medications: dose adjustments during intervention and side effects

Participants in the insulin arm moderately increased their dosage of insulin during the intervention period (after 21 months: median + 6 U at bedtime). Twelve of the participants in the insulin arm developed a need for insulin at meals and 10 in the sitagliptin arm needed rescue addition of repaglinid (Table S4 in File S1).

Three participants in the insulin arm experienced hypoglycaemia; two participants experienced a single episode and one experienced several episodes of severe hypoglycaemia.

### 3.4 | Weight evolution

Sitagliptin-treated participants reduced their body weight compared to baseline (after 21 months: mean −3.4 kg). By contrast, those treated with insulin increased their body weight (mean +1.9 kg). Differences in weight evolution between treatments were significant (*P* = .001 after 21 months; Table S5 in File S1).

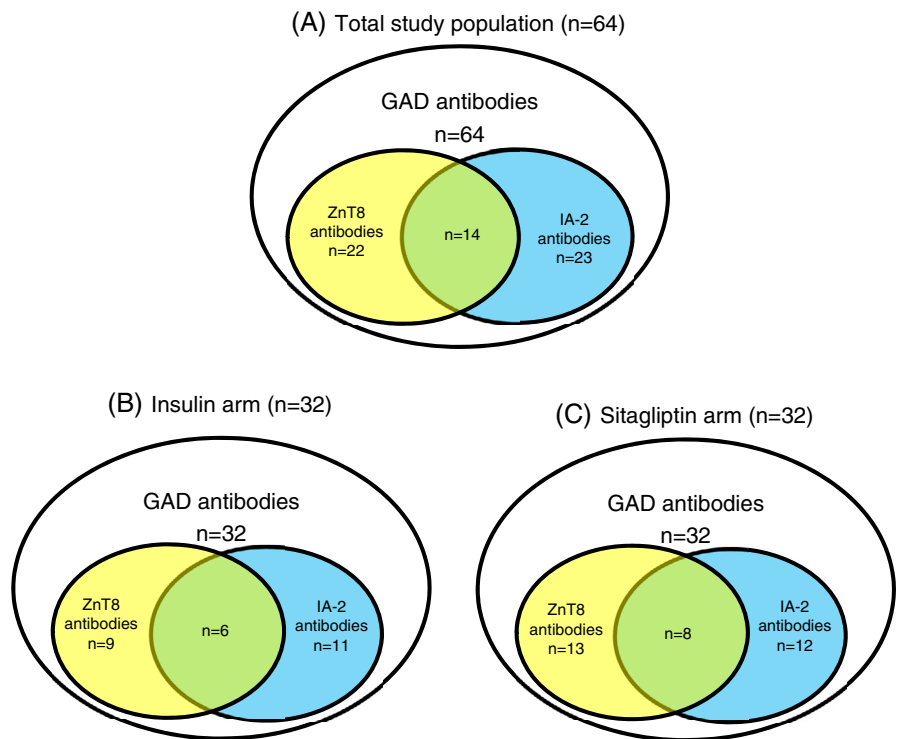
### 3.5 | Homeostatic model assessment of insulin resistance

Treatments did not influence HOMA2-IR (after 21 months: 1.77 in the insulin arm, 1.58 in the sitagliptin arm, both unchanged from levels at baseline: 1.74 and 1.52, respectively).

### 3.6 | Antibodies

A minority of participants (13/64) changed GAD antibody titre after 21 months vs baseline (defined as >15% increase or decrease in titre). There was no obvious difference in this regard between treatment arms (Table S6 in File S1). Forty-five of the 64 patients were positive for ZnT8 and/or IA-2 antibodies at baseline (Figure 1). Levels of ZnT8 antibodies decreased with time for six out of nine participants in the insulin arm and in eight out of 13 in the sitagliptin arm. Levels of IA-2 antibodies decreased in five out of 11 participants in the insulin arm and in seven out of 12 participants in the sitagliptin arm (Table S6 in File S1).

**FIGURE 1** Antibody positivity in the study population. Number of participants that were positive for glutamic acid decarboxylase (GAD) antibodies with or without positivity for zinc transporter 8 (ZnT8) and/or islet antigen 2 (IA-2) antibodies in (A), the whole study population, in (B), the insulin arm and in (C), the sitagliptin arm. Diagram regions depict the number of individuals with antibody positivity as follows: white, positivity for GAD antibodies (with or without positivity for ZnT8 and/or IA-2 antibodies); yellow, positivity for GAD antibodies and ZnT8 antibodies (with or without positivity for IA-2 antibodies); blue, positivity for GAD antibodies and IA-2 antibodies (with or without positivity for ZnT8 antibodies); green; positivity for all three antibodies. In the whole study population 33/64 participants had only GAD antibodies, in the insulin arm 18/32 had only GAD antibodies and in the sitagliptin arm 15/32 had only GAD antibodies



**3.7 | Metabolic control**

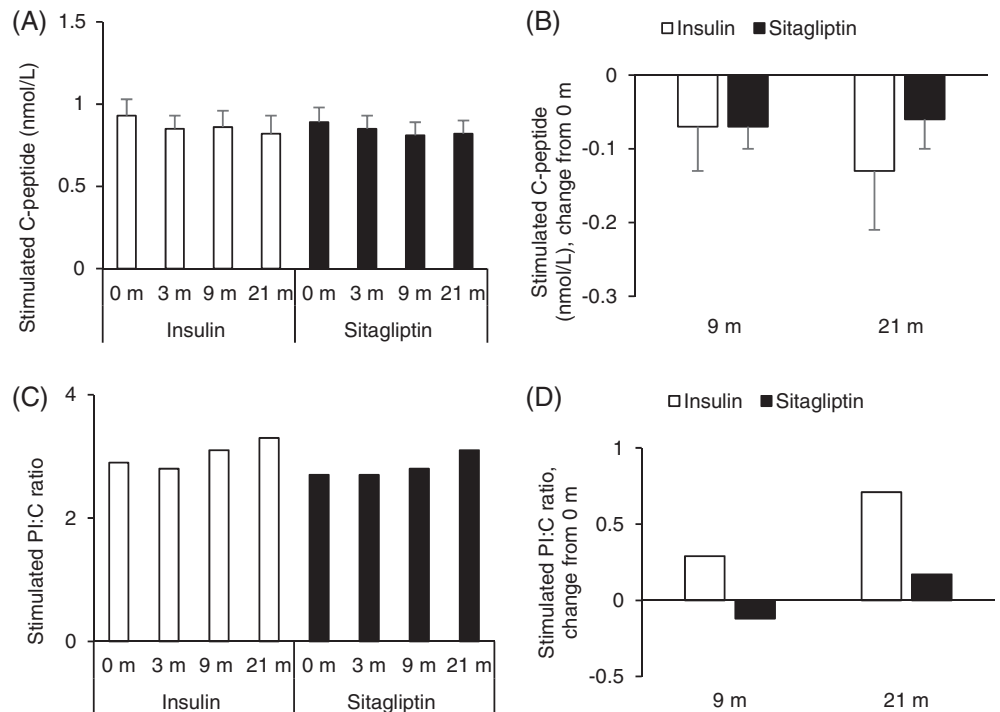
There was a modest decrease in HbA1c after 3 months of treatment in both arms (insulin arm: median -0.6% vs sitagliptin arm: median -7.9%). Also during the rest of the intervention the evolution of HbA1c levels was similar between treatment arms. Levels of fasting glucose were stable throughout the 21 months of intervention in both arms (Table S7 in File S1).

**3.8 |  $\beta$ -cell function during intervention**

The time course of fasting levels of C-peptide, insulin and proinsulin did not differ between treatments (Table S7 in File S1).

Stimulated C-peptide values during the intervention are shown in Figure 2A. Change in stimulated C-peptide at 21 months versus baseline (Figure 2B) did not differ between treatment arms ( $P = .45$ ). In addition, the change from baseline in incremental C-peptide (ie, the

**FIGURE 2** Stimulated hormone levels in the insulin and in the sitagliptin arm. Stimulated levels (ie, 6 minutes after the injection of glucagon) of (A) C-peptide and (C) proinsulin: C-peptide (PI:C) ratio at randomization (0) and after 3, 9 and 21 months (m) of intervention. Change from baseline in each treatment arm is given for (B) C-peptide and (D) PI: C ratios. Data are presented as mean  $\pm$  SEM (A and B) and median (C and D). The Mann-Whitney *U*-test was used to assess differences between treatments after 9 and 21 months of intervention. Interquartile range (25th and 75th percentiles) at 21 months, (C) insulin arm: 2.3 and 5.7, sitagliptin arm: 2.0 and 5.1, (D) insulin arm: 0.03 and 2.80, sitagliptin arm: -0.41 and 1.53



difference between fasting and stimulated levels, not shown in the figures) did not differ between treatments (insulin arm:  $-0.03 \pm 0.25$  nmol/L, sitagliptin arm:  $-0.06 \pm 0.24$  nmol/L;  $P = .84$ ). Stimulated proinsulin: C-peptide ratios are given in Figure 2C and Figure S1A in File S1. The change from baseline after 21 months (Figure 2D and Figure S1B in File S1) did not differ between arms ( $P = .10$ ).

The change from baseline in stimulated insulin (not shown in figures) tended to differ between treatment arms (insulin:  $4.4 \pm 4.0$   $\mu$ U/mL, sitagliptin:  $4.0 \pm 2.2$   $\mu$ U/mL at 21 months); however, this trend towards a stronger increase in the insulin versus the sitagliptin arm was not significant ( $P = .06$ ). No difference between treatments was seen for stimulated proinsulin ( $P = .64$  for difference, data not shown).

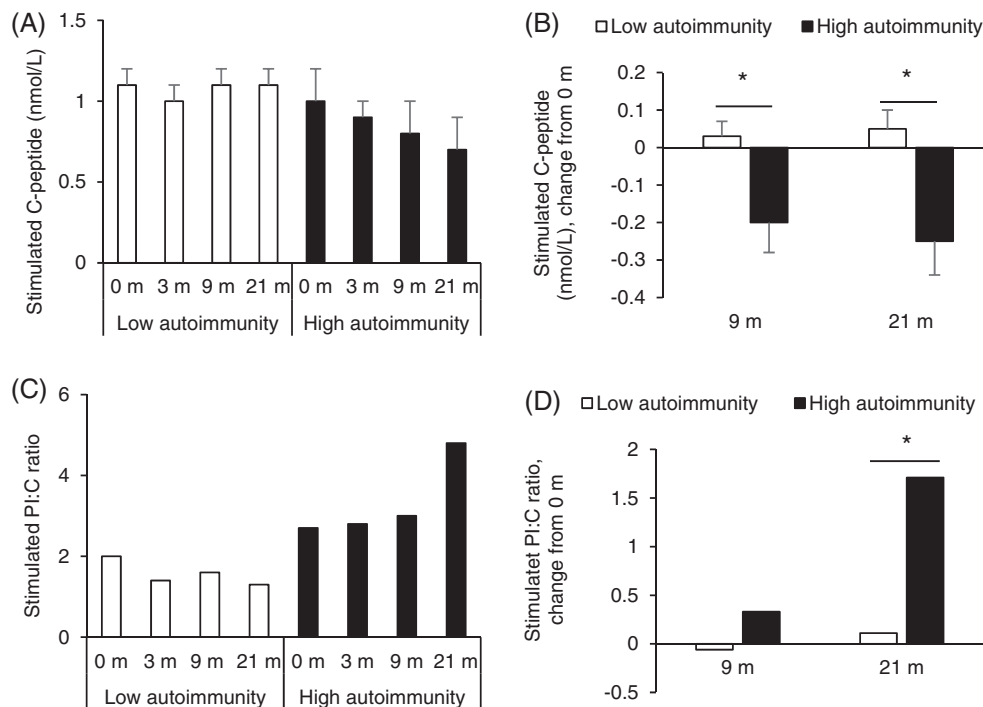
The time course of fasting levels of C-peptide, insulin and proinsulin did not differ among the three categories of autoimmunity (Table S8 in File S1).

Stimulated C-peptide levels in the low and high autoimmunity categories are shown in Figure 3A. The levels decreased successively (change from baseline) in the high versus the low category; the significance for difference between categories after 21 months was  $P = .015$  (Figure 3B). Proinsulin: C-peptide ratios during the

intervention are shown in Figure 3C and Figure S1C in File S1. This ratio became elevated (change from baseline) after 21 months in the high versus the low category ( $P = .007$  [Figure 3D and Figure S1D in File S1]). The median change from baseline in the middle category was  $0.29$  nmol/L ( $P = .012$  for difference versus the high category; not shown in the figure). There were no significant interaction effects between treatment and level of autoimmunity.

## 4 | DISCUSSION

In the present study we investigated  $\beta$ -cell function in people with LADA over time in relation to two clinically relevant alternatives of treatment. Focusing on  $\beta$ -cell function, the aim of the present study design was, in contrast to many other clinical trials,<sup>17</sup> to achieve similar metabolic control in the two arms of the study. A sizeable difference in metabolic control would raise the question of whether the degree of metabolic control per se could have influenced measures of  $\beta$ -cell function. The goal of comparable (and acceptable) metabolic control between arms of the study was achieved; a "glucotoxicity" effect should therefore not be relevant when interpreting the results. Further, in order to exclude the impact of ambient exposure to insulin



**FIGURE 3** Stimulated hormone levels in the lowest and highest category of autoimmunity. The figure shows stimulated levels (ie, 6 minutes after the injection of glucagon) of (A) C-peptide and (C) proinsulin: C-peptide (PI:C) ratio at randomization (0) and after 3, 9 and 21 months (m) of intervention. Change from baseline is given for (B) C-peptide and (D) PI:C ratio. Data are mean  $\pm$  SEM (A and B) and median (C and D). (B) Difference between the low and high autoimmunity categories after 9 and 21 months:  $*P = .014$  and  $P = .015$  (Kruskal-Wallis test and analysis of covariance, with the baseline value for stimulated C-peptide as a covariate. No interaction effect between treatment and level of autoimmunity). (D) Difference between the low and high autoimmunity categories after 21 months:  $*P = .007$  (Kruskal-Wallis test, no interaction effect (analysis of covariance) between treatment and autoimmunity). Interquartile range (25th and 75th percentiles) at 21 months (C), low autoimmunity: 1.4 and 3.4, high autoimmunity: 3.0 and 9.6, (D), low autoimmunity:  $-0.32$  and  $0.34$ , high autoimmunity:  $0.55$  and  $3.58$

or sitagliptin we performed tests of  $\beta$ -cell function after a 48-hour temporary withdrawal of sitagliptin and insulin.

Metformin is currently the first choice of treatment in adult-onset, non-insulin-requiring and usually overweight people with diabetes. It was therefore natural to design a protocol that included add-ons of study medications to metformin. Beneficial effects of metformin on  $\beta$ -cell function have been reported in clinical studies<sup>18</sup>; however, these could probably be explained by ameliorated glucose control which would counteract "glucotoxicity." In any case a putative  $\beta$ -cell effect would probably be equal for the insulin and sitagliptin arms of the present study, therefore, the use of metformin should not confound our results.

Sitagliptin and other DPP-4 inhibitors have become frequent add-on treatments in non-insulin-dependent persons who are not optimally controlled on metformin. Clinical interest in DPP-4 inhibitors as a treatment for LADA has arisen from reports of a possible effect on autoimmunity<sup>19</sup> and beneficial effects of sitagliptin on  $\beta$ -cell function when added to insulin in people with LADA.<sup>15</sup> In addition, an observational study of another DPP-4 inhibitor, saxagliptin, indicated a favourable effect on  $\beta$ -cell function,<sup>19</sup> as did other small studies.<sup>17</sup> In clinical practice, however, LADA treatment usually entails a choice between insulin on one hand and a per-oral antidiabetic drug on the other. Except for the Japanese study<sup>12</sup> no direct comparison has, to our knowledge, been carried out between insulin versus a per-oral drug in a randomized study. This lack of knowledge motivated the present study.

We chose the results of GSCTs as the primary endpoint. Such tests have been validated over many decades as reflective of  $\beta$ -cell function in people with type 2 as well as type 1 diabetes.<sup>20,21</sup> The tests are relatively easy to perform, an advantage in a multicentre study in which the capacities for testing varied among the centres. The tests were uniformly performed at normal or only slightly hyperglycaemic glucose levels; hence, a confounding influence of hypoglycaemia or marked hyperglycaemia on our measurements can be excluded. We acknowledge that additional testing of intravenous glucose and/or responses to a test meal would have added details of interest on the time dynamics of  $\beta$ -cell performance in our participants.

Both treatment arms were well accepted by the participants, and side effects were infrequent. In addition, the dropout rate was low. The weight loss during treatment with sitagliptin (on average 3.4 kg) was surprising as the effects of DPP-4 inhibitors are reportedly weight-neutral.<sup>22</sup> Participants in the study were mostly overweight and were recommended appropriate dieting and exercise; this may have influenced the evolution of body weight. The reduction in body weight could have helped to uphold an acceptable level of HbA1c in the sitagliptin-treated participants; however, we note that HOMA2-IR, a measure of insulin resistance, was not affected by treatment in either the sitagliptin or the insulin arm.

Demise of  $\beta$ -cells in people with LADA is well recognized, but the extent and the time scale of demise is variable.<sup>4,23</sup> This heterogeneity is well illustrated in the present study. Hence, high titres of GAD antibodies, in conjunction with the presence of ZnT8 and/or IA-2 antibodies, were associated with a marked and significant decrease in

C-peptide values, and an increased ratio of proinsulin: C-peptide, the latter indicating  $\beta$ -cell stress.<sup>24</sup> Importantly, adjusting for the impact of autoimmunity did not alter the findings of no difference in  $\beta$ -cell function between the insulin and the sitagliptin arms. The present study also fails to suggest in other respects (ie, similar development in antibody titres) an influence of study medication on the process of autoimmunity.

The recruitment criteria excluded both those with optimal and those with markedly deranged metabolic control. This restricts the study population in terms of its representativeness of the general population with LADA, but makes the study population more clinically relevant. Epidemiological studies in people with LADA indicate similarities to the present study population; age at onset of LADA and the degree of excess body weight were similar to findings from the large population-based health study in Nord-Trøndelag (HUNT) in Norway (which potentially includes all adults in the Nord-Trøndelag area).<sup>25</sup> A gender difference in GAD antibody titres (higher in women) reported in epidemiological studies<sup>1,26</sup> was also observed in the present study population (results not shown). The percentage of participants with LADA who were positive for more than one antibody was higher than in the HUNT epidemiological study.<sup>27</sup> This difference could relate to time between diagnosis and measurements. Measurements in the present study population were usually performed <1 year after diagnosis, whereas in epidemiological studies, such as the HUNT prevalence studies, time from diagnosis could be decades. A successive decrease in antibodies occurs with time<sup>27</sup> and, with regard to antibodies against IA-2 and ZnT8, this was also observed in the present study; thus the prevalence of more than one antibody decreases with the duration of LADA.

We did not observe differences in  $\beta$ -cell function between treatments during or after 21 months of intervention (a possible exception pertains to stimulated insulin data; however, as this was not accompanied by effects on C-peptide levels, this could be secondary to changes in insulin extraction over the liver). The question arises of whether our finding of no difference was attributable to a type 2 statistical error. We think that this is unlikely because there was no borderline significance,  $P = .1$  being closest for C-peptide values. Another concern could be that the intervention did not register differences between treatments because endpoints were not much affected in the whole study population; however, we registered a clear decline in C-peptide levels during the intervention in those participants who displayed strong markers of autoimmunity, thereby demonstrating that significant deterioration of  $\beta$ -cell function occurred in many participants during the timeframe of the study.

No deterioration of  $\beta$ -cell function was apparent in participants with low GAD antibody titres and absence of other antibodies. This finding is consistent with a recent report.<sup>28</sup> It implies that such patients are for many years at low risk of becoming insulin-dependent. Consequently, they could be treated similarly to those with type 2 diabetes and should not need insulin as a safety measure against a swift change to insulin dependence. Further studies are needed to establish a threshold in titres for no deterioration and to test for deterioration/no deterioration beyond the present time of observation.

Strengths of the present study include the comparatively large study population with LADA, especially when viewed in relation to the rather strict inclusion and exclusion criteria. Furthermore, the study population was well characterized and underwent a follow-up of  $\beta$ -cell-related variables that included a 48-hour temporary omission of study medication before the validated and standardized tests were undertaken. In addition, the dropout rate was low. Limitations of the study include the heterogeneity of autoimmune activity, which characterizes a typical LADA population. Also, we recognize that levels of GAD antibodies from identical samples may differ among laboratories<sup>29</sup>; the absolute levels reported should be viewed in that context.

In summary, we observed, to our knowledge for the first time, that  $\beta$ -cell function was similarly affected in insulin- and sitagliptin-treated individuals with LADA, regardless of the strength of autoimmunity. The results also imply that people with low levels of GAD antibodies and absence of other antibodies do not experience progressive deterioration of  $\beta$ -cell function over a 21-month timeframe. Taken together, the present findings could help clinicians' choices of treatment in people with LADA.

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## CONFLICT OF INTEREST

None declared.

## AUTHOR CONTRIBUTIONS

IKH supervised the study, followed participants, analysed results and took part in writing the manuscript. NR cleared formalities for starting the study and took part in recruiting patients. HFF recruited and followed participants and analyzed interim results. MA and KF recruited and followed participants. ZM analysed antibodies and hormones in the study. VG designed and initiated the study in Norway and took part in writing the manuscript. AB initiated the study in Sweden, recruited and followed patients and took part in writing the manuscript.

## ORCID

Ingrid K. Hals  <https://orcid.org/0000-0003-3753-0913>

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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