EFFECT OF OVERWEIGHT STATUS AT ONSET ON C-PEPTIDE LEVELS DURING FIRST 2 YEARS SINCE DIAGNOSIS IN CHILDREN WITH TYPE 1 DIABETES

by

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ABSTRACT

Background: Recently, the growing epidemic of obesity is mirrored in the increasing incidence rate of T1D in children. However, the role of overweight in the progress of T1D is still unknown.

Objective: To assess the relationship between the overweight status at onset and the insulin reserve in the first two years since diagnosis in children with T1D.

Methods: One hundred sixty-eight children newly diagnosed with T1D, aged from 1.5 to 18.9 years, with 2-years of follow-up, ≥4 autoantibodies measured at baseline, and onset C-peptide plus 3 or more follow-up measures were included in this study from the Children's Hospital of Pittsburgh Registry (2004-2006). Baseline demographic and clinical characteristics were compared between overweight and non-overweight subjects. The change and the rate of change of C-peptide were evaluated. The contribution of being overweight to C-peptide levels and change in C-peptide from onset over time were estimated using linear mixed models adjusting for other covariates.

Results: Among the 168 subjects with mean age at 9.7 years and mean onset C-peptide of 0.76ng/mL, 22% (36) were overweight at onset with BMI ⩾ 85th percentile. Onset C-peptide level of overweight subjects was higher than that of non-overweight (median: 0.88ng/mL vs. 0.50ng/mL, $P<0.0001$). The highest C-peptide levels (median: 1.86ng/mL vs. 1.47ng/mL, $P=0.30$) were observed a 3 months, followed by a continuous decline reaching the lowest level at

24 months (median: 0.29ng/mL vs. 0.18ng/mL, P=0.13). Linear mixed models suggest that the overall mean rate of change of the overweight subjects was 0.7865ng/mL/months (95% C.I.: (0.2277, 1.3452), P=0.0062) compared to the non-overweight subjects adjusting for other baseline covariates. The differences of mean C-peptide levels between these two groups decreased as time passed and reached similar levels at the end of the second year.

Conclusion: Compared to the non-overweight T1D children, overweight children had higher C-peptide levels at 3, 6, 12, and 18 months after diagnosis; however, at 24 months, this difference was not statistically significant.

Public health significance: Children with T1D who are overweight can benefit from the potential related target interventions to help them maintain or extend the duration of high C-peptide level after receiving treatment.

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1.0 INTRODUCTION

1.1 TYPE 1 DIABETES

1.1.1 Epidemiology

Type 1 diabetes (T1D), once known as juvenile diabetes or insulin-dependent diabetes, is defined as an autoimmune disease characterized by dysfunction of the pancreatic β cells [1], which results in deficiency of insulin secretion and/or defects in insulin action [2]. T1D is a worldwide public health burden for children and adolescents. Undoubtedly, T1D is one of the most common chronic diseases in America among people under the age of 20, and its long-term complications usually threaten the patients' quality of life. According to the findings from SEARCH, during 2008-2009, in the United States, over 18,000 people aged under 20 years were newly diagnosed with T1D [3]. People with T1D have a much higher risk for heart attack, stroke, hypertension, kidney failure, and other chronic diseases [2, 3]. The extreme gravity of T1D continues to inspire numerous researchers to investigate its etiology.

1.1.2 Risk factors

The most common recognition concerning T1D is that it is a classic autoimmune disorder characterized by destruction of the pancreatic β cells [3], which is known as the insulin-

producing cells. The residual β-cell function can be physiologically defined by the β-cell mass *in vivo*. A few studies show that at the time of the diagnosis of T1D the loss of β-cell mass can reach up to 90% [4, 5]. Yet, the exact mechanism that causes T1D is still unknown. The destruction is naturally initiated or mediated by islet-specific autoantibodies, including islet cell autoantibodies (ICA), glutamic acid decarboxylase autoantibodies (GAD), insulinoma associated 2 autoantibodies (IA2), insulin autoantibodies (IAA), and recently described zinc transporter autoantibodies (ZnT8A) [1]. Several researchers have shown that about 90% to 95% of the new onsets of T1D present with at least one positive autoantibody, which is believed to be a potential predictor of the later development of T1D [6-9]. Additionally, T1D is primarily seen in genetically susceptible people, such as those with specific HLA alleles [10], who may be exposed to some unknown environmental agent that may trigger the disease, such as vitamin D deficiency, viral infections, and stress [11]. Other risk factors, including age, gender, and race/ethnicity, are also considered to be associated with the presence of T1D in children and adolescents [1].

1.1.3 Overweight and type 1 diabetes

Over the recent decades, the growing epidemic of obesity [12] is mirrored in the worldwide increasing the incidence rate of T1D in youths [13-18]. An observational study from Finland gave both the evidence of excessive weight gain to the high risk of T1D in children [15], and the link between insulin resistance, autoimmune, and excessive weight gains, which is known as the “accelerator hypothesis” [19]. Nevertheless, the underlying relationship between overweight and residual β-cell function is still unproved.

1.2 C-PEPTIDE AS A SURROGATE MEASURE OF RESIDUAL BETA-CELL FUNCTION IN TYPE 1 DIABETES

The loss of β-cell function is displayed as the inadequate secretion of insulin that cannot maintain the normal glycemia level in pathophysiology. Yet, clinically, the direct measure of endogenous insulin, a marker of the residual function of the pancreas β cells, is limited [20]. On the other hand, a study shows that the measurement of C-peptide, which is co-secreted with endogenous insulin in a one-to-one molar ratio, can provide a valid and reliable measure of the residual β-cell function [20], and such a measurement has been widely used as the primary appropriate outcome in both clinical and observational studies of T1D [20-25].

At diagnosis of T1D, patients always have a lower value of C-peptide, reflecting a low preservation of the pancreas β-cell function, and such value of C-peptide is thought to be related to some physiological indices, including age at diagnosis, gender, islet-specific autoantibodies at baseline, base control of metabolism, and genotype [20, 26-29]. However, the relationship between overweight status at onset and C-peptide level over time, and, if any, the effect of overweight on the preservation of β-cell function, are still unknown. The objective of this study is to assess the relationship between the overweight status at onset and insulin reserve during the first two years of diagnosis in children with T1D by using C-peptide as the primary endpoint. From the perspective of public health implication, this study will provide a new direction of potential interventions that can benefit the overweight children with T1D.

2.0 RESEARCH DESIGN AND METHODS

2.1 SUBJECTS

The study subjects diagnosed with clinical T1D were identified from the diabetes clinic at Children's Hospital of Pittsburgh from January 1st, 2004 to December 31st, 2006, and accrued through the Children's Hospital of Pittsburgh Registry. All participants are children aged < 19 years. Inclusion criteria for this study were as follows: 1) informed consent, 2) diagnosis of T1D requiring insulin, and insulin treatment prior to hospital discharge, 3) available research laboratory results for at least four β-cell autoantibodies at baseline, including IAA, IA2, GAD, ICA human, and ZnT8A, and 4) available C-peptide at onset and at least three follow-ups.

2.2 DEMOGRAPHIC AND CLINICAL DATA

Clinical data at onset and follow-up within 3 months were obtained from hospital and research records including diagnosis date, blood draw date, age at diagnosis, gender, race, height, and weight. BMI percentiles and BMI z-scores were calculated based on the Centers for Disease Control and Prevention 2000 growth data, and overweight was defined as BMI $\geq 85^{th}$ percentile adjusting for age and gender. Insulin dose for treatment was recorded for each follow-up.

In the original records, among the subjects recruited in this study, some had more than one blood samples corresponding to each request return visit (Appendix A1). According to the protocol, the clinical record that has the closest days since diagnosis to the proposed follow-up window was retained regardless the records at the diagnosis (e.g., the clinical record with the closest days since diagnosis to 90 days was retain as the single record for 3 months, 180 days for 6 months). Multiple clinical records at diagnosis for each subject were combined into a single complete one as the onset record.

2.3 ASSAYS

Blood samples obtained at onset and/or follow-up within 3 months were assayed for glucose, glycated hemoglobin (HbA1c), C-peptide levels, and autoantibodies. IAA was measured only in samples that obtained within 1 week of diagnosis; GAD, IA2, ICA human, and ZnT8A were measured from blood samples obtained within 120 days. All the assays to be used have been evaluated in International and National workshops [30]. Sensitivities and specificities have been consistently 80-100% for ICA, GAD, and IA2, and been 60% and 93% for IAA [24]. Baseline autoantibodies were defined as the earliest valid clinical record for corresponding autoantibodies within the first 120 days since diagnosis.

C-peptide for onset, 3 months, and each 6 monthly follow-up for 2 years were measured by RIA kit (Linco Research, St. Charles, MO) with a lower detection limit of 0.1ng/mL, 4.7% inter-assay coefficient, and 4.6% intra-assay coefficient [24]. Levels of >5.0ng/mL were re-measured after dilutions. Levels below the detection of the device were replaced with the midpoint from 0 to the lower detection level [29].

2.4 STATISTICAL ANALYSIS

2.4.1 Descriptive statistics for covariates

The follow-up pattern for this study group was summarized. Demographic and clinical characteristics of study subjects were described by overweight status at onset. Data were summarized using means and SDs or medians and IQRs for continuous variables (e.g., age, C-peptide), and frequencies and proportions for categorical variables such as race, gender, and number of positive autoantibodies at baseline. Student t tests were conducted to compare means of continuous variables with normal distribution between overweight and non-overweight subjects at onset. Wilcoxon-Mann-Whitney tests were conducted if continuous variables were non-normally distributed. The proportions of categorical variables between overweight and non-overweight subjects were compared using Fisher's exact test.

Insulin dose-adjusted HbA1c (IDAA1c) is defined as follows: HbA1c (%) +[4*insulin dose (units/kg/24h)], which is a credible value to define the partial remission (PR) in T1D (IDAA1c$\leq$9) [21, 31].

Combinations of positive autoantibodies at baseline were counted by number of positive autoantibodies at baseline. Frequencies and proportions in each category were given.

2.4.2 C-peptide

Follow-up patterns for C-peptide were summarized by descriptive statistics and boxplots for onset and each follow-up visit. Changes in C-peptide from onset, from 3-month visit, and from each subsequent visit for corresponding follow-ups were evaluated. Rates of change of C-peptide

were calculated as follows: the window based rate of change = the change in C-peptide / the length between two follow-up periods in months, and the exact rate of change = the change in C-peptide / the difference of in days since diagnosis between two follow-up periods in months. For example, the window based rate of change of 3-month visit from onset = the change of C-peptide at 3 months from onset / 3 months. Trajectories of means and medians of C-peptide, change of C-peptide, and rate of change of C-peptide were also evaluated. Student t tests were used to assess the change or the rate of change being different from zero if the values were normally distributed. Wilcoxon signed rank tests or sign tests were conducted for such purpose if values were not normally distributed.

The same analytic procedures were performed for subgroup analysis, including comparisons between overweight and non-overweight subjects at onset, subgroups defined by their change in their overweight status from onset to 3-month visit, and among subjects with different number of positive autoantibodies at baseline. Additionally, Wilcoxon-Mann-Whitney tests were used to compare difference between two independent samples, and Kruskal-Wallis tests were used to compare difference between more than two independent samples, and Dwass, Steel, Critchlow-Fligner tests were performed for pairwise comparisons if multiple samples shown significantly difference. All are designed for nonparametric data.

Associations between follow-up C-peptide measures and baseline covariates were assessed with Spearman and Pearson correlations when both variables were continuous.

2.4.3 Linear mixed models

In linear mixed models, both fixed effects and random effects are included in a single model. They are able to model data in which the observations are not independent (i.e., repeated

measurements on the same units or measurements on clusters of related units). Random coefficients models, one type of mixed models, is an appropriate approach to modeling repeated measures data when the main interest of the study centers on the rate of change of the study endpoint over time among different study groups. It allows this rate of change to vary randomly between study units by fitting units and units*time interaction as random in the model, and regression curves can be fitted individually for each unit. Simply, the estimation of overall group effects and such group effects at each time points can be obtained in a single model. Moreover, the problems introduced by the presence of missing data are negligible since they can be assumed as missing at random.

In the current study, both univariate and multivariate linear mixed models (i.e., random coefficients models) were fitted for two endpoints: C-peptide levels from 3 months and forwards and change of C-peptide from onset for 3-month follow-up and forwards. Univariate linear mixed models were fitted for covariates including time (treated as continuous in days, continuous in months, and categorical in months), age at diagnosis (both continuous and categorical), gender, IDAA1c at 3 months, overweight status at onset, overweight status at 3 months, and number of positive autoantibodies at baseline. For multivariate linear mixed models, baseline model were first established using continuous variables, IDAA1c at 3 months, age at diagnosis, and time variable in days or in months, and categorical variables, gender (male as reference) and number of positive autoantibodies at baseline (0 or 1 as reference). The effects of overweight status at onset over time were evaluated based on the baseline model, and Pearson residuals were evaluated for each model. Comparisons between overweight and non-overweight subjects at each time were estimated. The models were shown as follows. The spaghetti plots were plotted for

both observed and fitted values for C-peptide and change of C-peptide from onset for each subject, and an overall spline was also presented.

All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC), and significance level was compared to 0.05.

Model A: treated time as continuous in days

$$y_{ij} = \mu + \alpha \cdot IDAA1c(3mth)_i + \beta \cdot I(female_i) + \gamma \cdot agedx_i + \tau \cdot I(2\ or\ more\ Abs\ at\ baseline_i) + m \cdot dsddx_{ij} + \theta \cdot I(overweight\ at\ onset_i) + \varphi \cdot I(overweight\ at\ onset_i) \cdot dsddx_{ij} + p_i + (pm)_i \cdot dsddx_{ij} + e_{ij}$$

where i is the subject index, j is the time index, dsddx is the days since diagnosis,

p_i = difference from average in the intercept term for the ith subject,

$(pm)_i$ = difference in slope for the ith subject, from the average slope,

$$e_i\ iid \sim N(0, \sigma^2), \begin{pmatrix} p_i \\ pm_i \end{pmatrix} \sim N(0, G), where\ G = \begin{pmatrix} \sigma_p^2 & \sigma_{p,pm} \\ \sigma_{p,pm} & \sigma_{pm}^2 \end{pmatrix}.$$

Model B: treated time as continuous in months

$$y_{ij} = \mu + \alpha \cdot IDAA1c(3mth)_i + \beta \cdot I(female_i) + \gamma \cdot agedx_i + \tau \cdot I(2\ or\ more\ Abs\ at\ baseline_i) + m \cdot follow - up_{ij} + \theta \cdot I(overweight\ at\ onset_i) + \varphi \cdot I(overweight\ at\ onset_i) \cdot follow - up_{ij} + p_i + (pm)_i \cdot follow - up_{ij} + e_{ij}$$

where i is the subject index, j is the time index,

p_i = difference from average in the intercept term for the ith subject,

$(pm)_i$ = difference in slope for the ith subject, from the average slope,

$$e_i\ iid \sim N(0, \sigma^2), \begin{pmatrix} p_i \\ pm_i \end{pmatrix} \sim N(0, G), where\ G = \begin{pmatrix} \sigma_p^2 & \sigma_{p,pm} \\ \sigma_{p,pm} & \sigma_{pm}^2 \end{pmatrix}.$$

Model C: treated time as categorical in months

$$y_{ij} = \mu + \alpha \cdot IDAA1c(3mth)_i + \beta \cdot I(female_i) + \gamma \cdot agedx_i + \tau \cdot I(2\ or\ more\ Abs\ at\ baseline_i) + m_j \cdot follow - up_{ij} + \theta \cdot I(overweight\ at\ onset_i) + \varphi_j \cdot I(overweight\ at\ onset_i) \cdot follow - up_{ij} + p_i + e_{ij}$$

where i is the subject index, j is the time index,

p_i = difference from average in the intercept term for the ith subject,

$$e_{ij}\ iid \sim N(0, \sigma^2), p_i \sim N(0, G), where\ G = \sigma_p^2.$$

Figure 1. Linear mixed models for C-peptide at 3 months and forwards and change of C-peptide from onset of 3-month visit and forwards

3.0 RESULTS

3.1 SUBJECTS

Among the total 201 complete new onsets, 198 participants had records at onset (<14 days since diagnosis), 192 participants had at least three antibodies measured at baseline, 184 participants had at least four antibodies measured at baseline, 182 participants had onset C-peptide and at least three follow-ups of C-peptide, and 168 participants had at least four antibodies measured at baseline and at least three follow-ups of C-peptide and the onset C-peptide (179 participants had at least three antibodies measured at baseline and at least three follow-ups of C-peptide and the onset C-peptide). The final 168 subjects (83.58% out of 201) were the analyzed subjects in this study. Approximately half of them (49.40%) had complete 2-year follow-up records. The onset records (<14 days) were obtained for all 168 participants. The mean length of follow-up for study subjects was 694.09 days. The 1-year loss-follow-up rate for this study was approximately 10.71%, and 17.26% for 2-year follow-up.

Table 1. The days since diagnosis in each visit time frame

Visits	N	Mean	SD	Median	25th Pctl	75th Pctl	Min	Max
<14 days	168	0	1	0	0	0	0	5
3 months	165	69	12	66	62	74	46	118
6 months	148	168	25	161	152	178	124	267
12 months	150	360	30	355	338	379	277	442
18 months	144	548	34	545	522	571	453	628
24 months	139	723	34	723	699	746	641	804

* Numbers are in days.

Table 2. Twelve follow-up patterns for study subjects (N=168)

<14 days	3 months	6 months	12 months	18 months	24 months	N (%)
X	X	X	X	X	X	83 (49.40)
X	X	X	X	X	.	26 (15.48)
X	X	X	X	.	X	19 (11.31)
X	X	X	.	X	X	14 (8.33)
X	X	X	.	X	.	1 (0.60)
X	X	X	.	.	X	2 (1.19)
X	X	.	X	X	X	17 (10.12)
X	X	.	X	X	.	1 (0.60)
X	X	.	X	.	X	1 (0.60)
X	X	.	.	X	X	1 (0.60)
X	.	X	X	X	.	1 (0.60)
X	.	X	X	.	X	2 (1.19)

* X indicates that there is a clinical record for that follow-up.

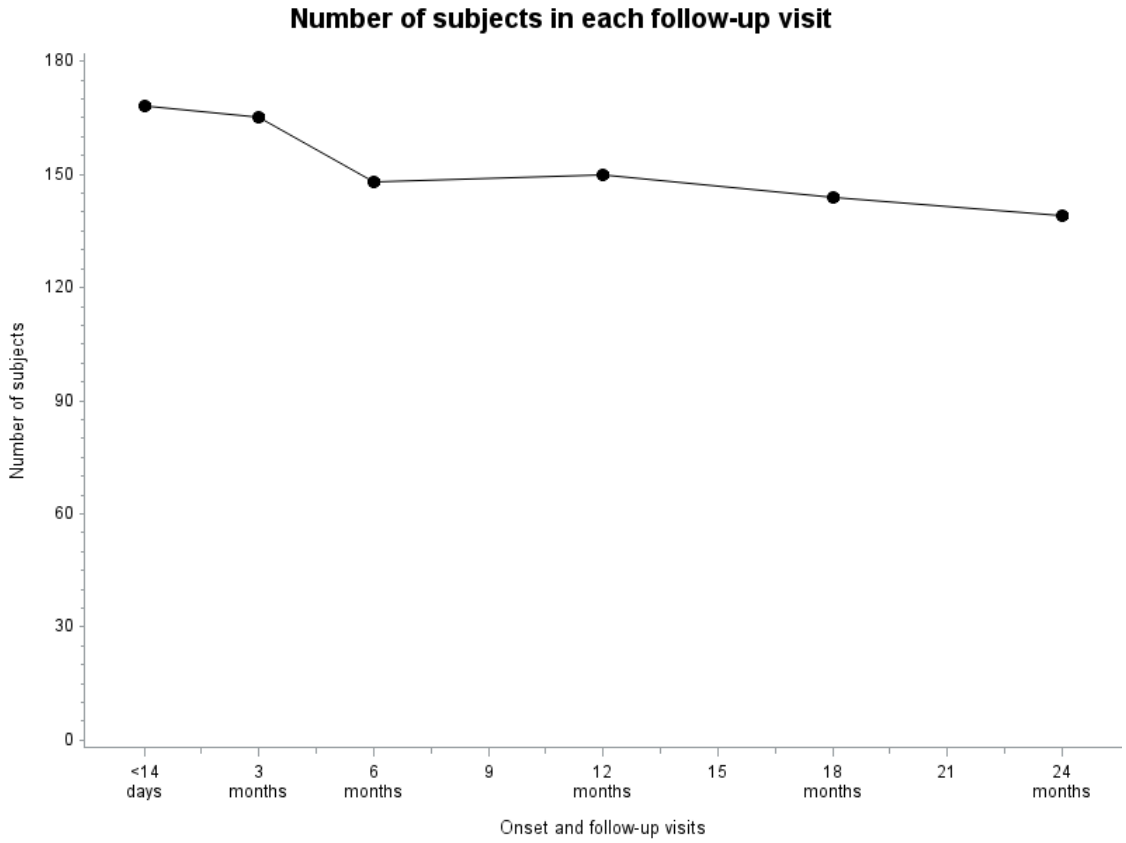

Figure 2. Number of subjects in each visit time frame

3.2 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF STUDY SUBJECTS AT BASELINE

The demographic and clinical characteristics at baseline of the 168 study subjects of 201 complete new onsets that with no less than 4 autoantibodies measured at baseline and more than 3 follow-ups of C-peptide besides diagnosis measure were shown in Table 3. The 11 participants that excluded from current analysis with 3 autoantibodies measured at baseline, which was included in previous study [32], were not significantly different from the 168 subjects in the demographic and clinical characteristics at baseline (See in Appendix A1).

Among the 168 subjects with mean age of 9.7 years, 92 (54.8%) were male, and 159 (94.6%) were white. The overall mean HbA1c level of the study subjects at onset was 11.86%, which was higher than the normal level for children under 19 (around 8%), and at 3 months after initiation of insulin treatment showed better controlled at 7.5%. The IDAA1c at 3-month visit was 9.27, which was on the boundary of partial remission of T1D. The mean BMI% for the study population is 51.4 at onset and increased to 73.5 at 3 months. The mean number of positive autoantibodies at baseline was 2.8. Only 16 (9.5%) participants had all negative autoantibodies at baseline, and 58 (34.5%) had 4 or 5 positive autoantibodies at baseline. The combinations of positive autoantibodies were shown in Appendix A1.

Thirty-six (22%) of the total 168 subjects were overweight at onset with BMI $\geq 85^{th}$ percentile. The mean BMI% of overweight subjects at onset was approximately 2.4 times higher than that of non-overweight subjects at onset (94.0 vs. 39.3). This ratio was shrunk to 1.4 at 3-month visit (94.2 vs. 68.2). All the baseline characteristics were not statistically significant different between the overweight subjects and non-overweight subjects at onset, regardless of the BMI related measures.

Table 3. Baseline demographic and clinical characteristics of study subjects by overweight status at onset (N=168)

Characteristics	Overall (N=168)	BMI ≥ 85th Pctl (N=36, 22%)	BMI < 85th Pctl (N=127, 78%)	*P value*
Demographic				
Age at diagnosis, yrs	9.7 ± 3.7	9.77 ± 3.85	10.07 ± 2.42	*0.64*[B]
Age group, yrs, n (%)				*0.29*
0-4	18 (10.71)	1 (2.78)	14 (11.02)	
5-9	68 (40.48)	16 (44.44)	51 (40.16)	
10-14	69 (41.07)	18 (50.00)	51 (40.16)	
≥ 15	13 (7.74)	1 (2.78)	11 (8.66)	
Gender, n (%)				*0.71*
Male	92 (54.76)	19 (52.78)	72 (56.69)	
Female	76 (45.24)	17 (47.22)	55 (43.31)	
Race, n (%)				*0.52*
White	159 (94.64)	33 (91.67)	121 (95.28)	
Black	8 (4.76)	3 (8.33)	5 (3.94)	
Other	1 (0.60)	0 (0.00)	1 (0.79)	
BMI at onset, kg/m^2	18.26 ± 4.42[a]	24.04 ± 4.86	16.62 ± 2.53	*<0.0001*[B]
BMI at 3 months, kg/m^2	19.78 ± 4.08[b]	24.60 ± 5.01[i]	18.47 ± 2.56[j]	*<0.0001*[B]
BMI % at onset	51.40 ± 32.61[a]	94.00 ± 4.75	39.33 ± 26.37	*<0.0001*[B]
BMI % at 3 months	73.48 ± 20.03[b]	94.16 ± 6.74[i]	68.17 ± 18.43[j]	*<0.0001*[B]
BMI z-score at onset	0.03 ± 1.29[a]	1.73 ± 0.51	-0.45 ± 1.00	*<0.0001*[B]
BMI z-score at 3 months	0.80 ± 0.76[b]	1.78 ± 0.53[i]	0.54 ± 0.56[j]	*<0.0001*[A]
Clinical				
HbA1c at onset	11.86 ± 2.37[a]	11.61 ± 2.15[g]	11.94 ± 2.44[h]	*0.53*[B]
HbA1c at 3 months	7.30 ± 0.83[d]	7.12 ± 0.98[i]	7.35 ± 0.75[h]	*0.22*[A]
IDAA1c at 3 months	9.27 ± 1.55[c]	9.04 ± 1.93[k]	9.33 ± 1.43[l]	*0.09*[B]
Positive antibodies at baseline, n (%)				*0.54*
0	16 (9.52)	4 (11.11)	11 (8.66)	
1	13 (7.74)	1 (2.78)	11 (8.66)	
2	27 (16.07)	5 (13.89)	22 (17.32)	
3	54 (32.14)	15 (41.67)	37 (29.13)	
4 or 5	58 (34.53)	11 (30.56)	46 (36.22)	
Autoantibodies at baseline, n (%)				
+ IAA	31(56.36)[e]	6 (54.55)[m]	25 (56.82)[n]	*1.00*
+ IA2	115 (68.45)	23 (63.89)	89 (70.08)	*0.54*
+ GAD	89 (52.98)	21 (58.33)	67 (52.76)	*0.56*
+ ICA human	135 (80.36)	28 (77.78)	103 (81.10)	*0.64*
+ ZnT8A	100 (60.24)[f]	23 (63.89)	74 (59.20)[j]	*0.70*

* Data are mean ± SD unless otherwise noted. P values for continuous covariates are obtained from [A]two-sample t-tests for normal distribution, and [B]Wilcoxon-Mann-Whitney tests for non-normal distribution. P values for categorical covariates are obtained from Fisher's exact tests. IDAA1c, insulin dose-adjusted HbA1c.
[a] Based on 163 subjects. [b] Based on 161 subjects. [c] Based on 146 subjects. [d] Based on 164 subjects. [e] Based on 55 subjects. [f] Based on 166 subjects. [g] Based on 35 subjects. [h] Based on 126 subjects. [i] Based on 33 subjects. [j] Based on 125 subjects. [k] Based on 30 subjects. [l] Based on 114 subjects. [m] Based on 11 subjects. [n] Based on 44 subjects.

3.3 C-PEPTIDE AT ONSET AND FOLLOW-UP VISITS

3.3.1 C-peptide levels at onset and each follow-up

The description of the C-peptide level at each follow-up was shown in Table 4. The mean level of C-peptide at onset in the study population was 0.76ng/mL (median: 0.57ng/mL). During the 2-year follow-up since diagnosis of T1D (Figure 3), the C-peptide of study subjects reached the highest level at 3 months as 1.92±1.58ng/mL (median: 1.53ng/mL) and continuously decreased at following visits, and attained the lowest level at the end of the two-year follow-up at 0.59±0.78ng/mL (median: 0.19ng/mL).

Table 4. C-peptide of study subjects at each visit time frame

Visits	N	Mean	SD	Median	25th Pctl	75th Pctl	Min	Max
<14 days	168	0.76	0.46	0.57	0.50	0.87	0.25	3.85
3 months	157	1.92	1.58	1.53	0.80	2.83	0.10	10.20
6 months	139	1.68	1.43	1.34	0.63	2.23	0.10	7.73
12 months	141	1.17	1.21	0.78	0.34	1.48	0.10	5.56
18 months	133	0.98	1.24	0.47	0.10	1.25	0.10	6.11
24 months	135	0.59	0.78	0.19	0.10	0.75	0.10	3.69

* Values are in ng/mL.

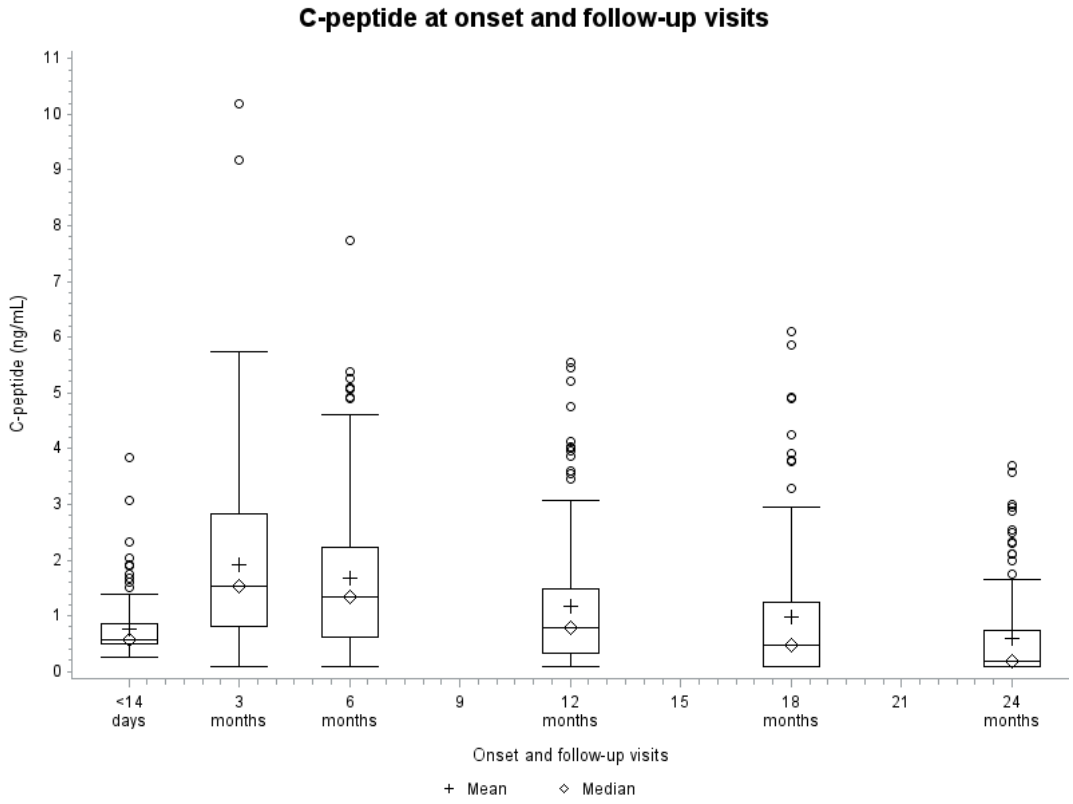

Figure 3. C-peptide of study subjects at each visit time frame

+ and ◇ are the mean and median values of C-peptide of the study subjects at each visit time frame. ∘ are the extreme values greater than the Q3+1.5IQR of each visit.

3.3.2 Change of C-peptide

The mean changes of C-peptide from onset of 3 months to 18 months indicated that the C-peptide level at 3 months to 18 months were greater than that at onset and the change rates decreased from 3 months to 18 months as 1.16ng/mL (median: 0.84ng/mL, P<0.0001), 0.94ng/mL (median: 0.67ng/mL, P<0.0001), 0.40ng/mL (median: 0.10ng/mL, P=0.15), and 0.24ng/mL (median: -0.11ng/mL, P=0.22), respectively; while at 24 months, the C-peptide level was less than onset with the mean change of -0.15ng/mL and median change of -0.40ng/mL (P<0.0001) (Table 5A). The increment of C-peptide from onset of 3 months was the largest one among all the 2-year follow-ups. However, such an increment lasted for a short time in current

study, since the C-peptide continuously decreased for the next follow-up periods (Table 5B), with mean change from 3-month visit of -0.21±1.33ng/mL (median: -0.29ng/mL, P<0.01), -0.81±1.44ng/mL (median: -0.67ng/mL, P<0.0001), -0.89±1.44ng/mL (median: -0.82ng/mL, P<0.0001), and -1.37±1.40ng/mL (median: -1.07ng/mL, P<0.0001) for 6-month to 24-month visit separately.

All the medians of change of each visit to the next follow-up were significantly different from 0 (all P<0.01) (Table 5C). Since diagnosis, C-peptide increased by 1.16ng/mL (median: 0.84ng/mL) within 3 months, and then started to decrease for each interval between two subsequent visits. The decline from 6-month visit to 12-month visit was the fastest one with 0.56ng/mL decrement (median: -0.42ng/mL). At 12-month and 24-month visit, the changes of C-peptide from previous visit were not as greater as previous ones (Figure 4).

Table 5. The change of C-peptide of each follow-up visit

Visits	N	Mean ± SD	Median [IQR]	*P value*
A. Change from onset				
3 months	157	1.16 ± 1.35	0.84 [0.16, 1.98]	*<0.0001*[c]
6 months	139	0.94 ± 1.32	0.67 [0.01, 1.52]	*<0.0001*[c]
12 months	141	0.40 ± 1.09	0.10 [-0.26, 0.73]	*0.15*[c]
18 months	133	0.24 ± 1.04	-0.11 [-0.40, 0.60]	*0.22*[c]
24 months	135	-0.15 ± 0.71	-0.40 [-0.45, 0.02]	*<0.0001*[c]
B. Change from 3 months				
6 months	129	-0.21 ± 1.33	-0.29 [-0.81, 0.38]	*<0.01*[b]
12 months	132	-0.81 ± 1.44	-0.67 [-1.50, -0.12]	*<0.0001*[b]
18 months	125	-0.89 ± 1.44	-0.82 [-1.90, -0.20]	*<0.0001*[a]
24 months	126	-1.37 ± 1.40	-1.07 [-2.31, -0.48]	*<0.0001*[c]
C. Change from each follow-up to subsequence				
Onset - 3 months	157	1.16 ± 1.35	0.84 [0.16, 1.98]	*<0.0001*[c]
3 months - 6 months	129	-0.21 ± 1.33	-0.29 [-0.81, 0.38]	*<0.01*[b]
6 months - 12 months	116	-0.56 ± 0.87	-0.42 [-1.11, -0.07]	*<0.0001*[b]
12 months - 18 months	111	-0.19 ± 0.74	-0.17 [-0.42, 0]	*<0.0001*[b]
18 months - 24 months	103	-0.33 ± 0.59	-0.14 [-0.47, 0]	*<0.0001*[c]

* Values are in ng/mL. [a] Based on t-test. [b] Based on Wilcoxon signed rank test. [c] Based on sign test.

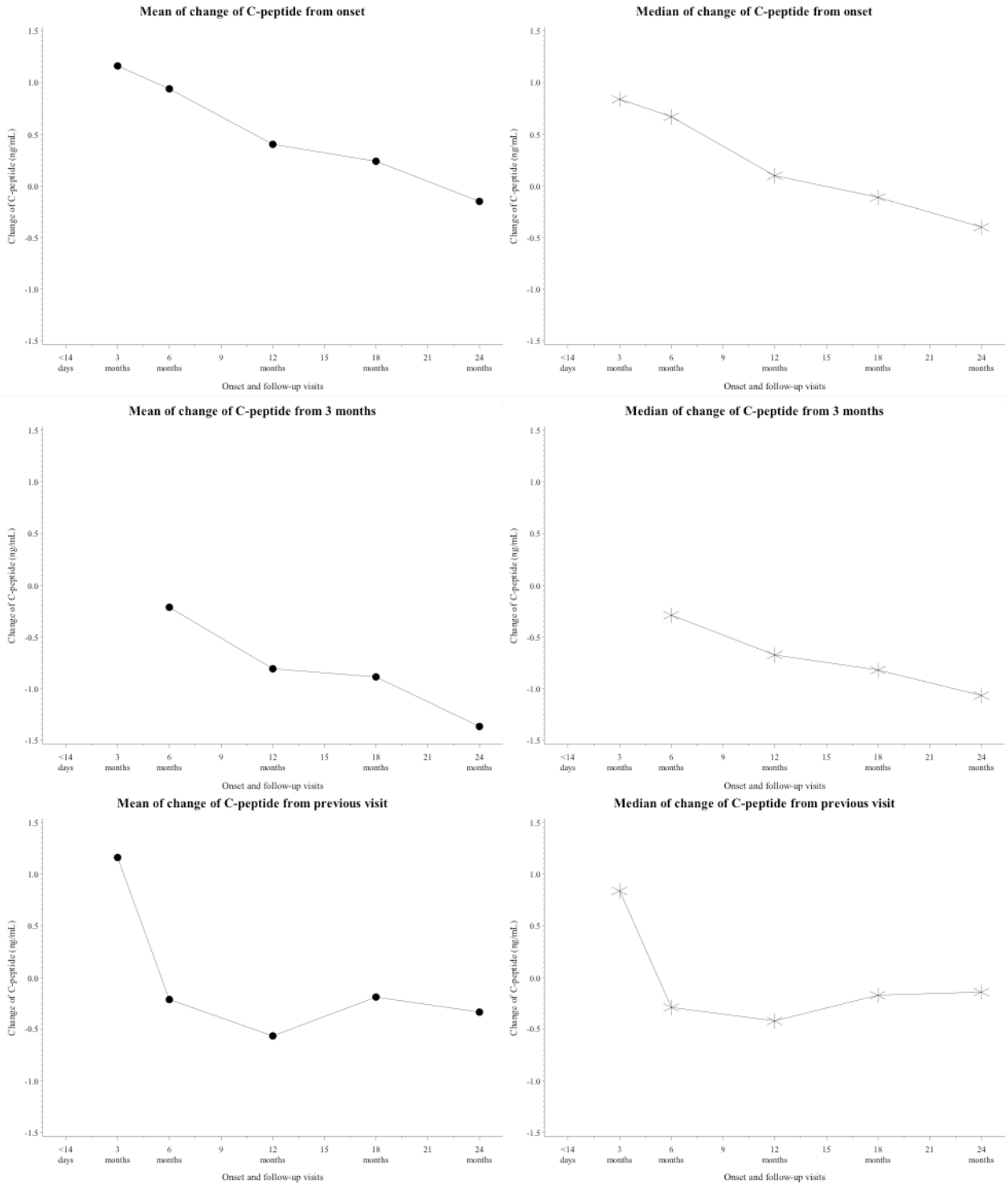

Figure 4. The change of C-peptide

3.3.3 The rate of change of C-peptide

The rates of change of C-peptide for each follow-up (calculated based on the length of follow-up window) were summarized in Table 6 and Figure 5. The mean increments of C-peptide from onset to 3-month, 6-month, 12-month, and 18-month visits were 0.39 (median: 0.28), 0.16 (median: 0.11), 0.03 (median: 0.01), and 0.01 (median: -0.01) per unit per month, respectively, and the median rate of change of 3-month and 6-month visits were significantly different from 0 ($P<0.0001$). While at 24-month window, by average, C-peptide decreased by 0.01ng/mL per month (median: -0.02, $P<0.0001$), which indicated the relapse of the function of beta cells in pancreas.

The mean decrements of C-peptide from 3-month, to 6-month, 12-month, 18-month, and 24-month follow-up visits were 0.07 (median: 0.10), 0.09 (median: 0.08), 0.06 (median: 0.05), and 0.07 (median: 0.05) per unit per month, respectively (all $P<0.01$ for mean or median values). Among all the follow-ups, the rate of change of C-peptide from 3-month visit to 12-month visit was the fastest one. After a year, the rate of decrement slowed down and stayed at a steady rate, but still significant.

All the median rates of change of each visit to the next one were significantly different from 0 (all $P<0.01$). Since diagnosis, on average, C-peptide increased by 0.39ng/mL per month within 3 months (0.28ng/mL per month in median), and then started to decrease for each interval between two subsequent visits and the rate was maintained at around -0.05ng/mL per month.

The summary for the rate of change divided by exact months interval was shown in Appendix A2, which present the same pattern here.

Table 6. The rate of change of C-peptide of each follow-up visit

Visits	N	Mean ± SD	Median [IQR]	*P value*
A. The rate of change from onset				
3 months	157	0.39 ± 0.45	0.28 [0.05, 0.66]	*<0.0001*[c]
6 months	139	0.16 ± 0.22	0.11 [0, 0.25]	*<0.0001*[c]
12 months	141	0.03 ± 0.09	0.01 [-0.02, 0.06]	*0.15*[c]
18 months	133	0.01 ± 0.06	-0.01 [-0.02, 0.03]	*0.22*[c]
24 months	135	-0.01 ± 0.03	-0.02 [-0.02, 0]	*<0.0001*[c]
B. The rate of change from 3 months				
6 months	129	-0.07 ± 0.44	-0.10 [-0.27, 0.13]	*<0.01*[b]
12 months	132	-0.09 ± 0.16	-0.08 [-0.17, -0.01]	*<0.0001*[b]
18 months	125	-0.06 ± 0.10	-0.05 [-0.13, -0.01]	*<0.0001*[a]
24 months	126	-0.07 ± 0.07	-0.05 [-0.11, -0.02]	*<0.0001*[c]
C. The rate of change from each follow-up to subsequence				
Onset - 3 months	157	0.39 ± 0.45	0.28 [0.05, 0.66]	*<0.0001*[c]
3 months - 6 months	129	-0.07 ± 0.44	-0.10 [-0.27, 0.13]	*<0.01*[b]
6 months - 12 months	116	-0.09 ± 0.14	-0.07 [-0.18, -0.01]	*<0.0001*[b]
12 months - 18 months	111	-0.03 ± 0.12	-0.03 [-0.07, 0]	*<0.0001*[b]
18 months - 24 months	103	-0.06 ± 0.10	-0.02 [-0.08, 0]	*<0.0001*[c]

* Values are calculated by the change in a subject / the length of window between two follow-ups in months, in ng/mL/month. [a] Based on t-test. [b] Based on Wilcoxon signed rank test. [c] Based on sign test.

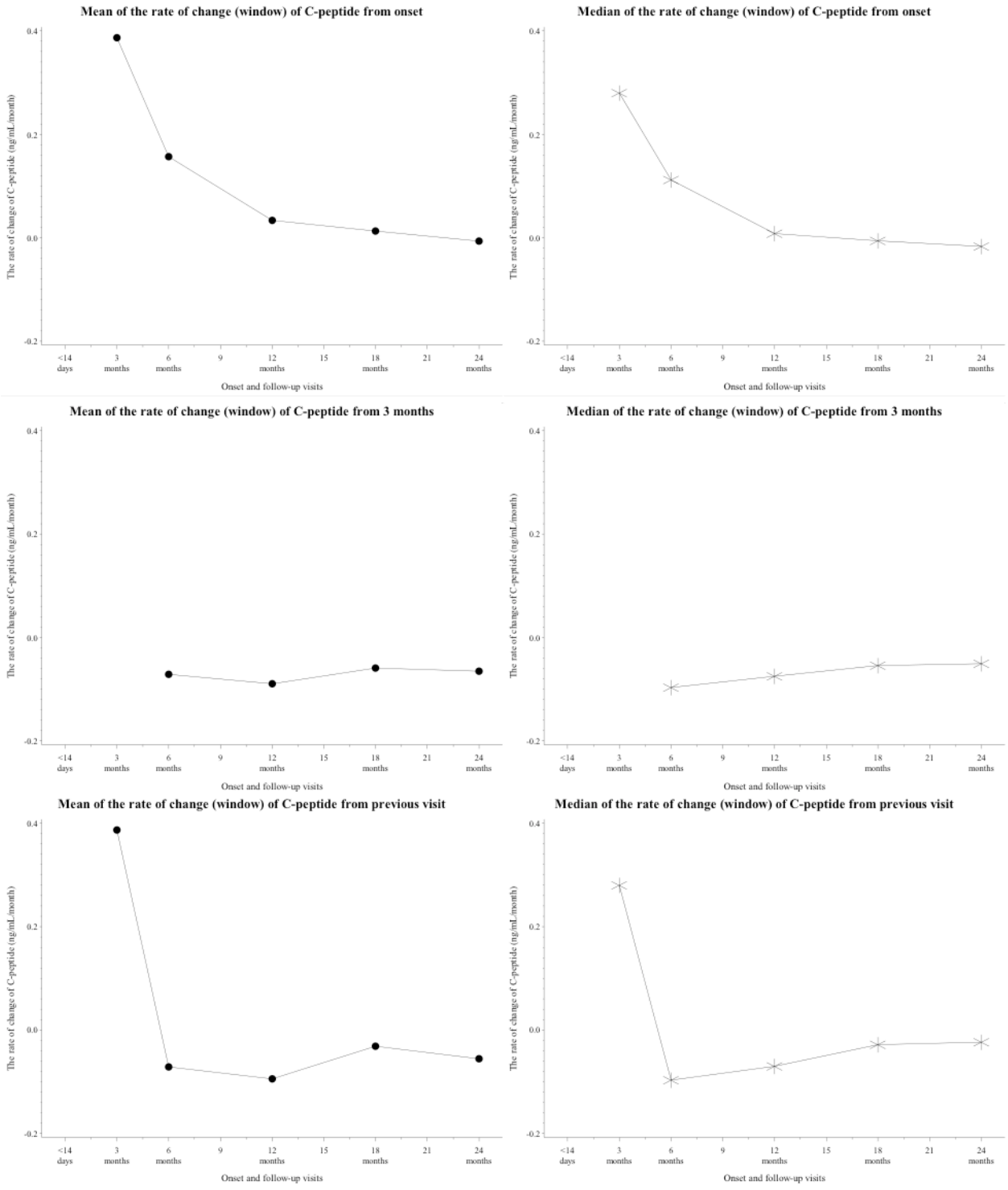

Figure 5. The rate of change of C-peptide

3.4 C-PEPTIDE AND OVERWEIGHT STATUS

3.4.1 C-peptide and overweight status at onset

C-peptide levels for each follow-up were summarized by overweight status at onset in Table 7. The patterns for overweight subjects and non-overweight subjects at onset were the same (Figure 6). The onset C-peptide level of overweight subjects at onset was higher than that of non-overweight subjects at onset (mean: 1.01ng/mL vs. 0.67ng/mL, median: 0.88ng/mL vs. 0.50ng/mL, $P<0.0001$). At 3 months, both attained their highest C-peptide level (mean: 2.53ng/mL vs. 1.74ng/mL, median: 1.86ng/mL vs. 1.47ng/mL, $P=0.30$). From then on, C-peptide level declined in both group and reached their lowest level (mean: 0.74ng/mL vs. 0.53ng/mL, median: 0.29ng/mL vs. 0.18ng/mL, $P=0.13$). However, overweight subjects had slight increase at 18 months (mean: 1.63ng/mL, median: 1.16ng/mL), which was significantly different from the continuously decreased C-peptide level for non-overweight subjects (mean: 0.81ng/mL, median: 0.45ng/mL) ($P<0.01$).

The change and the rate of change (calculated based on the length of follow-up window) of C-peptide for each follow-up period by overweight status at onset were summarized in Table 8 and 9. None show statistically significant difference between two groups except for the change from 18 months to 24 months, with the mean rates of -0.11ng/mL/month for overweight subjects at onset vs. -0.04ng/mL/months for non-overweight subjects at onset (median: -0.05 vs. -0.01 with $P=0.02$). Graphically, the change and rate of change from onset among overweight subjects were slightly greater than that of non-overweight subjects. On the contrary, C-peptide of non-overweight subjects decreased faster than overweight subjects from 3 months (Figure 6 and 7).

The summary for the rate of change divided by using exact months interval was shown in Appendix A3, which present the same pattern here.

Table 7. C-peptide over follow-up visits among overweight and non-overweight subjects at onset (N=168)

Visits	Overweight at onset			Non-overweight at onset			*P value*
	N	Mean ± SD	Median [IQR]	N	Mean ± SD	Median [IQR]	
<14 days	36	1.01 ± 0.64	0.88 [0.60, 1.15]	127	0.67 ± 0.37	0.50 [0.50, 0.72]	*<0.0001*
3 months	33	2.53 ± 2.44	1.86 [0.55, 3.42]	119	1.74 ± 1.19	1.47 [0.80, 2.54]	*0.30*
6 months	31	2.06 ± 1.65	1.58 [0.89, 3.64]	103	1.56 ± 1.32	1.29 [0.62, 2.15]	*0.19*
12 months	29	1.52 ± 1.50	1.00 [0.43, 2.03]	108	1.07 ± 1.10	0.73 [0.32, 1.42]	*0.16*
18 months	26	1.63 ± 1.69	1.16 [0.32, 2.46]	103	0.81 ± 1.04	0.45 [0.10, 0.95]	*<0.01*
24 months	31	0.74 ± 0.93	0.29 [0.11, 1.29]	100	0.53 ± 0.73	0.18 [0.10, 0.67]	*0.13*

* Values are in ng/mL. P values are obtained from Wilcoxon-Mann-Whitney test for two independent samples.

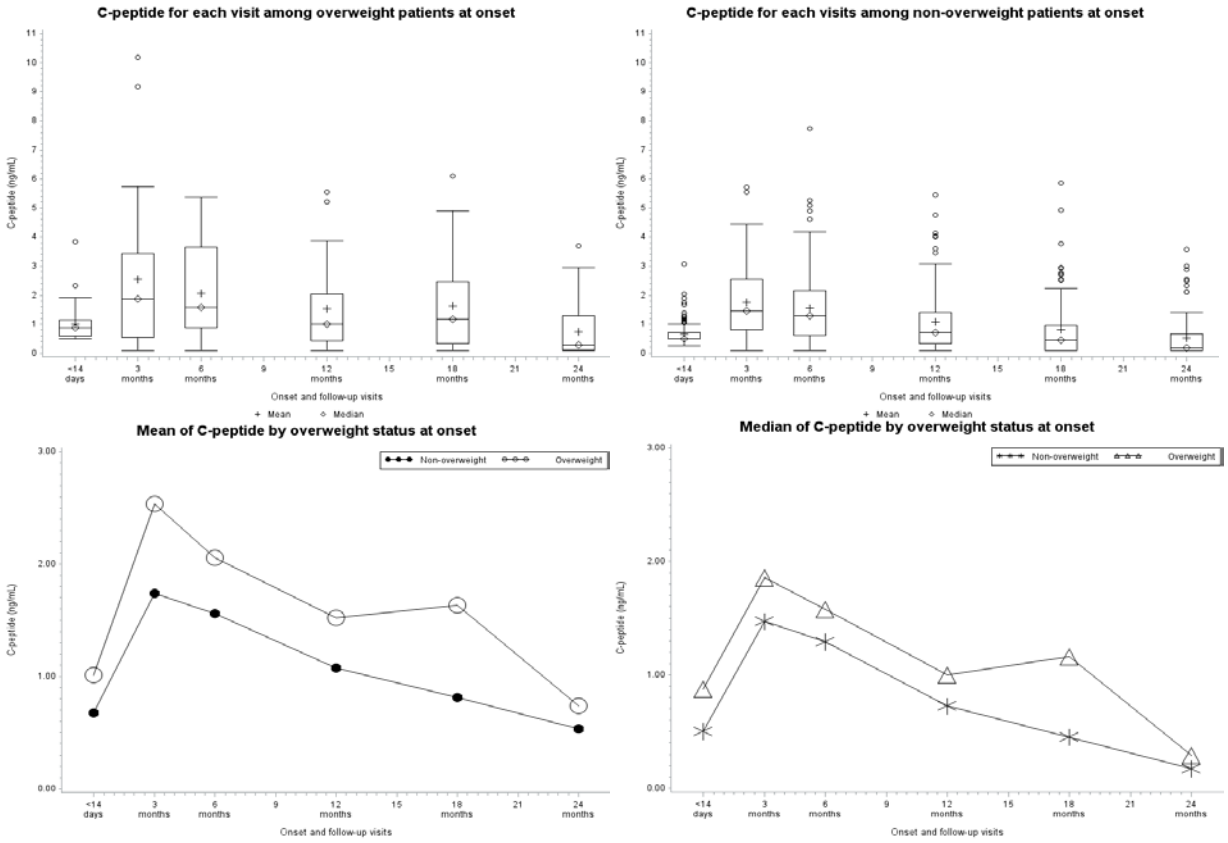

Figure 6. C-peptide over follow-up visits among overweight and non-overweight subjects at onset

Indexes from left to right, top to bottom are as follows: A: C-peptide for each visit among overweight subjects at onset, B: C-peptide for each visit among overweight subjects at onset, C: trajectory of mean values for C-peptide among overweight and non-overweight subjects, D: trajectory of median values for C-peptide among overweight and non-overweight subjects. + and ⋄ are the mean and median values of C-peptide of the study subjects at each visit time frame. ∘ are the extreme values greater than the Q3+1.5IQR of each visit. C-peptide levels are positively skewed with mean > median in every follow-up visit. C-peptide at onset (P<0.01) and 18-month follow-up visit (P<0.0001) are significantly different between overweight and non-overweight subjects at onset.

Table 8. Change of C-peptide among overweight and non-overweight subjects at onset (N=168)

Visits	Overweight at onset				Non-overweight at onset				*P value**
	N	**Mean ± SD**	**Median [IQR]**	***P value***	**N**	**Mean ± SD**	**Median [IQR]**	***P value***	
A. Change of C-peptide from onset									
3 months	33	1.54 ± 1.98	1.30 [-0.20, 2.14]	*0.04*[c]	119	1.06 ± 1.09	0.75 [0.22, 1.95]	*<0.0001*[b]	*0.69*
6 months	31	1.14 ± 1.55	0.69 [-0.03, 2.60]	*<0.001*[b]	103	0.88 ± 1.24	0.53 [0.06, 1.48]	*<0.0001*[c]	*0.61*
12 months	29	0.50 ± 1.19	0.11 [-0.16, 1.24]	*0.26*[c]	108	0.38 ± 1.06	0.11 [-0.26, 0.71]	*0.25*[c]	*0.63*
18 months	26	0.61 ± 1.26	0.21 [-0.40, 1.17]	*0.56*[c]	103	0.15 ± 0.97	-0.15 [-0.40, 0.38]	*0.11*[c]	*0.15*
24 months	31	-0.17 ± 0.84	-0.37 [-0.71, -0.02]	*<0.01*[c]	100	-0.13 ± 0.67	-0.40 [-0.40, 0.01]	*<0.0001*[c]	*0.38*
B. Change of C-peptide from 3 months									
6 months	28	-0.34 ± 1.59	-0.53 [-1.17, 0.49]	*0.26*[a]	96	-0.18 ± 1.28	-0.26 [-0.84, 0.38]	*0.02*[c]	*0.58*
12 months	27	-1.31 ± 1.90	-1.11 [-2.81, -0.12]	*<0.01*[a]	101	-0.66 ± 1.29	-0.60 [-1.36, -0.09]	*<0.0001*[b]	*0.15*
18 months	25	-1.04 ± 2.00	-0.83 [-2.24, -0.10]	*0.02*[a]	96	-0.84 ± 1.29	-0.82 [-1.71, -0.21]	*<0.0001*[b]	*0.69*
24 months	28	-1.62 ± 1.96	-1.59 [-2.70, -0.24]	*<0.001*[a]	94	-1.28 ± 1.18	-1.04 [-2.27, -0.51]	*<0.0001*[a]	*0.51*
C. Change of C-peptide from each follow-up to subsequence									
3 months	33	1.54 ± 1.98	1.30 [-0.20, 2.14]	*0.04*[c]	119	1.06 ± 1.09	0.75 [0.22, 1.95]	*<0.0001*[b]	*0.69*
6 months	28	-0.34 ± 1.59	-0.53 [-1.17, 0.49]	*0.26*[a]	96	-0.18 ± 1.28	-0.26 [-0.84, 0.38]	*0.02*[c]	*0.58*
12 months	24	-0.74 ± 1.06	-0.50 [-1.49, -0.15]	*<0.01*[a]	88	-0.50 ± 0.82	-0.38 [-1, -0.05]	*<0.0001*[b]	*0.35*
18 months	21	-0.12 ± 0.57	-0.02 [-0.38, 0.03]	*0.35*[a]	87	-0.20 ± 0.79	-0.17 [-0.42, 0]	*<0.0001*[b]	*0.61*
24 months	22	-0.63 ± 0.79	-0.32 [-0.83, 0]	*0.001*[c]	78	-0.24 ± 0.48	-0.07 [-0.37, 0]	*<0.0001*[c]	*0.02*

* Values are in ng/mL. P values are obtained from Wilcoxon-Mann-Whitney test for two independent samples unless otherwise noted. [a] Based on t-test. [b] Based on Wilcoxon signed rank test. [c] Based on sign test.

Table 9. The rate of change of C-peptide among overweight and non-overweight subjects at onset (N=168)

Visits	Overweight at onset				Non-overweight at onset				*P value**
	N	**Mean ± SD**	**Median [IQR]**	***P value***	**N**	**Mean ± SD**	**Median [IQR]**	***P value***	
A. Change of C-peptide from onset									
3 months	33	0.51 ± 0.66	0.43 [-0.07, 0.71]	*0.04*[c]	119	0.35 ± 0.36	0.25 [0.07, 0.65]	*<0.0001*[b]	*0.69*
6 months	31	0.19 ± 0.26	0.12 [-0.01, 0.43]	*<0.001*[b]	103	0.15 ± 0.21	0.09 [0.01, 0.25]	*<0.0001*[c]	*0.61*
12 months	29	0.04 ± 0.10	0.01 [-0.01, 0.10]	*0.26*[c]	108	0.03 ± 0.09	0.01 [-0.02, 0.06]	*0.25*[c]	*0.63*
18 months	26	0.03 ± 0.07	0.01 [-0.02, 0.07]	*0.56*[c]	103	0.01 ± 0.05	-0.01 [-0.02, 0.02]	*0.11*[c]	*0.15*
24 months	31	-0.01 ± 0.03	-0.02 [-0.03, 0]	*<0.01*[c]	100	-0.01 ± 0.03	-0.02 [-0.02, 0]	*<0.0001*[c]	*0.38*
B. Change of C-peptide from 3 months									
6 months	28	-0.11 ± 0.53	-0.18 [-0.39, 0.16]	*0.26*[a]	96	-0.06 ± 0.43	-0.09 [-0.28, 0.13]	*0.02*[c]	*0.58*
12 months	27	-0.15 ± 0.21	-0.12 [-0.31, -0.01]	*<0.01*[a]	101	-0.07 ± 0.14	-0.07 [-0.15, -0.01]	*<0.0001*[b]	*0.15*
18 months	25	-0.07 ± 0.13	-0.06 [-0.15, -0.01]	*0.02*[a]	96	-0.06 ± 0.09	-0.05 [-0.11, -0.01]	*<0.0001*[b]	*0.69*
24 months	28	-0.08 ± 0.09	-0.08 [-0.13, -0.01]	*<0.001*[a]	94	-0.06 ± 0.06	-0.05 [-0.11, -0.02]	*<0.0001*[a]	*0.39*[a]
C. Change of C-peptide from each follow-up to subsequence									
3 months	33	0.51 ± 0.66	0.43 [-0.07, 0.71]	*0.04*[c]	119	0.35 ± 0.36	0.25 [0.07, 0.65]	*<0.0001*[b]	*0.69*
6 months	28	-0.11 ± 0.53	-0.18 [-0.39, 0.16]	*0.26*[a]	96	-0.06 ± 0.43	-0.09 [-0.28, 0.13]	*0.02*[c]	*0.58*
12 months	24	-0.12 ± 0.18	-0.08 [-0.25, -0.03]	*<0.01*[a]	88	-0.08 ± 0.14	-0.06 [-0.17, -0.01]	*<0.0001*[b]	*0.35*
18 months	21	-0.02 ± 0.09	0 [-0.06, 0.01]	*0.35*[a]	87	-0.03 ± 0.13	-0.03 [-0.07, 0]	*<0.0001*[b]	*0.61*
24 months	22	-0.11 ± 0.13	-0.05 [-0.14, 0]	*0.001*[c]	78	-0.04 ± 0.08	-0.01 [-0.06, 0]	*<0.0001*[c]	*0.02*

* Values are calculated by the change in subject / the length of window between two follow-ups in months, in ng/mL/month. P values are obtained from Wilcoxon-Mann-Whitney test for two independent samples unless otherwise noted. [a] Based on t-test. [b] Based on Wilcoxon signed rank test. [c] Based on sign test.

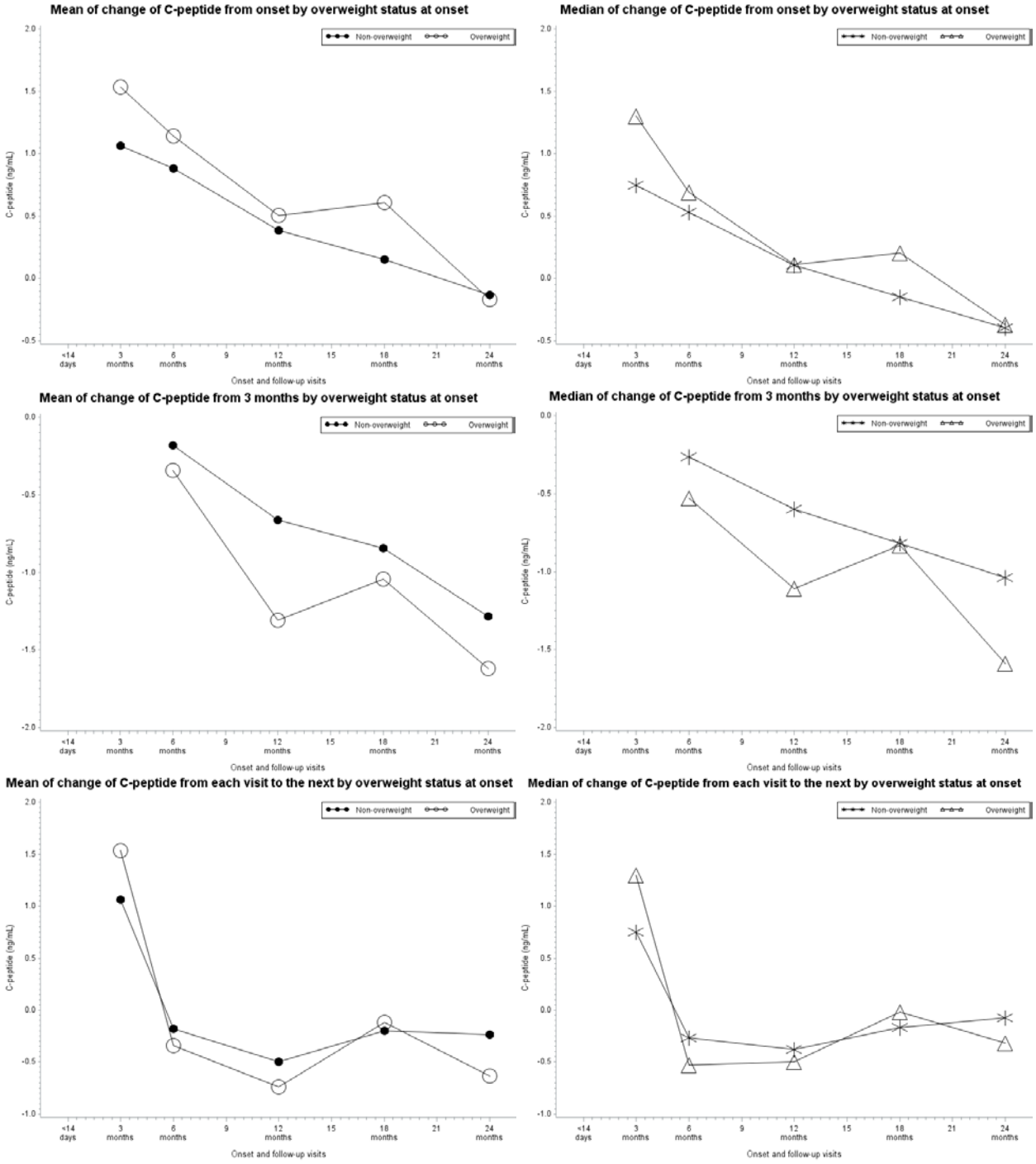

Figure 7. Change of C-peptide between two visits among overweight and non-overweight subjects at onset

Left three are the trajectory of mean value of change of C-peptide from onset, 3 months, and previous visit, respectively. Right three are the trajectory of median value of change of C-peptide from onset, 3 months, and previous visit.

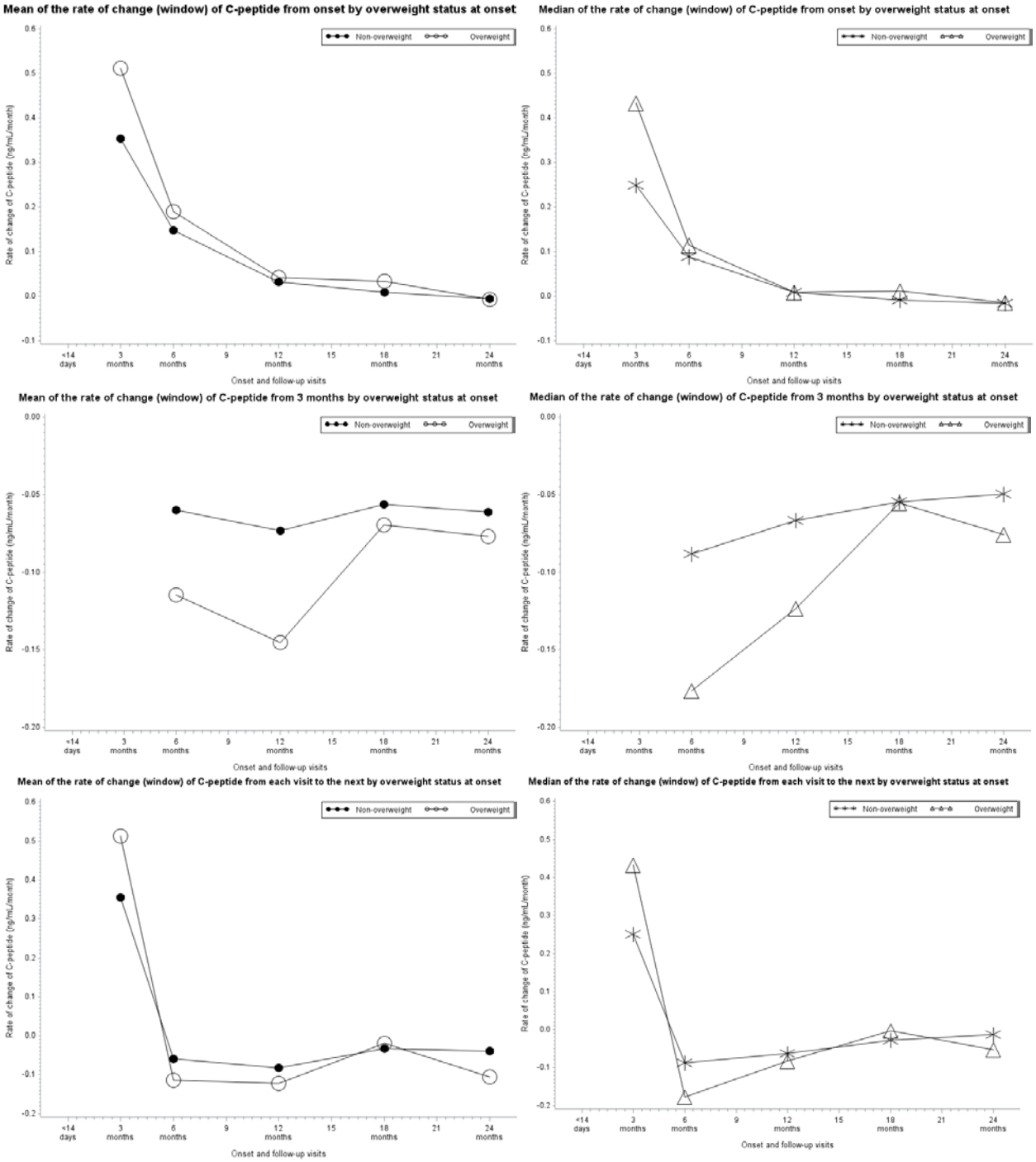

Figure 8. The rate of change of C-peptide between two visits among overweight and non-overweight subjects at onset

Left three are the trajectory of mean value of the rate of change of C-peptide from onset, 3 months, and previous visit, respectively. Right three are the trajectory of median value of the rate of change of C-peptide from onset, 3 months, and previous visit.

3.4.2 C-peptide and overweight status at onset and 3 months

Table 10. C-peptide over follow-up visits among overweight status at onset and 3-month visit (N=168)

Visits	Overweight status at onset and 3 months	N	Mean ± SD	Median [IQR]	*P value*	
					K-W	*K-W (exclude O - N)*
<14 days	O - O	29	0.99 ± 0.64	0.82 [0.63, 1.03]	*<0.0001*	*<0.0001***
	O - N	4	1.31 ± 0.80	1.21 [0.70, 1.92]		
	N - O	27	0.57 ± 0.12	0.50 [0.50, 0.61]		
	N - N	98	0.70 ± 0.41	0.50 [0.50, 0.77]		
3 months	O - O	29	2.30 ± 2.26	1.73 [0.45, 3.10]	*0.14*	*0.61*
	O - N	3	5.06 ± 3.66	3.81 [2.19, 9.18]		
	N - O	27	1.54 ± 0.99	1.16 [0.63, 2.45]		
	N - N	91	1.81 ± 1.24	1.49 [0.85, 2.59]		
6 months	O - O	24	2.01 ± 1.72	1.29 [0.64, 3.66]	*0.39*	*0.36*
	O - N	4	2.27 ± 1.79	1.59 [1.27, 3.26]		
	N - O	23	1.37 ± 1.34	0.81 [0.51, 2.09]		
	N - N	79	1.61 ± 1.33	1.34 [0.67, 2.20]		
12 months	O - O	23	1.48 ± 1.60	0.81 [0.34, 2.03]	*0.58*	*0.73*
	O - N	3	1.79 ± 1.53	1 [0.82, 3.56]		
	N - O	22	1.21 ± 1.55	0.68 [0.25, 1.40]		
	N - N	84	1.04 ± 0.97	0.76 [0.32, 1.50]		
18 months	O - O	22	1.55 ± 1.71	1.16 [0.30, 2.19]	*0.02*	*0.07*
	O - N	3	2.65 ± 1.62	2.69 [1.01, 4.24]		
	N - O	24	0.97 ± 1.50	0.34 [0.10, 1.01]		
	N - N	77	0.74 ± 0.85	0.45 [0.10, 0.90]		
24 months	O - O	24	0.72 ± 0.96	0.24 [0.10, 1.06]	*0.40*	*0.53*
	O - N	4	0.88 ± 0.97	0.55 [0.28, 1.49]		
	N - O	22	0.54 ± 0.84	0.17 [0.10, 0.48]		
	N - N	77	0.50 ± 0.67	0.16 [0.10, 0.66]		

Abbreviation: O – O: overweight – overweight, O – N: overweight – non-overweight, N – O: non-overweight – overweight, N – N: non-overweight – non-overweight.

*Values are in ng/mL. P values are obtained from Kruskal-Wallis test and Dwass, Steel, Critchlow-Fligner test of pairwise comparison.

** The differences among the three groups at onset exist between N – N vs. O – O (P<0.001), and N – O vs. O – O (P<0.0001).

Out of the 168 study subjects, 158 had available information of overweight status at both onset and 3-month follow-up. Out of the 127 non-overweight subjects at onset, 98 (62.0% of 158, 77.2% of 127) were still non-overweight at 3-month follow-up, and 27 (17.1% of 158, 21.3% of 127) became overweight at 3 months. Of the 36 overweight participants at onset, 29 (18.4% of 158, 80.6 % of 36) were still overweight at 3-month follow-up, and only 4 participants (2.5% of 158, 11.1% of 36) became non-overweight at 3-month follow-up (Table 10). Only at onset, the C-peptide levels were significantly different among the four groups ($P<0.0001$).

C-peptide changes in the four groups of subjects followed the same pattern: C-peptide levels increased from onset to 3-month follow up visit and reached the high point at 3 months, and then gradually decreased after that until 24-month follow-up (Figure 9). The figure also shows that the overweight – non-overweight subjects had the highest values across the 2-year follow-ups; however, since there were only 4 participants in this group, this probably due to the extreme variability of C-peptide level. Regardless of the overweight – non-overweight group, overweight subjects at onset that remained overweight at 3-month visit tended to have higher C-peptide levels than others. Additionally, subjects in overweight – overweight group also showed a same pattern as the overweight subjects at onset, of which C-peptide had a slightly jump-up at 18-month visit. Graphically, the rate of decrease of C-peptide from 3-month visit to the 24-month visit for subjects that were non-overweight at onset and changed to overweight at 3 months presented lower levels than that among the subjects remaining non-overweight at 3-month visit (Appendix A4).

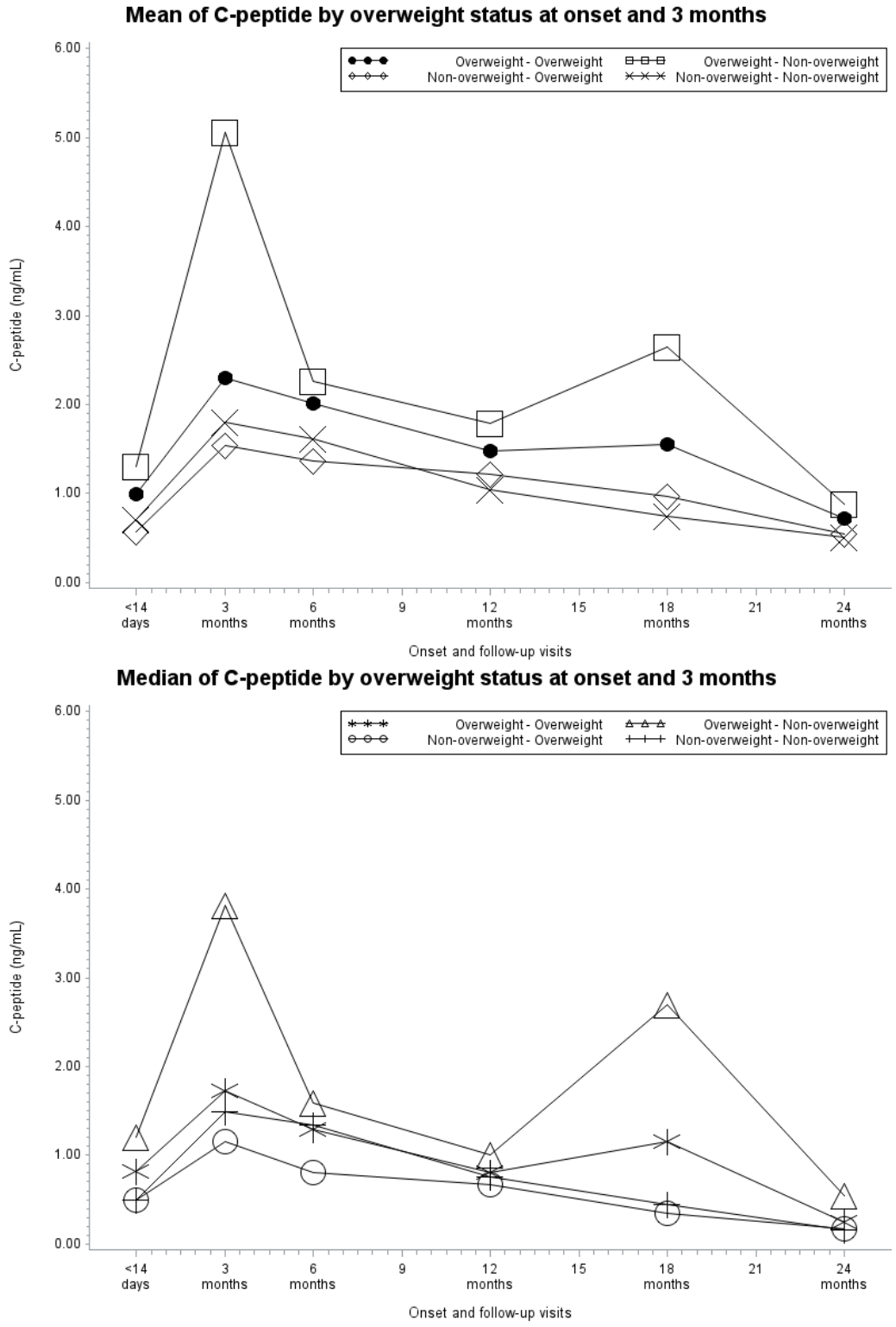

Figure 9. Trajectory plot for mean and median of C-peptide for each combination of overweight status at onset and 3-month visit

3.5 C-PEPTIDE AND BASELINE AUTOANTIBODIES

Table 11. C-peptide over follow-up visits among subjects with different number of positive antibodies at baseline (N=168)

Visits	# positive Abs at baseline	N	Mean ± SD	Median [IQR]	*P value*
<14 days	0	16	0.90 ± 0.83	0.61 [0.50, 0.92]	*0.92*
	1	13	0.73 ± 0.35	0.59 [0.50, 0.86]	
	2	27	0.69 ± 0.28	0.50 [0.50, 0.88]	
	3	54	0.76 ± 0.45	0.58 [0.50, 0.87]	
	4 or 5	58	0.75 ± 0.44	0.56 [0.50, 0.87]	
3 months	0	15	2.05 ± 2.44	1.16 [0.74, 2.38]	*0.43*
	1	11	1.68 ± 1.45	1.47 [0.60, 2.01]	
	2	26	2.25 ± 1.33	2.36 [0.94, 3.36]	
	3	51	1.73 ± 1.40	1.48 [0.69, 2.48]	
	4 or 5	54	1.94 ± 1.61	1.50 [0.77, 2.95]	
6 months	0	11	1.88 ± 1.24	1.83 [0.65, 2.69]	*0.60*
	1	11	2.03 ± 1.57	1.59 [0.99, 2.06]	
	2	25	1.81 ± 1.44	1.60 [0.58, 2.37]	
	3	46	1.65 ± 1.57	1.13 [0.67, 2.15]	
	4 or 5	46	1.51 ± 1.30	1.11 [0.49, 2.22]	
12 months	0	14	1.69 ± 1.50	1.42 [0.43, 2.35]	*0.20*
	1	12	1.63 ± 1.58	0.99 [0.71, 2.10]	
	2	26	1.17 ± 1.06	0.95 [0.34, 1.55]	
	3	41	1.02 ± 1.16	0.68 [0.26, 1.18]	
	4 or 5	48	1.04 ± 1.13	0.73 [0.29, 1.20]	
18 months	0	12	1.66 ± 1.77	1.18 [0.48, 2.02]	*0.13*
	1	9	1.43 ± 1.11	1.34 [0.56, 2.22]	
	2	21	1 ± 1.42	0.58 [0.11, 1.01]	
	3	44	0.86 ± 1.18	0.42 [0.10, 0.99]	
	4 or 5	47	0.82 ± 1.03	0.32 [0.10, 1.13]	
24 months	0	11	0.80 ± 0.85	0.53 [0.10, 1.15]	*0.51*
	1	13	0.93 ± 0.99	0.48 [0.10, 1.66]	
	2	22	0.63 ± 0.93	0.25 [0.10, 0.69]	
	3	43	0.48 ± 0.67	0.19 [0.10, 0.66]	
	4 or 5	46	0.52 ± 0.71	0.14 [0.10, 0.55]	

*Values are in ng/mL. P values are obtained from Kruskal-Wallis test.

The C-peptide levels by autoantibodies status were examined in Table 11 grouped by the number of positive autoantibodies at baseline. All groups followed the same change pattern that C-peptide reached the highest level at 3-month visit and then went down for following visits, and touched the lowest level at the end of the 2-year visit (Figure 10). In general, the less the number

of positive autoantibodies that the subjects had at baseline, the more C-peptide was produced as time passed and the slower the mean C-peptide level decreased from 3 months. (Appendix A5). Kruskal-Wallis Tests suggested that C-peptide levels were not significantly different among five autoantibody groups at onset and each follow up visit.

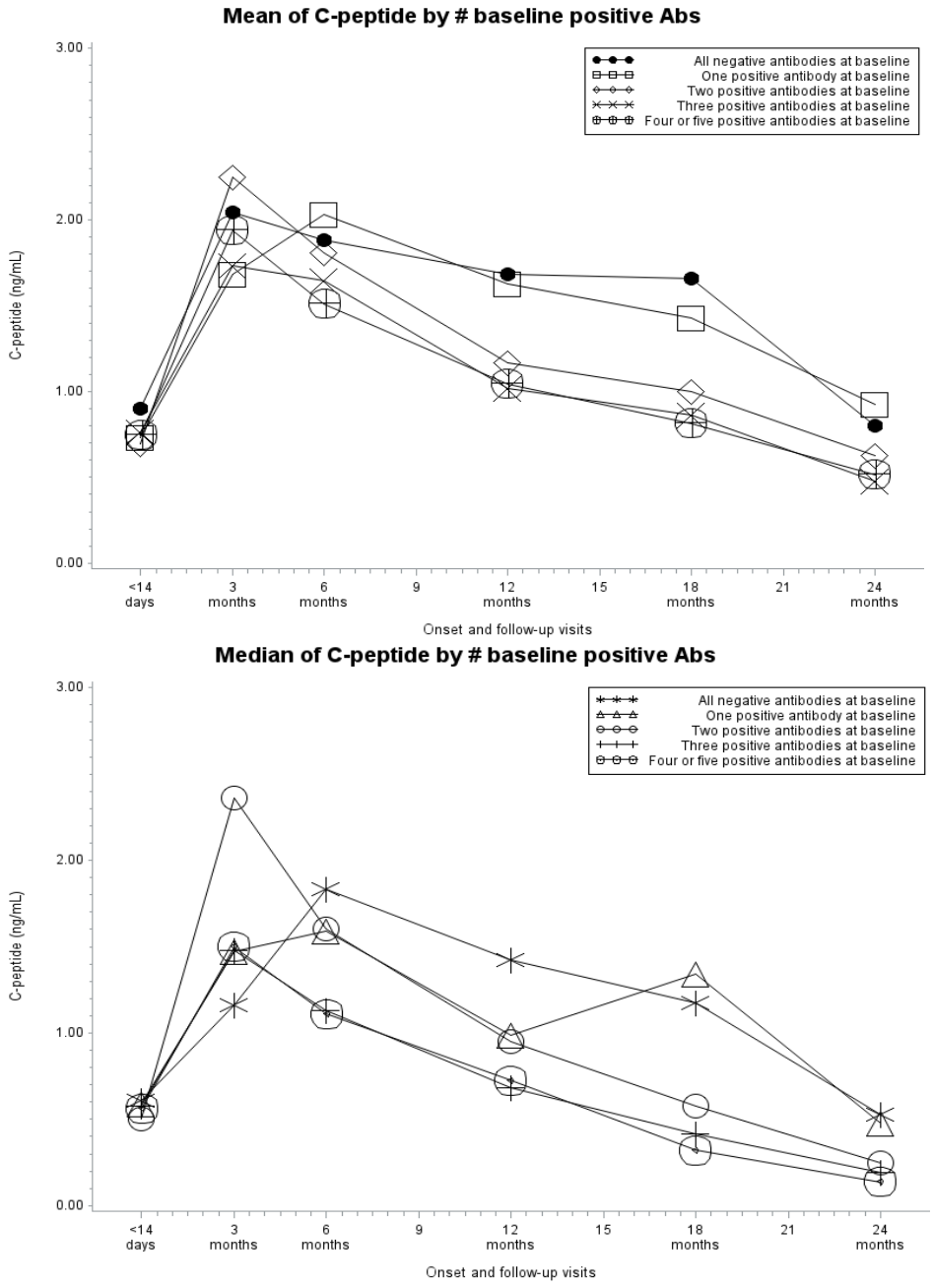

Figure 10. Trajectory plot of mean and median of C-peptide at each follow-up by different number of positive autoantibodies at baseline

3.6 CORRELATIONS BETWEEN COVARIATES AND FOLLOW-UP C-PEPTIDE MEASURES

The potential relationship between 2-year follow-up C-peptide levels and covariates, including age at diagnosis, gender, HbA1c at onset and 3 months, and IDAA1c at 3 months, were assessed in Table 12. Age at diagnosis was significantly positively correlated with each follow-up C-peptide. HbA1c level is a useful determinant of how well the glycemia level has been controlled in the recent past in diabetes. The higher the glucose concentration in blood is, the higher the level of HbA1c is. The correlation coefficients of HbA1c at onset suggested that HbA1c was only significantly correlated with onset C-peptide (Pearson=-0.21, $P<0.01$ and Spearman=-0.19, $P=0.01$) while 3-month HbA1c was significantly negatively correlated with all C-peptide in 2-year follow-up. Insulin dose-adjusted HbA1c (IDAA1c) is an indicator for partial remission in T1D [21], and can give more credible measure for the insulin reserve adjusting the effect of absorbed external insulin. In current study, 3-month IDAA1c shown a highly correlation with all C-peptide follow up levels [12]. Therefore, age at diagnosis, HbA1c at 3 months, and IDAA1c at 3 months could be potentially predictors for the C-peptide levels at each follow-up.

The correlations among age at diagnosis, HbA1c at 3 months, and IDAA1c at 3 months were also evaluated before fitting linear mixed models (Table 13). HbA1c at 3 months had statistical highly correlations with IDAA1c at 3-month follow-up (Pearson=0.72, $P<0.0001$, and Spearman=0.76, $P<0.0001$), whereas, there was no explicitly significant correlation between 3-month IDAA1c and age at diagnosis (Pearson=0.03, $P=0.75$, and Spearman=-0.0008, $P=0.99$).

Table 12. Correlations between C-peptide over time and other covariates at baseline

Covariate	Pearson Correlation Coefficients *(P value)* Spearman Correlation Coefficients *(P value)*					
	< 14 days	3 months	6 months	12 months	18 months	24 months
Age at diagnosis, yrs	0.24 *(<0.01)* 0.32 *(<0.0001)*	0.28 *(<0.01)* 0.30 *(0.0001)*	0.42 *(<0.0001)* 0.45 *(<0.0001)*	0.44 *(<0.0001)* 0.42 *(<0.0001)*	0.46 *(<0.0001)* 0.53 *(<0.0001)*	0.47527 *(<0.0001)* 0.49 *(<0.0001)*
HbA1c at onset	-0.21 *(<0.01)* -0.19 *(0.01)*	-0.05 *(0.51)* -0.01 *(0.92)*	0.05 *(0.56)* 0.12 *(0.15)*	0.11 *(0.18)* 0.14 *(0.10)*	0.05 *(0.59)* 0.14 *(0.12)*	0.10 *(0.25)* 0.19 *(0.03)*
HbA1c at 3 months	-0.27 *(<0.001)* -0.26 *(<0.001)*	-0.28 *(<0.001)* -0.27145 *(<0.001)*	-0.42 *(<0.0001)* -0.43 *(<0.0001)*	-0.37 *(<0.0001)* -0.42 *(<0.0001)*	-0.39 *(<0.0001)* -0.39 *(<0.0001)*	-0.32 *(<0.001)* -0.32 *(<0.001)*
IDAA1c at 3 months*	-0.18 *(0.03)* -0.21 *(0.01)*	-0.28 *(<0.001)* -0.27 *(<0.01)*	-0.37 *(<0.0001)* -0.36 *(<0.0001)*	-0.23 *(0.01)* -0.40 *(<0.0001)*	-0.18 *(0.05)* -0.29 *(<0.01)*	-0.15 *(0.10)* -0.26 *(<0.01)*

* IDAA1c, insulin dose-adjusted HbA1c.

^ P values are obtained from Kruskal-Wallis test.

Table 13. Correlations among all the continuous covariates

Pearson *(P value)* Spearman *(P value)*	Age at diagnosis, yrs	HbA1c at 3 months	IDAA1c at 3 months
Age at diagnosis, yrs		-0.21 *(<0.01)* -0.21 *(<0.01)*	0.03 *(0.75)* -0.0008 *(0.99)*
HbA1c at 3 months			0.72 *(<0.0001)* 0.76 *(<0.0001)*
IDAA1c at 3 months			

3.7 LINEAR MIXED MODELS FOR C-PEPTIDE AND CHANGE OF C-PEPTIDE FROM ONSET AT 3-MONTH VISIT AND FORWARD

The estimates of multivariate analysis for C-peptide and change of C-peptide from onset at 3 months and subsequent follow-ups using continuous visits as 3, 6, 12, 18, and 24 months were summarized in Table 14 and Table 15. The results for univariate models and multivariate analysis using time as days since diagnosis and categorical visits as 3, 6, 12, 18, and 24 months were presented in Appendix A6. All multivariate analysis for corresponding endpoint showed similar results.

In the univariate analysis for endpoint as C-peptide at 3 months and forward, the clinical meaningful covariates, including age at diagnosis, IDAA1c at 3 months, and follow-ups were individually significant predictors ($P<0.001$), while gender and number of positive autoantibodies at onset (0-1 vs. 2+) were slightly non-significant (both $P=0.08$). Overweight at onset individually was a significant predictor ($P=0.006$). In the multivariate analysis, all the baseline covariates and overweight at onset were shown statistically significant effect on the prediction of C-peptide at 3 months and subsequent follow-ups. It suggested that, for a given time period, on average, older female overweight children at diagnosis with less than one number of positive autoantibodies at baseline and lower value of IDAA1c at 3 months would have a higher level of C-peptide than younger male non-overweight children at onset with more than one number of positive autoantibodies at baseline and higher value of IDAA1c at 3 months. The estimates in Table 15 showed that adjusting for other baseline covariates, the mean levels of C-peptide at 3 months, 6 months, 12 months, and 18 months were significantly different between overweight and non-overweight subjects (with $P=0.0045$, 0.0033, 0.0030, and 0.0124 respectively). However, at 24 months after insulin treatment, they were not statistically

significant (P=0.1055). At 3 months, the difference of mean C-peptide levels between overweight and non-overweight subjects was as high as 0.7281ng/mL (95%C.I.: (0.2300, 1.2263)) and the differences became less in forward visits. The overall mean rate of change of C-peptide for overweight subjects was 0.7865ng/mL/months (95% C.I.: (0.2277, 1.3452), P=0.0062) compared to non-overweight subjects sharing the same information for other baseline covariates.

Table 14. Multivariate analysis for C-peptide and change of C-peptide from onset over 3 months and forward

Covariates	C-peptide at 3 months and forward		Change of C-peptide from onset of 3 months and forward	
	Fixed effects (SE)	*P value*	Fixed effects (SE)	*P value*
IDAA1c at 3 months	-0.1456 (0.04589)	*0.0019*	-0.1052 (0.04189)	*0.0132*
Female	0.4374 (0.1461)	*0.0033*	0.2189 (0.1333)	*0.1029*
Age at diagnosis	0.1428 (0.02017)	*<0.0001*	0.1068 (0.01840)	*<0.0001*
2 or more Abs+ at baseline	-0.4136 (0.1943)	*0.0352*	-0.2894 (0.1775)	*0.1054*
Follow-up visits (in months)	-0.05817 (0.006164)	*<0.0001*	-0.05771 (0.006144)	*<0.0001*
Overweight at onset	0.7865 (0.2825)	*0.0062*	0.4505 (0.2528)	*0.0771*
Overweight at onset * visits	-0.01945 (0.01333)	*0.1470*	-0.01756 (0.01329)	*0.1886*

*Model B: follow-up visits were coded as 3, 6, 12, 18, and 24 in months as continuous.

Table 15. Comparison of estimated means of C-peptide and change of C-peptide from onset between overweight and non-overweight subjects at each follow-up

Visits	C-peptide		Change of C-peptide from onset	
	Difference of means (95% C.I.)	*P value*	Difference of means (95% C.I.)	*P value*
3 months	0.7281 (0.2300, 1.2263)	*0.0045*	0.3978 (-0.04307, 0.8386)	*0.0766*
6 months	0.6698 (0.2264, 1.1132)	*0.0033*	0.3451 (-0.04352, 0.7337)	*0.0813*
12 months	0.5531 (0.1910, 0.9152)	*0.0030*	0.2397 (-0.07913, 0.5586)	*0.1394*
18 months	0.4364 (0.09613, 0.7767)	*0.0124*	0.1344 (-0.1853, 0.4541)	*0.4071*
24 months	0.3197 (-0.06845, 0.7079)	*0.1055*	0.02902 (-0.3616, 0.4197)	*0.8833*
Overall across time	0.7865 (0.2277, 1.3452)	*0.0062*	0.4505 (-0.04963, 0.9506)	*0.0771*

* For model B: follow-up visits were coded as 3, 6, 12, 18, and 24 in months as continuous. Values are in ng/mL. P values were obtained from t-test for testing the estimates are different from 0 or not.

In the univariate analysis for endpoint as change in C-peptide from onset at 3 months and forward, overweight at onset individually was no longer a significant predictor (P=0.1612) (Appendix A6). In the multivariate analysis, the change level of C-peptide from onset was significantly related to IDAA1c at 3 months, age at diagnosis, and visits. Overweight was borderline non-significance (P=0.0771). It suggested that adjusting for other baseline covariates, the difference of mean change level of C-peptide from onset between overweight and non-overweight subjects at 3 months (0.3978, 95%C.I.: (-0.04307, 0.8386)) was higher than any other visits. The overall mean increase of C-peptide from onset for overweight subjects could be 0.4504ng/mL (95% C.I.: (-0.04963, 0.9506)) faster per month than in non-overweight subjects that share the same values for other covariates.

The goodness-of-fit for each model was checked by spaghetti plots and Pearson residuals (Appendix A6). The plots in Figure 11 and 12 were comparing the observed values of C-peptide and change of C-peptide from onset at 3 months and forward follow-ups to the fitted values. While the model estimates were indeed very close to the observed values, there shows some underestimation using such a model.

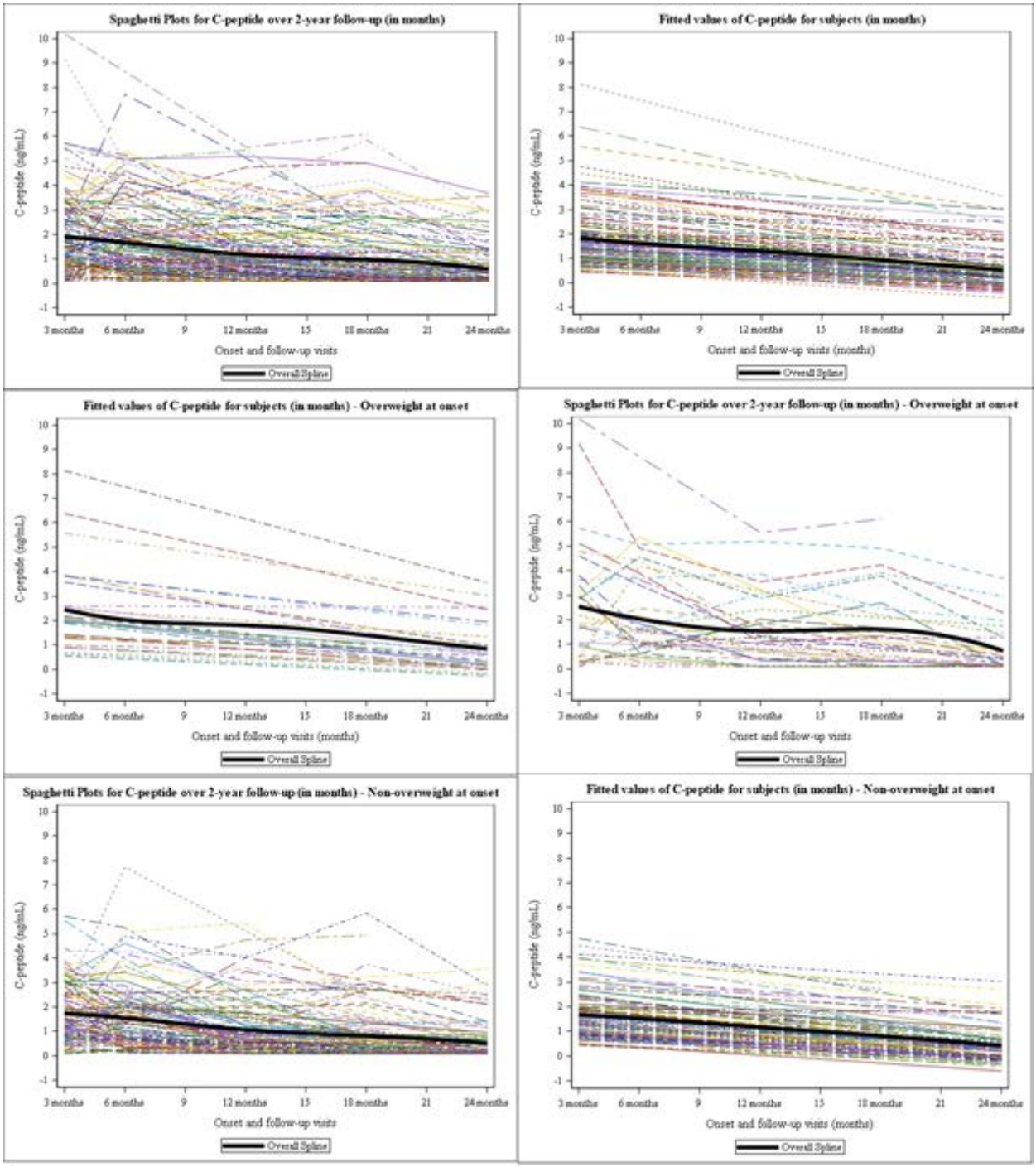

Figure 11. Spaghetti plots for actual and fitted values of C-peptide at 3 months and forward follow-ups in Model B

Left three are observed values for C-peptide. Right three are fitted values for C-peptide. Model B: follow-up visits were coded as 3, 6, 12, 18, and 24 in months as continuous.

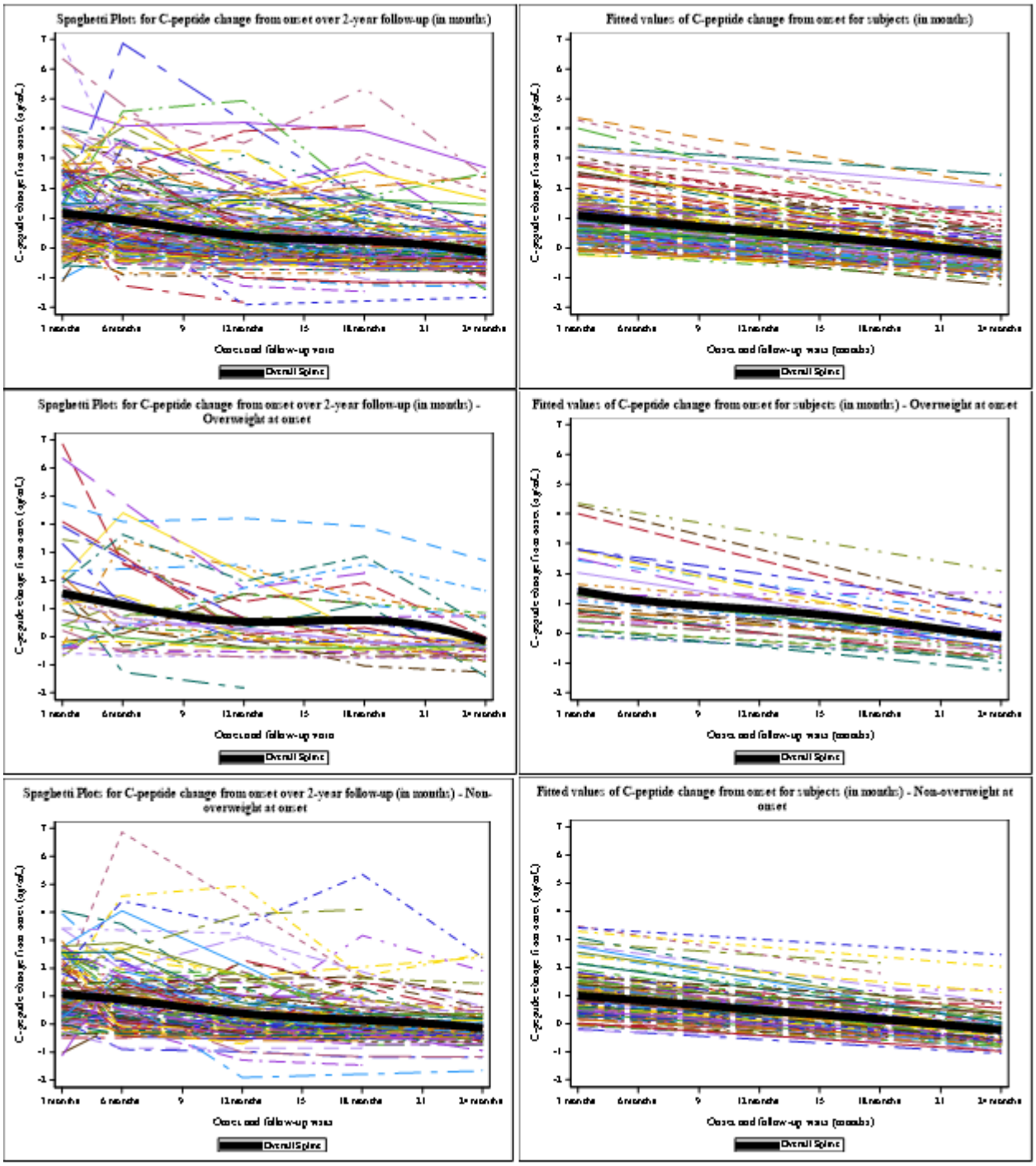

Figure 12. Spaghetti plots for actual and fitted values of change of C-peptide from onset in Model B

Left three are observed values for C-peptide. Right three are fitted values for C-peptide. Model B: follow-up visits were coded as 3, 6, 12, 18, and 24 in months as continuous.

4.0 DISCUSSION

The underlying fundamental defect of T1D is the continuous loss of insulin secretion after diagnosis, which is an indicator of the impairment of the pancreatic β cells [1]. In the light of such a rationale, interventions that can slow or stop the destruction of pancreas β cells would delay or prevent the clinical onset of T1D. Several studies have found that the prevalence of overweight at onset among T1D children is increasing [14, 15, 24, 33]. However, the underlying relationship between overweight and residual β-cell function is still unproved. In this study, C-peptide level, a surrogate endpoint for residual β-cell function, was used to investigate such a problem.

The primary finding of this study is that after adjusting for other baseline covariates, compared to non-overweight T1D children, overweight children had higher C-peptide levels at period of 3, 6, 12, and 18 months after diagnosis; however, at 24 months this difference was not significant. The physiological mechanism for overweight patients to have higher C-peptide levels remains unknown. One possible conjecture is that overweight patients are usually diagnosed earlier than non-overweight patients [34]. Under such circumstance, the residual function of pancreas β cells would be better in overweight patients than in non-overweight patients.

The mean BMI% for the study population was 51.4 at onset and increased to 73.5 at 3 months. In the progress of T1D, subjects with preclinical T1D suffer dramatic weight loss before they are diagnosed, which is one of the most common symptoms of T1D (61%) [35]. After

receiving insulin treatment, their physical levels will return to normal, which is consistent with the presence of “honeymoon phase.” To better detect the relationship of the progress of the disease and the overweight status that the T1D children normally are, further analysis using overweight status at 3 months will be conducted.

T1D is characterized by the autoimmune destruction of pancreas β cells. The production of islet autoantibodies reflects the magnitude of the autoimmunity. Among this study population, the mean number of positive autoantibodies at baseline was 2.8. Only 16 (9.5%) participants had all negative autoantibodies at baseline, and 58 (34.5%) had 4 or 5 positive autoantibodies at baseline. The statistical result of our study was consistent with earlier studies that high levels of islet antibodies are related to the rapid destruction of pancreas β cells and to the clinical onset of T1D [26, 36]. Additionally, through investigating the combination of all five positive autoantibodies in this study population, ICA human was mostly positive (80.4% of 168 subjects), which is in line with previous findings that the presence of ICA can be used as a surrogate marker to predict clinical T1D [37]. ZnT8A, a new marker discovered in recent years, also had a high present rate of 60.2% in this study. Various studies state that ZnT8A can improve the diagnosis and prediction of juvenile-onset T1D [38, 39]. Whether positive antibodies and overweight are associated or not need continued investigating.

This study was a longitudinal study where patients were followed from onset to two years after the initiation of insulin treatment. Such an optimal study design enabled the inference on the relationship of overweight and insulin reserve in T1D new-onsets over time. Linear mixed models are an appropriate method to analyze correlated longitudinal data. Since C-peptide values are right skewed in this population, the performance of the estimates for predictors may not be robust enough. However, since the primary goal of this study was to model the course of the C-

peptide across time, the results given by linear mixed models could give some useful reference here. In order to improve the goodness-of-fit of the models, the same linear mixed models were fitted using log-transformation C-peptide and log-ratio of C-peptide to onset as primary endpoints. The effects of overweight at onset showed the same pattern as has been discussed earlier, even though the overweight was no longer a statistically significant predictor (Appendix A7). Moreover, the Pearson residuals plots showed better fit using log-transformed C-peptide than the untransformed values of C-peptide (Appendix A7), but the interpretation of the transformed data is less clear. Additionally, in the current study, C-peptide levels at diagnosis were not included in the models because of the non-linear changes from onset to 3 months, and then from 3 months and beyond.

As there is still no cure for T1D, it is important to identify characteristics related to prolong preservation of insulin production in patients. Clinically, the diagnosis of T1D was followed by a short remission period at 3 months after being placed in insulin therapy. Maintaining or extending the honeymoon period could delay or prevent T1D children from developing severe complications. In this study, the overweight children had a higher level of insulin reserve than the non-overweight children across the entire two-year follow-up, especially in the honeymoon phase. These findings may well suggest that the overweight status at onset may be informative in developing effective interventions targeting on overweight patients with higher C-peptide to prolong their preservation of residual function of pancreas β cells.

APPENDIX A: SUPPLEMENTARY RESULTS

A.1 SUBJECTS AND FOLLOW-UPS

Table 16. Summary of duplicate records in each follow-up

Study window$^{\Delta}$	# participants	# single record	# multiple records*
Onset	198	42	156
3-month	194	193	1
6-month	176	165	11
12-month	181	169	12
18-month	168	152	16
24-month	169	158	11

* Some participants have more than 2 records in each study window.

Δ This study just concerns up to 2-year follow-up.

Table 17. Demographic and clinical characteristics of subjects having 3 autoantibodies measured at baseline (N=11)

Characteristics	Subjects with 3 autoantibodies measured at baseline (N=11)
Demographic	
Age at diagnosis, yrs	8.4 ± 3.8
Age group, yrs, n (%)	
0-4	18 (10.71)
5-9	68 (40.48)
10-14	69 (41.07)
≥ 15	13 (7.74)
Gender, n (%)	
Male	8 (72.73)
Female	3 (27.27)
Race, n (%)	
White	11 (100)
Black	0
Other	0
BMI at onset, kg/m^2	18.26 ± 6.21
BMI at 3 months, kg/m^2	19.48 ± 4.56
BMI % at onset	48.99 ± 38.67
BMI % at 3 months	72.80 ± 25.59
BMI z-score at onset	0.02 ± 1.70
BMI z-score at 3 months	0.86 ± 0.94
Clinical	
HbA1c at onset	11.75 ± 2.51
HbA1c at 3 months	7.35 ± 0.83
IDAA1c at 3 months	9.61 ± 1.59[a]
Positive antibodies at baseline, n (%)	
0	1 (9.09)
1	5 (45.45)
2	3 (27.27)
3	2 (18.18)
Autoantibodies at baseline, n (%)	
+ IAA	0
+ IA2	4 (36.36)
+ GAD	5 (45.45)
+ ICA human	8 (72.73)
C-peptide, ng/mL	
< 14 days	0.57 [0.50, 0.81]
3 months	0.88 [0.71, 2.38][a]
6 months	0.85 [0.60, 1.45][b]
12 months	0.47 [0.31, 1.00][b]
18 months	0.40 [0.10, 0.88][c]
24 months	0.10 [0.10, 0.10][d]

* Data are mean ± SD or median [IQR] unless otherwise noted.

[a] Based on 10 subjects. [b] Based on 9 subjects. [c] Based on 6 subjects. [d] Based on 8 subjects.

Table 18. Combinations of positive autoantibodies at baseline of study subjects (N=168)

	Positive Abs combination at baseline	N (%)	
All negative		16 (100)	16 (9.52)
One positive	IA2	2 (15.38)	13 (7.74)
	IAA	2 (15.38)	
	ICA human	4 (30.77)	
	GAD	5 (38.46)	
Two positive	IA2 and IAA	1 (3.70)	27 (16.07)
	IA2 and ICA human	8 (29.63)	
	IA2 and GAD	2 (7.41)	
	IA2 and ZnT8A	1 (3.70)	
	IAA and ICA human	2 (7.41)	
	ICA human and GAD	6 (22.22)	
	ICA human and ZnT8A	5 (18.52)	
	GAD and ZnT8A	2 (7.41)	
Three positive	IA2, IAA, and ICA human	3 (5.56)	54 (32.14)
	IA2, ICA human, and GAD	13 (24.07)	
	IA2, ICA human, and ZnT8A	27 (50)	
	IA2, GAD, and ZnT8A	1 (1.85)	
	IAA, ICA human, and ZnT8A	2 (3.70)	
	ICA human, GAD, and ZnT8A	8 (14.81)	
Four positive	IA2, IAA, ICA human, and GAD	4 (8.16)	49(29.17)
	IA2, IAA, ICA human, and ZnT8A	6 (12.24)	
	IA2, IAA, GAD, and ZnT8A	1 (2.04)	
	IA2, ICA human, GAD, and ZnT8A	37 (75.51)	
	IAA, ICA human, GAD, and ZnT8A	1 (2.04)	
Five positive	IA2, IAA, ICA human, GAD, and ZnT8A	9 (100)	9 (5.36)
Total		**168**	**168 (100)**

A.2 C-PEPTIDE OF EACH FOLLOW-UP

Table 19. Sixteen follow-up patterns for C-peptide (N=168)

<14 days	3 months	6 months	12 months	18 months	24 months	N (%)
X	X	X	X	X	X	63 (37.50)
X	X	X	X	X	.	21 (12.50)
X	X	X	X	.	X	21 (12.50)
X	X	X	X	.	.	3 (1.79)
X	X	X	.	X	X	14 (8.33)
X	X	X	.	X	.	2 (1.19)
X	X	X	.	.	X	5 (2.98)
X	X	.	X	X	X	16 (9.52)
X	X	.	X	X	.	5 (2.98)
X	X	.	X	.	X	3 (1.79)
X	X	.	.	X	X	4 (2.38)
X	.	X	X	X	X	3 (1.79)
X	.	X	X	X	.	2 (1.19)
X	.	X	X	.	X	3 (1.79)
X	.	X	.	X	X	2 (1.19)
X	.	.	X	X	X	1 (0.60)

* X indicates that there is a clinical record for that follow-up.

Table 20. The rate (exact) of change of fasting C-peptide of each follow-up visit

Visits	N	Mean ± SD	Median [IQR]	*P value*
A. The rate of change from onset				
3 months	157	0.52 ± 0.61	0.37 [0.08, 0.89]	*<0.0001*[c]
6 months	139	0.17 ± 0.25	0.10 [0, 0.28]	*<0.0001*[c]
12 months	141	0.03 ± 0.09	0.01 [-0.02, 0.06]	*0.15*[c]
18 months	133	0.01 ± 0.06	-0.01 [-0.02, 0.03]	*0.22*[c]
24 months	135	-0.01 ± 0.03	-0.02 [-0.02, 0]	*<0.0001*[c]
B. The rate of change from 3 months				
6 months	129	-0.07 ± 0.42	-0.08 [-0.28, 0.11]	*<0.01*[b]
12 months	132	-0.09 ± 0.15	-0.07 [-0.15, -0.01]	*<0.0001*[b]
18 months	125	-0.06 ± 0.09	-0.05 [-0.12, -0.01]	*<0.0001*[a]
24 months	126	-0.06 ± 0.06	-0.05 [-0.11, -0.02]	*<0.0001*[b]
C. The rate of change from each follow-up to subsequence				
Onset - 3 months	157	0.52 ± 0.61	0.37 [0.08, 0.89]	*<0.0001*[c]
3 months - 6 months	129	-0.07 ± 0.42	-0.08 [-0.28, 0.11]	*<0.01*[b]
6 months - 12 months	116	-0.09 ± 0.15	-0.06 [-0.17, -0.01]	*<0.0001*[b]
12 months - 18 months	111	-0.03 ± 0.11	-0.03 [-0.07, 0]	*<0.0001*[b]
18 months - 24 months	103	-0.05 ± 0.09	-0.02 [-0.08, 0]	*<0.0001*[c]

* Values are calculated by the change in subject / the difference of exact days since diagnosis between two follow up visits in months, in ng/mL/month. [a] Based on t-test. [b] Based on Wilcoxon signed rank test. [c] Based on sign test.

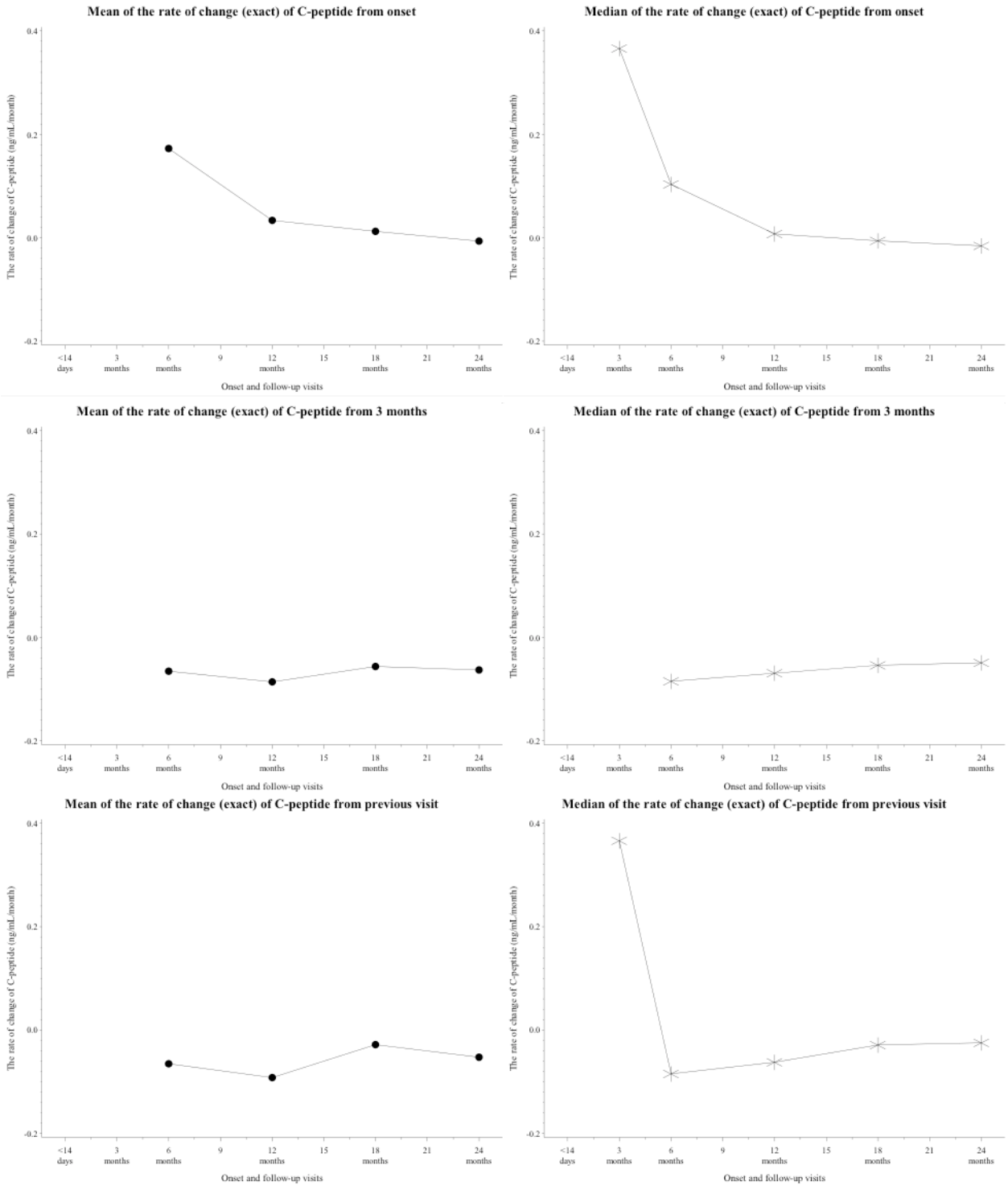

Figure 13. The rate of change (exact) of C-peptide

A.3 C-PEPTIDE BY OVERWEIGHT STATUS AT ONSET

Table 21. The rate of change (exact) of C-peptide among overweight and non-overweight subjects at onset (N=168)

Visits	Overweight at onset				Non-overweight at onset				*P value**
	N	**Mean ± SD**	**Median [IQR]**	***P value***	**N**	**Mean ± SD**	**Median [IQR]**	***P value***	
A. Change of C-peptide from onset									
3 months	33	0.66 ± 0.86	0.49 [-0.08, 0.89]	*0.04*[c]	119	0.49 ± 0.52	0.36 [0.10, 0.92]	*<0.0001*[b]	*0.77*
6 months	31	0.21 ± 0.29	0.13 [0, 0.49]	*<0.001*[b]	103	0.16 ± 0.23	0.10 [0.01, 0.28]	*<0.0001*[c]	*0.64*
12 months	29	0.04 ± 0.09	0.01 [-0.01, 0.11]	*0.26*[c]	108	0.03 ± 0.09	0.01 [-0.02, 0.06]	*0.25*[c]	*0.63*
18 months	26	0.03 ± 0.06	0.01 [-0.02, 0.06]	*0.06*[b]	103	0.01 ± 0.05	-0.01 [-0.02, 0.02]	*0.11*[c]	*0.14*
24 months	31	-0.01 ± 0.03	-0.02 [-0.03, 0]	*<0.01*[c]	100	-0.01 ± 0.03	-0.02 [-0.02, 0]	*<0.0001*[c]	*0.32*
B. Change of C-peptide from 3 months									
6 months	28	-0.09 ± 0.53	-0.13 [-0.31, 0.15]	*0.35*[a]	96	-0.06 ± 0.39	-0.08 [-0.28, 0.12]	*0.02*[c]	*0.73*
12 months	27	-0.14 ± 0.21	-0.11 [-0.28, -0.01]	*<0.01*[a]	101	-0.07 ± 0.13	-0.06 [-0.13, -0.01]	*<0.0001*[b]	*0.17*
18 months	25	-0.07 ± 0.13	-0.05 [-0.15, -0.01]	*0.01*[a]	96	-0.05 ± 0.08	-0.05 [-0.11, -0.01]	*<0.0001*[b]	*0.75*
24 months	28	-0.08 ± 0.09	-0.07 [-0.13, -0.01]	*0.0001*[a]	94	-0.06 ± 0.05	-0.05 [-0.10, -0.02]	*<0.0001*[a]	*0.35*[a]
C. Change of C-peptide from each follow-up to subsequence									
3 months	33	0.66 ± 0.86	0.49 [-0.08, 0.89]	*0.04*[c]	119	0.49 ± 0.52	0.36 [0.10, 0.92]	*<0.0001*[b]	*0.69*
6 months	28	-0.09 ± 0.53	-0.13 [-0.31, 0.15]	*0.35*[a]	96	-0.06 ± 0.39	-0.08 [-0.28, 0.12]	*0.02*[c]	*0.58*
12 months	24	-0.13 ± 0.22	-0.07 [-0.24, -0.03]	*<0.01*[c]	88	-0.08 ± 0.13	-0.06 [-0.16, -0.01]	*<0.0001*[b]	*0.35*
18 months	21	-0.02 ± 0.08	0 [-0.06, 0.01]	*0.23*[a]	87	-0.03 ± 0.12	-0.03 [-0.07, 0]	*<0.0001*[b]	*0.61*
24 months	22	-0.10 ± 0.12	-0.06 [-0.15, 0]	*0.001*[c]	78	-0.04 ± 0.08	-0.01 [-0.05, 0]	*<0.0001*[c]	*0.02*

* Values are calculated by the change in subject / the difference of exact days since diagnosis between two follow up visits in months, in ng/mL/month. P values are obtained from Wilcoxon-Mann-Whitney test for two independent samples unless otherwise noted. [a] Based on t-test. [b] Based on Wilcoxon signed rank test. [c] Based on sign test.

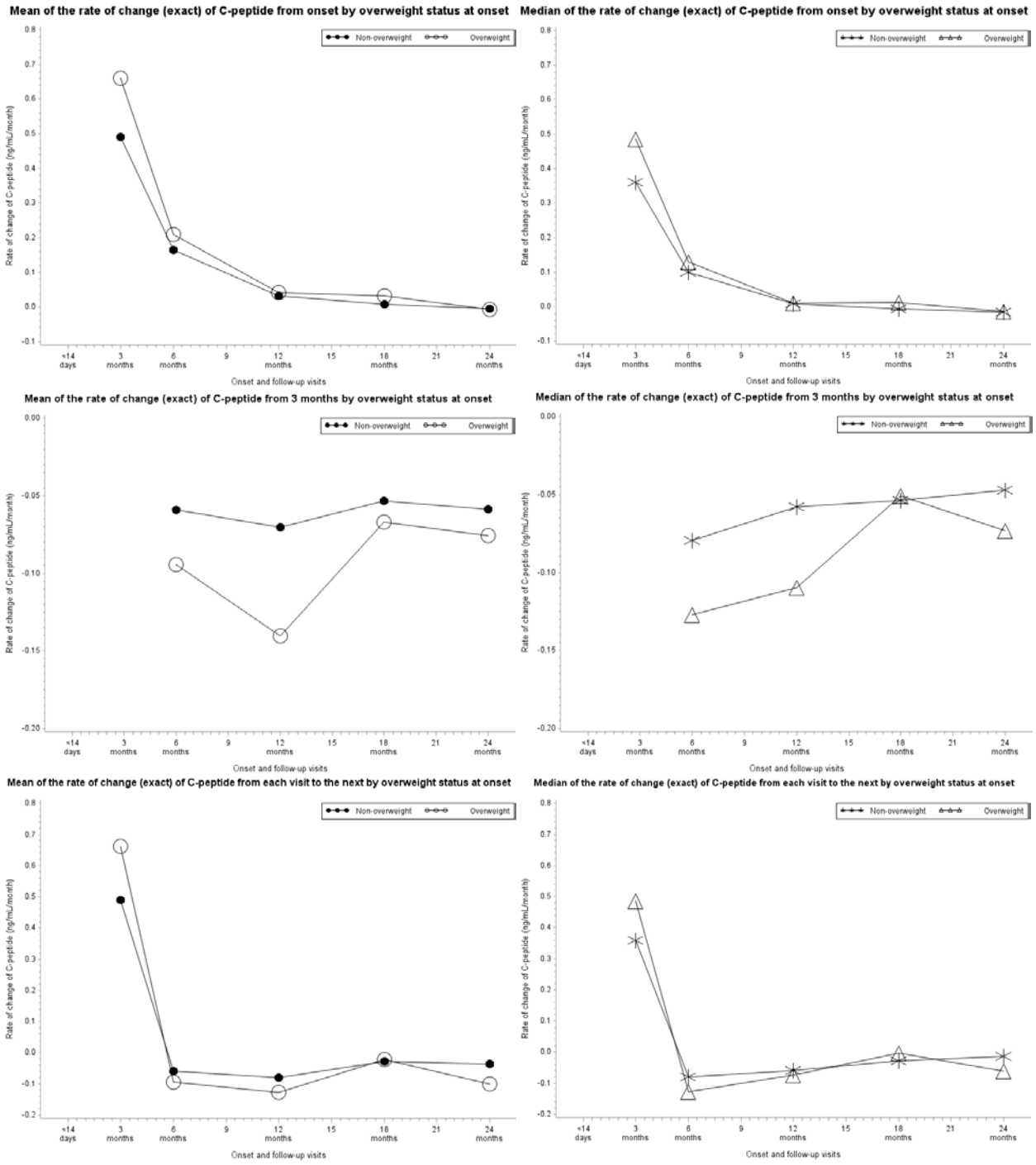

Figure 14. The rate of change (window) of C-peptide between two visits among overweight and non-overweight subjects at onset

Left three are the trajectory of mean value of the rate of change of C-peptide from onset, 3 months, and previous visit, respectively. Right three are the trajectory of median value of the rate of change of C-peptide from onset, 3 months, and previous visit.

A.4 C-PEPTIDE BY OVERWEIGHT STATUS AT ONSET AND 3 MONTHS

Table 22. The rate of change (window) of fasting C-peptide for each follow-up visit among overweight status at onset and 3-month visit (N=168)

A. The rate of change of C-peptide from onset

Visits	Overweight status at onset and 3 months	N	Mean ± SD	Median [IQR]	*P value*		
					Within	Between	Between (exclude O - N)
3 months	O - O	29	0.44 ± 0.61	0.31 [-0.10, 0.71]	*0.14*[c]	*0.19*	*0.82*
	O - N	3	1.27 ± 0.94	1.10 [0.43, 2.29]	*0.14*[a]		
	N - O	27	0.32 ± 0.33	0.22 [0.04, 0.65]	*<0.0001*[b]		
	N - N	91	0.37 ± 0.38	0.29 [0.09, 0.66]	*<0.0001*[b]		
6 months	O - O	24	0.19 ± 0.28	0.09 [-0.02, 0.47]	*<0.01*[b]	*0.89*	*0.74*
	O - N	4	0.16 ± 0.22	0.15 [0.01, 0.31]	*0.24*[a]		
	N - O	23	0.13 ± 0.22	0.05 [0, 0.22]	*0.02*[c]		
	N - N	79	0.15 ± 0.20	0.11 [0.02, 0.25]	*<0.0001*[c]		
12 months	O - O	23	0.04 ± 0.11	0.01 [-0.03, 0.13]	*0.68*[c]	*0.88*	*0.98*
	O - N	3	0.05 ± 0.05	0.04 [-0.01, 0.10]	*0.28*[a]		
	N - O	22	0.05 ± 0.13	0 [-0.03, 0.08]	*1.00*[c]		
	N - N	84	0.03 ± 0.08	0.01 [-0.02, 0.06]	*0.01*[b]		
18 months	O - O	22	0.03 ± 0.07	0 [-0.02, 0.07]	*0.83*[c]	*0.15*	*0.64*
	O - N	3	0.07 ± 0.04	0.07 [0.03, 0.11]	*0.10*[a]		
	N - O	24	0.02 ± 0.08	-0.02 [-0.02, 0.02]	*0.54*[c]		
	N - N	77	0 ± 0.04	-0.01 [-0.02, 0.02]	*0.11*[c]		
24 months	O - O	24	-0.01 ± 0.04	-0.02 [-0.03, 0.01]	*0.02*[c]	*0.76*	*0.56*
	O - N	4	-0.02 ± 0.03	-0.01 [-0.03, 0]	*0.13*[c]		
	N - O	22	0 ± 0.04	-0.02 [-0.02, 0]	*0.02*[c]		
	N - N	77	-0.01 ± 0.03	-0.02 [-0.02, 0]	*<0.0001*[c]		

B. The rate of change of C-peptide from 3-month visit

Visits	Overweight status at onset and 3 months	N	Mean ± SD	Median [IQR]	*P value*		
					Within	Between	Between (exclude O - N)
6 months	O - O	24	-0.03 ± 0.48	-0.10 [-0.32, 0.25]	*0.79*[a]	*0.18*	*0.87*
	O - N	3	-0.79 ± 0.61	-0.74 [-1.42, -0.20]	*0.16*[a]		
	N - O	23	-0.03 ± 0.44	-0.07 [-0.17, 0.18]	*0.78*[a]		
	N - N	73	-0.07 ± 0.42	-0.10 [-0.29, 0.13]	*0.10*[c]		
12 months	O - O	23	-0.12 ± 0.20	-0.09 [-0.25, 0.01]	*0.01*[a]	*0.06*	*0.32*
	O - N	3	-0.36 ± 0.24	-0.31 [-0.62, -0.15]	*0.12*[a]		
	N - O	22	-0.04 ± 0.17	-0.04 [-0.12, 0.06]	*0.35*[a]		
	N - N	78	-0.08 ± 0.13	-0.08 [-0.15, -0.02]	*<0.0001*[b]		
18 months	O - O	22	-0.05 ± 0.13	-0.05 [-0.14, 0.06]	*0.07*[a]	*0.08*	*0.41*
	O - N	2	-0.26 ± 0.10	-0.26 [-0.33, -0.19]	*0.17*[a]		
	N - O	24	-0.04 ± 0.09	-0.03 [-0.07, 0]	*0.06*[a]		
	N - N	71	-0.06 ± 0.08	-0.06 [-0.13, -0.02]	*<0.0001*[a]		

Table 21. The rate of change (window) of fasting C-peptide for each follow-up visit among overweight status at onset and 3-month visit (N=168) (continue)

24 months	O - O	24	-0.06 ± 0.08	-0.06 [-0.12, -0.01]	*0.001*[a]	*0.16*	*0.66*
	O - N	3	-0.19 ± 0.13	-0.16 [-0.33, -0.07]	*0.13*[a]		
	N - O	22	-0.05 ± 0.05	-0.04 [-0.10, -0.02]	*<0.001*[a]		
	N - N	71	-0.07 ± 0.06	-0.05 [-0.11, -0.03]	*<0.0001*[a]		

C. The rate of change of C-peptide from each visit to subsequence

Visits	**Overweight status at onset and 3 months**	**N**	**Mean ± SD**	**Median [IQR]**	***P value***		
					Within	**Between**	**Between (exclude O - N)**
3 months	O - O	29	0.44 ± 0.61	0.31 [-0.10, 0.71]	*0.14*[c]	*0.19*	*0.82*
	O - N	3	1.27 ± 0.94	1.10 [0.43, 2.29]	*0.14*[a]		
	N - O	27	0.32 ± 0.33	0.22 [0.04, 0.65]	*<0.0001*[b]		
	N - N	91	0.37 ± 0.38	0.29 [0.09, 0.66]	*<0.0001*[b]		
6 months	O - O	24	-0.03 ± 0.48	-0.10 [-0.32, 0.25]	*0.79*[a]	*0.18*	*0.87*
	O - N	3	-0.79 ± 0.61	-0.74 [-1.42, -0.20]	*0.16*[a]		
	N - O	23	-0.03 ± 0.44	-0.07 [-0.17, 0.18]	*0.78*[a]		
	N - N	73	-0.07 ± 0.42	-0.10 [-0.29, 0.13]	*0.10*[c]		
12 months	O - O	18	-0.12 ± 0.19	-0.06 [-0.25, -0.02]	*0.01*[a]	*0.54*	*0.82*
	O - N	3	-0.15 ± 0.07	-0.13 [-0.23, -0.10]	*0.06*[a]		
	N - O	19	-0.08 ± 0.10	-0.07 [-0.17, -0.01]	*<0.01*[a]		
	N - N	68	-0.08 ± 0.15	-0.06 [-0.16, -0.01]	*<0.0001*[b]		
18 months	O - O	18	-0.03 ± 0.10	-0.03 [-0.09, 0.01]	*0.27*[a]	*0.26*	*0.61*
	O - N	2	0.06 ± 0.08	0.06 [0, 0.11]	*0.49*[a]		
	N - O	20	-0.04 ± 0.15	0 [-0.05, 0.01]	*0.24*[c]		
	N - N	65	-0.04 ± 0.12	-0.03 [-0.08, 0]	*<0.0001*[b]		
24 months	O - O	18	-0.08 ± 0.11	-0.04 [-0.14, 0]	*0.01*[c]	*0.03*	*0.32*
	O - N	3	-0.28 ± 0.17	-0.32 [-0.43, -0.10]	*0.10*[a]		
	N - O	20	-0.05 ± 0.12	0 [-0.08, 0]	*0.09*[c]		
	N - N	57	-0.04 ± 0.06	-0.01 [-0.05, 0]	*<0.0001*[c]		

*Values are calculated by the change in subject / t the length of window between two follow-ups in months, in ng/mL/month. Between P values are obtained from Kruskal-Wallis test. Within P values are obtained from paired sample test. [a] Based on t-test. [b] Based on Wilcoxon signed rank test. [c] Based on sign test.

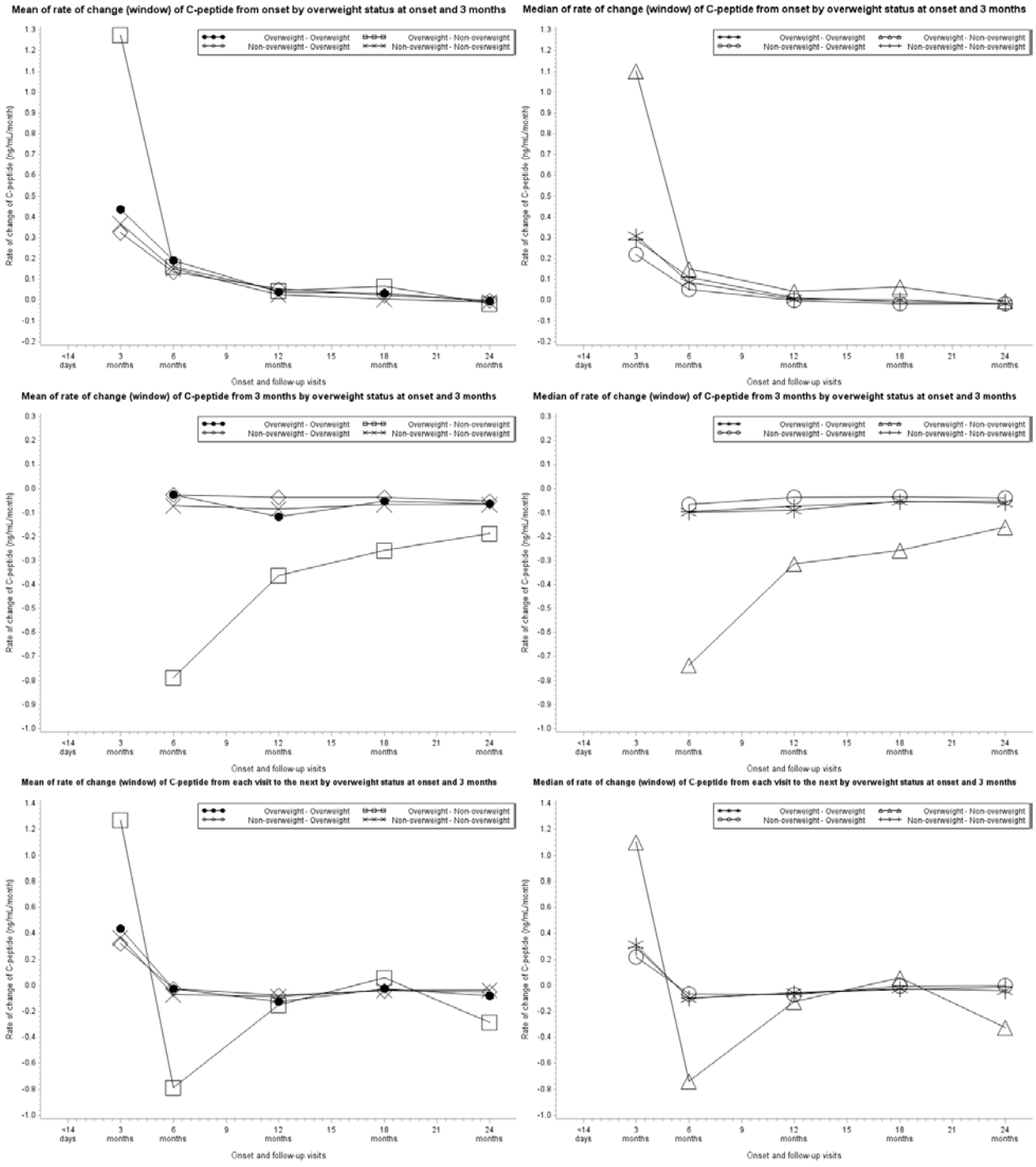

Figure 15. Trajectory plot for mean and median rate (window) of change of C-peptide by overweight status at onset and 3 months

Left three are the trajectory of mean value of the rate of change of C-peptide from onset, 3 months, and previous visit, respectively. Right three are the trajectory of median value of the rate of change of C-peptide from onset, 3 months, and previous visit. Values are calculated by the change in subject / the length of window between two follow-ups in months, in ng/mL/month.

Table 23. The rate of change (exact) of fasting C-peptide for each follow-up visit among overweight status at onset and 3-month visit (N=168)

A. The rate of change of C-peptide from onset

Visits	Overweight status at onset and 3 months	N	Mean ± SD	Median [IQR]	*P value*		
					Within	Between	Between (exclude O - N)
3 months	O - O	29	0.57 ± 0.80	0.40 [-0.12, 0.88]	*0.14*[c]	*0.23*	*0.80*
	O - N	3	1.59 ± 1.15	1.50 [0.48, 2.78]	*0.14*[a]		
	N - O	27	0.46 ± 0.48	0.26 [0.06, 0.94]	*<0.0001*[b]		
	N - N	91	0.50 ± 0.54	0.37 [0.12, 0.92]	*<0.0001*[b]		
6 months	O - O	24	0.21 ± 0.30	0.11 [-0.02, 0.47]	*0.06*[b]	*0.92*	*0.78*
	O - N	4	0.16 ± 0.26	0.15 [-0.01, 0.34]	*0.29*[a]		
	N - O	23	0.16 ± 0.27	0.06 [0, 0.23]	*0.02*[c]		
	N - N	79	0.16 ± 0.22	0.12 [0.02, 0.29]	*<0.0001*[c]		
12 months	O - O	23	0.04 ± 0.10	0.01 [-0.03, 0.13]	*0.68*[c]	*0.87*	*0.99*
	O - N	3	0.05 ± 0.06	0.04 [-0.01, 0.11]	*0.28*[a]		
	N - O	22	0.05 ± 0.13	0 [-0.03, 0.07]	*1.00*[c]		
	N - N	84	0.03 ± 0.08	0.01 [-0.02, 0.06]	*0.19*[c]		
18 months	O - O	22	0.03 ± 0.07	0 [-0.02, 0.06]	*0.83*[c]	*0.15*	*0.64*
	O - N	3	0.07 ± 0.04	0.06 [0.03, 0.11]	*0.10*[a]		
	N - O	24	0.02 ± 0.08	-0.02 [-0.02, 0.02]	*0.54*[c]		
	N - N	77	0 ± 0.04	-0.01 [-0.02, 0.02]	*0.11*[c]		
24 months	O - O	24	-0.01 ± 0.03	-0.02 [-0.03, 0]	*0.02*[c]	*0.72*	*0.50*
	O - N	4	-0.02 ± 0.03	-0.01 [-0.03, 0]	*0.13*[c]		
	N - O	22	0 ± 0.04	-0.02 [-0.02, 0]	*0.02*[c]		
	N - N	77	-0.01 ± 0.03	-0.02 [-0.02, 0]	*<0.0001*[c]		

B. The rate of change of C-peptide from 3-month visit

Visits	Overweight status at onset and 3 months	N	Mean ± SD	Median [IQR]	*P value*		
					Within	Between	Between (exclude O - N)
6 months	O - O	24	-0.01 ± 0.46	-0.08 [-0.26, 0.21]	*0.95*[a]	*0.21*	*0.82*
	O - N	3	-0.76 ± 0.70	-0.60 [-1.52, -0.16]	*0.20*[a]		
	N - O	23	-0.02 ± 0.46	-0.07 [-0.17, 0.16]	*0.85*[a]		
	N - N	73	-0.07 ± 0.37	-0.09 [-0.29, 0.10]	*0.10*[c]		
12 months	O - O	23	-0.11 ± 0.20	-0.07 [-0.25, 0.01]	*0.01*[a]	*0.06*	*0.32*
	O - N	3	-0.36 ± 0.27	-0.29 [-0.65, -0.13]	*0.14*[a]		
	N - O	22	-0.04 ± 0.17	-0.03 [-0.11, 0.07]	*0.31*[a]		
	N - N	78	-0.08 ± 0.12	-0.07 [-0.14, -0.02]	*<0.0001*[b]		
18 months	O - O	22	-0.05 ± 0.12	-0.05 [-0.12, 0.05]	*0.07*[a]	*0.08*	*0.40*
	O - N	2	-0.25 ± 0.11	-0.25 [-0.33, -0.18]	*0.19*[a]		
	N - O	24	-0.04 ± 0.09	-0.03 [-0.07, 0]	*0.05*[a]		
	N - N	71	-0.06 ± 0.08	-0.06 [-0.12, -0.02]	*<0.0001*[a]		
24 months	O - O	24	-0.06 ± 0.08	-0.06 [-0.12, -0.01]	*0.001*[a]	*0.15*	*0.67*
	O - N	3	-0.18 ± 0.12	-0.16 [-0.31, -0.08]	*0.12*[a]		
	N - O	22	-0.05 ± 0.05	-0.04 [-0.10, -0.02]	*<0.001*[a]		
	N - N	71	-0.06 ± 0.05	-0.05 [-0.11, -0.03]	*<0.0001*[a]		

Table 22. The rate of change (exact) of fasting C-peptide for each follow-up visit among overweight status at onset and 3-month visit (N=168) (continue)

C. The rate of change of C-peptide from each visit to subsequence

Visits	Overweight status at onset and 3 months	N	Mean ± SD	Median [IQR]	*P value*		
					Within	Between	Between (exclude O - N)
3 months	O - O	29	0.57 ± 0.80	0.40 [-0.12, 0.88]	*0.14*[c]	*0.23*	*0.80*
	O - N	3	1.59 ± 1.15	1.50 [0.48, 2.78]	*0.14*[a]		
	N - O	27	0.46 ± 0.48	0.26 [0.06, 0.94]	*<0.0001*[b]		
	N - N	91	0.50 ± 0.54	0.37 [0.12, 0.92]	*<0.0001*[b]		
6 months	O - O	24	-0.01 ± 0.46	-0.08 [-0.26, 0.21]	*0.95*[a]	*0.21*	*0.82*
	O - N	3	-0.76 ± 0.70	-0.60 [-1.52, -0.16]	*0.20*[a]		
	N - O	23	-0.02 ± 0.46	-0.07 [-0.17, 0.16]	*0.85*[a]		
	N - N	73	-0.07 ± 0.37	-0.09 [-0.29, 0.10]	*0.10*[c]		
12 months	O - O	18	-0.14 ± 0.24	-0.06 [-0.24, -0.02]	*0.01*[c]	*0.51*	*0.84*
	O - N	3	-0.15 ± 0.07	-0.12 [-0.23, -0.10]	*0.07*[a]		
	N - O	19	-0.07 ± 0.09	-0.07 [-0.16, -0.01]	*<0.01*[a]		
	N - N	68	-0.08 ± 0.14	-0.06 [-0.16, -0.01]	*<0.0001*[b]		
18 months	O - O	18	-0.03 ± 0.08	-0.02 [-0.08, 0.01]	*0.17*[a]	*0.24*	*0.58*
	O - N	2	0.05 ± 0.08	0.05 [0, 0.11]	*0.49*[a]		
	N - O	20	-0.03 ± 0.12	0 [-0.05, 0.01]	*0.10*[b]		
	N - N	65	-0.03 ± 0.11	-0.04 [-0.07, 0]	*<0.0001*[b]		
24 months	O - O	18	-0.08 ± 0.10	-0.05 [-0.14, 0]	*0.01*[c]	*0.03*	*0.27*
	O - N	3	-0.24 ± 0.13	-0.27 [-0.36, -0.10]	*0.08*[a]		
	N - O	20	-0.04 ± 0.12	-0.01 [-0.08, 0]	*0.04*[b]		
	N - N	57	-0.04 ± 0.06	-0.02 [-0.05, 0]	*<0.0001*[c]		

*Values are calculated by the change in subject / the difference of exact days since diagnosis between two follow up visits in months, in ng/mL/month. Between P values are obtained from Kruskal-Wallis test. Within P values are obtained from paired sample test. [a] Based on t-test. [b] Based on Wilcoxon signed rank test. [c] Based on sign test.

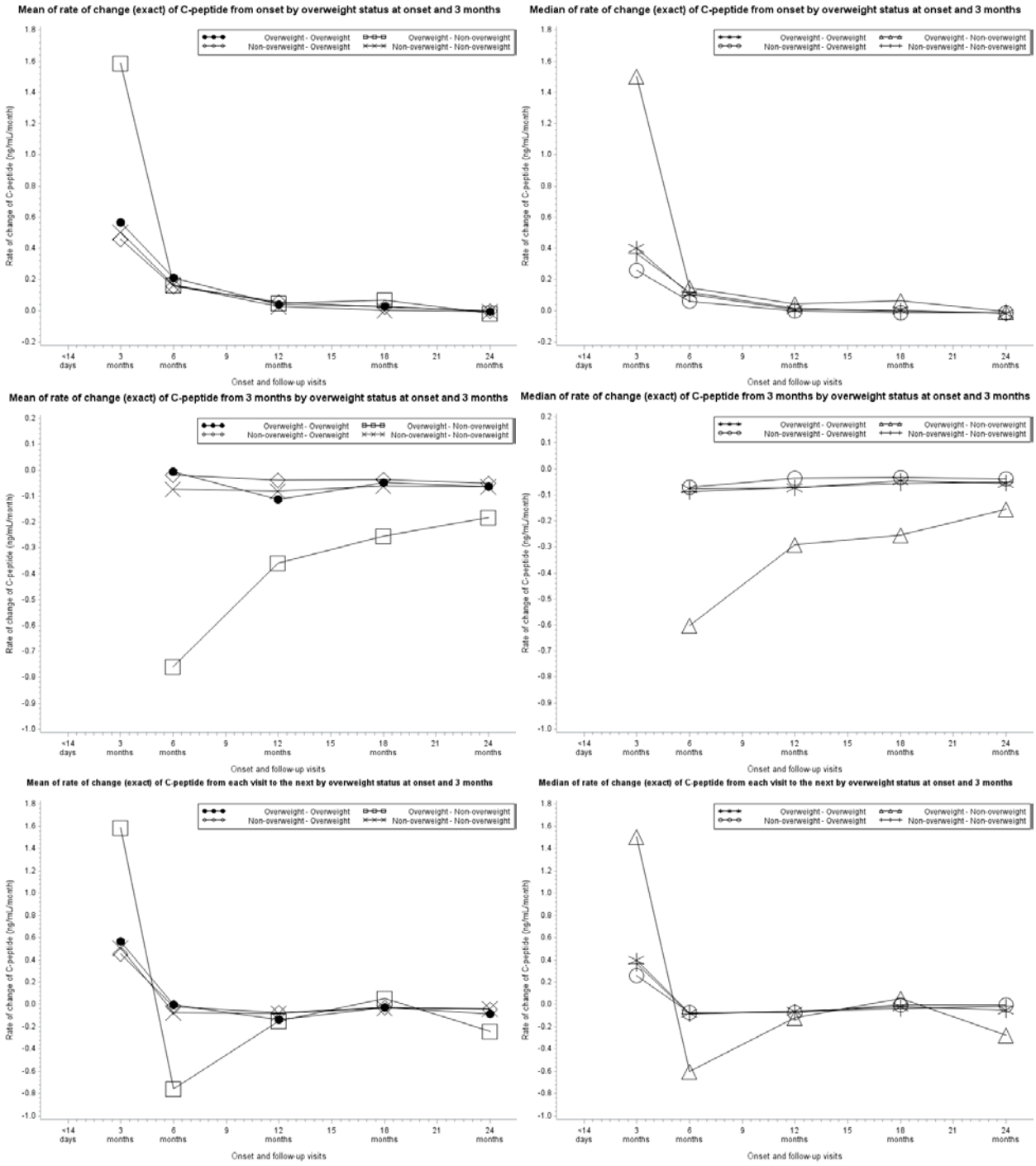

Figure 16. Trajectory plot for mean and median rate (exact) of change of C-peptide by overweight status at onset and 3 months

Left three are the trajectory of mean value of the rate of change of C-peptide from onset, 3 months, and previous visit, respectively. Right three are the trajectory of median value of the rate of change of C-peptide from onset, 3 months, and previous visit. Values are calculated by the change in subject / the difference of exact days since diagnosis between two follow up visits in months, in ng/mL/month.

A.5 C-PEPTIDE BY NUMBER OF POSITIVE AUTOANTIBODIES AT BASELINE

Table 24. The rate (window) of change of C-peptide among subjects with different number of positive antibodies at baseline (N=168)

A. Change of C-peptide from onset

Visits	# positive Abs at baseline	N	Mean ± SD	Median [IQR]	*P value*	
					Within	Between
3 months	0	15	0.38 ± 0.58	0.21 [0, 0.46]	*0.12*[c]	*0.31*
	1	11	0.31 ± 0.40	0.22 [0.03, 0.47]	*0.03*[a]	
	2	26	0.52 ± 0.42	0.55 [0.11, 0.85]	*<0.0001*[a]	
	3	51	0.32 ± 0.41	0.22 [0.03, 0.51]	*<0.001*[c]	
	4 or 5	54	0.40 ± 0.47	0.29 [0.07, 0.69]	*<0.0001*[c]	
6 months	0	11	0.20 ± 0.20	0.16 [0.03, 0.33]	*<0.01*[a]	*0.43*
	1	11	0.22 ± 0.24	0.16 [0.05, 0.25]	*0.001*[c]	
	2	25	0.19 ± 0.23	0.15 [0, 0.31]	*<0.001*[a]	
	3	46	0.15 ± 0.24	0.07 [0.02, 0.18]	*<0.001*[c]	
	4 or 5	46	0.12 ± 0.20	0.07 [-0.05, 0.26]	*<0.001*[b]	
12 months	0	14	0.06 ± 0.08	0.08 [-0.02, 0.14]	*0.04*[b]	*0.24*
	1	12	0.07 ± 0.12	0.02 [0, 0.11]	*0.15*[c]	
	2	26	0.04 ± 0.08	0.01 [-0.03, 0.08]	*0.56*[c]	
	3	41	0.02 ± 0.10	0 [-0.03, 0.05]	*1.00*[c]	
	4 or 5	48	0.02 ± 0.08	0.01 [-0.03, 0.04]	*0.56*[c]	
18 months	0	12	0.04 ± 0.05	0.03 [0, 0.07]	*0.02*[a]	*0.04*
	1	9	0.04 ± 0.04	0.03 [0, 0.08]	*0.04*[a]	
	2	21	0.02 ± 0.08	-0.02 [-0.02, 0.03]	*0.66*[c]	
	3	44	0.01 ± 0.06	-0.01 [-0.02, 0.02]	*0.10*[c]	
	4 or 5	47	0 ± 0.04	-0.01 [-0.02, 0.02]	*0.14*[c]	
24 months	0	11	0 ± 0.03	0 [-0.02, 0.02]	*0.74*[a]	*0.33*
	1	13	0.01 ± 0.04	0 [-0.02, 0.03]	*1.00*[c]	
	2	22	0 ± 0.04	-0.02 [-0.02, 0]	*0.02*[c]	
	3	43	-0.01 ± 0.03	-0.02 [-0.02, -0.01]	*<0.0001*[c]	
	4 or 5	46	-0.01 ± 0.03	-0.02 [-0.02, 0]	*<0.001*[c]	

B. Change of C-peptide from 3-month visit

Visits	# positive Abs at baseline	N	Mean ± SD	Median [IQR]	*P value*	
					Within	Between
6 months	0	10	0.08 ± 0.56	0.08 [-0.17, 0.15]	*0.64*[a]	*0.33*
	1	9	0.13 ± 0.44	0.03 [-0.17, 0.40]	*0.39*[a]	
	2	24	-0.14 ± 0.39	-0.16 [-0.33, 0.01]	*0.09*[a]	
	3	44	-0.05 ± 0.40	-0.13 [-0.22, 0.15]	*0.10*[c]	
	4 or 5	42	-0.14 ± 0.48	-0.09 [-0.43, 0.13]	*0.07*[a]	
12 months	0	13	-0.06 ± 0.21	-0.03 [-0.12, 0.07]	*0.30*[a]	*0.38*
	1	11	-0.01 ± 0.16	-0.03 [-0.11, 0.05]	*0.23*[c]	
	2	25	-0.12 ± 0.14	-0.09 [-0.20, -0.02]	*<0.001*[a]	
	3	38	-0.08 ± 0.16	-0.07 [-0.13, -0.03]	*<0.01*[a]	
	4 or 5	45	-0.11 ± 0.16	-0.07 [-0.17, -0.03]	*<0.0001*[b]	

Table 23. The rate (window) of change of C-peptide among subjects with different number of positive antibodies at baseline (N=168) (continue)

18 months	0	11	-0.04 ± 0.13	-0.07 [-0.10, 0.01]	*0.27*[a]	*0.06*
	1	8	0 ± 0.07	0.02 [-0.04, 0.04]	*0.94*[a]	
	2	20	-0.08 ± 0.11	-0.09 [-0.15, -0.03]	*<0.01*[a]	
	3	42	-0.04 ± 0.08	-0.05 [-0.10, -0.01]	*<0.001*[a]	
	4 or 5	44	-0.08 ± 0.10	-0.07 [-0.14, -0.03]	*<0.0001*[a]	
24 months	0	11	-0.03 ± 0.06	-0.05 [-0.05, -0.01]	*0.07*[c]	*0.22*
	1	11	-0.04 ± 0.07	-0.02 [-0.09, 0.02]	*0.12*[a]	
	2	21	-0.09 ± 0.07	-0.08 [-0.13, -0.03]	*<0.0001*[a]	
	3	40	-0.06 ± 0.06	-0.05 [-0.10, -0.02]	*<0.0001*[a]	
	4 or 5	43	-0.07 ± 0.07	-0.05 [-0.12, -0.02]	*<0.0001*[c]	

C. Change of C-peptide from each visit to subsequence

Visits	**# positive Abs at baseline**	**N**	**Mean ± SD**	**Median [IQR]**	***P value***	
					Within	**Between**
3 months	0	15	0.38 ± 0.58	0.21 [0, 0.46]	*0.12*[c]	*0.31*
	1	11	0.31 ± 0.40	0.22 [0.03, 0.47]	*0.03*[a]	
	2	26	0.52 ± 0.42	0.55 [0.11, 0.85]	*<0.0001*[a]	
	3	51	0.32 ± 0.41	0.22 [0.03, 0.51]	*<0.0001*[c]	
	4 or 5	54	0.40 ± 0.47	0.29 [0.07, 0.69]	*<0.0001*[c]	
6 months	0	10	-0.06 ± 0.21	-0.03 [-0.12, 0.07]	*0.64*[a]	*0.33*
	1	9	-0.01 ± 0.16	-0.03 [-0.11, 0.05]	*0.39*[a]	
	2	24	-0.12 ± 0.14	-0.09 [-0.20, -0.02]	*0.09*[a]	
	3	44	-0.08 ± 0.16	-0.07 [-0.13, -0.03]	*0.10*[c]	
	4 or 5	42	-0.11 ± 0.16	-0.07 [-0.17, -0.03]	*0.07*[a]	
12 months	0	10	-0.07 ± 0.12	-0.09 [-0.17, 0.01]	*0.10*[a]	*0.95*
	1	10	-0.06 ± 0.11	-0.05 [-0.13, -0.01]	*0.13*[a]	
	2	24	-0.11 ± 0.13	-0.07 [-0.17, -0.01]	*<0.001*[c]	
	3	36	-0.10 ± 0.14	-0.06 [-0.18, -0.03]	*<0.0001*[b]	
	4 or 5	36	-0.09 ± 0.17	-0.06 [-0.20, -0.01]	*<0.01*[a]	
18 months	0	10	-0.03 ± 0.17	-0.07 [-0.11, 0.09]	*0.58*[a]	*0.93*
	1	8	-0.05 ± 0.23	-0.03 [-0.09, 0.06]	*0.59*[a]	
	2	20	-0.03 ± 0.12	-0.04 [-0.07, 0]	*0.04*[b]	
	3	34	-0.03 ± 0.05	-0.03 [-0.06, 0]	*0.001*[a]	
	4 or 5	39	-0.03 ± 0.14	-0.02 [-0.09, 0]	*0.02*[b]	
24 months	0	8	-0.06 ± 0.08	0 [-0.14, 0]	*0.19*[c]	*0.19*
	1	9	-0.05 ± 0.10	-0.06 [-0.08, -0.03]	*0.16*[a]	
	2	17	-0.11 ± 0.14	-0.06 [-0.11, 0]	*<0.001*[c]	
	3	33	-0.04 ± 0.06	-0.01 [-0.04, 0]	*<0.01*[c]	
	4 or 5	36	-0.05 ± 0.10	-0.02 [-0.05, 0]	*<0.0001*[c]	

*Values are calculated by the change in subject / the length of window between two follow-ups in months, in ng/mL/month. Between P values are obtained from Kruskal-Wallis test. Within P values are obtained from paired sample test. [a] Based on t-test. [b] Based on Wilcoxon signed rank test. [c] Based on sign test.

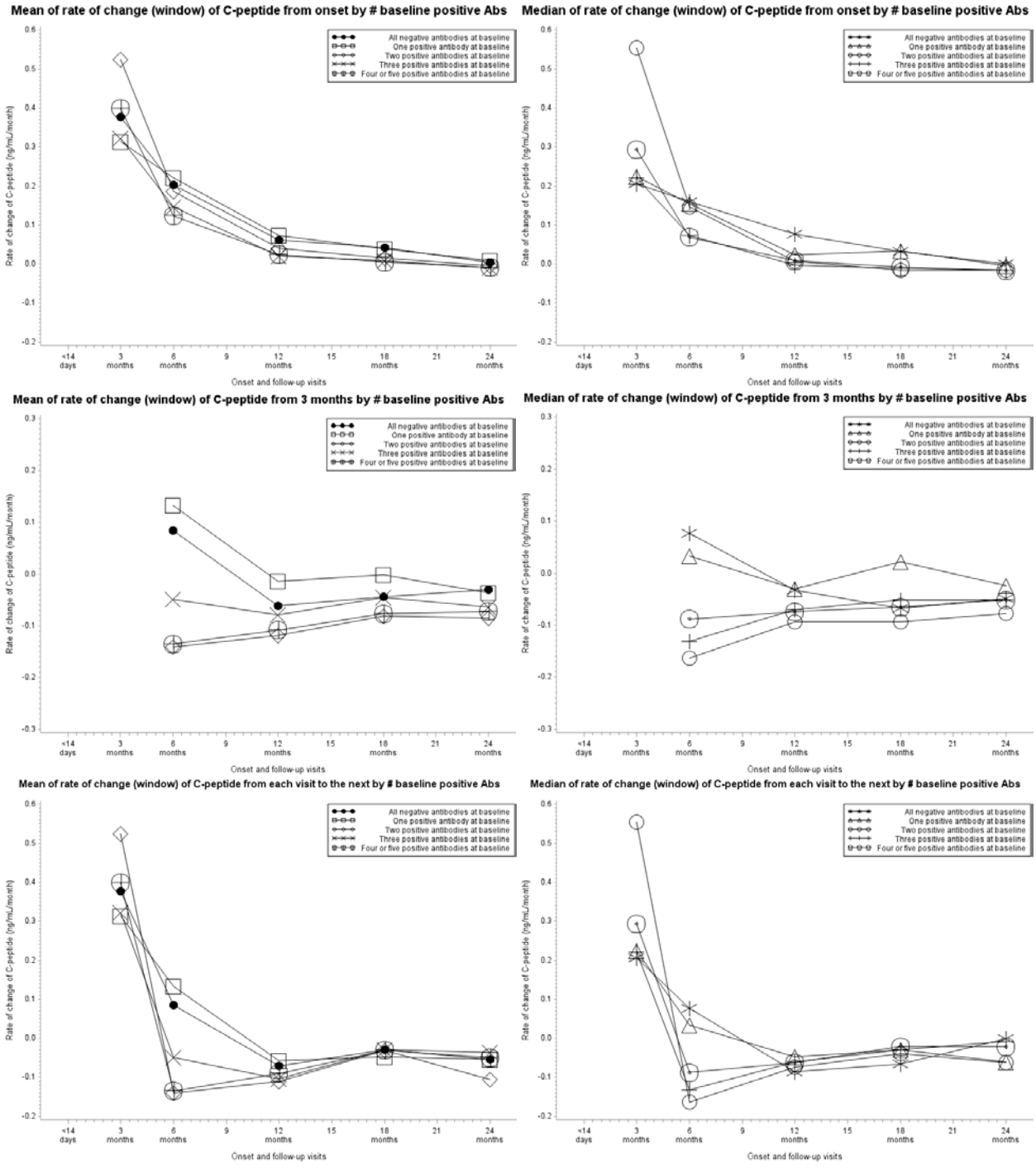

Figure 17. Trajectory plot for mean and median rate (window) of change of C-peptide for different number of positive autoantibodies at baseline

Left three are the trajectory of mean value of the rate of change of C-peptide from onset, 3 months, and previous visit, respectively. Right three are the trajectory of median value of the rate of change of C-peptide from onset, 3 months, and previous visit. Values are calculated by the change in subject / the length of window between two follow-ups in months, in ng/mL/month.

Table 25. The rate (exact) of change of C-peptide for each follow-up visit among subjects with different number of positive antibodies at baseline (N=168)

A. Change of C-peptide from onset

Visits	# positive Abs at baseline	N	Mean ± SD	Median [IQR]	*P value*	
					Within	Between
3 months	0	15	0.50 ± 0.73	0.29 [0, 0.58]	*0.12*[c]	*0.31*
	1	11	0.37 ± 0.40	0.31 [0.05, 0.58]	*0.01*[a]	
	2	26	0.74 ± 0.64	0.72 [0.16, 1.21]	*<0.0001*[a]	
	3	51	0.45 ± 0.60	0.28 [0.04, 0.88]	*<0.001*[c]	
	4 or 5	54	0.52 ± 0.59	0.37 [0.09, 0.84]	*<0.0001*[c]	
6 months	0	11	0.23 ± 0.23	0.18 [0.03, 0.43]	*<0.01*[a]	*0.40*
	1	11	0.23 ± 0.25	0.15 [0.06, 0.29]	*0.001*[c]	
	2	25	0.22 ± 0.28	0.16 [0, 0.35]	*<0.001*[a]	
	3	46	0.17 ± 0.26	0.08 [0.02, 0.20]	*<0.001*[c]	
	4 or 5	46	0.13 ± 0.20	0.07 [-0.05, 0.29]	*<0.001*[b]	
12 months	0	14	0.07 ± 0.09	0.08 [-0.01, 0.14]	*0.01*[a]	*0.23*
	1	12	0.07 ± 0.13	0.02 [0, 0.11]	*0.15*[c]	
	2	26	0.04 ± 0.09	0.01 [-0.03, 0.07]	*0.56*[c]	
	3	41	0.02 ± 0.09	0 [-0.03, 0.05]	*1.00*[c]	
	4 or 5	48	0.02 ± 0.08	0.01 [-0.03, 0.04]	*0.56*[c]	
18 months	0	12	0.04 ± 0.05	0.03 [0, 0.07]	*0.02*[a]	*0.05*
	1	9	0.04 ± 0.04	0.04 [0, 0.09]	*0.03*[a]	
	2	21	0.02 ± 0.08	-0.01 [-0.02, 0.03]	*0.66*[c]	
	3	44	0.01 ± 0.06	-0.01 [-0.02, 0.02]	*0.10*[c]	
	4 or 5	47	0 ± 0.04	-0.01 [-0.02, 0.02]	*0.14*[c]	
24 months	0	11	0 ± 0.03	0 [-0.02, 0.02]	*0.79*[a]	*0.26*
	1	13	0.01 ± 0.04	0 [-0.02, 0.03]	*1.00*[c]	
	2	22	0 ± 0.04	-0.02 [-0.02, 0]	*0.02*[c]	
	3	43	-0.01 ± 0.03	-0.02 [-0.02, -0.01]	*<0.0001*[c]	
	4 or 5	46	-0.01 ± 0.03	-0.02 [-0.02, 0]	*<0.001*[c]	

B. Change of C-peptide from 3-month visit

Visits	# positive Abs at baseline	N	Mean ± SD	Median [IQR]	*P value*	
					Within	Between
6 months	0	11	0.09 ± 0.55	0.07 [-0.15, 0.16]	*0.63*[a]	*0.27*
	1	11	0.13 ± 0.46	0.04 [-0.16, 0.30]	*0.40*[a]	
	2	25	-0.13 ± 0.40	-0.14 [-0.32, 0.01]	*0.14*[a]	
	3	46	-0.06 ± 0.38	-0.08 [-0.22, 0.14]	*0.10*[c]	
	4 or 5	46	-0.12 ± 0.42	-0.09 [-0.32, 0.09]	*0.07*[a]	
12 months	0	14	-0.06 ± 0.20	-0.03 [-0.11, 0.07]	*0.32*[a]	*0.41*
	1	12	-0.01 ± 0.15	-0.03 [-0.12, 0.04]	*0.23*[c]	
	2	26	-0.11 ± 0.13	-0.09 [-0.21, -0.03]	*<0.001*[a]	
	3	41	-0.07 ± 0.15	-0.07 [-0.12, -0.03]	*<0.01*[a]	
	4 or 5	48	-0.11 ± 0.15	-0.07 [-0.17, -0.02]	*<0.0001*[c]	
18 months	0	12	-0.04 ± 0.11	-0.06 [-0.10, 0.01]	*0.24*[a]	*0.06*
	1	9	0 ± 0.07	0.02 [-0.04, 0.04]	*0.95*[a]	
	2	21	-0.08 ± 0.10	-0.08 [-0.15, -0.03]	*<0.01*[a]	
	3	44	-0.04 ± 0.08	-0.05 [-0.09, -0.01]	*<0.001*[a]	
	4 or 5	47	-0.07 ± 0.09	-0.06 [-0.13, -0.02]	*<0.0001*[a]	

Table 24. The rate (exact) of change of C-peptide for each follow-up visit among subjects with different number of positive antibodies at baseline (N=168) (continue)

24 months	0	11	-0.03 ± 0.05	-0.05 [-0.05, -0.01]	*0.07*[c]	*0.21*
	1	13	-0.03 ± 0.07	-0.02 [-0.09, 0.02]	*0.13*[a]	
	2	22	-0.08 ± 0.06	-0.07 [-0.13, -0.03]	*<0.0001*[a]	
	3	43	-0.06 ± 0.06	-0.05 [-0.10, -0.02]	*<0.0001*[a]	
	4 or 5	46	-0.07 ± 0.07	-0.05 [-0.11, -0.02]	*<0.0001*[c]	

C. Change of C-peptide from each visit to subsequence

Visits	**# positive Abs at baseline**	**N**	**Mean ± SD**	**Median [IQR]**	***P value***	
					Within	**Between**
3 months	0	15	0.50 ± 0.73	0.29 [0, 0.58]	*0.12*[c]	*0.31*
	1	11	0.37 ± 0.40	0.31 [0.05, 0.58]	*0.01*[a]	
	2	26	0.74 ± 0.64	0.72 [0.16, 1.21]	*<0.0001*[a]	
	3	51	0.45 ± 0.60	0.28 [0.04, 0.88]	*<0.001*[c]	
	4 or 5	54	0.52 ± 0.59	0.37 [0.09, 0.84]	*<0.0001*[c]	
6 months	0	11	0.09 ± 0.55	0.07 [-0.15, 0.16]	*0.63*[a]	*0.27*
	1	11	0.13 ± 0.46	0.04 [-0.16, 0.30]	*0.40*[a]	
	2	25	-0.13 ± 0.40	-0.14 [-0.32, 0.01]	*0.14*[a]	
	3	46	-0.06 ± 0.38	-0.08 [-0.22, 0.14]	*0.10*[c]	
	4 or 5	46	-0.12 ± 0.42	-0.09 [-0.32, 0.09]	*0.07*[a]	
12 months	0	14	-0.07 ± 0.11	-0.08 [-0.16, 0]	*0.08*[a]	*0.94*
	1	12	-0.05 ± 0.10	-0.05 [-0.11, -0.01]	*0.14*[a]	
	2	26	-0.11 ± 0.13	-0.07 [-0.18, -0.01]	*<0.001*[c]	
	3	41	-0.09 ± 0.12	-0.06 [-0.17, -0.03]	*<0.0001*[a]	
	4 or 5	48	-0.10 ± 0.20	-0.05 [-0.18, -0.01]	*<0.001*[c]	
18 months	0	12	-0.02 ± 0.14	-0.06 [-0.10, 0.08]	*0.73*[a]	*0.94*
	1	9	-0.03 ± 0.20	-0.03 [-0.09, 0.06]	*0.72*[a]	
	2	21	-0.03 ± 0.11	-0.04 [-0.06, 0]	*0.03*[b]	
	3	44	-0.03 ± 0.05	-0.03 [-0.06, 0]	*0.001*[a]	
	4 or 5	47	-0.03 ± 0.12	-0.02 [-0.08, 0]	*0.02*[b]	
24 months	0	11	-0.05 ± 0.07	0 [-0.12, 0]	*0.22*[b]	*0.18*
	1	13	-0.03 ± 0.14	-0.05 [-0.07, -0.03]	*0.07*[c]	
	2	22	-0.10 ± 0.13	-0.06 [-0.11, 0]	*<0.001*[c]	
	3	43	-0.04 ± 0.07	-0.01 [-0.05, 0]	*<0.01*[c]	
	4 or 5	46	-0.05 ± 0.09	-0.02 [-0.06, 0]	*<0.0001*[c]	

*Values are calculated by the change in subject / the difference of exact days since diagnosis between two follow-ups in months, in ng/mL/month. Between P values are obtained from Kruskal-Wallis test. Within P values are obtained from paired sample test. [a] Based on t-test. [b] Based on Wilcoxon signed rank test. [c] Based on sign test.

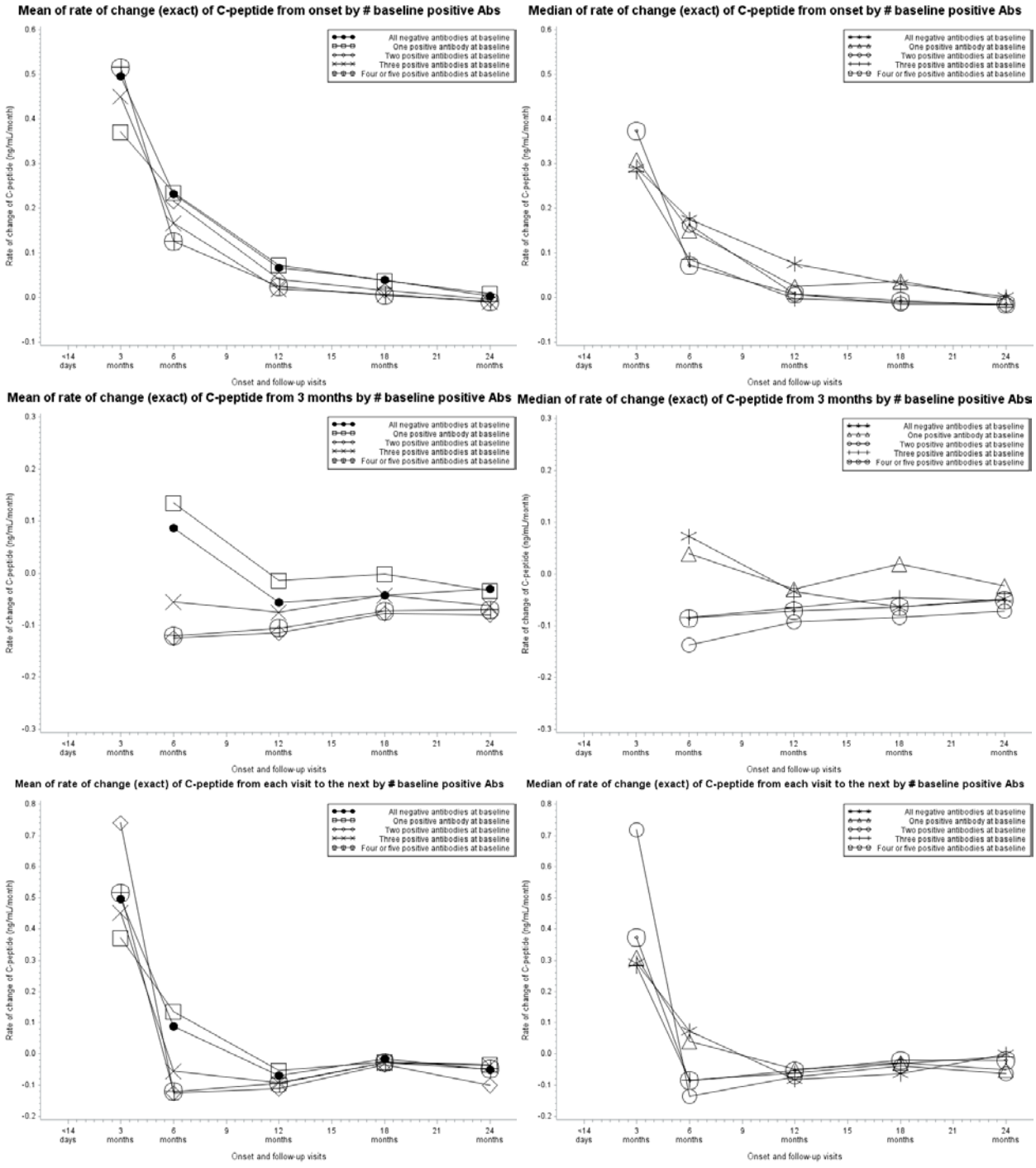

Figure 18. Trajectory plot for mean and median rate (exact) of change of C-peptide for different number of positive autoantibodies at baseline

Left three are the trajectory of mean value of the rate of change of C-peptide from onset, 3 months, and previous visit, respectively. Right three are the trajectory of median value of the rate of change of C-peptide from onset, 3 months, and previous visit. Values are calculated by the change in subject / the difference of exact days since diagnosis between two follow-ups in months, in ng/mL/month.

A.6 LINEAR MIXED MODELS (ACTUAL SCALE OF C-PEPTIDE)

Table 26. Variables considered in univariate random coefficients models for C-peptide and change of C-peptide from onset at 3 months and forward

Variables	C-peptide		Change of C-peptide from onset	
	Coefficient	***P value***	**Coefficient**	***P value***
Age at diagnosis (years)	0.1345	*<0.0001*	0.1054	*<0.0001*
Puberty (prepuberty is reference)	0.8332	*<0.0001*	0.6557	*<0.0001*
Age at diagnosis (categorical)				
0-4	(ref)		(ref)	
5-9	0.3773	*<0.0001*	0.3095	*<0.0001*
10-14	1.1171		0.8735	
≥ 15	1.4810		1.1807	
Gender (male is reference)	0.2939	*0.0798*	0.08335	*0.5444*
IDAA1c at 3-month visit	-0.1952	*0.0009*	-0.1387	*0.0034*
Days since diagnosis	-0.00198	*<0.0001*	-0.00197	*<0.0001*
Visits (3, 6, 12, 18, and 24 months)	-0.06237	*<0.0001*	-0.06187	*<0.0001*
Visits (categorical)				
3 months	(ref)		(ref)	
6 months	-0.1768	*<0.0001*	-0.1786	*<0.0001*
12 months	-0.7518		-0.7543	
18 months	-1.3216		-0.9390	
24 months	-0.9436		-1.3176	
Overweight status at onset (Non-overweight is reference)	0.5553	*0.0057*	0.2321	*0.1612*
Overweight status at 3 months (Non-overweight is reference)	0.2516	*0.1639*	0.1953	*0.1864*
Overweight status at onset and 3-month visit				
Overweight – Overweight	0.4992	*0.0343*	0.2261	*0.4537*
Overweight – Non-overweight	1.1123		0.5252	
Non-overweight – Overweight	-0.01722		0.1183	
Non-overweight – Non-overweight	(ref)		(ref)	
# positive baseline Abs				
0	(ref)		(ref)	
1	-0.2572	*0.3783*	-0.09219	*0.2958*
2	-0.3343		-0.1333	
3	-0.5188		-0.3887	
4 or 5	-0.5606		-0.4095	
2 or more positive Abs at baseline	-0.3833	*0.0836*	-0.3049	*0.0921*

Table 27. Multivariate analysis for C-peptide and change of C-peptide from onset over 3 months and forward (Model A and C)

Covariates	C-peptide at 3 months and forward		Change of C-peptide from onset of 3 months and forward	
	Fixed effects (SE)	*P value*	Fixed effects (SE)	*P value*
Model A: Times are days since diagnosis				
IDAA1c at 3 months	-0.1554 (0.04687)	*0.0012*	-0.1141 (0.04247)	*0.0082*
Female	0.4419 (0.1491)	*0.0036*	0.2231 (0.1351)	*0.1010*
Age at diagnosis	0.1436 (0.02059)	*<0.0001*	0.1071 (0.01865)	*<0.0001*
2 or more Abs+ at baseline	-0.3948 (0.1986)	*0.0489*	-0.2694 (0.1802)	*0.1372*
Days since diagnosis	-0.00184 (0.000198)	*<0.0001*	-0.00183 (0.000198)	*<0.0001*
Overweight at onset	0.7655 (0.2720)	*0.0056*	0.4354 (0.2427)	*0.0751*
Overwt_onset * dsddx	-0.00059 (0.000428)	*0.1711*	-0.00055 (0.000428)	*0.2036*
Model C: Times are follow-ups in months coded as 3, 6, 12, 18, and 24 in months as categorical				
IDAA1c at 3 months	-0.1992 (0.04835)	*<0.0001*	-0.1399 (0.04257)	*0.0013*
Female	0.4584 (0.1536)	*0.0034*	0.2317 (0.1354)	*0.0894*
Age at diagnosis	0.1488 (0.02109)	*<0.0001*	0.1106 (0.01862)	*<0.0001*
2 or more Abs+ at baseline	-0.3131 (0.2048)	*0.1286*	-0.2282 (0.1806)	*0.2085*
6 months	-0.1079 (0.1192)	*<0.0001*	-0.1118 (0.1189)	*<0.0001*
12 months	-0.6630 (0.1167)		-0.6673 (0.1165)	
18 months	-0.9003 (0.1195)		-0.8975 (0.1192)	
24 months	-1.1975 (0.1206)		-1.1941 (0.1202)	
Overweight at onset	0.8214 (0.2353)	*0.0056*	0.5052 (0.2190)	*0.1943*
Overweight at onset * 6 months	-0.3225 (0.2544)	*0.2058*	-0.3006 (0.2538)	*0.2387*
Overweight at onset * 12 months	-0.4936 (0.2559)		-0.4891 (0.2553)	
Overweight at onset * 18 months	-0.1926 (0.2628)		-0.1845 (0.2621)	
Overweight at onset * 24 months	-0.5381 (0.2577)		-0.5065 (0.2570)	

Table 28. Comparison of estimated means of C-peptide and change of C-peptide from onset between overweight and non-overweight subjects at each follow-up (Model C)

Visits	C-peptide		Change of C-peptide from onset	
	Mean difference (95% C.I.)	*P value*	Mean difference (95% C.I.)	*P value*
3 months	0.8214 (0.3586, 1.2843)	*0.0005*	0.5052 (0.07460, 0.9358)	*0.0216*
6 months	0.4989 (0.01230, 0.9854)	*0.0445*	0.2046 (-0.2510, 0.6602)	*0.3780*
12 months	0.3278 (-0.1621, 0.8178)	*0.1891*	0.01609 (-0.4430, 0.4752)	*0.9451*
18 months	0.6288 (0.1256, 1.1320)	*0.0145*	0.3207 (-0.1524, 0.7937)	*0.1835*
24 months	0.2833 (-0.2098, 0.7763)	*0.2593*	-0.00130 (-0.4637, 0.4611)	*0.9956*
Overall across time	0.5120 (0.1526, 0.8715)	*0.0056*	0.2090 (-0.1079, 0.5260)	*0.1943*

* For model C: Times are follow-ups in months coded as 3, 6, 12, 18, and 24 in months as categorical Values are in ng/mL. P values were obtained from t-test for testing the estimates are different from 0 or not.

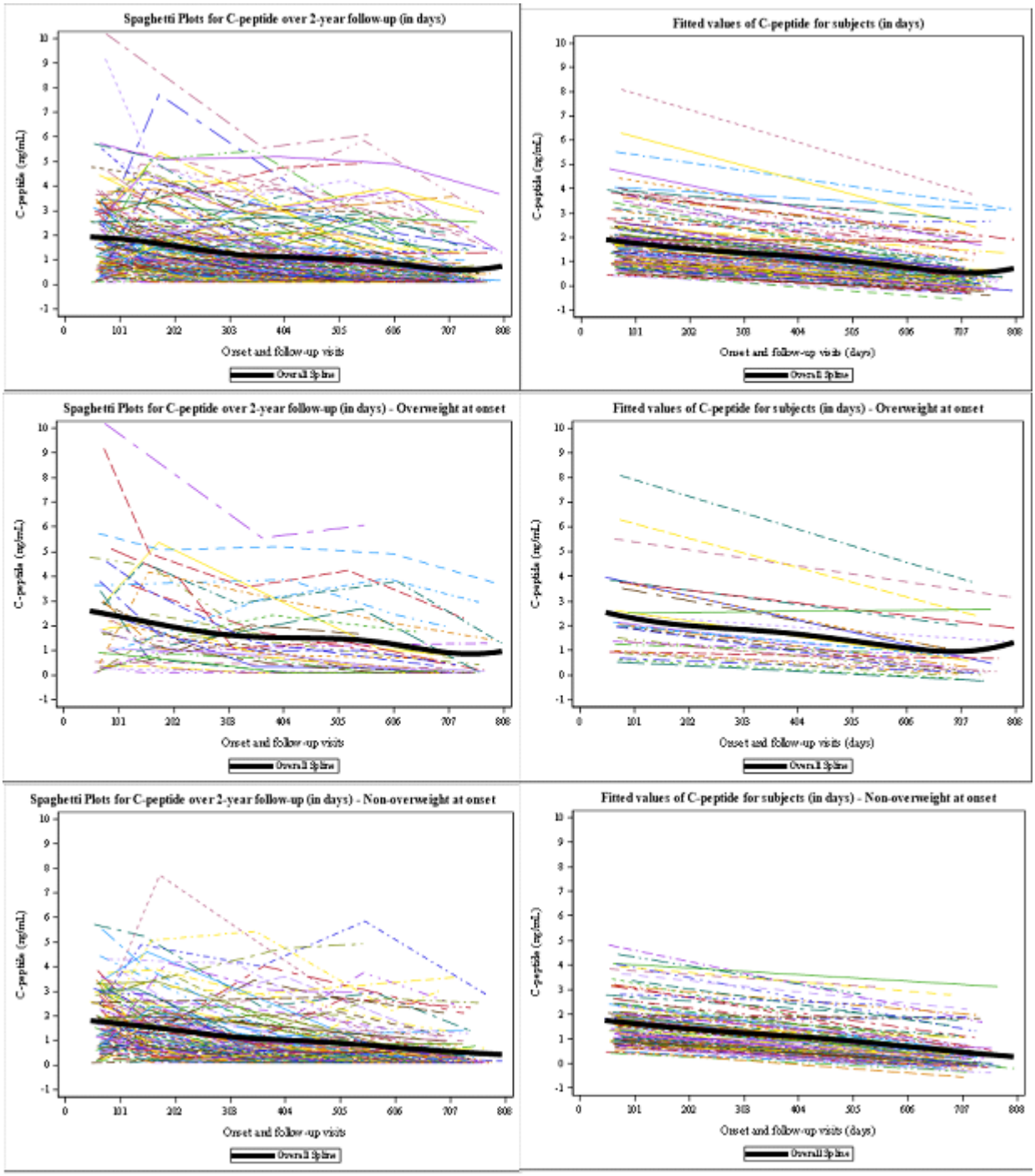

Figure 19. Spaghetti plots for observed and fitted values of C-peptide in Model A

Left three are observed values for C-peptide. Right three are fitted values for C-peptide. Model A: time was treated as days since diagnosis.

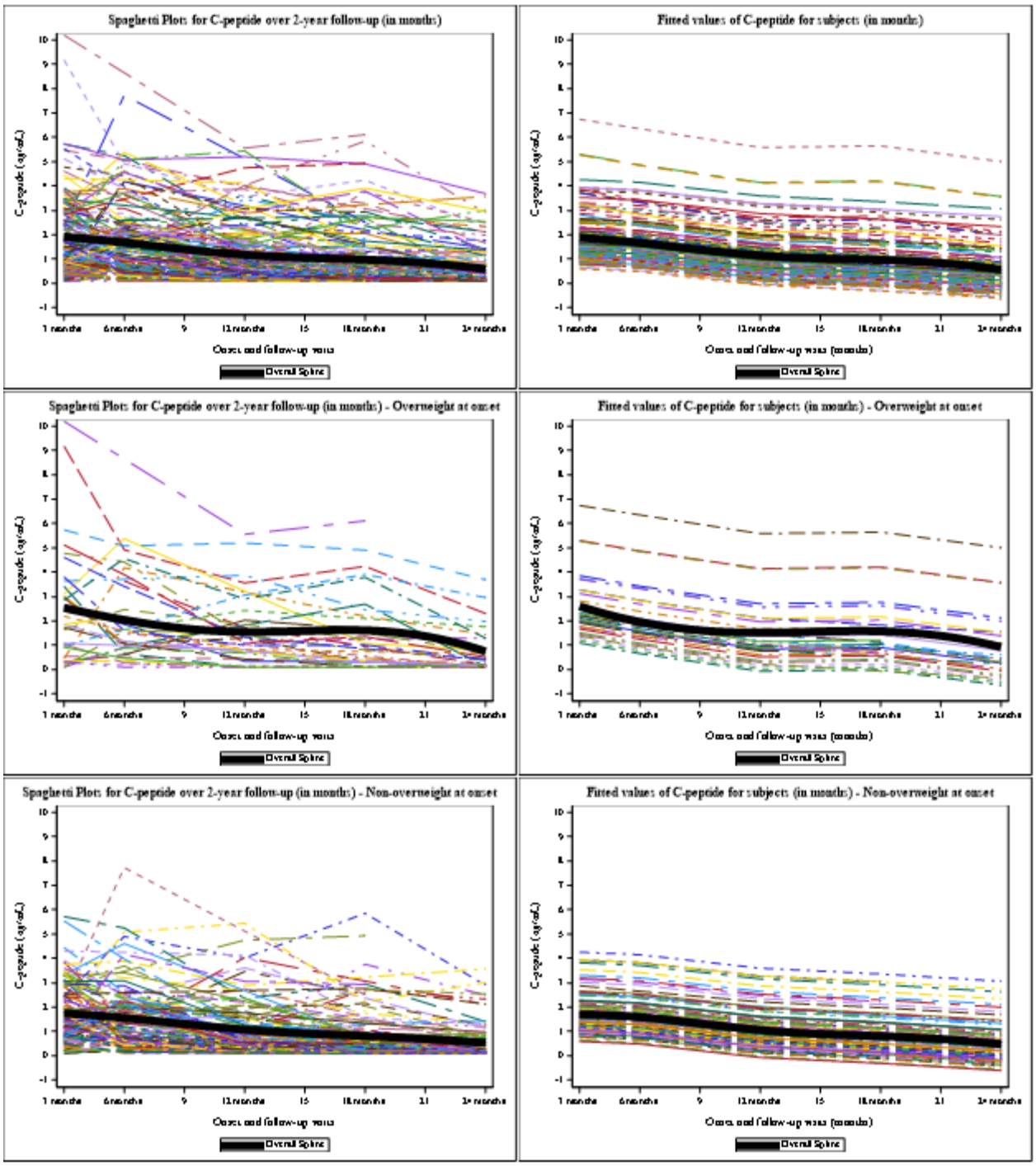

Figure 20. Spaghetti plots for observed and fitted values of C-peptide in Model C

Left three are observed values for C-peptide. Right three are fitted values for C-peptide. Model C: time was treated as follow-ups in months as categorical, coded as 3, 6, 12, 18, and 24.

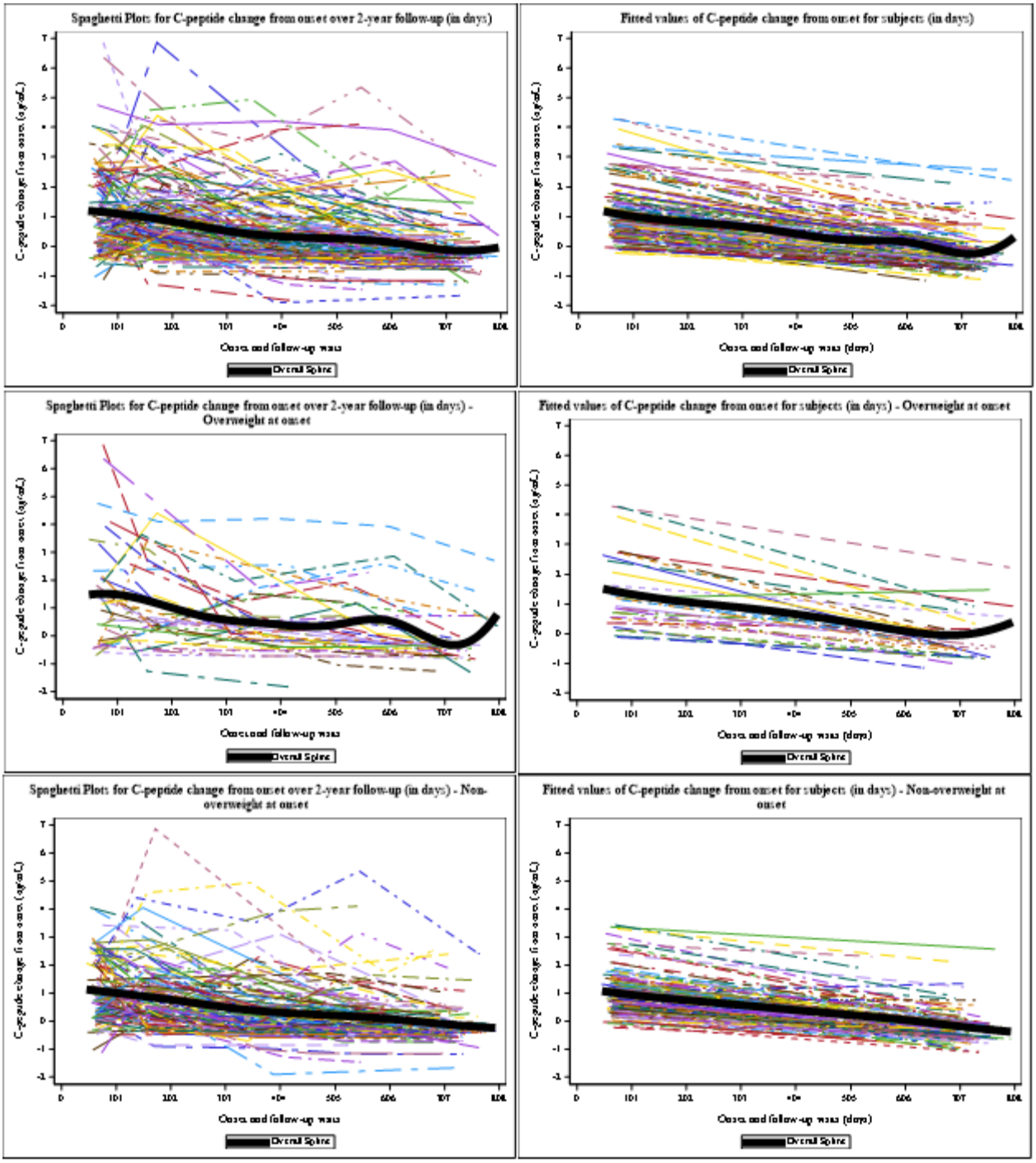

Figure 21. Spaghetti plots for observed and fitted values of change of C-peptide from onset in Model A

Left three are observed values for change of C-peptide from onset. Right three are fitted values for change of C-peptide from onset. Model A: time was treated as days since diagnosis.

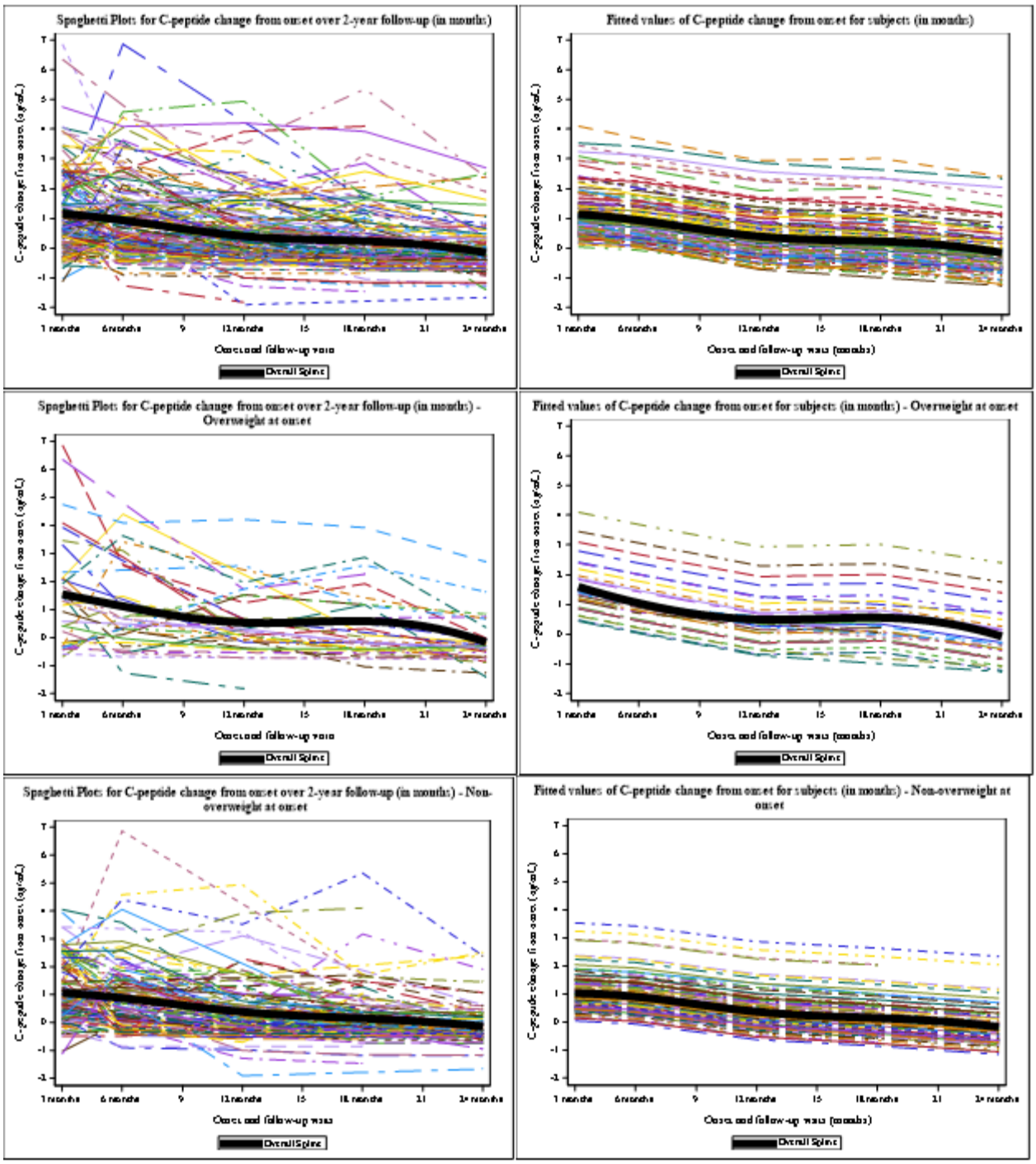

Figure 22. Spaghetti plots for observed and fitted values of change of C-peptide from onset in Model C

Left three are observed values for change of C-peptide from onset. Right three are fitted values for change of C-peptide from onset. Model C: time was treated as follow-ups in months as categorical, coded as 3, 6, 12, 18, and 24.

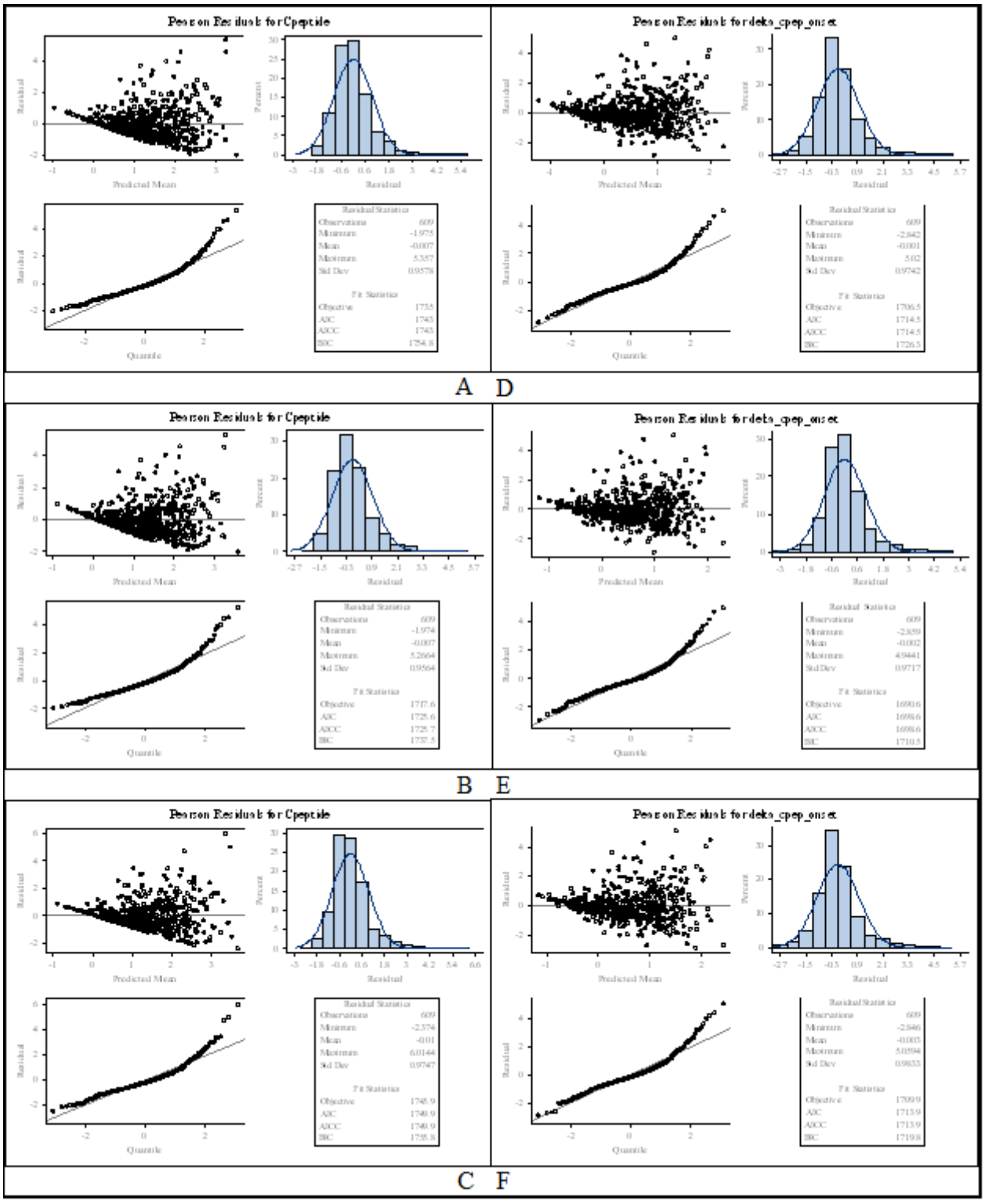

Figure 23. Pearson residuals plots of models A, B, and C for C-peptide and change of C-peptide from onset at 3 months and subsequent follow-ups

A, B, and C were Pearson residuals plots of random coefficients models A, B, and C separately for C-peptide at 3 months and forward follow-ups. D, E, and F were Pearson residuals plots of random coefficients models A, B, and C separately for change of C-peptide from onset at 3 months and forward follow-ups.

A.7 LINEAR MIXED MODEL (LOG-TRANSFORMED C-PEPTIDE)

Table 29. Multivariate analysis for log-transformed C-peptide and log-ratio of C-peptide to onset of each follow-up

Covariates	Log-transformed C-peptide at 3 months and forward		Log-ratio of C-peptide of each follow-up to onset	
	Fixed effects (SE)	*P value*	Fixed effects (SE)	*P value*
Model A: Times are days since diagnosis				
IDAA1c at 3 months	-0.2207 (0.03587)	*<0.0001*	-0.1553 (0.03683)	*<0.0001*
Female	0.2849 (0.1141)	*0.0137*	0.1092 (0.1171)	*0.3527*
Age at diagnosis	0.1184 (0.01567)	*<0.0001*	0.07974 (0.01608)	*<0.0001*
2 or more Abs+ at baseline	-0.1850 (0.1522)	*0.2265*	-0.1623 (0.1562)	*0.3007*
Days since diagnosis	-0.00249 (0.000192)	*<0.0001*	-0.00249 (0.000191)	*<0.0001*
Overweight at onset	0.08269 (0.1692)	*0.6258*	-0.2476 (0.1740)	*0.1571*
Overwt_onset * dsddx	0.000426 (0.000419)	*0.3110*	0.000432 (0.000417)	*0.3024*
Model B: Times are follow-ups in months coded as 3, 6, 12, 18, and 24 as continuous				
IDAA1c at 3 months	-0.2172 (0.03614)	*<0.0001*	-0.1518 (0.03692)	*<0.0001*
Female	0.2761 (0.1149)	*0.0176*	0.10000 (0.1174)	*0.3957*
Age at diagnosis	0.1185 (0.01580)	*<0.0001*	0.07999 (0.01613)	*<0.0001*
2 or more Abs+ at baseline	-0.1968 (0.1535)	*0.2019*	-0.1735 (0.1567)	*0.2702*
Follow-up visits (in months)	-0.07859 (0.005983)	*<0.0001*	-0.07847 (0.005953)	*<0.0001*
Overweight at onset	0.04695 (0.1755)	*0.7895*	-0.2842 (0.1799)	*0.1167*
Overweight at onset * visits	0.01536 (0.01302)	*0.2402*	0.01562 (0.01295)	*0.2302*
Model C: Times are follow-ups in months coded as 3, 6, 12, 18, and 24 as categorical				
IDAA1c at 3 months	-0.2192 (0.03651)	*<0.0001*	-0.1535 (0.03707)	*<0.0001*
Female	0.3115 (0.1161)	*0.0082*	0.1322 (0.1179)	*0.2642*
Age at diagnosis	0.1352 (0.01597)	*<0.0001*	0.09545 (0.01620)	*<0.0001*
2 or more Abs+ at baseline	-0.2861 (0.1549)	*0.0669*	-0.2580 (0.1572)	*0.1030*
6 months	-0.1456 (0.1015)	*<0.0001*	-0.1465 (0.1014)	*<0.0001*
12 months	-0.7129 (0.09944)		-0.7138 (0.09932)	
18 months	-1.1933 (0.1018)		-1.1912 (0.1017)	
24 months	-1.6300 (0.1027)		-1.6251 (0.1025)	
Overweight at onset	0.05248 (0.1874)	*0.0547*	-0.2820 (0.1888)	*0.6724*
Overweight at onset * 6 months	0.1512 (0.2167)	*0.2033*	0.1685 (0.2164)	*0.1902*
Overweight at onset * 12 months	0.1067 (0.2180)		0.1105 (0.2177)	
Overweight at onset * 18 months	0.5046 (0.2238)		0.5082 (0.2235)	
Overweight at onset * 24 months	0.3069 (0.2194)		0.3272 (0.2192)	

* In model C, 3 months was the reference.

Table 30. Estimated mean ratio of C-peptide and the ratio of C-peptide ratio to onset for each follow-up between overweight and non-overweight subjects

Visits	Ratio of C-peptide		Ratio of C-peptide ratio to onset	
	Mean difference (95% C.I.)	*P value*	Mean difference (95% C.I.)	*P value*
Model B: Times are follow-ups in months coded as 3, 6, 12, 18, and 24 as continuous				
3 months	1.0975 (0.8103, 1.4866)	*0.5452*	0.7887 (0.5773, 1.0774)	*0.1346*
6 months	1.1492 (0.8732, 1.5127)	*0.3183*	0.8265 (0.6233, 1.0960)	*0.1840*
12 months	1.2602 (0.9528, 1.6670)	*0.1042*	0.9077 (0.6842, 1.2042)	*0.4990*
18 months	1.3820 (0.9652, 1.9788)	*0.0769*	0.9968 (0.6974, 1.4249)	*0.9860*
24 months	1.5154 (0.9407, 2.4415)	*0.0869*	1.0947 (0.6829, 1.7549)	*0.7047*
Overall across time	1.0481 (0.7406, 1.4833)	*0.7895*	0.7526 (0.5271, 1.0745)	*0.1167*
Model C: Times are follow-ups in months coded as 3, 6, 12, 18, and 24 as categorical				
3 months	1.0539 (0.7291, 1.5235)	*0.7796*	0.7543 (0.5203, 1.0934)	*0.1362*
6 months	1.2258 (0.8301, 1.8103)	*0.3051*	0.8928 (0.6030, 1.3218)	*0.5702*
12 months	1.1725 (0.7916, 1.7367)	*0.4263*	0.8424 (0.5673, 1.2509)	*0.3944*
18 months	1.7456 (1.1646, 2.6164)	*0.0071*	1.2538 (0.8344, 1.8840)	*0.2756*
24 months	1.4325 (0.9644, 2.1274)	*0.0749*	1.0462 (0.7026, 1.5578)	*0.8235*
Overall across time	1.3051 (0.9945, 1.7129)	*0.0547*	0.9426 (0.7153, 1.2421)	*0.6724*

* P values were obtained from t-test for testing the estimates are different from 0 or not.

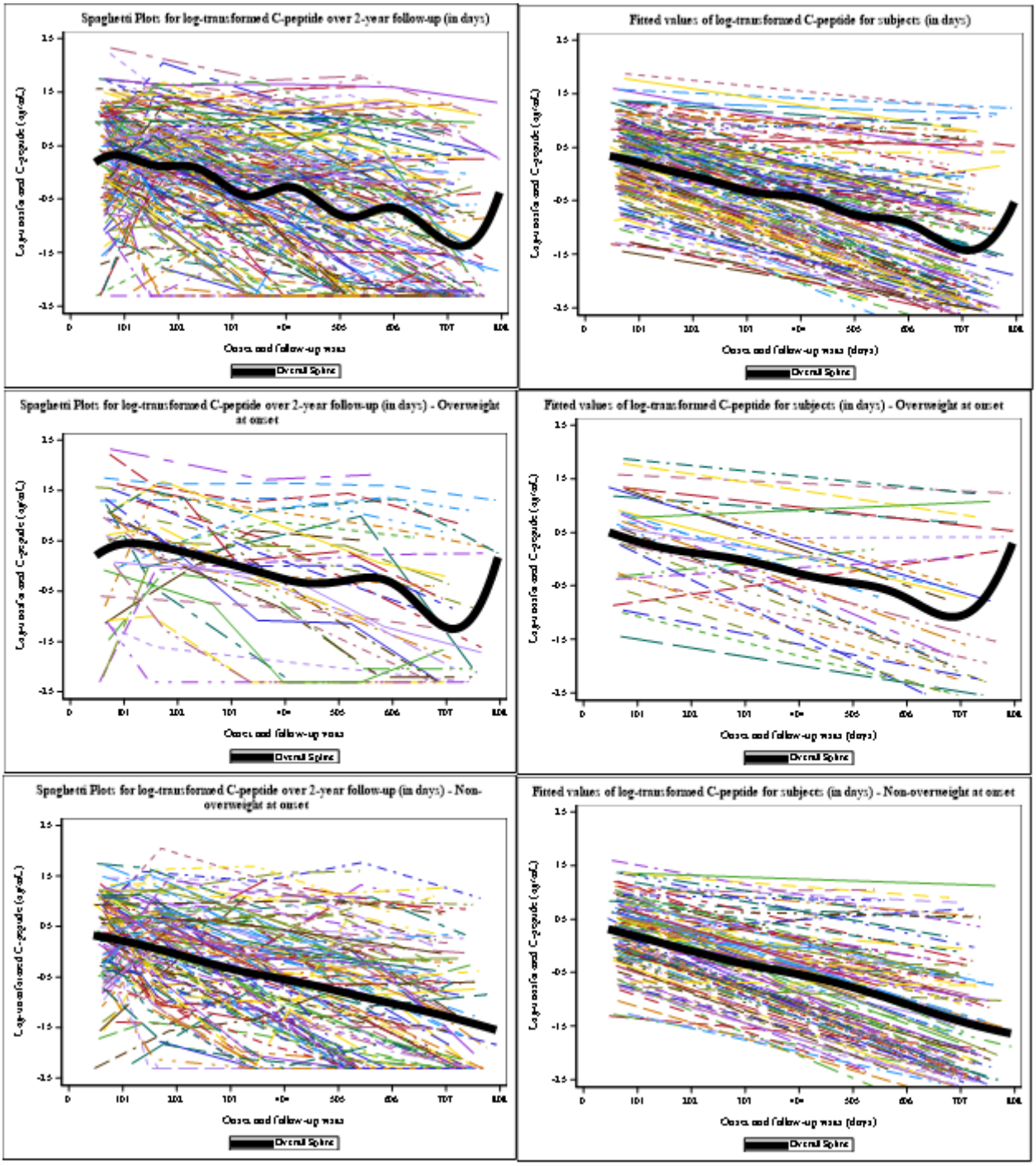

Figure 24. Spaghetti plots for observed and fitted values of log-transformed C-peptide in Model A

Left three are observed values for log-transformed C-peptide at 3 months and forward. Right three are fitted values for log-transformed C-peptide at 3 months and forward. Model A: time was treated as days since diagnosis.

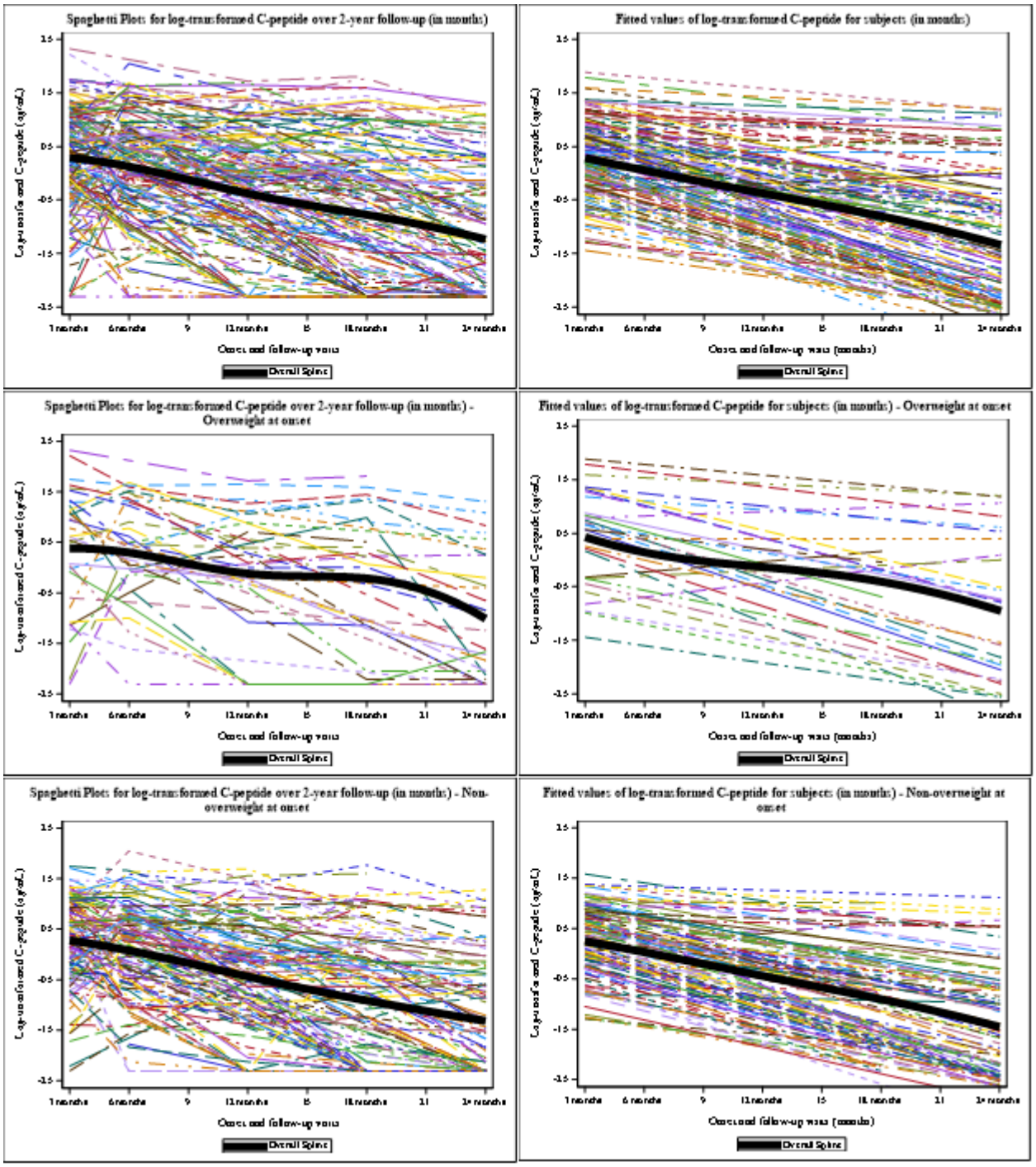

Figure 25. Spaghetti plots for observed and fitted values of log-transformed C-peptide in Model B

Left three are observed values for log-transformed C-peptide at 3 months and forward. Right three are fitted values for log-transformed C-peptide at 3 months and forward. Model B: time was treated as continuous in 3, 6, 12, 18, and 24 months.

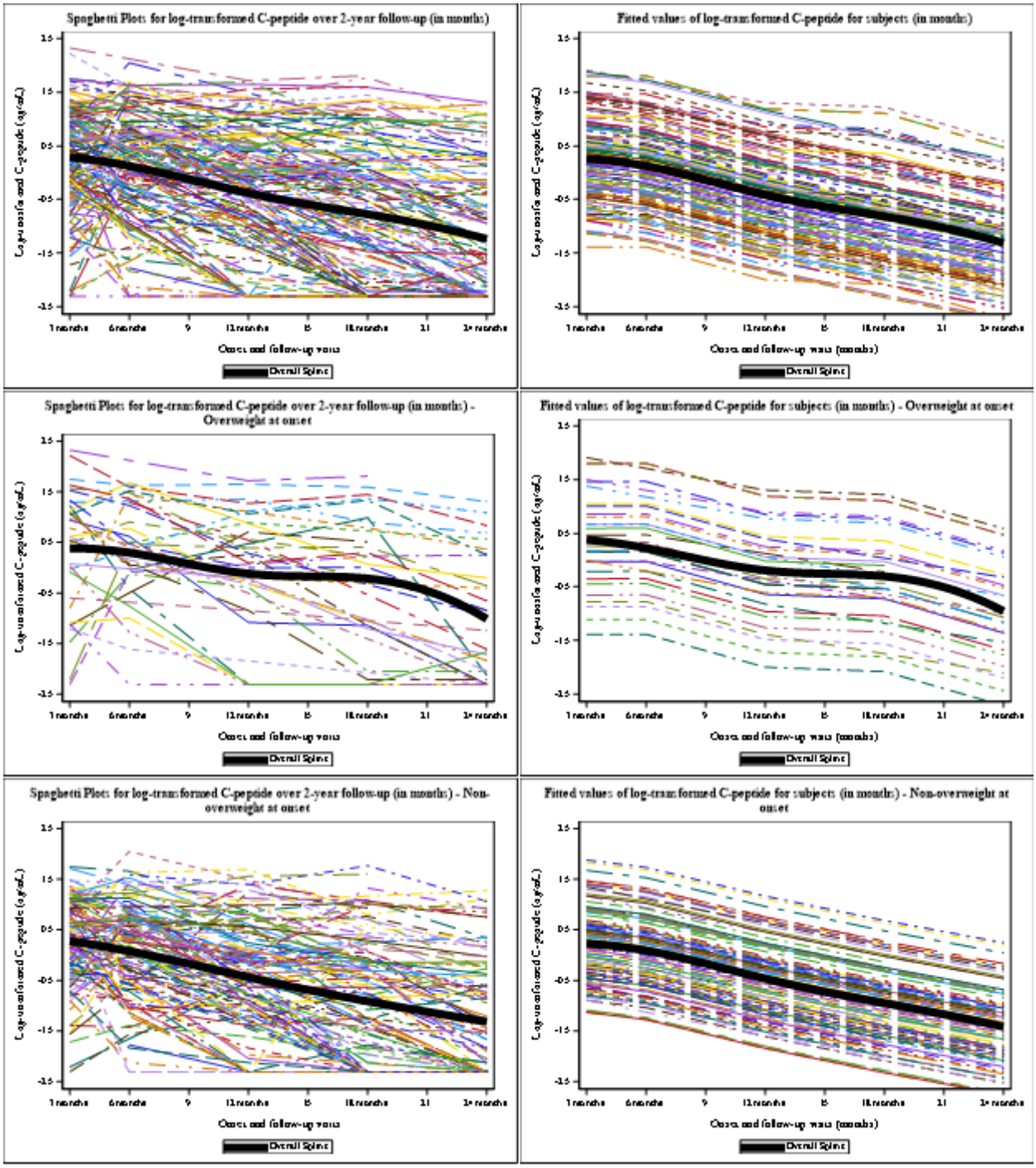

Figure 26. Spaghetti plots for observed and fitted values of log-transformed C-peptide in Model C

Left three are observed values for log-transformed C-peptide at 3 months and forward. Right three are fitted values for log-transformed C-peptide at 3 months and forward. Model C: time was treated as categorical in 3, 6, 12, 18, and 24 months.

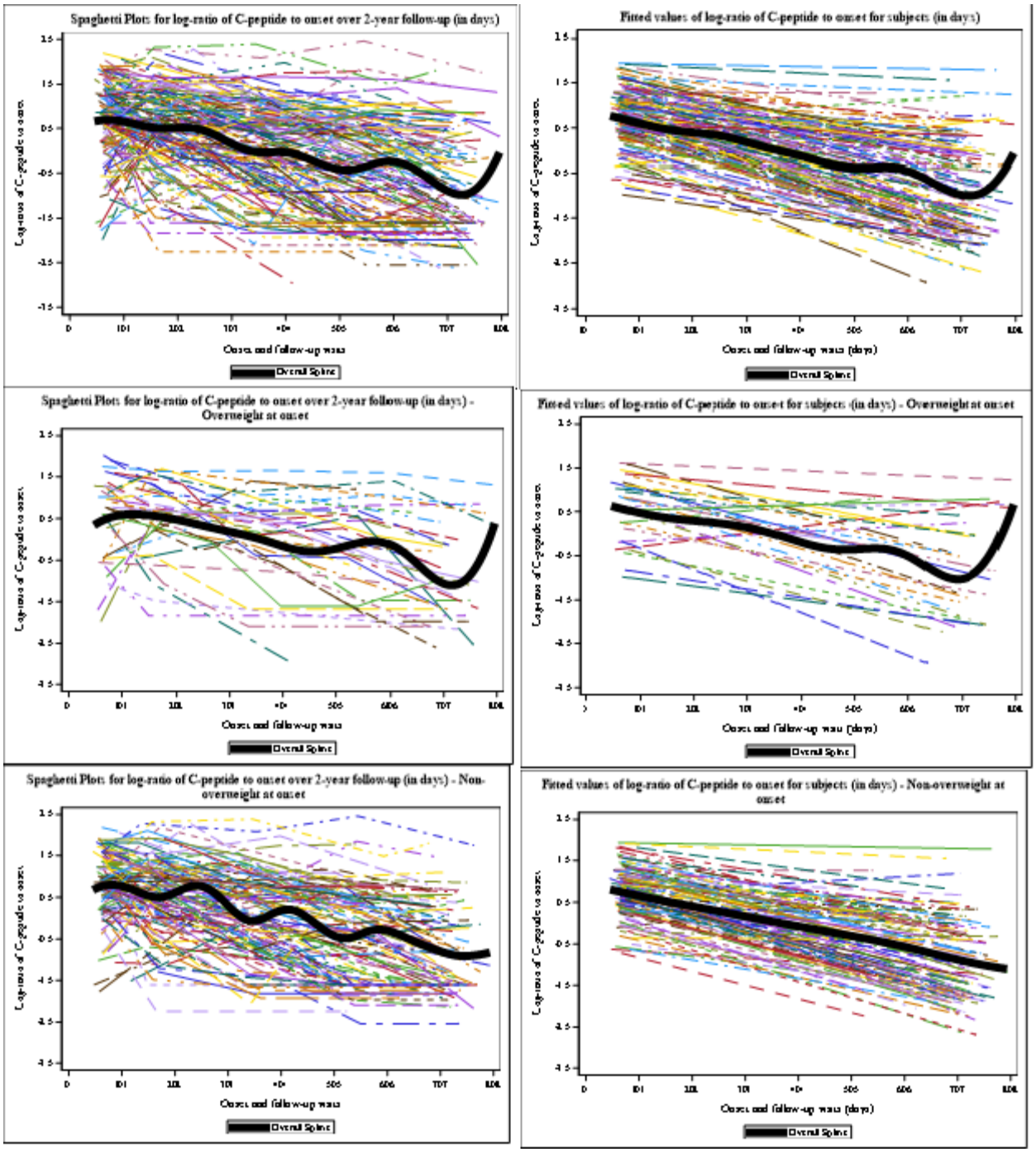

Figure 27. Spaghetti plots for observed and fitted values of log-ratio of C-peptide to onset in Model A

Left three are observed values for log-ratio of C-peptide to onset at 3 months and forward. Right three are fitted values for log-ratio of C-peptide to onset at 3 months and forward. Model A: time was treated as days since diagnosis.

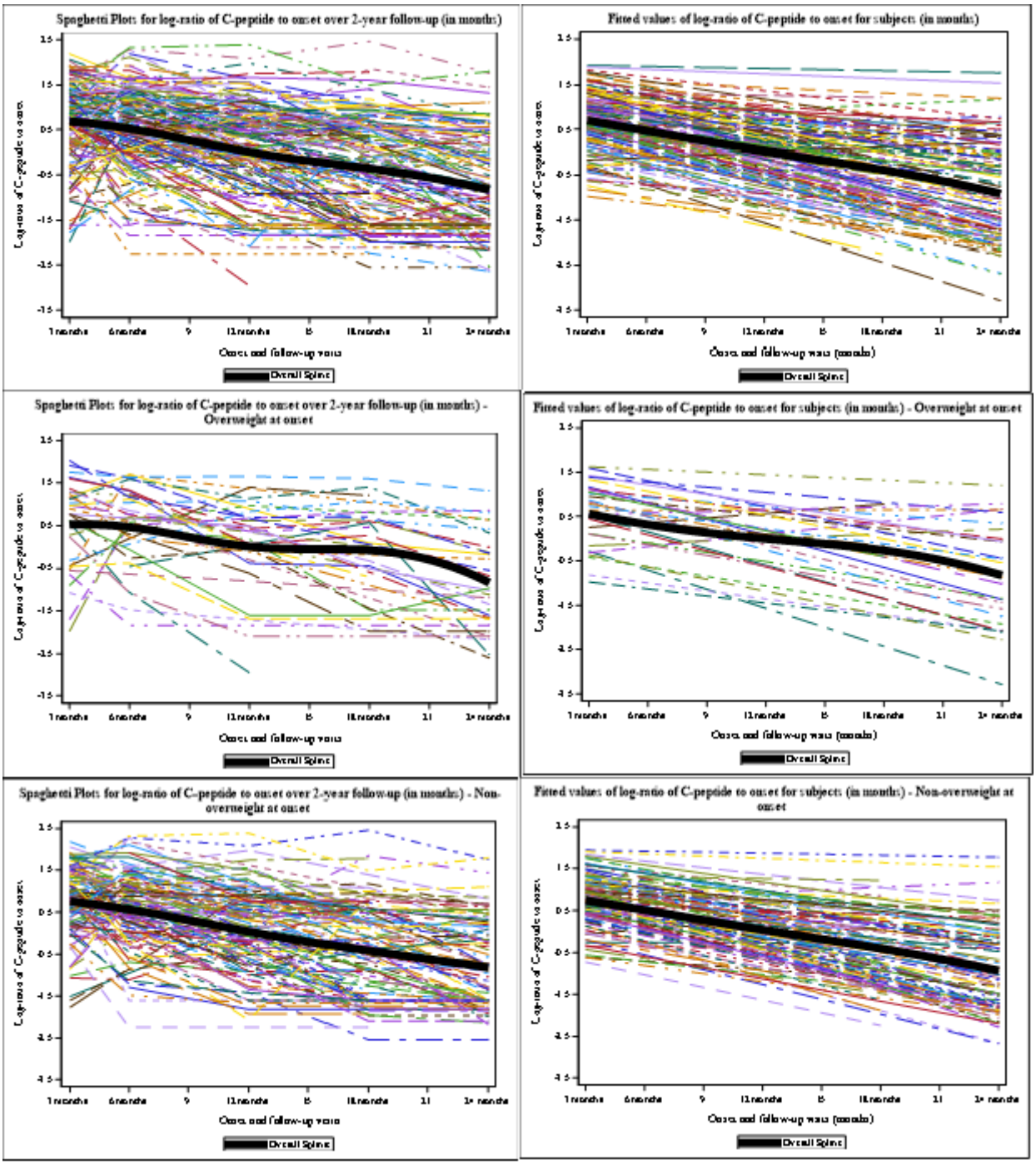

Figure 28. Spaghetti plots for observed and fitted values of log-ratio of C-peptide to onset in Model B

Left three are observed values for log-ratio of C-peptide to onset at 3 months and forward. Right three are fitted values for log-ratio of C-peptide to onset at 3 months and forward. Model B: time was treated as continuous in 3, 6, 18, and 24 months.

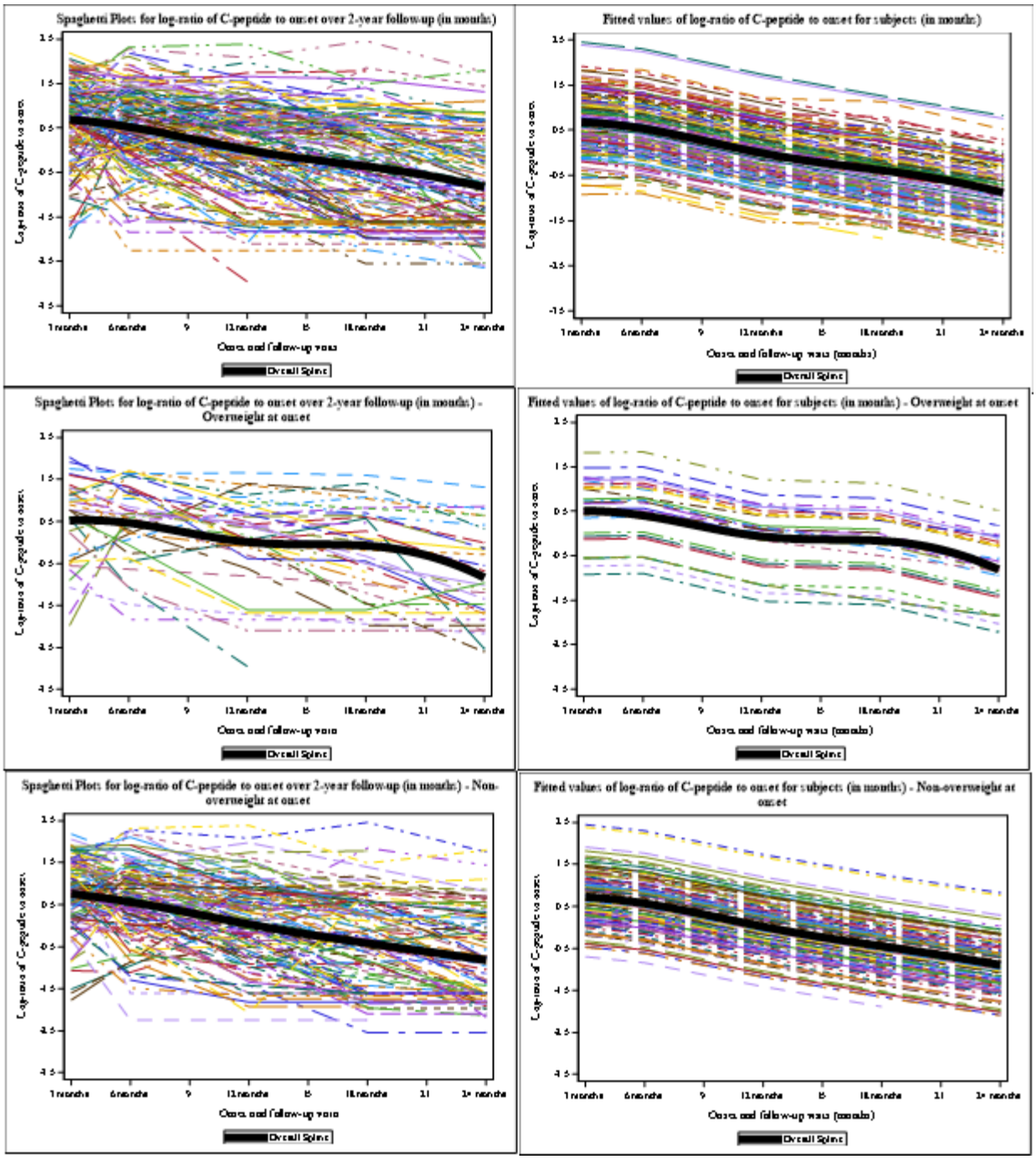

Figure 29. Spaghetti plots for observed and fitted values of log-ratio of C-peptide to onset in Model C

Left three are observed values for log-ratio of C-peptide to onset at 3 months and forward. Right three are fitted values for log-ratio of C-peptide to onset at 3 months and forward. Model C: time was treated as categorical in 3, 6, 18, and 24 months.

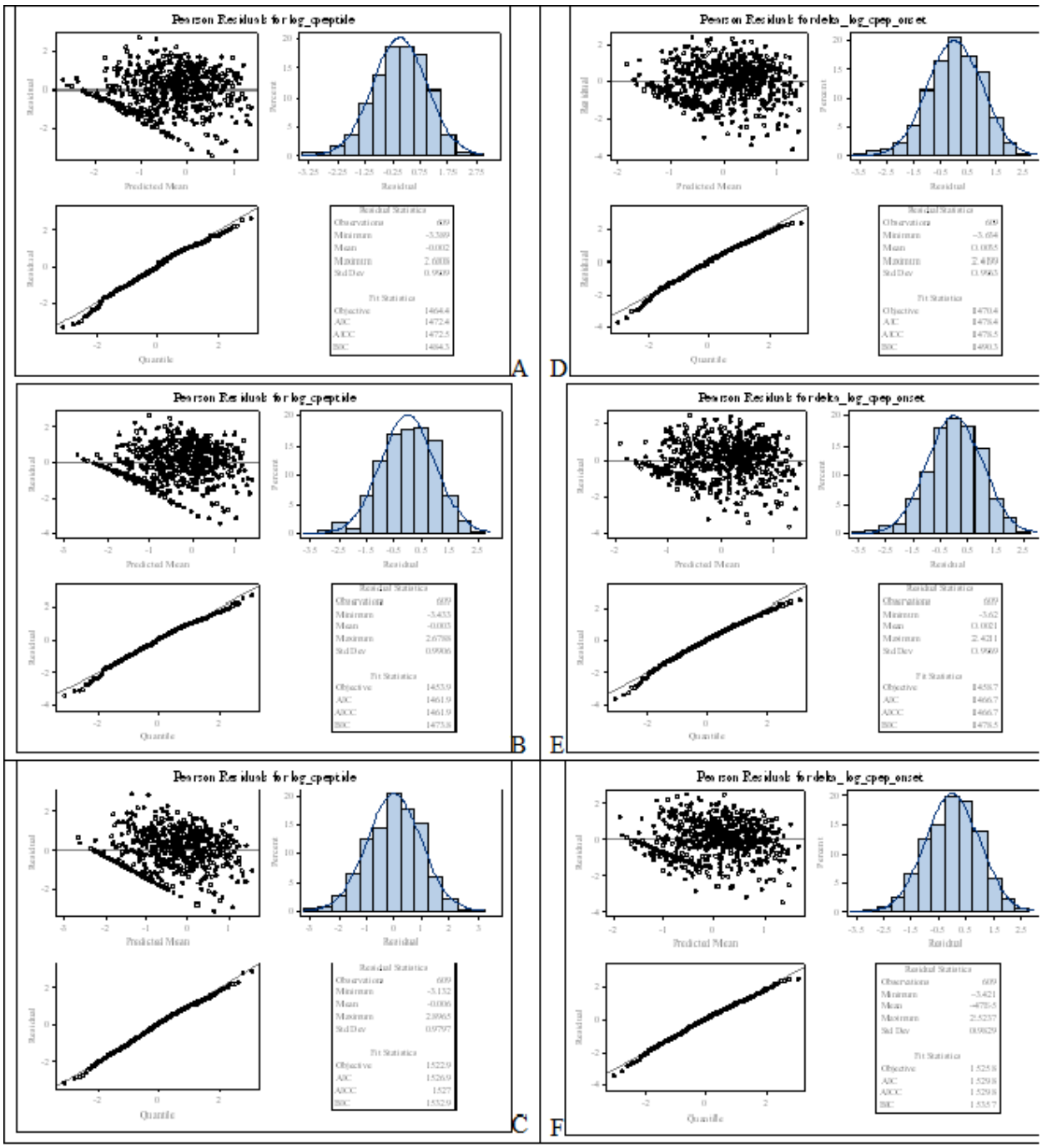

Figure 30. Pearson residuals plots of models A, B, and C for log-transformed C-peptide and log-ratio of C-peptide to onset at 3 months and subsequent follow-ups

A, B, and C were Pearson residuals plots of random coefficients models A, B, and C separately for log-transformed C-peptide at 3 months and forward follow-ups. D, E, and F were Pearson residuals plots of random coefficients models A, B, and C separately for log-ratio of C-peptide to onset at 3 months and forward follow-ups.

APPENDIX B: SAS CODE FOR TESTING AND MODELING

1. Paired-sample test (t-test, signed rank test, and sign test; normality test)

```
*--- Paired-sample t-test and nonparametric tests in each visit ;
%macro onesample_test(testvar, levelvar, timevar, title);
      proc sort data=thesis.Analysis_cpeptide;
            by &levelvar;
      run;
      proc univariate data=thesis.Analysis_cpeptide normal;
            var &testvar;
            by &levelvar ;
            class &timevar;
            where &levelvar ne .;
            *histogram/normal kernel(l=2);
            qqplot/normal(l=1 mu=est sigma=est) square;
            title &title;
      run;
%mend;
title;
```

2. Two-sample test (t-test, and nonparametric test)

```
*--- Summary statistics ;
%macro sumry_grp(testvar, levelvar, timevar, title);
      proc sort data=thesis.Analysis_cpeptide;
            by &levelvar;
      run;
      proc means nolabels data=thesis.Analysis_cpeptide n nmiss mean std
median p25 p75 min max maxdec=2;
            var &testvar ;
            class &timevar ;
            by &levelvar;
            title &title ;
      run;
      title;
%mend;

*--- Two-sample t-test ;
%macro ttest_2sample(testvar, levelvar, timevar, title);
      proc sort data=thesis.Analysis_cpeptide;
            by &timevar;
      run;
      proc ttest data=thesis.Analysis_cpeptide ;
            class &levelvar ;
            var &testvar ;
            by &timevar ;
```

```
            title &title ;
      run;
%mend ;

*--- Wilcoxon-Mann-Whitney test: nonparametric two-sample t-test ;
%macro npar_2sample(testvar, levelvar, timevar, title);
      proc sort data=thesis.Analysis_cpeptide;
            by &timevar;
      run;
      proc npar1way data=thesis.Analysis_cpeptide wilcoxon;
            class &levelvar ;
            var &testvar ;
            by &timevar ;
            title &title ;
      run;
%mend ;
title;
```

3. Multiple-sample test (Nonparametric ANOVA test, and DSCF comparisons)

```
*--- Summary statistics ;
%macro sumry_grp(testvar, levelvar, timevar, title);
      proc sort data=thesis.Analysis_cpeptide;
            by &levelvar;
      run;
      proc means nolabels data=thesis.Analysis_cpeptide n nmiss mean std
median p25 p75 min max maxdec=2;
            var &testvar ;
            class &timevar ;
            by &levelvar;
            title &title ;
      run;
      title;
%mend;

*--- Kruskal Wallis test (nonparametric ANOVA test) and multiple comparisons
;
%macro npar_ANOVA(testvar, levelvar, timevar, title);
      proc sort data=thesis.Analysis_cpeptide;
            by &timevar;
      run;
      proc npar1way data=thesis.Analysis_cpeptide Wilcoxon dscf;
            class &levelvar ;
            var &testvar ;
            by &timevar ;
            title &title ;
      run;
%mend ;
```

4. Trajectory plot for mean and median by group

```
*--- Print out the descriptive stats ;
%macro traj_grp_cpep(testvar, levelvar, group, stats);

      proc means data=thesis.Analysis_cpeptide maxdec=2 noprint;
            var &testvar;
            class plotwindow;
```

```
            where &levelvar=&group;
            output out=cpep_&group &stats=&stats;
      run;
      data cpep_&group;
            set cpep_&group;
            if plotwindow ne .;
            &levelvar=&group;
      run;
      proc sort;
            by plotwindow &levelvar ;
      run;
%mend;

*--- Merge all the dataset for each group ;
%macro merge_grp_cpep(datasets, levelvar);
      data cpep_grp;
            set &datasets;
            by plotwindow &levelvar;
      run;
%mend;

*--- Trajectory for median ;
%macro p50plot_grp_cpep(levelvar, frm, lower, upper, step, ylabel, title);
      goptions reset=all;
      SYMBOL1 V=star C=black I=join h=4;
      SYMBOL2 V=triangle C=black I=join h=4;
      SYMBOL3 V=circle C=black I=join h=4;
      SYMBOL4 V=plus C=black I=join h=4;
      SYMBOL5 V=- C=black I=join h=4;
      legend label=none down=3 position=(top right inside) mode=share
cborder=black;
      axis1 label=(angle=90 &ylabel) order=(&lower to &upper by &step);
      axis2 offset=(6, 6) label=('Onset and follow-up visits') order=(0 to 24
by 3) split=' ';
      proc gplot data=cpep_grp;
            plot median*plotwindow=&levelvar/haxis=axis2 vaxis=axis1 noframe
legend=legend;
            format &levelvar &frm ;
            title &title;
      run;
      quit;
      title;
%mend;

*--- Trajectory for mean ;
%macro meanplot_grp_cpep(levelvar, frm, lower, upper, step, ylabel, title);
      goptions reset=all;
      SYMBOL1 V=dot C=black I=join h=2;
      SYMBOL2 V=square C=black I=join h=4;
      SYMBOL3 V=diamond C=black I=join h=4;
      SYMBOL4 V=x C=black I=join h=4;
      SYMBOL5 V=+ C=black I=join h=4;
      legend label=none down=3 position=(top right inside) mode=share
cborder=black;
      axis1 label=(angle=90 &ylabel) order=(&lower to &upper by &step);
      axis2 offset=(6, 6) label=('Onset and follow-up visits') order=(0 to 24
by 3) split=' ';
```

```
      proc gplot data=cpep_grp;
            plot mean*plotwindow=&levelvar/haxis=axis2 vaxis=axis1 noframe
legend=legend;
            format &levelvar &frm ;
            title &title;
      run;
      quit;
      title;
%mend;
```

5. Box plots with mean and median

```
*--- Box plots by group ;

proc sort data=thesis.Analysis_cpeptide;
      by plotwindow;
run;

%macro box_grp(var, levelvar, group, ylabel, lower, upper, scale, title);
      *--- Use the UNIVARIATE procedure to determine the mean and median
values ;
            proc univariate data=thesis.Analysis_cpeptide noprint;
                  var &var;
                  where &levelvar=&group ;
                  by plotwindow;
                  output mean=mean median=median out=stat;
            run;
      *--- Merge the mean and median values back into the original data set
by drawwindow ;
            data all;
                  merge thesis.Analysis_cpeptide stat;
                  by plotwindow;
                  if &levelvar=&group;
                  keep plotwindow &var mean median;
            run;
      *--- Reshape the data set to create a category variable to use to
generate the legend ;
            data reshape;
                  set all;
                  length zvar $8;
                  newy=&var; zvar='Box'; output;
                  newy=mean; zvar='Mean'; output;
                  newy=median; zvar='Median'; output;
            run;
      *--- Create the graph ;
            goptions reset=all interpol=boxt;
            symbol1 width=1.85 bwidth=7 value=circle c=black;
            symbol2 interpol=none color=black height=1.8 value=plus;
            symbol3 interpol=none color=black height=1.8 value=diamond;
            axis1 label=(angle=90 &ylabel) order=(&lower to &upper by
&scale);
            axis2 offset=(6,6) label=('Onset and follow-up visits') order=(0
to 24 by 3) split=' ';
            legend order=('Mean' 'Median') repeat=1 label=none;
            proc gplot data = reshape;
                  title &title;
                  plot newy*plotwindow=zvar /vaxis=axis1 haxis=axis2 noframe
```

```
legend=legend;
            run;
            quit;
            title;
%mend;
```

6. Spaghetti plots

```
*--- Spaghetti plot for overall ;
%macro spaghetti_overall(dependent_var, indep_var, xvalues, xlabel, yvalues,
ylabel, title);
      options nobyline;
      title &title;
      proc sgplot data = thesis.Analysis_cpeptide;
            where drawwindow ne 0;
            series x = &indep_var y = &dependent_var / group = pid ;
            pbspline y=&dependent_var x=&indep_var / nomarkers lineattrs =
(color= black pattern = 1 thickness = 5) legendlabel='Overall Spline'
name='spline1';
            xaxis values = &xvalues label=&xlabel;
            yaxis values = &yvalues label=&ylabel;
            keylegend 'spline1';
      run;
      title;
      quit;
%mend;

*--- Spaghetti plot by group ;
%macro spaghetti_grp(dependent_var, indep_var, group_var, grp_value, xvalues,
xlabel, yvalues, ylabel, title);
      options nobyline;
      title &title;
      proc sgplot data = thesis.Analysis_cpeptide;
            by &group_var;
            where drawwindow ne 0 and &group_var=&grp_value;
            series x = &indep_var y = &dependent_var / group = pid ;
            pbspline y=&dependent_var x=&indep_var / nomarkers lineattrs =
(color= black pattern = 1 thickness = 5) legendlabel='Overall Spline'
name='spline1';
            xaxis values = &xvalues label=&xlabel;
            yaxis values = &yvalues label=&ylabel;
            keylegend 'spline1';
      run;
      title;
      quit;
%mend;
```

7. Random coefficient model

```
*--- Fit model, save the fitted values into out_dataset, and check residuals;
%macro model_randcoef(class_var, dependent_var, fixed_var, random_var,
covtype, title1, title2);
      title1 &title1;
      title2 &title2;
      proc mixed data=thesis.complete_visit_cpeptide order=data covtest ;
            *--- Define categorical variables ;
```

```
            class &class_var ;
            *--- Fit the mean structure ;
            model &dependent_var = &fixed_var /solution ddfm=kr
                  outp=&out_dataset residual;
            where drawwindow ne 0 ;
            *--- Fit random effects ;
            random &random_var / subject=pid type=&covtype g v vcorr;
      run;
      title;
%mend;
```

8. Spaghetti plot for fitted values

```
*--- Spaghetti plot for overall fitted value ;
%macro spaghetti_overall_fitted(dataset, dependent_var, indep_var, xvalues,
xlabel, ylabel, title);
      proc sort data=&dataset ;
            by &indep_var ;
      run;

      options nobyline;
      title &title;
      proc sgplot data = &dataset;
            where drawwindow ne 0;
            series x = &indep_var y = &dependent_var / group = pid ;
            xaxis values = &xvalues label=&xlabel;
            yaxis label=&ylabel;
      run;
      title;
      quit;
%mend;
*--- Spaghetti plot for fitted value by group ;
%macro spaghetti_grp_fitted(dataset, group_var, grp_value, dependent_var,
indep_var, xvalues, xlabel, ylabel, title);
      proc sort data=&dataset ;
            by &indep_var ;
      run;

      options nobyline;
      title &title;
      proc sgplot data = &dataset;
            where drawwindow ne 0 and &group_var=&grp_value ;
            series x = &indep_var y = &dependent_var / group = pid ;
            xaxis values = &xvalues label=&xlabel;
            yaxis label=&ylabel;
      run;
      title;
      quit;
%mend;
```

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