RELATIONSHIP BETWEEN OVERWEIGHT STATUS AT ONSET AND INSULIN RESERVE IN CHILDREN WITH TYPE 1 DIABETES

by

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University of Pittsburgh, 2012

Background: The incidence rate of Type 1 Diabetes (T1DM) is increasing in line with the obesity epidemic in children. It remains unknown whether being overweight is associated with greater insulin reserve at the onset of the disease and how this impacts disease progression.

Objective: To evaluate the relationship between overweight status at onset and insulin reserve in children and adolescents with T1DM.

Methods: This study is a prospective follow-up study of new onset T1DM patients (aged 1.5 to 18.9 years) identified from the Children' Hospital of Pittsburgh (CHP) Registry. This study invited all children aged <19 years, newly diagnosed with T1DM from 2004 to 2006 at CHP to participate. The main outcome of interest was C-peptide levels at the onset of T1DM and at 3-month, 6-month, 12-month, 18-month, and 24-month follow up visits. We evaluated the independent associations between C-peptide levels and overweight status at baseline using generalized estimating equation (GEE) regression models.

Results: Complete data were available in 179 subjects. The patients were on average 9.5 years old at enrollment, with 22% (39) overweight, defined as body mass index $\geq 85^{th}$ percentile for the same gender and age. Mean C-peptide levels in the study population was 0.65ng/ml at baseline. C-peptide levels increased at the 3-month follow up visit and then gradually decreased at subsequent follow-up visits. GEE models suggested a statistically significant interaction between overweight status at baseline and follow-up visits after adjustment of potential confounders. C-

peptide concentrations were significantly higher for overweight compared to non-overweight participants at 3-month, 6-month, 18-month follow up visits and borderline significant at the 12-month follow up visit.

Conclusion: Overweight T1DM children had higher C-peptide levels as compared to non-overweight T1DM children at 3-month, 6-month, 12-month, and 18-month follow up visits; however, at 24-months after initiation of treatment, the C-peptide levels between overweight and non-overweight groups were not statistically significant different any longer.

Public health significance: Overweight patients with T1DM may potentially benefit from target preventive strategies that will help them maintain or prolong the high C-peptide levels after initiation of treatment.

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1.0 INTRODUCTION

1.1 TYPE 1 DIABETES MELLITUS

Diabetes mellitus is a group of metabolic conditions characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin action [1]. Diabetes and its complications impose a major public health burden in the United States. As of 2011, 25.8 million people (8.3% of the population) have diabetes and the total national diabetes associated costs is $174 billion, including $116 billion for direct medical costs and $58 billion for indirect costs [2]. Diabetes is classified into four categories: Type 1 diabetes (T1DM), Type 2 diabetes (T2DM), gestational diabetes mellitus (GDM), and diabetes from other causes.

The incidence rate of T1DM is increasing in line with the obesity epidemic [3-6]. T1DM, which occurs in 5 to 10% of those with diabetes, results from a cellular mediated autoimmune destruction of the β-cells of the pancreas [1]. The exact mechanism that causes T1DM is still unknown. Risk factors that are found to be associated with T1DM include autoimmune, genetic, and environmental [2]. T1DM mostly affects children and young adults [2], a time of rapid growth. In developed countries, diabetes is the most prevalent chronic disease of children after asthma[7]. An increase in insulin secretion during adolescence in healthy individuals has been well documented, and is thought to occur in response to the increased insulin resistance seen during this stage. Insulin secretion is profoundly impaired at the time of diagnosis of T1DM.

Studies have shown between 67% and 90% loss of normal beta cell mass at the time of diagnosis of T1DM [8, 9]. At present it is not well understood whether it follows a gradual or steep course. Moreover, the impact of obesity on this decline is unknown. One of the principal limitations of human studies is that it is not currently possible to measure beta cell mass in vivo. Instead, indirect methods based on biochemical and secretory responses are used to approximate beta call mass. Markers of the beta cell destruction include, but are not limited to, islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase, and autoantibodies to the tyrosine phosphatase IA-2.

There are limitations to evaluate plasma insulin as a measure of insulin secretion. Insulin has a short half-life and peripheral clearance makes the peripheral insulin concentrations an inaccurate reflection of insulin secretion [10, 11]. C-peptide, which is co-produced with insulin, can be measured more accurately and with higher reproducibility and greater sensitivity as a marker of beta-cell function. C-peptide has been recommended as the appropriate outcome measure for future clinical trials aimed at preserving beta-cell function [12].

1.2 C-PEPTIDE AS A MARKER OF BETA-CELL FUNCTION

Evaluation of the C-peptide levels remains the most widely used surrogate measure of functional beta cell mass in children with T1DM. Pro-insulin is first secreted with an A-chain, C-peptide, a B-chain, and a signal sequence. The signal sequence is first cut off and leaves A-chain, C-peptide, and B-chain. Then the C-peptide is cut off, leaving the A-chain and B-chain to form insulin. C-peptide also facilitates the efficient assembly, folding, and processing of insulin in the endoplasmic reticulum. C-peptide and insulin are then stored in secretory granules of the

pancreatic beta cells and both are eventually released to the portal circulation. Most investigations have used C-peptide responses to secretagogues as a measure of insulin secretion. It is of great value in furthering the understanding of the pathophysiology and treatment effects of diabetes.

The pancreases of patients with T1DM are unable to produce insulin as needed, and, therefore, they will have a decreased level of C-peptide with respect to their needs. The level of C-peptide secretion at diagnosis is of clinical importance. Data from the Diabetes Control and Complications Trail demonstrated that a stimulated C-peptide level of ≥0.2 pmol/ml was correlated with improved metabolic control and a decreased rate of complications when compared to patients with C-peptide levels below this threshold [12]. Several factors have been found to affect insulin secretion at diagnosis and the loss of beta-cell function afterwards in T1DM patients. The factors include age at diagnosis, degree of metabolic control, immune status measured by antibody levels, and genetics [13-15]. It is not clear what the effect of overweight is, if any, on insulin reserve.

1.3 STATEMENT OF THE PROBLEM

There is increasing interest on residual β-cell function in patients with T1DM and its preservation. Different factors have been shown to influence C-peptide insulin reserve levels. The phenotype of children with T1DM is changing. One study suggested a higher prevalence of overweight among T1DM children compared to non-diabetic controls [16]. At onset, 25% of children with T1DM are overweight despite frequent weight loss [17]. Moreover, after a 3-year-duration, one third of children <10 years and 1 in 2 children of 10-18 years are overweight.

Although the effect of childhood overweight has been studied extensively, it is still unknown whether overweight is associated with greater insulin reserve at presentation of the disease and how it impacts its progression.

2.0 STUDY OBJECTIVES AND HYPOTHESES

The aim of this study is to evaluate the relationship between overweight and insulin reserve in children and adolescents diagnosed with T1DM.The specific study objectives were:

Objective 1: To describe C-peptide levels at onset of T1DM and at each follow-up visit.

Hypothesis 1: C-peptide levels increase at the first follow-up visit following diagnosis and start of treatment. C-peptide levels decrease after the first follow-up.

Objective 2: To compare C-peptide levels at onset of the disease between overweight and non-overweight patients.

Hypothesis 2: Overweight patients with T1DM have greater C-peptide levels at onset of the disease than those who were not overweight.

Objective 3: To compare C-peptide levels at follow up visits between overweight and non-overweight patients.

Hypothesis 3: Overweight patients with T1DM have greater C-peptide levels at follow up visits than those who were not overweight.

Objective 4: To compare C-peptide levels between three weight transitioning groups: 1) those who were overweight at onset and remained overweight at 1st follow up visit, 2) those who were not overweight at onset and remained not overweight at 1st follow up visit and 3) those who were not overweight at onset and became overweight at 1st follow up visit.

Hypothesis 4: Overweight patients who were overweight at onset and remained overweight at 1st follow up visit have higher c-peptide levels compared to the two other groups at 1st follow up visit and subsequent visits.

3.0 MATERIALS AND METHODS

3.1 STUDY POPULATION

This study population was derived from the Children' Hospital of Pittsburgh (CHP) Registry. The CHP Registry enrolls patients with T1DM and their first-degree relatives (FDRs) and then follows them systematically. This process has been ongoing since the 1970's. The CHP registry starts by identifying children who were diagnosed at CHP or seen within 1 year of diagnosis at CHP. These T1DM cases are referred to as probands and used to identify the FDRs who will then be enrolled in the CHP cohort. Each year, the registry has identified and enrolled 120 to 140 new-onset type 1 diabetes cases which has represented approximately 70% of those cases diagnosed in Allegheny County, PA .

All children aged <19 years, diagnosed with T1DM from January 1st, 2004 through December 31st, 2006 at Children's Hospital of Pittsburgh were invited to participate in the study. After providing informed consent their medical data was reviewed for eligibility into the study.

The criteria for inclusion in this study were: 1) treated with insulin therapy prior to hospital discharge, 2) returned for follow-up at least 3 times after diagnosis during a 2 year period, and 3) had at least three β-cell antibodies (GAD, human ICA, IA2, and IAA) measured at onset of the disease (Figure 1). All cases of secondary diabetes and type 2 diabetes, diagnosed on the basis of clinical criteria, were excluded. The study was reviewed and approved by the

Institutional Review Board of the University of Pittsburgh. Informed consent was obtained for all study participants.

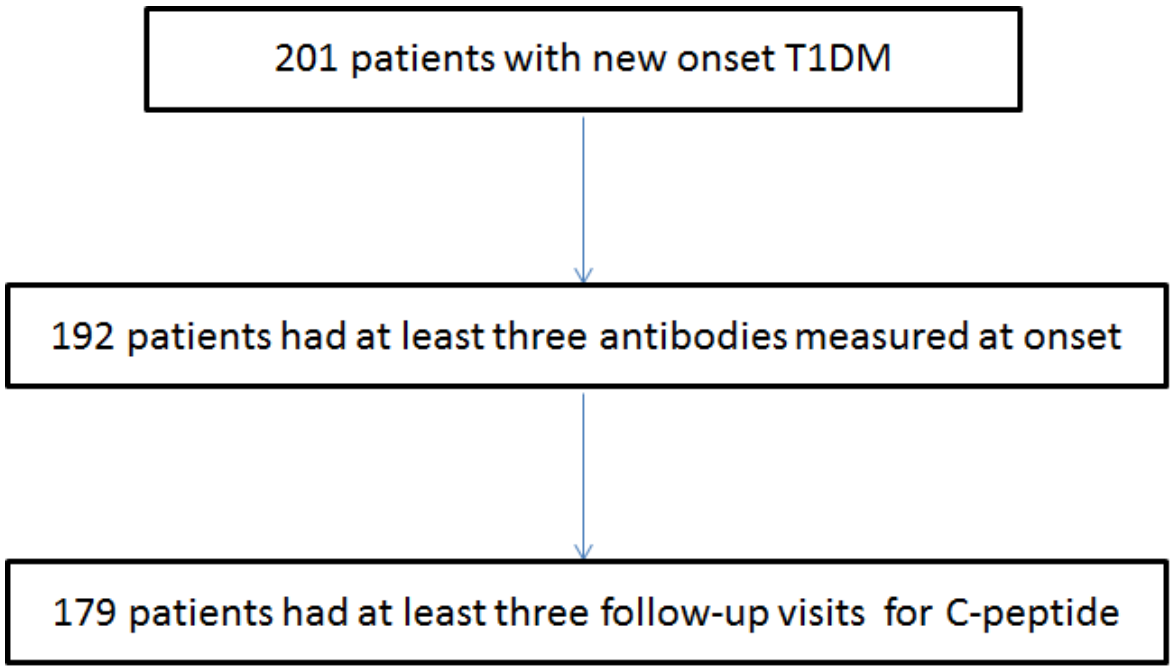

Figure 1.Flow chart of the study population

3.2 ANALYTICAL APPROACH

3.2.1 Outcome variable

The main outcome variable of interest of this study was C-peptide levels at the onset of T1DM and at each follow-up visit through up to 867 days after the diagnosis. Baseline C-peptide levels were obtained from clinical records at Children's Hospital of Pittsburgh during admission. The lowest limit of detection was 0.5 ng/ml. Follow up C-peptide levels were determined using

Human C-peptide radioimmunoassay Kit (Linco Research, St. Charles, Missouri). The lowest level of C-peptide that can be detected by this kit is 0.1ng/ml when using a 100 uL sample volume. The limit of linearity is 5.0ng/ml. Any results greater than 5.0 ng/ml were repeated on dilution. Two levels of controls provided by this kit and two levels of in-house controls were used for assay stability over time. For C-peptide levels below the detection levels, we used half of the lowest limit of detection as the C-peptide levels for analysis.

Follow-up visits

As part of the study, participants were scheduled to come back for five follow-up visits at 3, 6, 12, 18 and 24 months after onset. However, some participants only showed up for several of the suggested follow-up visits, and some participants had appointments that varied from the exact prescribed times in the protocol and did not come back for follow-up at the exact specified times as suggested by the protocol (Table 1). To account for those situations, we created follow-up windows centered on the specified follow-up visit times and placed the follow-up visit into the appropriate follow-up window. Thus, windows were defined as 3-months (1-4 months), 6-months (4-9 months), 12-months (9-15 months), 18-months (15-21 months), and 24-months (21 and above months) (Figure 2). If a patient made more than one visits during one follow-up window, we considered the first visit during the interval as valid and ignored his/her other visits during this follow-up window. Table 2 shows the number of participants that came back for follow up at during each created follow up visit window and the time interval between onset and each follow up visit window. In general, when participants did schedule a follow-up visit, the date ended to fall near the center of the protocol specified windows. For patients who came back for their 3-month follow-up visit, the average of days from onset to the 3-month follow-up visit was 68 days. For patients with a 6-month follow-up visit, the average of days from onset was

168 days. For the 12-month follow-up visit, the average of days from onset was 359 days. For the 18-month follow-up visits, the average number of days from onset to 18-month follow-up visit was 548 days. For the 24-month follow-up visits, the average number of days from onset to 24-month follow-up visit was 725 days.

Table 1.Days between onset of T1DM and each follow-up visit (3-month windows)

Follow-up visits	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
3-month follow-up	169	69	14	66	62	74	40	136
6-month follow-up	133	167	19	161	153	176	137	226
9-month follow-up	16	280	33	276	251	313	232	318
12-month follow-up	132	357	23	355	339	374	320	409
15-month follow-up	26	450	27	448	430	464	412	497
18-month follow-up	116	544	24	543	524	565	503	593
21-month follow-up	40	635	30	633	606	661	595	684
24-month follow-up	133	734	35	728	708	756	685	867

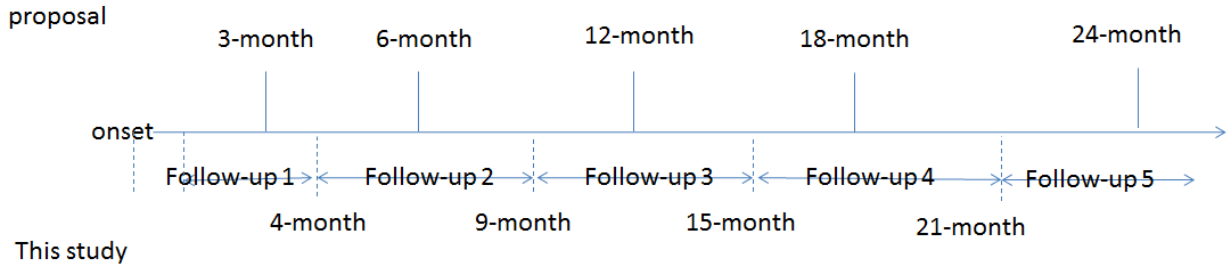

Figure 2. Follow-up windows

Table 2.Days between onset of type 1 diabetes and each follow-up visit (6-month window)

Follow-up visit	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
3-month follow-up	165	68	10	66	62	73	40	100
6-month follow-up	147	168	26	161	152	178	124	267
12-month follow-up	152	359	31	355	338	379	276	453
18-month follow-up	143	548	38	544	522	572	461	636
24-month follow-up	145	725	39	723	699	746	641	867

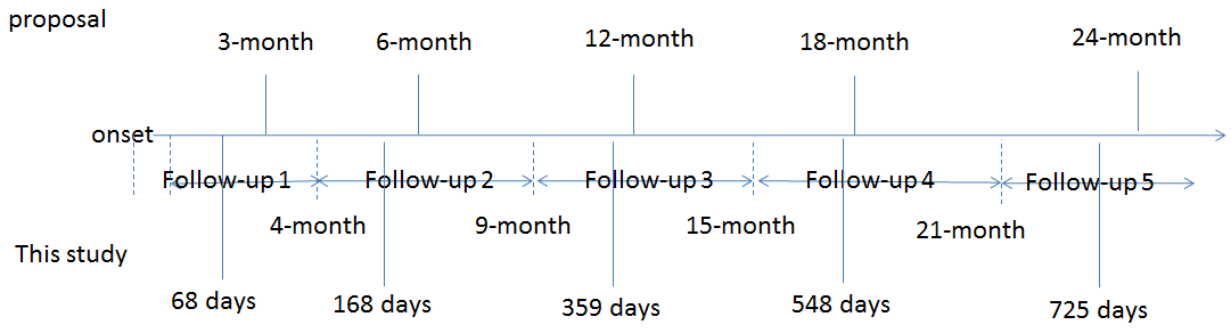

Figure 3.Follow-up windows with the mean of time interval in days

3.2.2 Potential Confounding Variables

Data including gender, race, date of birth, date of onset of T1DM, height, weight and HbA1c at onset and at their first clinic visit were obtained from hospital and research medical records. Height and weight were obtained at onset of the diabetes and at their initial follow-up visit to the clinic. BMI percentiles and BMI z-scores were calculated using the Centers for Disease Control and Prevention 2000 growth data [18].

Blood samples were obtained within 1 week of diagnosis for insulin autoantibodies (IAA) or within 3 months for human islet cell antibodies (human ICA), antibodies to glutamic acid decarboxylase (GAD), and antibodies to insulinoma-associated protein 2 (IA-2). Blood samples were stored frozen at -20°C before antibody testing. ICAs were detected by a modification of an immunoperoxidase method on human blood group O (H) fresh frozen pancreas [19]. This assay is sensitive to Juvenile Diabetes Foundation (JDF) units using the JDF serum as standard. The specificity of this assay varied from 77 to 100%, and sensitivity between 88 and 99% in the JDF proficiency workshops conducted by the University of Florida in Gainesville from 1991 to 1996.

The IAA assay was performed only on those subjects with serum available within 7 days of diagnosis. The radioimmunoassay used ^{125}I-mono-iodinated insulin obtained from New England Nuclear and protein A separation using the assay described by Williams et al [20]. This assay detected 16% of subsets of T1DM and was 100% specific in the Diabetes Autoantibody Standardization Program workshop in 2000, which was average for participating laboratories. The intra-assay coefficient of variation was 8% and the inter-assay coefficients of variation (CVs) were 8.9, 6.5, and 21.6%, respectively, for low, medium, and high controls.

GAD65 autoantibodies were detected by a radiobinding assay using ^{35}S-[Met]-labeled recombinant human GAD65 produced in vitro with the TNT reticulocyte R transcription/translation kit as described by [21]. The GAD65 construct used for this study was donated by Dr. Åke Lernmark, whereas the IA-2 construct (ICA512bdc) was provided by Dr. George Eisenbarth. The results were expressed as an index

$$\text{index} = \text{sample cpm} - \frac{\text{negative control cpm}}{\text{positive control cpm}} - \text{negative control cpm}$$

as previously reported. Of the results from the proficiency workshops organized by the University of Florida in Gainesville (1995, 1996, and 1997) and the Diabetes Autoantibody Standardization Program workshop (2000) organized by the World Health Organization, the data were 76–100% sensitivity, 90–100% specificity (100% specificity three times), and 100% validity for GAD autoantibodies; and 48–78.5% sensitivity, 98–100% specificity, 87.5% validity, and 91.6% consistency in the 1996 and 2000 workshops for IA-2 autoantibodies.

3.2.3 Covariate

The main factor of interest was overweight status at the onset of T1DM. Overweight was defined as body mass index (BMI) $\geq 85^{th}$ percentile for age and gender. We created a two-level categorical variable: overweight and non-overweight, according to the patient's standardized BMI percentile at the onset of type 1 diabetes. We also collected information on the participants' BMI at 3-month follow up visit.

3.2.4 Analytical approach and statistical analysis

The statistical analysis started with descriptive analysis. The first step was to describe the demographic and clinical characteristics of this population. For continuous variables (e.g., age, C-peptide), we evaluated mean, median and standard deviation measures. For categorical variables such as race, gender, and obese status at onset, we assessed the proportion of each category within each variable. We compared the means of continuous variables between overweight and non-overweight participants with t-test or Wilcoxon-Mann-Whitney test. We compared the proportions of categorical variables between overweight and non-overweight participants with chi-square test.

We then summarized the C-peptide levels at baseline, 3-month follow-up, 6-month follow-up, 12-month follow-up, 18-month follow-up, and 24-month follow-up in the whole study population. In addition, we described the C-peptide levels by overweight status at onset and antibody status. To understand the change of C-peptide, we calculated the rate of change in C-peptide from baseline to each follow-up visit. The rate of change was calculated as the difference between C-peptide levels at baseline and each follow-up visit divided by the time interval (in

months) between baseline and the corresponding follow-up visit. Taking 3-month follow-up as an example, we used the following formula to calculate the rate of C-peptide change.

$$rate\ of\ C-peptide\ change = \frac{(C-peptide\ at\ 3-month\ follow-up\ -\ C-peptide\ at\ onset)}{months\ between\ onset\ and\ 3-month\ follow-up}$$

In addition, we calculated the rate of change in C-peptide from 3-month follow-up through subsequent follow-up visits with the same method. Taking the rate of C-peptide change from 3-month follow-up to the 24-month follow-up as an example, we used the following formula to calculate the rate of C-peptide change.

$$rate\ of\ C-peptide\ change = \frac{(C-peptide\ at\ 24-month\ follow-up\ -\ C-peptide\ at\ 3-month)}{months\ between\ 3-month\ and\ 24-month\ follow-up}$$

We then compared the rate of change in C-peptide between overweight and non-overweight groups by t-test.

The current study is a longitudinal study that measured the same participant's C-peptide at onset and follow up visits. To account for the correlation between repeated measurements of C-peptide in the same participant, we used GEE to evaluate the independent associations between C-peptide levels and overweight status at baseline. The potential confounding variables included age at onset, gender, A1C at onset, A1C at 3-month, and the number of positive antibodies. The GEE is a method to analyze correlated data that would typically be modeled as a generalized linear model. GEE models are particularly suitable when the correlation is of no substantive interest and is merely a nuisance parameter. In the current study, using the C-peptide levels as separate observations violates the independence assumption for generalized linear

model. Therefore, we fit GEE to account for the correlations between C-peptides within the same participant. The GEE is used to estimate the parameters of a generalized linear model with possible correlation between outcomes. GEE measures differences in the response for a unit change in the predictor, averaged over the whole population, rather than on a given individual. In GEE with onset/follow-up visit as a categorical variable, we could test the pairwise comparison in C-peptide between overweight and non-overweight groups at each visit.

In the current study, we were also interested in the effect of a continuous time variable on the C-peptide levels. Preliminary descriptive analysis showed a non-linear trend of C-peptide over the two years period after onset of T1DM. The nonlinear trend made it difficult to establish the relationship between time and C-peptide levels with conventional linear regression methods. Therefore, we considered fractional polynomial GEE models to account for the non-linear curves and generate plot for the prediction of C-peptide levels. Fractional polynomial is a variant on polynomial regression with powers estimated in a fixed lower range of fractional powers. Fractional polynomials may be used with any generalized linear model [22]. By incorporating a variety of functional forms, fractional polynomial models have more flexibility than conventional polynomial models and can accommodate the non-linear relationships between exposure and outcomes. The fractional polynomial models combine the low- and high-order polynomials into one model and produce a range of curves with flexible shapes [23].

We fit two different fractional polynomial GEE models with a continuous time variable. In the first model, we treated the time variable as a continuous variable indicating onset or subsequent follow-up visits. The values of the time variable are 0, 3, 6, 12, 18, and 24. In the second fractional polynomial GEE mode, we modeled time as a continuous variable indicating the days between onset and each participant's follow-up. The values range from 0 to 867. After

fitting the models, we produced the plots with predicted C-peptides for overweight and non-overweight participants respectively. We also fit GEE models with time as a categorical variable, which indicates onset or follow-up visit (0, 3, 6, 12, 18, and 24). In the GEE model with time as a categorical variable, we were able to test the pairwise comparison in C-peptide between overweight and non-overweight groups at each visit. We tested for the interaction between overweight and time in all three models.

4.0 RESULTS

4.1 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE POPULATION AT BASELINE

Complete data, including blood available for antibody testing as well as c-peptide measurements for at least 3 follow up visits, were available in 179 subjects. Their demographic and clinical characteristics are shown in Table 2. At baseline, 22% (39) of patients were overweight and 78% (138) of patients were non-overweight. In this study population, 58% (103) of patients were males and 42% (76) of patients were females. The proportions of males and females were similar in overweight and non-overweight participants. In this study population, 95% (170) of patients were Whites. The patients were on average 9.5 years old at enrollment for the current study. At 3-month visit, 37% (65) of patients were overweight. At baseline, overweight participants had significantly higher C-peptide concentrations than non-overweight participants. The overweight and non-overweight group did not differ in age, gender, race, Hemoglobin A1C (HbA1c) at onset and 3-months, or the number of follow-up visits.

Table 3.Demographic and clinical characteristics according to overweight status at onset

	Total population (n=179)	Overweight at baseline (n=39)	Non-overweight at baseline (n=138)
Demographic characteristics			
Age (years) #	9.5 (3.7)	10.0 (2.6)	9.5 (3.9)
Male (%)	103 (58%)	22 (56%)	80 (58%)
White (%)	170 (95%)	36 (92%)	132 (95%)
BMI (kg/m^2) at baseline #	18.21 (4.51)	24.23 (4.95) *	16.51 (2.48)
BMI percentile at baseline #	51.02 (32.78)	94.36 (4.72) *	38.77 (26.24)
BMI z-score at baseline #	0.02 (1.30)	1.77 (0.51) *	-0.47 (1.00)
Overweight at baseline (%)	39 (22%)	-	-
BMI (kg/m^2) at 3-month #	19.80 (4.08)	24.54 (4.87) *	18.48 (2.61)
BMI percentile at 3-month #	73.98 (19.98)	94.28 (6.47) *	68.30 (18.75)
BMI z-score at 3-month #	0.82 (0.77)	1.78 (0.50) *	0.55 (0.59)
Overweight at 3-month (%)	65 (37%)	-	-
Clinical characteristics			
HbA1c at baseline #	11.86 (2.34)	11.44 (2.19)	11.99 (2.38)
HbA1c at 3-month #	7.34 (0.82)	7.12 (0.97)	7.40 (0.78)
C-peptide at baseline	0.55 (0.25-85)	0.88 (0.57-1.31) ^	0.50 (0.25-0.70)
C-peptide at 3-month follow-up	1.47 (0.76-2.61)	2.29 (0.74-3.53)	1.39 (0.76-2.45)
C-peptide at 6-month follow-up	1.29 (0.62-2.18)	1.54 (0.92-3.64)	1.18 (0.61-2.09)
C-peptide at 12-month follow-up	0.75 (0.32-1.46)	0.93 (0.43-2.03)	0.70 (0.30-1.40)
C-peptide at 18-month follow-up	0.47 (0.05-1.25)	1.16 (0.34-2.58) ^	0.44 (0.05-0.95)
C-peptide at 24-month follow-up	0.16 (0.05-0.66)	0.25 (0.08-1.06)	0.14 (0.05-0.55)
Number of follow-up visits for C-peptide #	4.20 (0.71)	4.18 (0.76)	4.21 (0.71)

Numbers in parenthesis, standard deviation
* P-value<0.05 from t-test
^ P-value<0.05 from Wilcoxon-Mann-Whitney

4.2 FOLLOW-UP WINDOWS

As part of the inclusion criteria, participants in this study had to have at least three follow up visits with C-peptide measurements (Table 4). In our study, 18% of the participants came back for three C-peptide follow-up visits, 45% came back for 4 C-peptide follow-up visits, and 37% came back for 5 follow-up visits.

Table 4.Distribution of study participants' number of follow-up visits

Number of follow-up visits	Frequency	Percent (%)
3	31	18
4	81	45
5	67	37

4.3 ANTIBODIES STATUS

This study measured levels of GAD, human ICA, IA2, and IAA. The participants in the current analysis have three or four antibodies measured. Forty-four percent (n=78) of participants had three or more positive antibodies and nine percent (n=17) of the participants have no positive antibodies (Table 5). Since only 14 participants had four positive antibodies, we combined the participants with three or four positive antibodies together for future analysis. Table 6 and Table 7 show the distribution of antibodies for participants with one-positive-antibody and two/three-antibodies, respectively.

Table 5.Distribution of the number of positive antibodies

The number of positive antibodies	Frequency (percent)
0 positive antibodies	17 (9%)
1 positive antibodies	25 (14%)
2 positive antibodies	59 (33%)
3 positive antibodies	64 (36%)
4 positive antibodies	14 (8%)

Table 6.Distribution of antibodies for participants with only one-positive-antibody

Antibody		Frequency (percent)
Distribution of combination of antibodies		
One-positive-antibody group (n=25)	**GAD**	8 (32%)
	Human ICA	11 (44%)
	IA2	4 (16%)
	IAA	2 (8%)

Table 7.Distribution of the antibodies for participants with two/three-positive-antibody group

Antibody		Frequency (percent)
Distribution of combination of antibodies		
Two-positive-antibody group (n=59)	GAD and human ICA	16 (27%)
	GAD and IA2	3 (5%)
	GAD and IAA	0
	Human ICA and IA2	35 (59%)
	Human ICA and IAA	4 (7%)
	IA2 and IAA	1 (2%)
Three-positive-antibody group (n=64)	Human ICA, IA2, and IAA	10 (15%)
	GAD, IA2, and IAA	1 (2%)
	GAD, human ICA, and IAA	1 (2%)
	GAD, human ICA, and IA2	52 (81%)
Distribution of each antibody		
Two-positive-antibody group (n=59)	GAD	19 (16%)
	Human ICA	55 (47%)
	IA2	39 (33%)
	IAA	5 (4%)
Three-positive-antibody group (n=64)	GAD	54 (28%)
	Human ICA	63 (33%)
	IA2	63 (33%)
	IAA	12 (6%)

4.4 C-PEPTIDE LEVELS AT BASELINE AND EACH FOLLOW-UP

4.4.1 C-peptide at baseline and follow-up visits

The mean of C-peptide levels in the study population was 0.65ng/ml at baseline. Figure 4 shows the observed C-peptide levels at onset and follow-up visits. The C-peptide levels increased at first follow-up visit, when was approximately 3-month after the participants received treatment of T1DM. The C-peptide levels then gradually decreased at subsequent follow-up visits. At the last follow-up visit, about two years after the onset of T1DM, the C-peptide levels decreased to 0.52ng/ml (Table 8).

Table 8.C-peptide at baseline and follow-up visits

	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
baseline	179	0.65	0.53	0.55	0.25	0.85	0.25	3.85
3-month follow-up	165	1.86	1.56	1.47	0.76	2.61	0.05	10.20
6-month follow-up	147	1.62	1.42	1.29	0.62	2.18	0.05	7.73
12-month follow-up	152	1.12	1.19	0.75	0.32	1.46	0.05	5.56
18-month follow-up	143	0.96	1.24	0.47	0.05	1.25	0.05	6.11
24-month follow-up	145	0.52	0.77	0.16	0.05	0.66	0.05	3.69

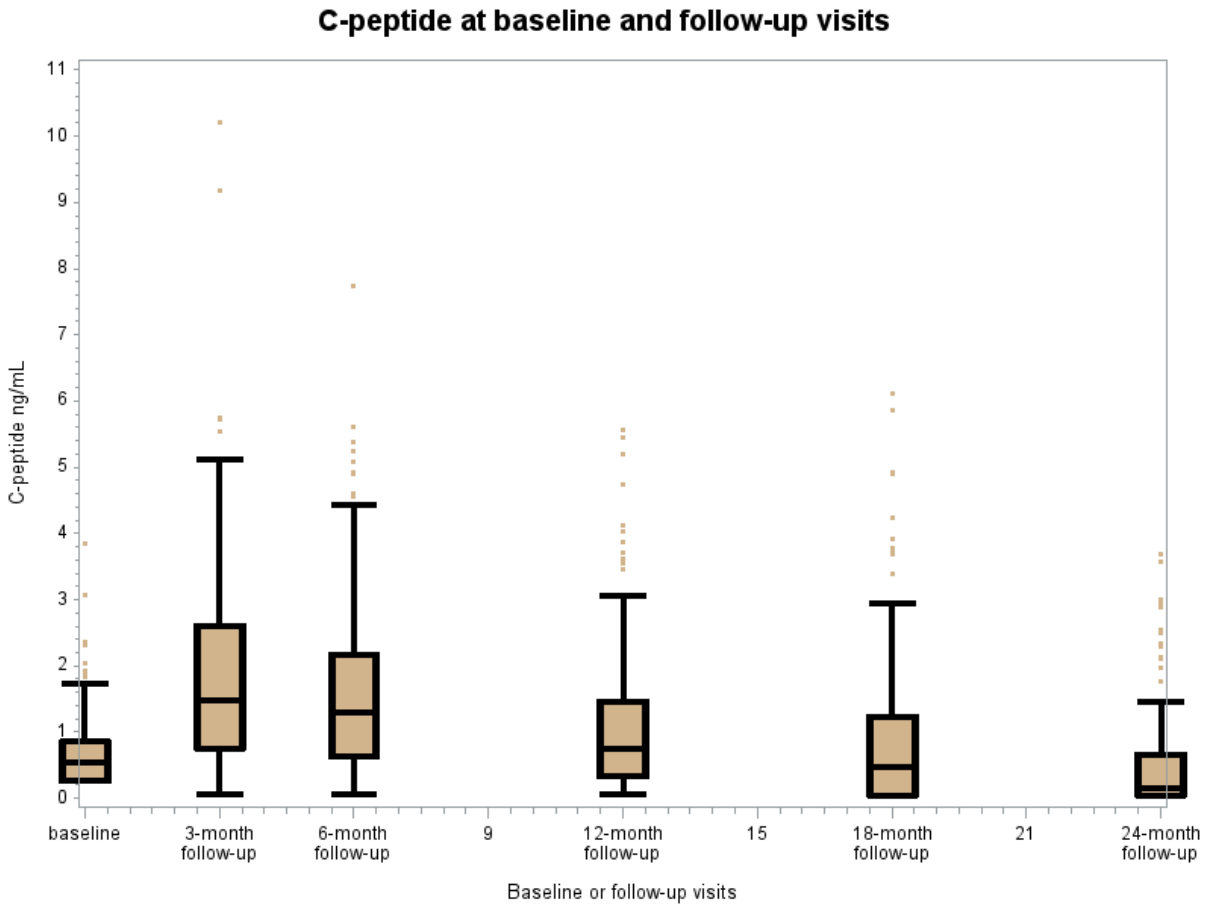

Figure 4.C-peptide at baseline and follow-up visits

4.4.2 C-peptide levels by antibody status

We also examined the C-peptide level changes by antibody status (Table 9-13). The C-peptide level change patterns were similar among all antibody status groups (Figure 5). In general, the less positive number of antibodies, the more C-peptide was produced. Kruskal-Wallis Test results suggest that C-peptide levels were not significantly different between antibody groups at onset and each follow up visit (Table 14)

At 3-month follow-up visit, two participants had C-peptide level above 8ng/ml. The high C-peptide level may indicate clinical significance and the medical records were reviewed by the

research physician on the study. Table 15 displays those two participants' antibody status and C-peptide level.

Table 9.C-peptide at baseline and follow-up visits for participants with all negative antibodies

	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
baseline	17	0.71	0.88	0.25	0.25	0.80	0.25	3.85
3-month follow-up	16	1.84	2.41	1.05	0.65	1.75	0.28	10.20
6-month follow-up	12	1.61	1.31	1.15	0.64	2.55	0.05	4.18
12-month follow-up	15	1.54	1.52	1.40	0.38	2.35	0.05	5.56
18-month follow-up	12	1.65	1.78	1.18	0.48	2.02	0.05	6.11
24-month follow-up	11	0.74	0.91	0.42	0.05	1.44	0.05	2.96

Table 10.C-peptide at baseline and follow-up visits for participants with one positive antibody

	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
baseline	25	0.64	0.47	0.57	0.25	0.86	0.25	2.35
3-month follow-up	23	1.69	1.26	1.40	0.71	2.38	0.18	4.62
6-month follow-up	21	1.79	1.51	1.44	0.85	2.00	0.25	5.61
12-month follow-up	24	1.33	1.29	0.95	0.46	1.75	0.05	5.44
18-month follow-up	17	1.23	1.18	0.88	0.27	1.74	0.05	3.79
24-month follow-up	20	0.71	0.86	0.34	0.05	1.23	0.05	3.01

Table 11.C-peptide levels at baseline and follow-up visits for participants with two positive antibodies

	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
baseline	59	0.69	0.53	0.56	0.25	0.87	0.25	3.08
3-month follow-up	57	2.06	1.44	1.84	0.86	3.01	0.11	5.74
6-month follow-up	53	1.81	1.61	1.48	0.62	2.18	0.05	7.73
12-month follow-up	50	1.15	1.18	0.76	0.36	1.40	0.05	5.20
18-month follow-up	50	0.97	1.33	0.52	0.05	1.06	0.05	5.85
24-month follow-up	47	0.53	0.87	0.13	0.05	0.67	0.05	3.69

Table 12.C-peptide at baseline and follow-up visits for participants with ≥three positive antibodies

	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
baseline	78	0.62	0.46	0.55	0.25	0.82	0.25	2.32
3-month follow-up	69	1.74	1.51	1.23	0.70	2.59	0.05	9.18
6-month follow-up	61	1.41	1.23	1.01	0.49	2.15	0.05	5.38
12-month follow-up	63	0.92	1.05	0.65	0.24	1.10	0.05	4.13
18-month follow-up	64	0.74	1.01	0.30	0.05	0.99	0.05	4.24
24-month follow-up	67	0.43	0.63	0.16	0.05	0.48	0.05	2.54

Table 13.C-peptide at baseline and follow-up visits for participants with ≥one positive antibody

	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
baseline	162	0.64	0.48	0.56	0.25	0.85	0.25	3.08
3-month follow-up	149	1.86	1.45	1.53	0.77	2.64	0.05	9.18
6-month follow-up	135	1.62	1.44	1.29	0.61	2.15	0.05	7.73
12-month follow-up	137	1.07	1.15	0.74	0.31	1.33	0.05	5.44
18-month follow-up	131	0.89	1.17	0.44	0.05	1.24	0.05	5.85
24-month follow-up	134	0.51	0.76	0.16	0.05	0.55	0.05	3.69

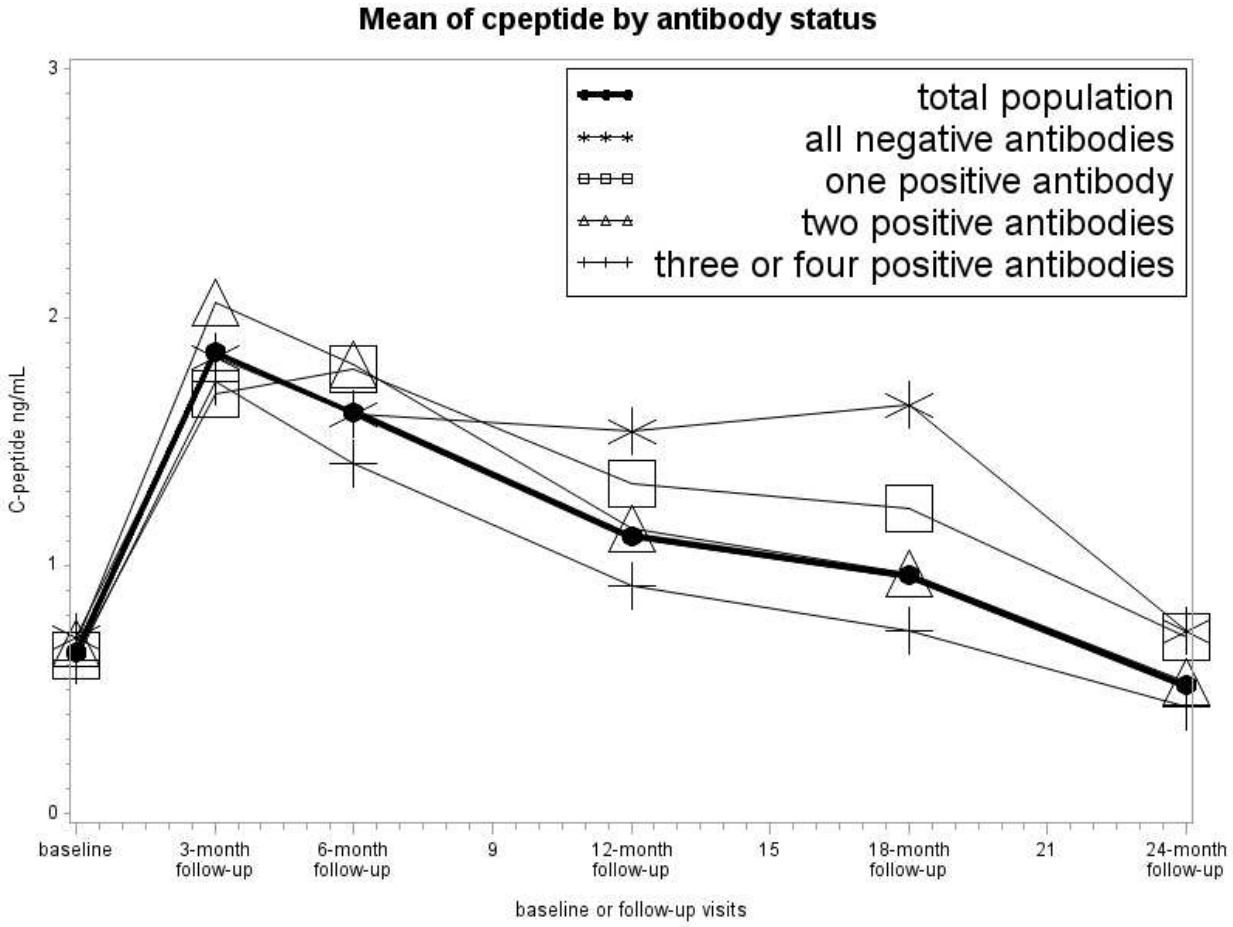

Figure 5.C-peptide at baseline and follow-up visits by antibody status

Table 14.Comparison of C-peptide levels between antibody groups

	P-value
Onset	0.81
3-month follow up	0.36
6-month follow up	0.52
12-month follow up	0.18
18-month follow up	0.06
24-month follow up	0.50

Table 15.Antibody status and C-peptide of participants with C-peptide> 8ng/mL at follow-up 1

PID	C-peptide at follow-up 1	GAD	IA2	ICA	IAA
498701017	10.20	-	-	-	-
140	9.18	+	+	+	-

PID	C-peptide at baseline	C-peptide at follow-up 1	C-peptide at follow-up 2	C-peptide at follow-up 3	C-peptide at follow-up 4	C-peptide at follow-up 5
498701017	3.85	10.20		5.56	6.11	
140	2.32	9.18	4.92	3.56	4.24	2.3

4.4.3 Difference in C-peptide levels between baseline and follow-up visits

Overall, C-peptide levels increased after the patients received treatment. At approximately 3-month after the treatment, the C-peptide increased by 1.20 ng/mL on average. Then C-peptide gradually decreased. Two-year after the treatment, the C-peptide levels returned to a level lower than that at onset (Table 16). We also examined the C-peptide level changes by different antibody status (Table 17-21). The C-peptide changes follow a similar pattern among different antibody status (Figure 6). Kruskal-Wallis Test results suggested that the only significant differences in C-peptide changes between antibody groups was at 18-month follow up visits (Table 22).

Table 16.C-peptide change from baseline

	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
3-month follow-up	165	1.20	1.31	0.91	0.26	2.06	-1.13	6.86
6-month follow-up	147	0.98	1.30	0.67	0.10	1.47	-1.26	6.86
12-month follow-up	152	0.46	1.08	0.16	-0.20	0.84	-1.90	5.19
18-month follow-up	143	0.29	1.03	-0.01	-0.20	0.67	-2.09	5.60
24-month follow-up	145	-0.10	0.73	-0.20	-0.48	0.05	-1.78	2.76

Table 17.C-peptide change from baseline for participants with all negative antibodies

	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
3-month follow-up	16	1.13	1.71	0.65	0.14	1.50	-0.41	6.36
6-month follow-up	12	1.15	1.25	0.80	0.19	2.06	-0.20	3.41
12-month follow-up	15	0.80	1.00	1.15	-0.12	1.73	-0.75	2.10
18-month follow-up	12	0.89	0.90	0.70	0.23	1.33	-0.20	2.58
24-month follow-up	11	0.20	0.75	-0.20	-0.45	0.67	-0.67	1.63

Table 18.C-peptide change from baseline for participants with one positive antibody

	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
3-month follow-up	23	1.05	1.06	0.92	0.22	1.81	-0.34	3.95
6-month follow-up	21	1.18	1.30	0.88	0.40	1.34	-0.35	4.83
12-month follow-up	24	0.68	1.18	0.37	0.02	1.07	-0.59	5.19
18-month follow-up	17	0.54	0.90	0.31	-0.20	0.86	-0.45	2.86
24-month follow-up	20	0.11	0.81	-0.20	-0.20	0.42	-0.94	2.76

Table 19.C-peptide change from baseline for participants with two positive antibodies

	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
3-month follow-up	57	1.38	1.31	1.28	0.38	2.45	-1.03	4.75
6-month follow-up	53	1.12	1.49	0.69	0.14	1.47	-0.90	6.86
12-month follow-up	50	0.47	1.22	0.11	-0.15	0.78	-1.90	4.21
18-month follow-up	50	0.29	1.32	-0.17	-0.36	0.64	-2.09	5.60
24-month follow-up	47	-0.16	0.87	-0.27	-0.56	-0.02	-1.78	2.70

Table 20.C-peptide change from baseline for participants with ≥three positive antibodies

	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
3-month follow-up	69	1.13	1.31	0.83	0.28	1.86	-1.13	6.86
6-month follow-up	61	0.76	1.11	0.48	-0.09	1.46	-1.26	4.40
12-month follow-up	63	0.28	0.93	0.07	-0.27	0.63	-1.87	3.36
18-month follow-up	64	0.12	0.76	-0.10	-0.22	0.46	-1.15	3.17
24-month follow-up	67	-0.16	0.57	-0.20	-0.52	-0.02	-1.40	1.90

Table 21.C-peptide change from baseline for participants with ≥one positive antibody

	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
3-month follow-up	149	1.21	1.27	0.93	0.28	2.06	-1.13	6.86
6-month follow-up	135	0.97	1.31	0.67	0.07	1.47	-1.26	6.86
12-month follow-up	137	0.42	1.09	0.11	-0.20	0.73	-1.90	5.19
18-month follow-up	131	0.24	1.03	-0.07	-0.24	0.63	-2.09	5.60
24-month follow-up	134	-0.12	0.72	-0.20	-0.49	-0.01	-1.78	2.76

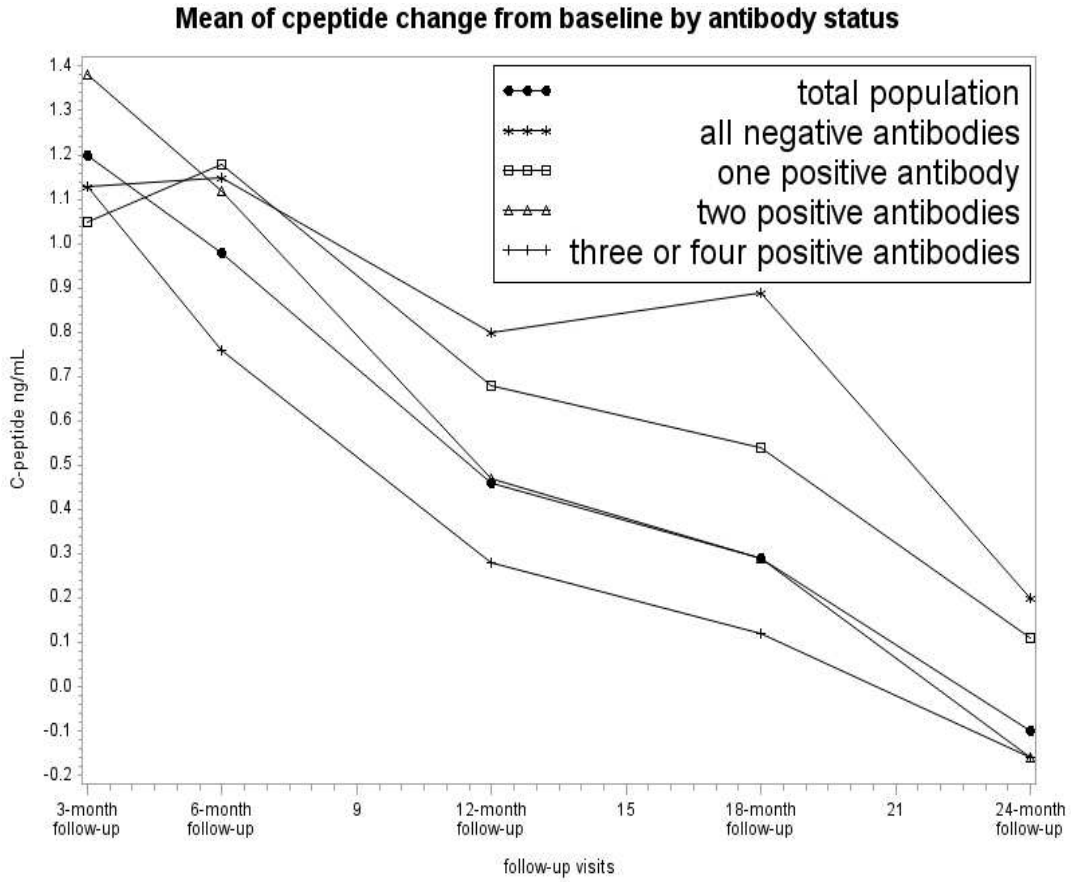

Figure 6.C-peptide change from baseline by antibody status

Table 22.Comparision of C-peptide change from baseline

	P-value
3-month follow up	0.50
6-month follow up	0.47
12-month follow up	0.14
18-month follow up	0.02
24-month follow up	0.16

4.5 OVERWEIGHT AT BASELINE AND 3-MONTH FOLLOW-UP

Out of the 136 non-overweight participants at baseline, 105 participants were still non-overweight at 3-month follow-up, and 31 participants became overweight at the 3-month follow-up. Of the 38 overweight participants at baseline, 34 were still overweight at 3-month follow-up visit, and only 4 participants became non-overweight at 3-month follow-up (Table 23). C-peptide changes in the four groups of participants follow the same pattern: C-peptide levels increased at 3-month follow up visit and gradually decreased after that until 24-month follow up visit (Table 24-27). The group presenting as overweight and remaining overweight at 3-month tended to have higher C-peptide levels (Figure 7). The overweight / non-overweight group has only four participants and this probably accounts for the extreme variability of C-peptide results.

Table 23.Overweight status at baseline and 3-month follow-up visit

Overweight status at baseline and 3-month follow-up	Frequency (percent)
Overweight – Overweight	34 (20%)
Overweight – Non-overweight	4 (2%)
Non- overweight – Overweight	31 (18%)
Non- overweight – Non- overweight	105 (60%)

Note: frequency missing=5

Table 24.C-peptide at baseline and follow-up visits overweight – overweight participants (n=34)

Baseline/Follow-up visits	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
Baseline	34	1.02	0.68	0.88	0.63	1.24	0.25	3.85
3-month follow-up	32	2.38	2.19	2.12	0.50	3.26	0.05	10.20
6-month follow-up	29	2.14	2.14	1.49	0.89	3.64	0.05	5.61
12-month follow-up	29	1.49	1.49	0.93	0.34	2.03	0.05	5.56
18-month follow-up	24	1.60	1.60	1.16	0.32	2.33	0.05	6.11
24-month follow-up	27	0.70	0.70	0.20	0.05	1.29	0.05	3.69

Table 25.C-peptide at baseline and follow-up visits overweight – non-overweight participants (n=4)

Baseline/Follow-up visits	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
Baseline	4	1.24	0.88	1.20	0.57	1.92	0.25	2.32
3-month follow-up	3	5.06	3.66	3.81	2.19	9.18	2.19	9.18
6-month follow-up	4	2.27	1.79	1.59	1.27	3.26	0.96	4.92
12-month follow-up	3	1.79	1.53	1.00	0.82	3.56	0.82	3.56
18-month follow-up	3	2.65	1.52	2.69	1.01	4.24	1.01	4.24
24-month follow-up	4	0.88	0.97	0.55	0.28	1.48	0.12	2.30

Table 26.C-peptide at baseline and follow-up visits non-overweight – overweight participants (n=31)

Baseline/Follow-up visits	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
Baseline	31	0.43	0.22	0.25	0.25	0.61	0.25	1.01
3-month follow-up	29	1.48	0.98	1.16	0.74	2.22	0.28	3.47
6-month follow-up	26	1.24	1.31	0.69	0.38	1.77	0.05	5.08
12-month follow-up	25	1.07	1.50	0.46	0.14	1.03	0.05	5.44
18-month follow-up	24	0.92	1.52	0.27	0.05	0.96	0.05	5.85
24-month follow-up	25	0.47	0.83	0.05	0.05	0.45	0.05	3.01

Table 27.C-peptide at baseline and follow-up visits non-overweight – non-overweight participants (n=105)

Baseline/Follow-up visits	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
Baseline	105	0.58	0.46	0.50	0.25	0.73	0.25	3.08
3-month follow-up	96	1.71	1.22	1.40	0.79	2.46	0.05	5.72
6-month follow-up	84	1.55	1.27	1.33	0.67	2.11	0.05	7.73
12-month follow-up	90	1.00	0.94	0.76	0.31	1.44	0.05	4.13
18-month follow-up	88	0.72	0.88	0.45	0.05	0.92	0.05	3.76
24-month follow-up	85	0.42	0.56	0.16	0.05	0.55	0.05	2.54

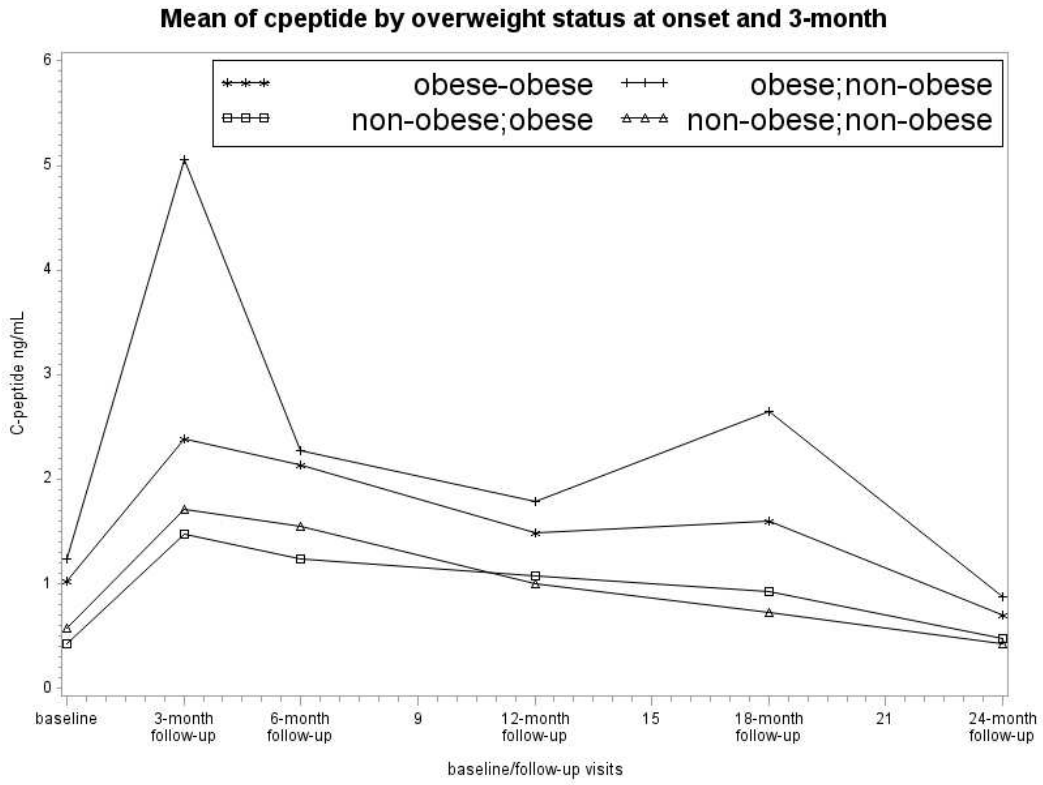

Figure 7.C-peptide at onset and follow up visits by overweight status change

4.6 C-PEPTIDE CHANGES BY OVERWEIGHT STATUS AT BASELINE

At baseline and each follow-up visit, participants who were overweight at baseline always had higher C-peptide level than their non-overweight counterparts. As to the trend of C-peptide changes, overweight and non-overweight participants had a similar pattern of change: the C-peptide levels increased after they got treatment, reaching peak level at 3-month follow-up, and then gradually decreased to a level similar to that at the onset of type 1 diabetes after about two years (Table 28-29, Figure 5). Kruskal-Wallis Test results suggested that at baseline and 18-month follow up visits, the C-peptide levels were significantly different between overweight and non-overweight participants (Table 30).

Table 28.C-peptide at baseline and follow-up visits for non-overweight participants at baseline

Baseline/Follow-up visits	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
Baseline	138	0.55	0.42	0.50	0.25	0.70	0.25	3.08
3-month follow-up	127	1.67	1.17	1.39	0.76	2.45	0.05	5.72
6-month follow-up	111	1.49	1.30	1.18	0.61	2.09	0.05	7.73
12-month follow-up	117	1.03	1.08	0.70	0.30	1.40	0.05	5.44
18-month follow-up	114	0.79	1.05	0.44	0.05	0.95	0.05	5.85
24-month follow-up	112	0.47	0.71	0.14	0.05	0.55	0.05	3.57

Table 29.C-peptide at baseline and follow-up visits for overweight participants at baseline

Baseline/Follow-up visits	N	Mean	Std Dev	Median	25th percentile	75th percentile	Min	Max
Baseline	39	1.02	0.70	0.88	0.57	1.31	0.25	3.85
3-month follow-up	36	2.58	2.36	2.29	0.74	3.53	0.05	10.20
6-month follow-up	34	2.12	1.69	1.54	0.92	3.64	0.05	5.61
12-month follow-up	33	1.49	1.50	0.93	0.43	2.03	0.05	5.56
18-month follow-up	28	1.67	1.67	1.16	0.34	2.58	0.05	6.11
24-month follow-up	32	0.71	0.93	0.25	0.08	1.06	0.05	3.69

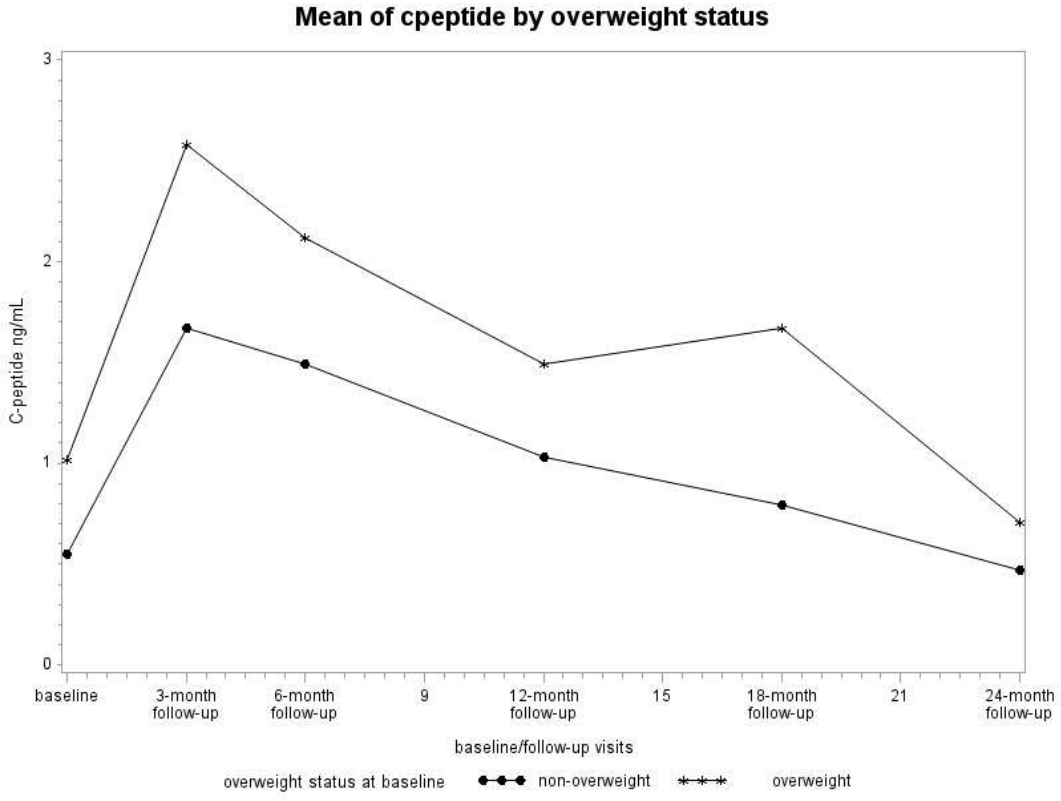

Figure 8.C-peptide at baseline and follow-up visits by overweight status at baseline

Table 30.Comparison of C-peptide levels between overweight and non-overweight groups at onset and follow up visits

	P-value
C-peptide at baseline	<0.01
C-peptide at 3-month follow-up	0.08
C-peptide at 6-month follow-up	0.06
C-peptide at 12-month follow-up	0.14
C-peptide at 18-month follow-up	<0.05
C-peptide at 24-month follow-up	0.09

4.7 RATE OF CHANGE IN C-PEPTIDE

The rates of change in C-peptide from baseline to each follow-up visit were always in the same direction for baseline overweight and non-overweight participants. The rates of increase were always equal or higher in magnitude in baseline overweight participants than baseline non-overweight participants. The rate of change from baseline to 18-month follow-up is borderline significant (p=0.05). None of the other rates was significantly different between those two groups (Table 31). Similar to the change from baseline to follow-ups, the rates of change from 3-month follow-up to subsequent follow-up visits were always in the same direction for baseline overweight and non-overweight participants. The rates of decline were always greater in baseline overweight participants than baseline non-overweight participants. Except the rate from 3-month follow-up to 12-month follow-up, which was borderline significant (p=0.06), the other rates were not statistically significant (Table 32).

Table 31.Rate of change in C-peptide from baseline

	Change rate of C-peptide in over weight	Change rate of C-peptide in non-over weight	P-value
3-month follow-up	0.69 *	0.52 *	0.25
6-month follow-up	0.22 *	0.18 *	0.38
12-month follow-up	0.04 *	0.04 *	0.94
18-month follow-up	0.03 *	0.01 *	0.05
24-month follow-up	-0.01	0	0.31

*P<0.05 for one sample t-test testing if the rate is significantly different from 0

Table 32.Rate of change in C-peptide from 3-month follow-up

	Change rate of C-peptide in overweight	Change rate of C-peptide in non-overweight	P-value
6-month follow-up	-0.10	-0.06	0.68
12-month follow-up	-0.14 *	-0.07 *	0.06
18-month follow-up	-0.07 *	-0.06 *	0.61
24-month follow-up	-0.08 *	-0.06 *	0.22

*P<0.05 for one sample t-test testing if the rate is significantly different from 0

4.8 CORRELATION BETWEEN HBA1C AT BASELINE AND A1C AT 1ST FOLLOW-UP

HbA1c levels is a useful determinant of how well the blood glucose level has been controlled in the recent past and may be used to monitor the effects of diet, exercise, and drug therapy on blood glucose in diabetic patients. The higher the glucose concentration in blood is, the higher the level of HbA1c is. Levels of HbA1c reflect the average glucose levels over the prior six to eight weeks. The Pearson correlation coefficient of HbA1c at baseline and HbA1c at three-month was 0.159, with p-value of 0.04. The Spearman correlation coefficient of HbA1c at baseline and HbA1c at three-month was 0.138, with p-value of 0.08 (Table 33).

Table 33.Correlation between HbA1c at baseline and HbA1c at follow-up 1

Method	**Correlation coefficient**	**P-value**
Pearson	0.159	0.04
Spearman	0.138	0.08

4.9 CORRELATION BETWEEN AGE AT ONSET, HBA1C AT BASELINE, AND HBA1C AT 1ST FOLLOW-UP AND C-PEPTIDE

Age at onset and HbA1c at 1st follow-up were correlated with C-peptide at baseline and follow-up visits. HbA1c at baseline was only correlated with C-peptide at baseline (Table 34). As mentioned above, HbA1c is a determinant of the glucose control over the prior six to eight weeks. Participants received treatment at onset, therefore, HbA1c level was more informative in the current study.

Table 34.Correlation between age at onset, A1C at baseline, and A1C at 1st follow-up and C-peptide

	C-peptide at baseline	C-peptide at 3-month follow-up	C-peptide at 6-month follow-up	C-peptide at 12-month follow-up	C-peptide at 18-month follow-up	C-peptide at 24-month follow-up
Age at onset						
Pearson correlation coefficient (P-value)	0.337 (<0.01)	0.296 (<0.01)	0.445 (<0.01)	0.465 (<0.01)	0.491 (<0.01)	0.477 (<0.01)
Spearman correlation coefficient (P-value)	0.381 (<0.01)	0.306 (<0.01)	0.469 (<0.01)	0.426 (<0.01)	0.534 (<0.01)	0.487 (<0.01)
A1C at baseline						
Pearson correlation coefficient (P-value)	-0.203 (<0.01)	-0.078 (0.32)	0.029 (0.73)	0.103 (0.21)	0.052 (0.54)	0.102 (0.23)
Spearman correlation coefficient (P-value)	-0.170 (0.02)	-0.040 (0.61)	0.097 (0.24)	0.114 (0.16)	0.118 (0.16)	0.189 (0.02)
A1C at follow-up 1						
Pearson correlation coefficient (P-value)	-0.270 (<0.01)	-0.290 (<0.01)	-0.415 (<0.01)	-0.409 (<0.01)	-0.426 (<0.01)	-0.380 (<0.01)
Spearman correlation coefficient (P-value)	-0.245 (<0.01)	-0.293 (<0.01)	-0.423 (<0.01)	-0.472 (<0.01)	-0.440 (<0.01)	-0.409 (<0.01)

4.10 GEE MODEL

GEE model with time interval as a categorical (Table 35) suggested a statistically significant interaction between overweight status and baseline/follow-up visits after adjustment of potential confounders, including age at diagnosis, gender, HbA1c at 3-month visit, and antibody group. Therefore, we conducted a pairwise comparison to compare the C-peptide levels between overweight and non-overweight at each time point (Table 36). C-peptide concentrations were significantly different for overweight and non-overweight participants at 3-month, 6-month, 18-month follow up visits. They were borderline significantly different for 12-month follow up visit. The C-peptide levels were not different at onset or 24-month visit.

The general form generated from the GEE:

$$C-peptide = 0.292 * overweight\ status\ at\ onset + 1.093 * 3-month\ follow-up\ visit + 0.978 * 6-month\ follow-up + 0.482 * 12-month\ follow-up + 0.214 * 18-month\ follow-up + (-0.045) * 24-month\ follow-up + 0.111 * age\ at\ diagnosis + 0.272 * female + (-0.328) * HbA1c\ at\ 3-month + (-0.417) * one-positive-antibody + (-0.252) * two-positive-antibodies + (-0.581) * three-or-four\ antibodies + 0.546 * interaction\ of\ 3-month\ follow\ up\ and\ overweight\ status + 0.313 * interaction\ of\ 6-month\ follow\ up\ and\ overweight\ status + 0.09 * interaction\ of\ 12-month\ follow\ up\ and\ overweight\ status + 0.4 * interaction\ of\ 18-month\ follow\ up\ and\ overweight\ status + (-0.099) * interaction\ of\ 24-month\ follow\ up\ and\ overweight\ status$$

Table 35.Regression coefficients (β) for the GEE model with time interval as a categorical variable

Variable	β	Standard Error	95% confidence interval	p-value
Overweight at baseline	0.292	0.192	-0.083, 0.668	0.127
3-month follow-up visit	1.093	0.101	0.895,1.291	<0.01
6-month follow-up visit	0.978	0.106	0.771, 1.185	
12-month follow-up visit	0.482	0.104	0.279, 0.685	
18-month follow-up visit	0.214	0.104	0.01, 0.418	
24-month follow-up visit	-0.045	0.105	-0.251, 0.16	
Age	0.111	0.016	0.08, 0.142	<0.01
Female	0.272	0.111	0.05, 0.49	0.01
A1C at three month	-0.328	0.069	-0.463, -0.193	<0.01
One positive antibody	-0.417	0.231	-0.87, 0.037	<0.01
Two positive antibodies	-0.252	0.203	-0.65, 0.146	
Three or four antibodies	-0.581	0.194	-0.963, -0.2	
Interaction: 3-month follow up visit*overweight	0.546	0.221	0.113, 0.98	0.04
Interaction: 6-month follow up visit*overweight	0.313	0.225	-0.128, 0.753	
Interaction: 12-month follow up visit*overweight	0.09	0.227	-0.356, 0.535	
Interaction: 18-month follow up visit*overweight	0.4	0.234	-0.059, 0.859	
Interaction: 24-month follow up visit*overweight	-0.099	0.228	-0.546, 0.348	

Table 36.Pairwise comparison of C-peptide levels from GEE model

Baseline/follow up visits	P-value
Baseline	0.13
3-month follow up	<0.01
6-month follow up	<0.01
12-month follow up	0.06
18-month follow up	<0.01
24-month follow up	0.35

We have conducted fractional polynomial GEE models to gain flexibility to fit the non-linear trend of C-peptide over time. Two fractional polynomial GEE models were conducted: 1) we considered the days between onset to each follow up visits as the continuous time variable, 2) we considered a continuous time interval variable to indicate the baseline and follow up visits (0, 3, 6, 12, 18, 24).

The best-fitting model for the first fractional polynomial GEE model is a second-degree fractional polynomial model with powers of -0.5 and 0.5. The following formulas show the transformation of the continuous time interval variable to the time variables used in the best-fitting model:

$$time\ variable\ 1 = \left(\frac{time\ interval + 1}{100}\right)^{-0.5} - 0.580464678$$

$$time\ variable\ 2 = \left(\frac{time\ interval + 1}{100}\right)^{0.5} - 0.722757711$$

The first model, similar to the last GEE model, suggested a statistically significant interaction between time and overweight status at baseline (Table 37). Therefore, we presented the prediction of C-peptide over time for overweight and non-overweight participants respectively (Figure 9). Overtime, overweight and non-overweight participants have similar prediction patterns of C-peptide levels. C-peptide increased after onset, reached peak level at round 3-month, and then gradually declined to a level lower than that in onset at about 24-month after onset.

The general form generated from the GEE:

$$C-peptide = 0.856 * overweight\ status\ at\ onset + (-0.185) * FP2\ time\ (-0.5)$$
$$+ (-0.699) * FP2time\ (0.5) + 0.111 * age\ at\ diagnosis + 0.271 * female$$
$$+ (-0.337) * HbA1c\ at\ 3-month + (-0.426) * one-positive-antibody$$
$$+ (-0.261) * two-positive-antibodies + (-0.563) * three-or$$
$$-four\ antibodies + (-0.085)$$
$$* interaction\ of\ FP2\ time\ (-0.5)\ and\ overweight\ status + (-0.305)$$
$$* interaction\ of\ FP2\ time\ (0.5)\ and\ overweight\ status$$

Table 37.Regression coefficients (β) for the GEE model with days between onset and follow up visits as continuous time variable

Variable	β	Standard Error	95% confidence interval	p-value
Overweight at baseline	0.856	0.141	0.309, 0.862	<0.01
FP2 time (-0.5)*	-0.185	0.013	-0.212, -0.159	<0.01
FP2 time (0.5)	-0.699	0.055	-0.806, -0.159	<0.01
Age	0.111	0.016	0.08, 0.142	<0.01
Female	0.271	0.113	0.05, 0.492	0.016
A1C at three month	-0.337	0.07	-0.473, -0.2	<0.01
One positive antibody	-0.426	0.234	-0.884, 0.032	<0.01
Two positive antibodies	-0.261	0.206	-0.664, 0.143	
Three or four antibodies	-0.563	0.196	-0.949, -0.179	
FP2 time (-0.5)*overweight	-0.085	0.029	-0.142, -0.027	0.013
FP2 time (0.5)*overweight	-0.305	0.118	-0.537, -0.073	

*FP2(power) represents the second-degree fractional polynomial models

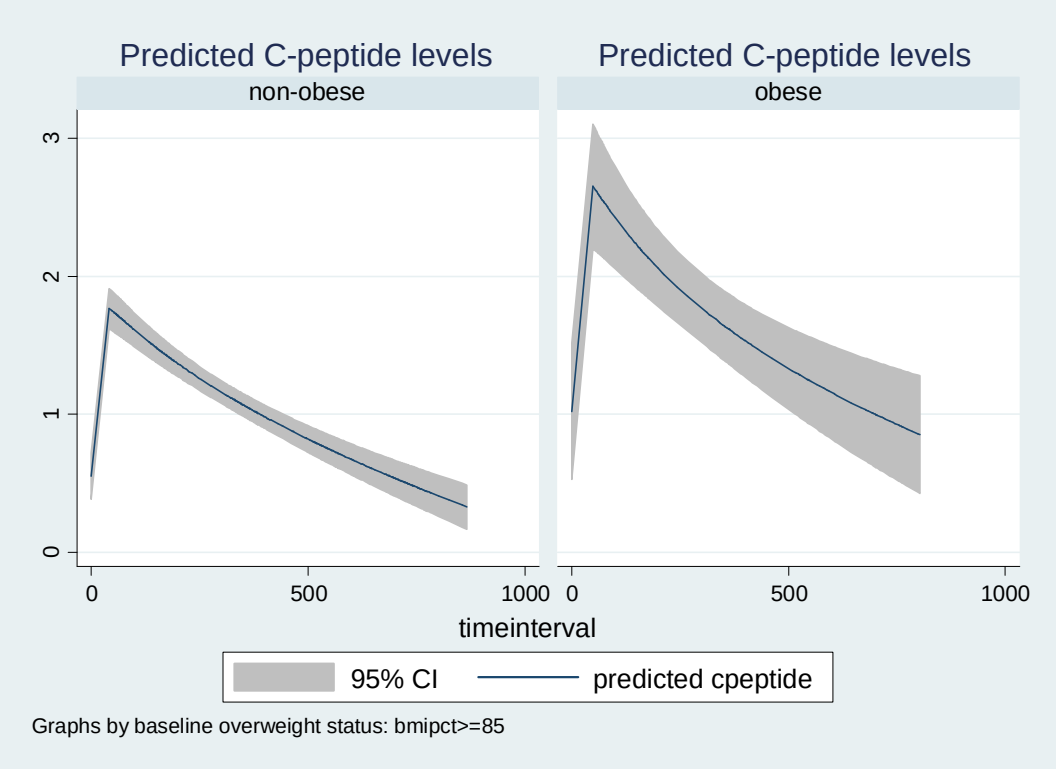

Figure 9.Prediction of C-peptide for overweight and non-overweight participants from fractional polynomial GEE model with days between onset and follow up visits as continuous time variable

In the second fractional polynomial GEE model, we modeled a variable indicating baseline and follow up visits (0, 3, 6, 12, 18, 24) as a continuous time variable. The best-fitting fractional polynomial GEE model is a second-degree fractional polynomial model with powers of -1 and -1. The following formulas show the transformation of the follow up variable to the time variables used in the best-fitting model:

$$time\ variable\ 1 = \left(\frac{follow\ up + 3}{10}\right)^{-1} - 0.7699805068$$

$$time\ variable\ 2 = \left(\frac{follow\ up + 3}{10}\right)^{-1} * \ln\left(\frac{follow\ up + 3}{10}\right) - 0.2012652665$$

Similar to the last model, it shows a significant interaction between time variables and overweight status. Therefore, we presented the prediction of C-peptide over time for overweight and non-overweight participants respectively (Figure 10). Overtime, overweight and non-overweight participants have similar prediction patterns of C-peptide levels. C-peptide increased after onset, reached peak level at round 3-month, and then gradually declined to a level lower than that in onset at about 24-month after onset (Table 38).

The general form generated from the GEE:

$$C-peptide = 0.575 * overweight\ status\ at\ onset + 2.395 * FP2\ time\ 1\ (-1) + 1.611 * FP2time2\ (-1) + 0.111 * age\ at\ diagnosis + 0.272 * female + (-0.33) * HbA1c\ at\ 3-month + (-0.427) * one-positive-antibody + (-0.258) * two-positive-antibodies + (-0.588) * three-or-four\ antibodies + 0.948 * interaction\ of\ FP2\ time\ (-0.5)\ and\ overweight\ status + 0.641 * interaction\ of\ FP2\ time\ (0.5)\ and\ overweight\ status$$

Table 38.Regression coefficients (β) for the GEE model with a continuous time variable indicating baseline and follow up visits

Variable	β	Standard Error	95% confidence interval	p-value
Overweight at baseline	0.575	0.142	0.296, 0.853	<0.01
FP2 time 1 (-1)*	2.395	0.172	2.058, 2.732	<0.01
FP2 time 2 (-1)	1.611	0.112	1.391, 1.83	<0.01
Age	0.111	0.016	0.08, 0.142	<0.01
Female	0.272	0.111	0.054, 0.49	0.02
A1C at three month	-0.33	0.069	-0.466, -0.195	<0.01
One positive antibody	-0.427	0.232	-0.882, 0.028	<0.01
Two positive antibodies	-0.258	0.204	-0.658, 0.141	
Three or four antibodies	-0.588	0.195	-0.971, -0.206	
FP2 time 1 (-1)*overweight	0.948	0.374	0.216, 1.68	0.03
FP2 time 2 (0.5)*overweight	0.641	0.242	0.166, 1.116	

*FP2(power) represents the second-degree fractional polynomial models

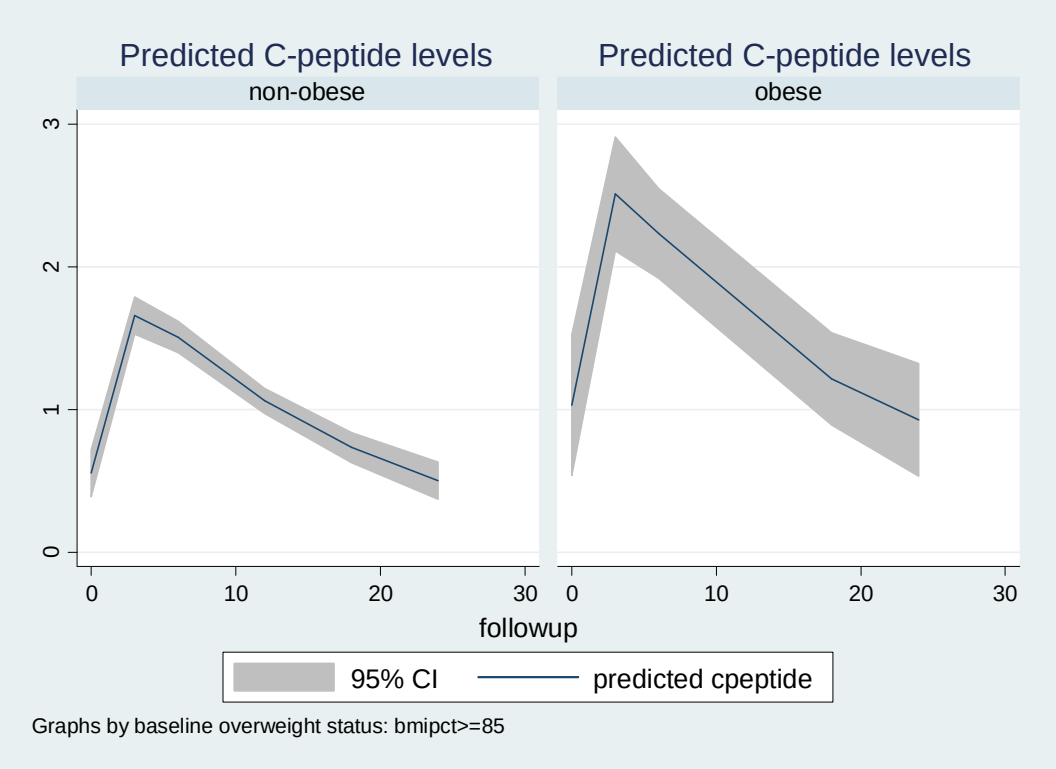

Figure 10.Prediction of C-peptide for overweight and non-overweight participants from fractional polynomial GEE model with a continuous time variable indicating baseline and follow up visits

5.0 DISCUSSION

In this follow-up of patients with new onset T1DM diagnosed between the ages of 1.5 and 18.9 years, C-peptide levels over two years following initiation of treatment were different among overweight and non-overweight. Overweight T1DM children had higher C-peptide levels as compared to non-overweight T1DM children at 3-month, 6-month, 12-month, and 18-month follow up visits; however, at 24-month after initiation of treatment, the C-peptide levels between overweight and non-overweight groups were not statistically significant different.

Studies suggest that patients with recent-onset T1DM still have approximately 20% of their β-cell function [24-26]. T1DM has been considered as a "relapsing-remitting" disease due to the period after onset as a last surge of the regulatory immune response before the eventual impairment of the residual β-cell function. C-peptide levels can be considered as indicator of residual insulin secretion. Whether overweight at onset has a role in the pathophysiology of T1DM after onset remains unknown. However, we had the opportunity to investigate the C-peptide levels in overweight and non-overweight patients in a longitudinal study. Our results point to a significant correlation of the overweight status at onset and the preservation of β-cell function. After adjustment of potential confounders (age at diagnosis, gender, HbA1c at 3-month visit, and antibody group), C-peptide levels between overweight and non-overweight groups were not statistically different at onset. However, after the initiation of treatment, overweight patients had higher C-peptide levels at 3-month, 6-month, 12-month, and 18-month visits than

non-overweight patients. At 24-month follow up visit, the differences in C-peptide levels between overweight and non-overweight groups were not different any more. The mechanism for overweight patients to have higher C-peptide levels remains unknown. One possibility is that because overweight patients are usually diagnosed with T1DM earlier than non-overweight patients [27], the overweight newly diagnosed T1DM patients may be at better situation than the non-overweight newly diagnosed T1DM patients. This may explain why the treatment helped the overweight patients to present higher C-peptide levels.

T1DM is characterized by destruction of β-cell function, which is thought to be driven by an autoimmune process, reflected in the production of islet antibodies. Our study is in line with earlier studies that high levels of islet antibodies are associated with rapid development of β-cell destruction and low C-peptide concentrations [14, 28]. At 18-month follow up visit, the C-peptide levels between antibody groups were significantly different ($p<0.02$). Due to the small sample size in some antibody groups, we were not able to investigate further the impact of antibody groups in the association between C-peptide levels and overweight status.

The current study has a couple of limitations; HLA-DQ haplotypes information was not included in the analyses and this may impact on our results. The destruction of β-cell function has been linked to certain HLA-DR and DQ haplotypes. Individuals with DQ2/8 are at increased risk of T1DM, while individuals with DQ6 are at decreased risk [29]. However, a recent study suggested the HLA-DQ was more important for the development of T1DM than for the destructive process after onset. For some antibody and weight change subgroups, there is limited sample size to perform analyses and this precludes us from further examining their impact.

The strength of this study is the study design, which enables to follow-up the participants from onset to approximately two years after the initiation of treatment. The longitudinal design

of the study allows us to make causal inference on the relationship between overweight status at onset and insulin reserve in the new onset T1DM patients. This cohort, which included all children diagnosed between 2004 and 2006 who consented to participate in the study, is thought to be representative of the population in the Allegheny area. This study represents a large group of newly childhood-onset T1DM patients.

Public health significance: Clinical diagnosis of T1DM is often followed by a transient remission period, usually referred to as honeymoon period. This honeymoon period is directly related to residual β-cell function at onset [30]. Knowledge of the residual β-cell function after onset allows the risks of adverse side effects associated with treatments weighed against the benefit of recovering residual β-cell function. It is also useful to develop effective intervention programs. Research on preservation of the residual β-cell function in T1DM patients is therefore of public health significance. Our study suggested the overweight status at baseline may be informative to develop effective intervention strategies. Given overweight patients had higher C-peptide levels after the initiation of treatment, it may be useful to develop intervention strategies to target on this group of patients to maintain or prolong the period of high C-peptide levels after initiation of treatment.

6.0 CONCLUSION

The current longitudinal study suggested that in patients with new onset T1DM patients diagnosed between the ages of 1.5 and 18.9 years, C-peptide levels over two years following initiation of treatment were different among overweight and non-overweight. Overweight T1DM children had higher C-peptide levels as compared to non-overweight T1DM children at 3-month, 6-month, 12-month, and 18-month follow up visits; however, at 24-month after initiation of treatment, the C-peptide levels between overweight and non-overweight groups were not statistically significant different any more.

The greater C-peptide levels among overweight patients 3 month to 18 month after initiation of treatment may be due to the early diagnosis of T1DM in overweight patients. Our study suggested that overweight patients may benefit from target preventive strategies that will help them maintain or prolong the high C-peptide levels after initiation of treatment.

APPENDIX A: SAS CODE USED FOR THE ANALYSIS

```
libname   library  "F:\biostat" ;
filename  format   "F:\biostat\jod_formats_2011_11_07.sas" ;
%include format ;
run ;

/*** # of missing for antibodies ***/
     data cpeptide; set library.cpep_wide_2012_02_29; run;

proc freq data=cpeptide; table pos_antibodies_gad
pos_antibodies_ica pos_antibodies_ia2 pos_antibodies_iaa; run;

data cpeptide; set cpeptide; if pos_antibodies_gad="" then
gadmiss=1; else     gadmiss=0;run;
data cpeptide; set cpeptide; if pos_antibodies_ica="" then
icamiss=1; else     icamiss=0;run;
data cpeptide; set cpeptide; if pos_antibodies_ia2="" then
ia2miss=1; else     ia2miss=0;run;
data cpeptide; set cpeptide; if pos_antibodies_iaa="" then
iaamiss=1; else     iaamiss=0;run;
data cpeptide; set cpeptide;
antimiss=sum(gadmiss,icamiss,ia2miss,iaamiss);run;
data  cpeptide; set cpeptide; label antimiss="Number of missing
value for antibody"; run;

proc freq data=cpeptide; table gadmiss icamiss ia2miss iaamiss;
run;
proc freq data=cpeptide; table antimiss;run;

/*** sum of antibodies ***/
data cpeptide; set cpeptide;
antisum=sum(pos_antibodies_gad,
pos_antibodies_ica,pos_antibodies_ia2,pos_antibodies_iaa );
totalanti=4-antimiss;
run;
data cpeptide; set cpeptide; label antisum="Number of positive
antibody each participant has";run;
```

```
data cpeptide; set cpeptide; label totalanti="Number of antibody
each participant has measured";run;
proc freq data=cpeptide; table antisum;run;
proc freq data=cpeptide; table totalanti;run;

data cpeptide; set cpeptide; if antisum=0 then antisum_gr=0;
else if antisum=1 then antisum_gr=1; else if antisum=2 then
antisum_gr=2;else if antisum>0 then antisum_gr=3;run;
data cpeptide; set cpeptide; if antisum=0 then antisum_gr1=0;
else if antisum=1 then antisum_gr1=1; else if antisum>0 then
antisum_gr1=2;run;

proc format; value posianti 0="all negative antibodies" 1="1
positive antibody" 2="2 positive antibodies" 3="3 or 4 positive
antibodies"; run;
proc format; value posiantigr 0="all negative antibodies" 1="1
positive antibody" 2=">=2 positive antibodies"; run;

proc freq data=cpeptide; table antisum_gr; format antisum_gr
posianti.;run;
proc freq data=cpeptide; table antisum_gr1;format antisum_gr1
posiantigr.;run;

/*** study population: AGS=1, AGSdisp=1, count of antibodies>=3
***/

data cpeptidesub1; set cpeptide; where AGS=1 and AGSdisp=1 and
totalanti>=3; run; /* 192 obs */

proc freq data=cpeptidesub1;
table pos_antibodies_gad pos_antibodies_ica pos_antibodies_ia2
pos_antibodies_iaa;
run;

proc freq data=cpeptidesub1; table antisum_gr; format antisum_gr
posianti.;run;
proc freq data=cpeptidesub1; table antisum_gr1;format
antisum_gr1 posiantigr.;run;

/***** Create follow-up dates, c-peptide ****/
data cpeptidesub1;
  set cpeptidesub1;
  newcpepfu1=cpepfu2;
  newcpepfu2=cpepfu3;
  newcpepfu3=cpepfu4;
  newcpepfu4=cpepfu5;
  newcpepfu5=cpepfu6;
```

```
  newcpepfu6=cpepfu7;
 run;

data cpeptidesub1;
  set cpeptidesub1;
  newblooddrawdatefu1=blooddrawdatefu2;
  newblooddrawdatefu2=blooddrawdatefu3;
  newblooddrawdatefu3=blooddrawdatefu4;
  newblooddrawdatefu4=blooddrawdatefu5;
  newblooddrawdatefu5=blooddrawdatefu6;
  newblooddrawdatefu6=blooddrawdatefu7;
  format newblooddrawdatefu1-newblooddrawdatefu6 date9.;
run;

data cpeptidesub1;
  set cpeptidesub1;
  if newcpepfu1=0.1 and CpepRangefu2=-1 then nnewcpepfu1=0.05;
  else nnewcpepfu1=newcpepfu1;
run;

data cpeptidesub1;
  set cpeptidesub1;
  if newcpepfu2=0.1 and CpepRangefu3=-1 then nnewcpepfu2=0.05;
  else nnewcpepfu2=newcpepfu2;
run;

data cpeptidesub1;
  set cpeptidesub1;
  if newcpepfu3=0.1 and CpepRangefu4=-1 then nnewcpepfu3=0.05;
  else nnewcpepfu3=newcpepfu3;
run;

data cpeptidesub1;
  set cpeptidesub1;
  if newcpepfu4=0.1 and CpepRangefu5=-1 then nnewcpepfu4=0.05;
  else nnewcpepfu4=newcpepfu4;
run;

data cpeptidesub1;
  set cpeptidesub1;
  if newcpepfu5=0.1 and CpepRangefu6=-1 then nnewcpepfu5=0.05;
  else nnewcpepfu5=newcpepfu5;
run;

data cpeptidesub1;
  set cpeptidesub1;
  if newcpepfu6=0.1 and CpepRangefu7=-1 then nnewcpepfu6=0.05;
```

```
  else nnewcpepfu6=newcpepfu6;
run;

data cpeptidesub1; set cpeptidesub1;
 array old_c(6) nnewcpepfu1-nnewcpepfu6;
 array new_c(6) cpepfu_n1-cpepfu_n6;
 array b(6) newblooddrawdatefu1-newblooddrawdatefu6;
 array f(6) followup1-followup6;
 array d(6) diff1-diff6;
 array old_r (6) CpepRangefu1-CpepRangefu6;
 array new_r (6) rCpepRangefu1-rCpepRangefu6;
 format followup1-followup6 date9.;

 do i=1 to 6;
 if b(i) ne . then do;
 d(i)=b(i)-insulin_cpeptide_date;
 if 0<d(i)<=(4*365/12) and new_c(1)=. then do;
 new_c(1)=old_c(i);
 f(1)=b(i);
 new_r(1)=old_r(i);
 end;
 if (4*365/12)<d(i)<=(9*365/12) and new_c(2)=. then do;
 new_c(2)=old_c(i);
 f(2)=b(i);
 new_r(2)=old_r(i);
 end;
 if (9*365/12)<d(i)<=(15*365/12) and new_c(3)=. then do;
 new_c(3)=old_c(i);
 f(3)=b(i);
 new_r(3)=old_r(i);
 end;
 if (15*365/12)<d(i)<=(21*365/12) and new_c(4)=. then do;
 new_c(4)=old_c(i);
 f(4)=b(i);
 new_r(4)=old_r(i);
 end;
 if (21*365/12)<d(i)<=(36*365/12) and new_c(5)=. then do;
 new_c(5)=old_c(i);
 f(5)=b(i);
 new_r(5)=old_r(i);
 end;
 if (36*365/12)<d(i) and new_c(6)=. then do;
 new_c(6)=old_c(i);
 f(6)=b(i);
 new_r(6)=old_r(i);
 end;
```

```
 end;
 end;
 run;

data cpeptidesub1; set cpeptidesub1;
 lengthwfu1=followup1-insulin_cpeptide_date;
 lengthwfu2=followup2-insulin_cpeptide_date;
 lengthwfu3=followup3-insulin_cpeptide_date;
 lengthwfu4=followup4-insulin_cpeptide_date;
 lengthwfu5=followup5-insulin_cpeptide_date;
run;

data cpeptidesub1; set cpeptidesub1;
 cpepdiff1=cpepfu_n1-cpeptide;
 cpepdiff2=cpepfu_n2-cpeptide;
 cpepdiff3=cpepfu_n3-cpeptide;
 cpepdiff4=cpepfu_n4-cpeptide;
 cpepdiff5=cpepfu_n5-cpeptide;
run;

data  cpeptidesub1; set cpeptidesub1; label followup1="Followup
date fall in follow-up window 1: up to 4.5 months after
baseline"; run;
data  cpeptidesub1; set cpeptidesub1; label followup2="Followup
date fall in follow-up window 2: >4.5-10.5 months after
baseline"; run;
data  cpeptidesub1; set cpeptidesub1; label followup3="Followup
date fall in follow-up window 3: >10.5-16.5 months after
baseline"; run;
data  cpeptidesub1; set cpeptidesub1; label followup4="Followup
date fall in follow-up window 4: >16.5-22.5 months after
baseline"; run;
data  cpeptidesub1; set cpeptidesub1; label followup5="Followup
date fall in follow-up window 5: >22.5 months after baseline";
run;

data  cpeptidesub1; set cpeptidesub1; label cpepfu_n1="cpeptide:
follow-up window 1"; run;
data  cpeptidesub1; set cpeptidesub1; label cpepfu_n2="cpeptide:
follow-up window 2"; run;
data  cpeptidesub1; set cpeptidesub1; label cpepfu_n3="cpeptide:
follow-up window 3"; run;
data  cpeptidesub1; set cpeptidesub1; label cpepfu_n4="cpeptide:
follow-up window 4"; run;
data  cpeptidesub1; set cpeptidesub1; label cpepfu_n5="cpeptide:
follow-up window 5"; run;
```

```
data  cpeptidesub1; set cpeptidesub1; label
cpepdiff1="Difference between cpeptide at baseline and follow-up
window 1"; run;
data  cpeptidesub1; set cpeptidesub1; label
cpepdiff2="Difference between cpeptide at baseline and follow-up
window 2"; run;
data  cpeptidesub1; set cpeptidesub1; label
cpepdiff3="Difference between cpeptide at baseline and follow-up
window 3"; run;
data  cpeptidesub1; set cpeptidesub1; label
cpepdiff4="Difference between cpeptide at baseline and follow-up
window 4"; run;
data  cpeptidesub1; set cpeptidesub1; label
cpepdiff5="Difference between cpeptide at baseline and follow-up
window 5"; run;

data  cpeptidesub1; set cpeptidesub1; label lengthwfu1="Days
between baseline and follow-up window 1"; run;
data  cpeptidesub1; set cpeptidesub1; label lengthwfu2="Days
between baseline and follow-up window 2"; run;
data  cpeptidesub1; set cpeptidesub1; label lengthwfu3="Days
between baseline and follow-up window 3"; run;
data  cpeptidesub1; set cpeptidesub1; label lengthwfu4="Days
between baseline and follow-up window 4"; run;
data  cpeptidesub1; set cpeptidesub1; label lengthwfu5="Days
between baseline and follow-up window 5"; run;

/***** count how many follow ups each participant has ****/
data  cpeptidesub1;
  set cpeptidesub1;
  followup=5-nmiss (of lengthwfu1-lengthwfu5);
run;
proc freq data=cpeptidesub1;
 table followup;
run;

/**** study population: exclude patients with no baseline c-
peptide or =<2 follow-up cpeptide ***/
data  cpeptidesub1;
  set cpeptidesub1;
  where followup>=3;
run;

/**** Table 1: Days between onset of T1DM and each follow-up
visit (8 follow-up windows) ***/
data a;
set cpeptidesub1;
```

```
run;

data a;
 set a;
 newcpepfu8=.;
 newcpepfu7=.;
 newblooddrawdatefu8=.;
 newblooddrawdatefu7=.;
run;

data a (keep=pid cpeptide newcpepfu1-newcpepfu8
insulin_cpeptide_date newblooddrawdatefu1-newblooddrawdatefu8 );
 set a;
run;

data a; set a;
 array old_c(8) newcpepfu1-newcpepfu8;
 array new_c(8) cpepfu_n1-cpepfu_n8;
 array b(8) newblooddrawdatefu1-newblooddrawdatefu8;
 array f(8) followup1-followup8;
 array d(8) diff1-diff8;
 format followup1-followup8 date9.;

 do i=1 to 8;
 if b(i) ne . then do;
 d(i)=b(i)-insulin_cpeptide_date;
 if 0<d(i)<=(4.5*365/12) and new_c(1)=. then do;
 new_c(1)=old_c(i);
 f(1)=b(i);
 end;
 if (4.5*365/12)<d(i)<=(7.5*365/12) and new_c(2)=. then do;
 new_c(2)=old_c(i);
 f(2)=b(i);
 end;
 if (7.5*365/12)<d(i)<=(10.5*365/12) and new_c(3)=. then do;
 new_c(3)=old_c(i);
 f(3)=b(i);
 end;
 if (10.5*365/12)<d(i)<=(13.5*365/12) and new_c(4)=. then do;
 new_c(4)=old_c(i);
 f(4)=b(i);
 end;
 if (13.5*365/12)<d(i)<=(16.5*365/12) and new_c(5)=. then do;
 new_c(5)=old_c(i);
 f(5)=b(i);
 end;
 if (16.5*365/12)<d(i)<=(19.5*365/12) and new_c(6)=. then do;
```

```
 new_c(6)=old_c(i);
 f(6)=b(i);
 end;
 if (19.5*365/12)<d(i)<=(22.5*365/12) and new_c(7)=. then do;
 new_c(7)=old_c(i);
 f(7)=b(i);
 end;
 if (22.5*365/12)<d(i) and new_c(8)=. then do;
 new_c(8)=old_c(i);
 f(8)=b(i);
 end;

 end;
 end;
 run;

 data a; set a;
 lengthwfu1=followup1-insulin_cpeptide_date;
 lengthwfu2=followup2-insulin_cpeptide_date;
 lengthwfu3=followup3-insulin_cpeptide_date;
 lengthwfu4=followup4-insulin_cpeptide_date;
 lengthwfu5=followup5-insulin_cpeptide_date;
 lengthwfu6=followup6-insulin_cpeptide_date;
 lengthwfu7=followup7-insulin_cpeptide_date;
 lengthwfu8=followup8-insulin_cpeptide_date;
run;

proc means data=a N NMISS MIN MAX MEAN MEDIAN RANGE STD
maxdec=2;
 var  lengthwfu1 lengthwfu2 lengthwfu3 lengthwfu4 lengthwfu5
lengthwfu6 lengthwfu7 lengthwfu8;
run;

/**** Table 2: Days between onset of type 1 diabetes and each
follow-up visit (6-month window) ***/
/****** follow-up windows *****/
proc means data=cpeptidesub1 N MEAN STD MEDIAN P25 P75 MIN MAX
maxdec=2;
 var  lengthwfu1 lengthwfu2 lengthwfu3 lengthwfu4 lengthwfu5;
run;

proc means data=cpeptidesub1 N NMISS MEAN MEDIAN P75 P25 RANGE
STD maxdec=2;
 var  lengthwfu1 lengthwfu2 lengthwfu3 lengthwfu4 lengthwfu5;
 class overweight_bs;
 format overweight_bs overweight.;
run;
```

```
/**** Table 3: Demographic and clinical characteristics
according to overweight status at onset *****/
/****** obese/non-obese at baseline *****/
data cpeptidesub1; set cpeptidesub1; if BMIPCT>=85 then
overweight_bs=1; else if BMIPCT>0 then overweight_bs=0; run;
data  cpeptidesub1; set cpeptidesub1; label
overweight_bs="Baseline overweight status: BMIpct>=85"; run;
proc format; value overweight 1="overweight" 0="non-overweight";
run;
proc freq data=cpeptidesub1; table overweight_bs; format
overweight_bs overweight.;run;
/*** Demo characteristics **/
/*** gender ***/
proc freq data=cpeptidesub1; table gender; run;
proc sort data=cpeptidesub1; by overweight_bs; run;
proc freq data=cpeptidesub1; table gender*overweight_bs/chisq;
format overweight_bs overweight.;run;

/**** race ****/
proc freq data=cpeptidesub1; table race; run;
proc sort data=cpeptidesub1; by overweight_bs; run;
proc freq data=cpeptidesub1; table race*overweight_bs/chisq;
format overweight_bs overweight.;run;

/**** age ****/
proc means data=cpeptidesub1 N NMISS MIN MAX MEAN MEDIAN RANGE
STD P25 P75 fw=5 maxdec=2; var age_baseline; run;
proc sort data=cpeptidesub1; by overweight_bs; run;
proc means data=cpeptidesub1 N NMISS MIN MAX MEAN MEDIAN RANGE
STD P25 P75 fw=5 maxdec=2; var age_baseline; by overweight_bs;
run;
proc ttest data=cpeptidesub1; var age_baseline; class
overweight_bs; format overweight_bs overweight.;run;

/*** bmi at baseline ***/
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var bmi;
run;
proc sort data=cpeptidesub1; by overweight_bs; run;
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var bmi;
by overweight_bs; format overweight_bs overweight.; run;
proc ttest data=cpeptidesub1; var bmi; class overweight_bs;
format overweight_bs overweight.;run;

/*** bmi percentile at baseline ***/
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var
bmipct; run;
```

```
proc sort data=cpeptidesub1; by overweight_bs; run;
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var
bmipct; by overweight_bs; format overweight_bs overweight.; run;
proc ttest data=cpeptidesub1; var bmipct; class overweight_bs;
format overweight_bs overweight.;run;

/*** bmi z-score at baseline ***/
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var bmiz;
run;
proc sort data=cpeptidesub1; by overweight_bs; run;
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var bmiz;
by overweight_bs; format overweight_bs overweight.; run;
proc ttest data=cpeptidesub1; var bmiz; class overweight_bs;
format overweight_bs overweight.;run;

/*** bmi at 3month ***/
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var
bmi_3months; run;
proc sort data=cpeptidesub1; by overweight_bs; run;
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var
bmi_3months; by overweight_bs; format overweight_bs overweight.;
run;
proc ttest data=cpeptidesub1; var bmi_3months; class
overweight_bs; format overweight_bs overweight.;run;

/*** bmi percentile at 3month ***/
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var
bmipct_3months; run;
proc sort data=cpeptidesub1; by overweight_bs; run;
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var
bmipct_3months; by overweight_bs; format overweight_bs
overweight.; run;
proc ttest data=cpeptidesub1; var bmipct_3months; class
overweight_bs; format overweight_bs overweight.;run;

/*** bmi z-score at 3month ***/
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var
bmiz_3months; run;
proc sort data=cpeptidesub1; by overweight_bs; run;
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var
bmiz_3months; by overweight_bs; format overweight_bs
overweight.; run;
proc ttest data=cpeptidesub1; var bmiz_3months; class
overweight_bs; format overweight_bs overweight.;run;

/**** overweight at 3-month ***/
```

```
data cpeptidesub1; set cpeptidesub1; if BMIPCT_3months>=85 then
overweight_fu1=1; else if BMIPCT_3months>0 then
overweight_fu1=0; run;
data  cpeptidesub1; set cpeptidesub1; label overweight_fu1="3-
month overweight status: BMIpct>=85"; run;
proc freq data=cpeptidesub1; table overweight_fu1; format
overweight_fu1 overweight.;run;

/***** hba1c at baseline *****/
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var A1C;
run;
proc sort data=cpeptidesub1; by overweight_bs; run;
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var A1C;
by overweight_bs; format overweight_bs overweight.; run;
proc ttest data=cpeptidesub1; var A1C; class overweight_bs;
format overweight_bs overweight.;run;

/***** hba1c at 3-month *****/
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var
A1C_3months; run;
proc sort data=cpeptidesub1; by overweight_bs; run;
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var
A1C_3months; by overweight_bs; format overweight_bs overweight.;
run;
proc ttest data=cpeptidesub1; var A1C_3months; class
overweight_bs; format overweight_bs overweight.;run;

/**** number of follow-up ***/
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var
followup; run;
proc sort data=cpeptidesub1; by overweight_bs; run;
proc means data=cpeptidesub1 N MEAN STD fw=5 maxdec=2; var
followup; by overweight_bs; format overweight_bs overweight.;
run;
proc ttest data=cpeptidesub1; var followup; class overweight_bs;
format overweight_bs overweight.;run;

/***** c-peptide at baseline *****/
proc means data=cpeptidesub1 N MEAN STD median p25 p75 fw=5
maxdec=2; var cpeptide; run;
proc univariate data=cpeptidesub1;
 var cpeptide;
 histogram cpeptide;
run;

proc sort data=cpeptidesub1; by overweight_bs; run;
```

```
proc means data=cpeptidesub1 N MEAN STD median p25 p75 fw=5
maxdec=2; var cpeptide; by overweight_bs; format overweight_bs
overweight.;run;

proc npar1way data=cpeptidesub1 wilcoxon;
  class overweight_bs;
  var cpeptide;
run;

/***** c-peptide at follow up visits *****/
proc means data=cpeptidesub1 N MEAN STD median p25 p75 fw=5
maxdec=2;
  var cpepfu_n1 cpepfu_n2 cpepfu_n3 cpepfu_n4 cpepfu_n5;
run;

proc sort data=cpeptidesub1; by overweight_bs; run;
proc means data=cpeptidesub1 N MEAN STD median p25 p75 fw=5
maxdec=2;
  var cpepfu_n1 cpepfu_n2 cpepfu_n3 cpepfu_n4 cpepfu_n5;
  by overweight_bs;
  format overweight_bs overweight.;
run;

proc npar1way data=cpeptidesub1 wilcoxon;
  class overweight_bs;
  var cpepfu_n1 cpepfu_n2 cpepfu_n3 cpepfu_n4 cpepfu_n5;
run;

/***** Table 4: The distribution of study participants' number
of follow-up visits ****/
proc freq data=cpeptidesub1;
 table followup;
run;

/******* Table 5: Distribution of the number of positive
antibodies ****/
proc freq data=cpeptidesub1; table totalanti;run;

proc freq data=cpeptidesub1; table antisum;run;

/**** Table 6: Distribution of antibodies for participants with
only one-positive-antibody  ****/
proc freq data=cpeptidesub1;
table pos_antibodies_gad pos_antibodies_ica pos_antibodies_ia2
pos_antibodies_iaa;
where antisum_gr=1;
run;
```

```
/***** Table 7: Distribution of the antibodies for participants
with two/three-positive-antibody group ***/
data antibody2;
 set cpeptidesub1;
 if pos_antibodies_gad=1 and pos_antibodies_ica=1 then
antibody=1;
 else if pos_antibodies_gad=1 and pos_antibodies_ia2=1 then
antibody=2;
 else if pos_antibodies_gad=1 and pos_antibodies_iaa=1 then
antibody=3;
 else if pos_antibodies_ica=1 and pos_antibodies_ia2=1 then
antibody=4;
 else if pos_antibodies_ica=1 and pos_antibodies_iaa=1 then
antibody=5;
 else if pos_antibodies_ia2=1 and pos_antibodies_iaa=1 then
antibody=6;
 where antisum=2;
run;
proc format;
 value antibody  1="GAD and ICA"
                 2="GAD and IA2"
                     3="GAD and IAA"
                     4="ICA and IA2"
                     5="ICA and IAA"
                     6="IA2 and IAA";
run;
proc freq data=antibody2;
 table antibody;
 format antibody antibody.;
 where antisum=2;
run;

data antibody3;
 set cpeptidesub1;
 if pos_antibodies_gad=0 or pos_antibodies_gad=. then
antibody3=1;
 else if pos_antibodies_ica=0 or pos_antibodies_ica=. then
antibody3=2;
 else if pos_antibodies_ia2=0 or pos_antibodies_ia2=. then
antibody3=3;
 else if pos_antibodies_iaa=0 or pos_antibodies_iaa=. then
antibody3=4;
run;
proc format;
 value antibodyr  1="ICA,IA2, and IAA "
                  2="GAD,IA2, and IAA"
```

```
                        3="GAD,ICA, and IAA"
                        4="GAD,ICA, and IA2";
run;
proc freq data=antibody3;
table antibody3;
where antisum=3;
format antibody3 antibodyr.;
run;

/****** Table 8: Distribution of the antibodies for participants
with two/three-positive-antibody group ****/
proc means data=cpeptidesub1 N   MEAN STD MEDIAN Q1 Q3 MIN MAX
maxdec=2;
  var  cpeptide cpepfu_n1 cpepfu_n2 cpepfu_n3 cpepfu_n4
cpepfu_n5;
run;

/****** Figure 4.C-peptide at baseline and follow-up visits
*****/
data cpeptide (keep=PID overweight_bs cpeptide cpepfu_n1
cpepfu_n2 cpepfu_n3 cpepfu_n4 cpepfu_n5);
 set cpeptidesub1;
run;
proc sort data=cpeptide; by PID; run;
proc transpose data=cpeptide out=cpeptidelong
(rename=(col1=cpeptidelong));
var  cpeptide cpepfu_n1 cpepfu_n2 cpepfu_n3 cpepfu_n4 cpepfu_n5;
by PID;
copy overweight_bs;
run;

data cpeptidelong;
 set cpeptidelong;
 by PID;
 retain overweight_bs;
run;

data cpeptidelong;
 set cpeptidelong;
 if _NAME_="cpeptide" then group=0;
 else if _NAME_="cpepfu_n1" then group=3;
 else if _NAME_="cpepfu_n2" then group=6;
 else if _NAME_="cpepfu_n3" then group=12;
 else if _NAME_="cpepfu_n4" then group=18;
 else if _NAME_="cpepfu_n5" then group=24;
run;
proc sort data=cpeptidelong; by group; run;
```

```
proc format;
 value group 0="baseline"
             3="3-month follow-up"
                6="6-month follow-up"
                12="12-month follow-up"
                18="18-month follow-up"
                24="24-month follow-up";
run;

goptions reset=all interpol=boxt;
symbol value=dot height=0.4 interpol=boxtf width=3 bwidth=5
co=BL cv=TAN;
axis1 label=(angle=90 'C-peptide ng/mL');
axis2 label=("Baseline or follow-up visits")order=(0 to 24 by 3)
split=' ';
proc gplot data = cpeptidelong;
  title1 "C-peptide at baseline and follow-up visits";
  plot cpeptidelong*group /vaxis=axis1 haxis=axis2;
  format group group.;
run;

/****** Table 9-12: C-peptide levels by antibody status *****/
proc sort data=cpeptidesub1; by antisum_gr; run;
proc means data=cpeptidesub1 N   MEAN STD MEDIAN Q1 Q3 MIN MAX
maxdec=2;
var  cpeptide cpepfu_n1 cpepfu_n2 cpepfu_n3 cpepfu_n4 cpepfu_n5;
by antisum_gr;
format antisum_gr posianti.;
run;

/***** Table 13: C-peptide at baseline and follow-up visits for
participants with ≥one positive antibody ****/
data cpeptidesub1;
 set cpeptidesub1;
 if antisum_gr>0 then atleastone=1;
 else atleastone=0;
run;
proc format;
 value onepos 1="at least one positive antibody"
              0="all negative antibody";
run;
proc freq data=cpeptidesub1;
 table atleastone;
 format atleastone onepos.;
run;
proc means data=cpeptidesub1 N   MEAN STD MEDIAN Q1 Q3 MIN MAX
maxdec=2;
```

```
var  cpeptide cpepfu_n1 cpepfu_n2 cpepfu_n3 cpepfu_n4 cpepfu_n5;
where atleastone=1;
format atleastone onepos.;
run;   * at least 1 positive antibody ;

/**** Table 14. Comparison of C-peptide levels between antibody
groups ***/
proc npar1way data=cpeptidesub1;
  class antisum_gr;
  var cpeptide;
run;

proc npar1way data=cpeptidesub1;
  class antisum_gr;
  var cpepfu_n1;
run;

proc npar1way data=cpeptidesub1;
  class antisum_gr;
  var cpepfu_n2;
run;

proc npar1way data=cpeptidesub1;
  class antisum_gr;
  var cpepfu_n3;
run;

proc npar1way data=cpeptidesub1;
  class antisum_gr;
  var cpepfu_n4;
run;

proc npar1way data=cpeptidesub1;
  class antisum_gr;
  var cpepfu_n5;
run;

/****** Figure 5. plot for c-peptide at baseline and follow-up
visits by antibody status *****/
DATA cpepplot;
   INPUT  fu   cpeptide antisumgr;
   DATALINES;
0       0.65 0
3       1.86 0
6       1.62 0
12       1.12 0
18       0.96 0
```

```
24        0.52 0
0        0.71 1
3        1.84 1
6        1.61 1
12        1.54 1
18        1.65 1
24        0.74 1
0        0.64 2
3        1.69 2
6        1.79 2
12        1.33 2
18        1.23 2
24        0.71 2
0        0.69 3
3        2.06 3
6        1.81 3
12        1.15 3
18        0.97 3
24        0.53 3
0        0.62 4
3        1.74 4
6        1.41 4
12        0.92 4
18        0.74 4
24        0.43 4
run;
PROC PRINT; RUN;

proc format;
 value antisumgr 0="total population"
                 1="all negative antibodies"
                     2="one positive antibody"
                     3="two positive antibodies"
                     4="three or four positive antibodies";
 value  fu       0="baseline"
                 3="3-month follow-up"
                     6="6-month follow-up"
                     12="12-month follow-up"
                     18="18-month follow-up"
                     24="24-month follow-up";
run;

SYMBOL1 V=dot   C=black I=join h=2;
SYMBOL2 V=star   C=black   I=join h=5;
SYMBOL3 V=square   C=black   I=join h=5;
SYMBOL4 V=triangle   C=black   I=join h=5;
SYMBOL5 V=plus      C=black   I=join h=5;
```

```
legend label=none value=(h=2)
       position=(top right inside) mode=share cborder=black;

axis1 label=(angle=90 'C-peptide ng/mL') order=(0 1 2 3);
axis2 label=('baseline or follow-up visits') order=(0 to 24 by
3) split=' ';
proc gplot data=cpepplot;
plot cpeptide*fu=antisumgr/haxis=axis2 vaxis=axis1
legend=legend;
format antisumgr antisumgr. fu fu.;title "Mean of cpeptide by
antibody status";
run;quit;

/*** Table 15.Antibody status and C-peptide of participants with
C-peptide> ng/mL at follow-up 1 **/
data cpeptide8 (keep=pid cpeptide newcpepfu_n1 newcpepfu_n2
newcpepfu_n3 newcpepfu_n4 newcpepfu_n5 antisum_gr
pos_antibodies_gad pos_antibodies_ica pos_antibodies_ia2
pos_antibodies_iaa);
 set cpeptidesub1;
 where cpepfu_n1>=8;
run;

proc freq data=cpeptide8;
 table  antisum_gr pos_antibodies_gad pos_antibodies_ica
pos_antibodies_ia2 pos_antibodies_iaa;
 format antisum_gr posianti.;
run;

/***** Table 16.C-peptide change from baseline  ****/
proc means data=cpeptidesub1 N   MEAN STD MEDIAN Q1 Q3 MIN MAX
maxdec=2;
 var  cpepdiff1 cpepdiff2 cpepdiff3 cpepdiff4 cpepdiff5;
run;

/**** Table 17-20.C-peptide change from baseline by antibody
status ****/
proc sort data=cpeptidesub1; by antisum_gr; run;
proc means data=cpeptidesub1 N   MEAN STD MEDIAN Q1 Q3 MIN MAX
maxdec=2;
var  cpepdiff1 cpepdiff2 cpepdiff3 cpepdiff4 cpepdiff5;
by antisum_gr;
format antisum_gr posianti.;
run;
```

```
/*** Table 21.C-peptide change from baseline for participants
with ≥one positive antibody ***/
proc means data=cpeptidesub1 N   MEAN STD MEDIAN Q1 Q3 MIN MAX
maxdec=2;
var  cpepdiff1 cpepdiff2 cpepdiff3 cpepdiff4 cpepdiff5;
where atleastone=1;
format atleastone onepos.;
run;

/**** Table 22. Comparison of C-peptide change between antibody
groups ***/
proc npar1way data=cpeptidesub1;
  class antisum_gr;
  var cpepdiff1;
run;

proc npar1way data=cpeptidesub1;
  class antisum_gr;
  var cpepdiff2;
run;

proc npar1way data=cpeptidesub1;
  class antisum_gr;
  var cpepdiff3;
run;

proc npar1way data=cpeptidesub1;
  class antisum_gr;
  var cpepdiff4;
run;

proc npar1way data=cpeptidesub1;
  class antisum_gr;
  var cpepdiff5;
run;

/****** figure 6. plot for c-peptide change from baseline at
each follow-up visits by antibody status *****/
DATA cpepplot2;
   INPUT  fugr   cpepdiff antisumgr;
   DATALINES;
3      1.20  0
6      0.98  0
12      0.46  0
18      0.29  0
24      -0.10 0
3      1.13  1
```

```
6       1.15  1
12       0.80  1
18       0.89  1
24       0.20  1
3       1.05  2
6       1.18  2
12       0.68  2
18       0.54  2
24       0.11  2
3       1.38  3
6       1.12  3
12       0.47  3
18       0.29  3
24       -0.16 3
3       1.13  4
6       0.76  4
12       0.28  4
18       0.12  4
24       -0.16 4
run;

data cpepplot2;
 set cpepplot2;
 label antisumgr="antibody group";
run;

PROC PRINT; RUN;

proc format;
 value antisumgr 0="total population"
                 1="all negative antibodies"
                     2="one positive antibody"
                     3="two positive antibodies"
                     4="three or four positive antibodies";
value  fugr      3="3-month follow-up"
                     6="6-month follow-up"
                     12="12-month follow-up"
                     18="18-month follow-up"
                     24="24-month follow-up";
run;

SYMBOL1 V=dot   C=black I=join;
SYMBOL2 V=star   C=black   I=join;
SYMBOL3 V=square   C=black   I=join;
SYMBOL4 V=triangle   C=black   I=join;
SYMBOL5 V=plus   C=black   I=join;
```

```
legend label=none value=(h=2)
       position=(top right inside) mode=share cborder=black;

axis1 label=(angle=90 'C-peptide ng/mL');
axis2 label=('follow-up visits') order=(3 to 24 by 3) split=' ';
proc gplot data=cpepplot2;
plot cpepdiff*fugr=antisumgr/haxis=axis2 vaxis=axis1
legend=legend;
format antisumgr antisumgr. fugr fugr.;title "Mean of cpeptide
change from baseline by antibody status";
run;quit;

/*** Table 23- 27, C-peptide by overweight status at onset and
3-month **/
proc means data=cpeptidesub1 N NMISS MIN MAX MEAN MEDIAN RANGE
STD P25 P75 fw=5 maxdec=2; var BMIPCT; run;
proc means data=cpeptidesub1 N NMISS MIN MAX MEAN MEDIAN RANGE
STD P25 P75 fw=5 maxdec=2; var BMIPCT_3months; run;

data cpeptidesub1; set cpeptidesub1; if BMIPCT_3months>=85 then
overweight_fu1=1; else if BMIPCT_3months>0 then
overweight_fu1=0; run;
data  cpeptidesub1; set cpeptidesub1; label overweight_fu1="3-
month overweight status: BMIpct>=85"; run;
proc freq data=cpeptidesub1; table overweight_fu1; format
overweight_fu1 overweight.;run;

data cpeptidesub1; set cpeptidesub1;
 if overweight_bs=1 and overweight_fu1=1 then
obesity_status_c=1;
 else if overweight_bs=1 and overweight_fu1=0 then
obesity_status_c=2;
 else if overweight_bs=0 and overweight_fu1=1 then
obesity_status_c=3;
 else if overweight_bs=0 and overweight_fu1=0 then
obesity_status_c=4;
run;

proc format;
  value overweightc 1="obese-obese"
                    2="obese;non-obese"
                    3="non-obese;obese"
                    4="non-obese;non-obese";
run;

proc freq data=cpeptidesub1; table obesity_status_c; format
obesity_status_c overweightc.;run;
```

```
proc means data=cpeptidesub1 N MEAN STD MEDIAN p25 p75 Min max
maxdec=2;
 var  cpeptide cpepfu_n1 cpepfu_n2 cpepfu_n3 cpepfu_n4
cpepfu_n5;
 class obesity_status_c;
 format obesity_status_c overweightc.;
run;

/*** Figure 7.C-peptide at onset and follow up visits by
overweight status change **/
DATA plot5;
   INPUT  fu   cpeptide obesity_status_c;
   DATALINES;
0      1.02 1
3      2.38 1
6      2.14 1
12      1.49 1
18      1.60 1
24      0.70 1
0      1.24 2
3      5.06 2
6      2.27 2
12      1.79 2
18      2.65 2
24      0.88 2
0      0.43 3
3      1.48 3
6      1.24 3
12      1.07 3
18      0.92 3
24      0.47 3
0      0.58 4
3      1.71 4
6      1.55 4
12      1.00 4
18      0.72 4
24      0.42 4
run;

PROC PRINT; RUN;

data plot5;
 set plot5;
 label obesity_status_c="overweight status at baseline and 3-
month";
run;
```

```
proc format;
  value overweightc 1="obese-obese"
                    2="obese;non-obese"
                    3="non-obese;obese"
                    4="non-obese;non-obese";
run;

goptions reset=all;
SYMBOL1 V=star   C=black   I=join;
SYMBOL2 V=plus   C=black   I=join;
SYMBOL3 V=square   C=black   I=join;
SYMBOL4 V=triangle   C=black   I=join;

legend label=none value=(h=2)
       position=(top right inside) mode=share cborder=black;

axis1 label=(angle=90 'C-peptide ng/mL');
axis2 label=('baseline/follow-up visits') order=(0 to 24 by 3)
split=' ';
proc gplot data=plot5;
plot cpeptide*fu=obesity_status_c/haxis=axis2 vaxis=axis1
legend=legend;
format obesity_status_c overweightc.  fu fu.;
title "Mean of cpeptide by overweight status at onset and 3-
month";
run;quit;

/**** Comparison of C-peptide between overweight change groups
***/
proc npar1way data=cpeptidesub1;
  class obesity_status_c;
  var cpeptide;
run;

proc npar1way data=cpeptidesub1;
  class obesity_status_c;
  var cpepfu_n1;
run;

proc npar1way data=cpeptidesub1;
  class obesity_status_c;
  var cpepfu_n2;
run;

proc npar1way data=cpeptidesub1;
  class obesity_status_c;
```

```
  var cpepfu_n3;
run;

proc npar1way data=cpeptidesub1;
  class obesity_status_c;
  var cpepfu_n4;
run;

proc npar1way data=cpeptidesub1;
  class obesity_status_c;
  var cpepfu_n5;
run;

/***** Table 28-29: cpeptide by obesity at baseline ****/
proc means data=cpeptidesub1 N MEAN STD MEDIAN p25 p75 Min max
maxdec=2;
 var  cpeptide cpepfu_n1 cpepfu_n2 cpepfu_n3 cpepfu_n4
cpepfu_n5;
 class overweight_bs;
 format overweight_bs overweight.;
run;

DATA plot;
   INPUT  fu   cpeptide overweight_bs;
   DATALINES;
0      1.02 1
3      2.58 1
6      2.12 1
12      1.49 1
18      1.67 1
24      0.71 1
0      0.55 0
3      1.67 0
6      1.49 0
12      1.03 0
18      0.79 0
24      0.47 0
run;

data plot;
 set plot;
 label overweight_bs="overweight status at baseline";
run;

PROC PRINT; RUN;

proc format;
```

```
 value fu 0="baseline"
          3="3-month follow-up"
            6="6-month follow-up"
            12="12-month follow-up"
            18="18-month follow-up"
            24="24-month follow-up";
run;

SYMBOL1 V=dot   C=black I=l;
SYMBOL2 V=star   C=black   I=l;

axis1 label=(angle=90 'C-peptide ng/mL');
axis2 label=('baseline/follow-up visits') order=(0 to 24 by 3)
split=' ';
proc format; value ob 1="overweight" 0="non-overweight"; run;
proc gplot data=plot;
plot cpeptide*fu=overweight_bs/haxis=axis2 vaxis=axis1;
format overweight_bs ob.  fu fu.;
title "Mean of cpeptide by overweight status";
run;quit;

/*** Table 30.Comparison of C-peptide levels between overweight
and non-overweight groups at onset and follow up visits **/
proc npar1way data=cpeptidesub1 wilcoxon;
  class overweight_bs;
  var cpeptide cpepfu_n1 cpepfu_n2 cpepfu_n3 cpepfu_n4
cpepfu_n5;
run;

/**** Table 31-32  change rate of c-peptide ****/
data cpeptidesub1;
 set cpeptidesub1;
 cpepfu21=cpepfu_n2-cpepfu_n1;
 cpepfu31=cpepfu_n3-cpepfu_n1;
 cpepfu41=cpepfu_n4-cpepfu_n1;
 cpepfu51=cpepfu_n5-cpepfu_n1;
 lengthwfu21=lengthwfu2-lengthwfu1;
 lengthwfu31=lengthwfu3-lengthwfu1;
 lengthwfu41=lengthwfu4-lengthwfu1;
 lengthwfu51=lengthwfu5-lengthwfu1;
run;

data cpeptidesub1;
 set cpeptidesub1;
 cpeprate1=cpepdiff1/(lengthwfu1/30.41667);
 cpeprate2=cpepdiff2/(lengthwfu2/30.41667);
 cpeprate3=cpepdiff3/(lengthwfu3/30.41667);
```

```
 cpeprate4=cpepdiff4/(lengthwfu4/30.41667);
 cpeprate5=cpepdiff5/(lengthwfu5/30.41667);
 cpeprate21=cpepfu21/(lengthwfu21/30.41667);
 cpeprate31=cpepfu31/(lengthwfu31/30.41667);
 cpeprate41=cpepfu41/(lengthwfu41/30.41667);
 cpeprate51=cpepfu51/(lengthwfu51/30.41667);
run;

proc means data=cpeptidesub1 N MEAN   maxdec=2;
 var  cpeprate1 cpeprate2 cpeprate3 cpeprate4 cpeprate5
cpeprate21 cpeprate31 cpeprate41 cpeprate51;
 class overweight_bs;
 format overweight_bs overweight.;
run;

proc univariate data=cpeptidesub1;
 var cpeprate1;
 histogram cpeprate1;
run;
proc univariate data=cpeptidesub1;
 var cpeprate2;
 histogram cpeprate2;
run;
proc univariate data=cpeptidesub1;
 var cpeprate3;
 histogram cpeprate3;
run;
proc univariate data=cpeptidesub1;
 var cpeprate4;
 histogram cpeprate4;
run;
proc univariate data=cpeptidesub1;
 var cpeprate5;
 histogram cpeprate5;
run;

proc ttest data=cpeptidesub1;
 var cpeprate1 cpeprate2 cpeprate3 cpeprate4 cpeprate5;
 class overweight_bs;
 format overweight_bs overweight.;
run;
proc ttest data=cpeptidesub1;
 var cpeprate21 cpeprate31 cpeprate41 cpeprate51;
 class overweight_bs;
 format overweight_bs overweight.;
run;
```

```
proc sort data=cpeptidesub1; by overweight_bs; run;
proc ttest data=cpeptidesub1 h0=0;
 var  cpeprate1;
 by overweight_bs;
 format overweight_bs overweight.;
run;

proc ttest data=cpeptidesub1 h0=0;
 var  cpeprate2;
 by overweight_bs;
 format overweight_bs overweight.;
run;

proc ttest data=cpeptidesub1 h0=0;
 var  cpeprate3;
 by overweight_bs;
 format overweight_bs overweight.;
run;

proc ttest data=cpeptidesub1 h0=0;
 var  cpeprate4;
 by overweight_bs;
 format overweight_bs overweight.;
run;

proc ttest data=cpeptidesub1 h0=0;
 var  cpeprate5;
 by overweight_bs;
 format overweight_bs overweight.;
run;

proc ttest data=cpeptidesub1 h0=0;
 var  cpeprate21;
 by overweight_bs;
 format overweight_bs overweight.;
run;

proc ttest data=cpeptidesub1 h0=0;
 var  cpeprate31;
 by overweight_bs;
 format overweight_bs overweight.;
run;

proc ttest data=cpeptidesub1 h0=0;
 var  cpeprate41;
 by overweight_bs;
 format overweight_bs overweight.;
```

```
run;

proc ttest data=cpeptidesub1 h0=0;
 var  cpeprate51;
 by overweight_bs;
 format overweight_bs overweight.;
run;

/*** Table 33. correlation between A1C at baseline and A1C at 3-
month ***/
proc corr data=cpeptidesub1 pearson spearman;
 var A1C A1C_3months;
run;

/***** Table 34. correlation between age at onset and c-peptide
*****/
proc corr data=cpeptidesub1 pearson spearman;
 var age_baseline cpeptide;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var age_baseline cpepfu_n1;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var age_baseline cpepfu_n2;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var age_baseline cpepfu_n3;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var age_baseline cpepfu_n4;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var age_baseline cpepfu_n5;
run;

/***** correlation between A1C at onset and c-peptide *****/
proc corr data=cpeptidesub1 pearson spearman;
 var A1C cpeptide;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var A1C cpepfu_n1;
```

```
run;

proc corr data=cpeptidesub1 pearson spearman;
 var A1C cpepfu_n2;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var A1C cpepfu_n3;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var A1C cpepfu_n4;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var A1C cpepfu_n5;
run;

/***** correlation between A1C-3 month at onset and c-peptide
*****/
proc corr data=cpeptidesub1 pearson spearman;
 var A1C_3months cpeptide;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var A1C_3months cpepfu_n1;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var A1C_3months cpepfu_n2;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var A1C_3months cpepfu_n3;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var A1C_3months cpepfu_n4;
run;

proc corr data=cpeptidesub1 pearson spearman;
 var A1C_3months cpepfu_n5;
run;

/***** distribution of follow-up time *****/
data longdata;
 set library.long_2012_02_29;
```

```
run;

data longdata;
 set longdata;
 if pos_antibodies_gad=. then gadmiss=1;
 if pos_antibodies_ia2=. then ia2miss=1;
 if pos_antibodies_iaa=. then iaamiss=1;
 if pos_antibodies_ica=. then icamiss=1;
 antimisssum=sum (gadmiss,ia2miss,iaamiss,icamiss);
run;

proc freq data=longdata;
 table antimisssum;
run;

data longdata;
 set longdata;
 where  AGS=1 and AGSdisp=1;
run;

data longdata;
 set longdata;
 where antimisssum=1 or antimisssum=.;
run;

data longdata;
 set longdata;
 fu=blooddrawdatefu-insulin_cpeptide_date;
run;

proc univariate data=longdata;
 histogram fu;
run;

/***** get long data set for GEE model ****/
data cpeptidesub1a (keep=PID  cpeptide cpepfu_n1 cpepfu_n2
cpepfu_n3 cpepfu_n4 cpepfu_n5
                            lengthwfu1 lengthwfu2 lengthwfu3
lengthwfu4 lengthwfu5);
   set cpeptidesub1;
run;

data cpeptidesub1a;
 set cpeptidesub1a;
 lengthwfu0=0;
run;
```

```
proc sort data=cpeptidesub1a; by PID; run;
proc transpose data=cpeptidesub1a out=cpeptidelong
prefix=cpeptide;
     by PID;
     var cpeptide cpepfu_n1 cpepfu_n2 cpepfu_n3 cpepfu_n4
cpepfu_n5;
run;
data cpeptidelong (rename=(cpeptide1=cpeptide));
   set cpeptidelong;
   by PID;
   time=input(substr(_name_, 9), 1.);
   if time=. then time=0; else time=time;
   drop _name_ _LABEL_;
run;

proc sort data=cpeptidesub1a; by PID; run;
proc transpose data=cpeptidesub1a out=fulong prefix=fu;
     by PID;
     var lengthwfu0 lengthwfu1 lengthwfu2 lengthwfu3 lengthwfu4
lengthwfu5;
run;
data fulong (rename=(fu1=timeinterval));
   set fulong;
   by PID;
   time=input(substr(_name_, 10), 1.);
   drop _name_ _LABEL_;
run;

proc sort data=cpeptidelong; by PID time;run;
proc sort data=fulong; by PID time;run;
data cpeptidegee;
 merge cpeptidelong fulong;
 by PID time;
run;

data cpeptidesub1_gee (keep= PID gender age_baseline race
antisum_gr antisum_gr1 pos_antibodies_gad pos_antibodies_ica
 pos_antibodies_ia2 pos_antibodies_iaa totalanti antisum
overweight_bs obesity_status_c A1C A1C_3months);
 set cpeptidesub1;
run;

proc sort data=cpeptidegee; by PID;run;
proc sort data=cpeptidesub1_gee; by PID;run;
data cpeptide_long;
 merge cpeptidegee cpeptidesub1_gee;
 by PID;
```

```
run;

data cpeptide_long;
 set cpeptide_long;
 if time=0 then followup=0;
 else if time=1 then followup=3;
 else if time=2 then followup=6;
 else if time=3 then followup=12;
 else if time=4 then followup=18;
 else if time=5 then followup=24;
run;

proc freq data=cpeptide_long;
 table followup;
run;

data library.cpeptide_long;
 set cpeptide_long;
run;
```

APPENDIX B: STATA CODE USED FOR GEE MODELS

```
/****** time variable - categorical, 0,3,6,12,18,24 ******/
/****** c-peptide: obesity *****/
xtgee  cpeptide  i.followup i.overweight_bs, i(pid) t(followup)
testparm i.followup

/***** cpeptide: obesity and age ****/
xtgee  cpeptide  i.followup i.overweight_bs age_baseline, i(pid) t(followup)
testparm i.followup

/***** cpeptide: obesity, age, and gender ****/
xtgee  cpeptide  i.followup i.overweight_bs age_baseline i.gender, i(pid) t(followup)
testparm i.followup

/***** cpeptide: obesity, age, gender, A1C at onset ****/
xtgee  cpeptide  i.followup i.overweight_bs age_baseline i.gender a1c, i(pid) t(followup)
testparm i.followup

/***** cpeptide: obesity, age, gender, and a1c at 3-month ****/
xtgee  cpeptide  i.followup i.overweight_bs age_baseline i.gender a1c_3months, i(pid)
t(followup)
testparm i.followup

/***** cpeptide: obesity, age, gender, A1C at onset, and a1c at 3-month ****/
xtgee  cpeptide  i.followup i.overweight_bs age_baseline i.gender a1c a1c_3months, i(pid)
t(followup)
testparm i.followup

/***** cpeptide: obesity, age, gender, a1c at 3-month, and number of positive antibodies ****/
xtgee  cpeptide  i.followup i.overweight_bs age_baseline i.gender a1c_3months i.antisum_gr,
i(pid) t(followup)
testparm i.followup
testparm i.antisum_gr

/*** test for interaction ****/
xtgee  cpeptide i.followup##i.overweight_bs age_baseline i.gender a1c_3months i.antisum_gr,
i(pid) t(followup)
```

```
pwcompare i.followup#i.overweight_bs, pv

testparm i.followup
testparm i.antisum_gr
testparm followup#overweight_bs

/**** predict cpeptide and make plots for obese and non-obese ***/
predict yhat
predict yhat2, xb
predict std,stdp
generate upper_limit= yhat+1.96* std
generate low_limit= yhat-1.96* std

twoway (scatter yhat  followup if overweight_bs==1, msymbol(S)) (rcap upper_limit low_limit followup if overweight_bs==1)
twoway (scatter yhat  followup if overweight_bs==0, msymbol(S)) (rcap upper_limit low_limit followup if overweight_bs==0), ysca (r(-2 (2) 6))

/****** time variable - continuous, 0,3,6,12,18,24 ******/
use "C:\Users\J\Desktop\cpeptide_long.dta"
/****** c-peptide: obesity *****/
xtgee  cpeptide  followup  i.overweight_bs, i(pid) t(followup)

/***** cpeptide: obesity and age ****/
xtgee  cpeptide  followup i.overweight_bs age_baseline, i(pid) t(followup)

/***** cpeptide: obesity, age, and gender ****/
xtgee  cpeptide  followup i.overweight_bs age_baseline i.gender, i(pid) t(followup)

/***** cpeptide: obesity, age, gender, A1C at onset ****/
xtgee  cpeptide  followup overweight_bs age_baseline i.gender a1c, i(pid) t(followup)

/***** cpeptide: obesity, age, gender, and a1c at 3-month ****/
xtgee  cpeptide  followup i.overweight_bs age_baseline i.gender a1c_3months, i(pid) t(followup)

/***** cpeptide: obesity, age, gender, A1C at onset, and a1c at 3-month ****/
xtgee  cpeptide  followup i.overweight_bs age_baseline i.gender a1c a1c_3months, i(pid) t(followup)

/***** cpeptide: obesity, age, gender, a1c at 3-month, and number of positive antibodies ****/
xtgee  cpeptide  followup i.overweight_bs age_baseline i.gender a1c a1c_3months i.antisum_gr, i(pid) t(followup)
testparm _Iantisum*

/*** test for interaction ****/
```

```
xtgee cpeptide c.followup##i.overweight_bs age_baseline i.gender a1c a1c_3months i.antisum_gr, i(pid) t(followup)


/****** time variable - continuous, 0,3,6,12,18,24, fractional polynomial GEE ******/
/****** c-peptide: obesity *****/
xi:fracpoly xtgee  cpeptide followup  i.overweight_bs, i(pid) t(followup)
testparm Ifoll*

/***** cpeptide: obesity and age ****/
xi:fracpoly xtgee  cpeptide  followup i.overweight_bs age_baseline, i(pid)
testparm Ifoll*

/***** cpeptide: obesity, age, and gender ****/
xi: fracpoly xtgee  cpeptide  followup i.overweight_bs age_baseline i.gender, i(pid)
testparm Ifoll*

/***** cpeptide: obesity, age, gender, A1C at onset ****/
xi: fracpoly xtgee  cpeptide  followup i.overweight_bs age_baseline i.gender a1c, i(pid)
testparm Ifoll*

/***** cpeptide: obesity, age, gender, and a1c at 3-month ****/
xi: fracpoly xtgee cpeptide followup i.overweight_bs age_baseline i.gender a1c_3months, i(pid)
testparm Ifoll*

/***** cpeptide: obesity, age, gender, a1c at onset, and a1c at 3-month ****/
xi: fracpoly xtgee cpeptide followup i.overweight_bs age_baseline i.gender a1c a1c_3months, i(pid)
testparm Ifoll*

/***** cpeptide: obesity, age, gender, a1c at 3-month, and number of positive antibodies ****/
xi: fracpoly xtgee cpeptide followup i.overweight_bs age_baseline i.gender a1c_3months i.antisum_gr, i(pid)
testparm Ifoll*
testparm _Iantisum_*

label values overweight_bs obese

sort overweight_bs
twoway (fpfitci cpeptide followup), by (overweight_bs) title("Predicted C-peptide levels")


/***** test for interaction cpeptide: obesity, age, gender, a1c at onset, and a1c at 3-month ****/
generate time1=((followup+3)/10)^(-1) - .7699805068
generate time2=((followup+3)/10)^(-1)*ln((followup+3)/10) -.2012652665
```

```
xi: xtgee cpeptide c.time1##i.overweight_bs c.time2##i.overweight_bs i.overweight_bs age_baseline i.gender a1c_3months i.antisum_gr, i(pid)

testparm _Ioverweigh_1#c.tim* overweight_bs#c.ti*
testparm _Iantisum*
/**** predict cpeptide and make plots for obese and non-obese ***/
drop yhat yhat2 std upper_limit low_limit

predict yhat
predict yhat2, xb
predict std,stdp
generate upper_limit= yhat+1.96* std
generate low_limit= yhat-1.96* std

twoway (scatter yhat followup if overweight_bs==1, msymbol(S)) (rcap upper_limit low_limit followup if overweight_bs==1),title("Overweight")
twoway (scatter yhat followup if overweight_bs==0, msymbol(S)) (rcap upper_limit low_limit followup if overweight_bs==0),title("Non-Overweight") ysca (r(-2 (2) 6))

/********* time variable - continuous, days between baseline and each fu ******/
use "C:\Users\J\Desktop\cpepgee.dta"
/****** c-peptide: obesity *****/
xtgee cpeptide timeinterval overweight_bs, i(pid)

fracpoly xtgee cpeptide timeinterval overweight_bs, i(pid)
fracpoly,compare
testparm Itime*

/**** predict cpeptide and make plots for obese and non-obese ***/
predict yhat
predict yhat2, xb
predict std,stdp
generate upper_limit= yhat+1.96* std
generate low_limit= yhat-1.96* std

twoway (scatter yhat timeinterval if overweight_bs==1, msymbol(S)) (rcap upper_limit low_limit timeinterval if overweight_bs==1)
twoway (scatter yhat timeinterval if overweight_bs==0, msymbol(S)) (rcap upper_limit low_limit timeinterval if overweight_bs==0)

label define obese 0 "non-obese" 1 "obese"
label values overweight_bs obese

sort overweight_bs
twoway (fpfitci cpeptide timeinterval), by (overweight_bs) title("Predicted C-peptide levels")
```

```
/***** cpeptide: obesity and age ****/
fracpoly xtgee  cpeptide  timeinterval   overweight_bs  age_baseline, i(pid)
testparm Itime*

/***** cpeptide: obesity, age, and gender ****/
xi:fracpoly xtgee  cpeptide  timeinterval   overweight_bs  age_baseline i.gender, i(pid)
testparm Itime*

/***** cpeptide: obesity, age, gender, A1C at onset ****/
xi: fracpoly xtgee  cpeptide  timeinterval   overweight_bs  age_baseline i.gender a1c, i(pid)
testparm Itime*

/***** cpeptide: obesity, age, gender, A1C at onset, and a1c at 3-month ****/
xi: fracpoly xtgee cpeptide timeinterval overweight_bs age_baseline i.gender a1c a1c_3months,
i(pid)
testparm Itime*

/***** cpeptide: obesity, age, gender, a1c at 3-month, and number of positive antibodies ****/
xi: fracpoly xtgee cpeptide timeinterval overweight_bs age_baseline i.gender a1c_3months
i.antisum_gr, i(pid)
testparm Itime*
testparm _Iantisum*

sort overweight_bs
twoway (fpfitci cpeptide timeinterval), by (overweight_bs) title("fractional-polynomial
prediction plots")

/*** test for interaction ****/
drop time1 time2
generate time1=((timeinterval+1)/100)^(-.5)-.5784942298
generate time2=((timeinterval+1)/100)^(.5)-1.728625712

xi: xtgee cpeptide time1 time2 overweight_bs c.time1#overweight_bs c.time2#overweight_bs
age_baseline i.gender a1c_3months i.antisum_gr, i(pid)
testparm overweight_bs#c.*
testparm _Iantisum*

drop yhat yhat2 std upper_limit low_limit

predict yhat
predict yhat2, xb
predict std,stdp
generate upper_limit= yhat+1.96* std
generate low_limit= yhat-1.96* std
```

```
twoway (scatter yhat  timeinterval if overweight_bs==1, msymbol(S)) (rcap upper_limit low_limit  timeinterval if overweight_bs==1),title("Overweight")
twoway (scatter yhat  timeinterval if overweight_bs==0, msymbol(S)) (rcap upper_limit low_limit  timeinterval if overweight_bs==0),title("Non-Overweight")
```

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