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Long Term Insulin Independence and Improvement in Insulin Secretion after Supplemental Islet Infusion under Exenatide and Etanercept

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Abstract

Background—Progressive graft dysfunction (GDF) and loss of insulin independence (II) has been invariably observed in islet transplant recipients under the 'Edmonton protocol'. To reestablish II we performed supplemental islet infusions (SI) in recipients of allogeneic islet transplant alone, displaying GDF. To improve the engraftment and long-term graft function of SI, exenatide (EXN) and etanercept treatment at islet infusion, and long-term EXN treatment were tested in a non-randomized pilot clinical trial.

Methods—Patients with GDF received SI under Edmonton-like immunosuppression with daclizumab induction, either without interventions (SI-control; n=5) or with EXN and etanercept treatment (SI-EXN; n=4). Clinical and metabolic profiles were assessed over 18-month follow-up.

Results—Long-term II (18 months) was observed in 100% of SI-EXN and in 20% of SI-Control (p=0.04). SI-EXN subjects demonstrated restoration of function better than that seen following initial islet infusions. Comparison of SI-EXN and SI-Control groups demonstrated better responses in SI-EXN subjects at three months post SI. Over the 18 months of follow-up, function was sustained in the SI-EXN subjects better than in SI-Controls. Acute effects of exenatide during MMTT and IVGTT results in improved first and second phase insulin release in response to intravenous glucose (IVGTT), and suppressed postprandial hyperglucagonemia after mixed meal tolerance test (MMTT).

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Conclusion—These results suggest that the combination of exenatide and etanercept improve engraftment and long term islet survival and function in subjects undergoing SI. This data however must be interpreted with some caution due to small sample size, lack of randomization and sequential comparison with historical controls.

Keywords

islet; islet transplantation; insulin independence; graft dysfunction; Exenatide; metabolism; diabetes; glucagon; insulin; etanercept

INTRODUCTION

A current limitation of islet transplantation (ITx) under the 'Edmonton Protocol' is that insulin independence (II) is not sustained and exogenous insulin reintroduction is required in virtually all ITx recipients over time to maintain normoglycemia (1-4). Despite graft dysfunction (GDF), benefits of stable control, lack of severe hypoglycemia (3) and improved quality of life remain (5).

To maintain these benefits and to re-establish II in subjects already on immunosuppression, SI have been performed at our center in ITx recipients with GDF (4); results demonstrated some success. Recently, two new agents, exenatide and etanercept, became available that warranted testing in ITx.

Exenatide, like GLP-1 and its analogues, enhances glucose-stimulated insulin secretion and inhibits glucagon secretion (6,7). In experimental models, GLP-1 analogues protected islet β -cells from stress and immunosuppressive drug-induced apoptosis *in vitro* and *in vivo* (8,9). In rodents, they promoted pancreatic progenitor cell differentiation and improved β -cell function and viability (10-12). In human ITx recipients, islets responded to GLP-1 treatment during glucose challenge (13). In two other groups, exenatide administration in ITx recipients with GDF resulted in improved responses to glucose and meal challenges and reduction in exogenous insulin requirements or maintenance of II (14,15). Hence, exenatide has the potential to improve existing islet function, prevent loss of functional islet mass and possibly even stimulate islet regeneration.

Etanercept is a dimeric fusion protein consisting of the extracellular binding portion of the human tumor necrosis factor (TNF) receptor linked to the Fc portion of human IgG1.

Etanercept binds and deactivates TNF α , a key regulator of inflammatory immune responses and has been shown to be toxic to human β -cells (16). Etanercept in rodent models improved functional outcome of marginal mass islet allografts (17). In human islet allotransplantation, its use was associated with II in two different marginal mass studies; one combined with antithymocyte globulin and daclizumab (18) and another with exenatide (19). Hence using etanercept to decrease the inflammatory response from the ITx procedure and promote better islet engraftment, has the potential to improve short and long-term islet survival and function.

The aim of this study was to assess the impact of EXN and etanercept as part of the peri-transplant induction regimen, and long term EXN administration, on islet engraftment and short and long term function in subjects undergoing SI.

MATERIALS AND METHODS

Definitions

Islet transplant completion—Achievement of II after sequential ITx usually observed after two islet infusions. At 3-month post-completion the best function after ITx is generally observed in II subjects with stable glycemic control (20).

Graft Dysfunction (GDF)—C-peptide positivity, but elevation of, fasting capillary glucose more than 140 mg/dL or 2-hr-postprandial capillary glucose more than 180 mg/dL, on three or more occasions in a week, or two sequential monthly HbA1c values more than 6.5% (21).

Insulin independence(II)—C-peptide positivity and absence of GDF.

Supplemental Islet Infusions (SI)—Additional single islet infusion performed in subjects with GDF.

This study was a non-randomized pilot clinical trial. The protocols were approved and monitored by the Institutional Review Board and signed informed consent was obtained from all subjects.

Historical subjects (SI-Control) consisted of five islet recipients transplanted in an Edmonton-like protocol as previously described (4). The EXN subjects (SI-EXN) consisted of one subject from this same protocol and three subjects from the Immune Tolerance Network (ITN) protocol (2). These subjects were commenced on daily EXN treatment for GDF and were eligible for SI if they did not re-achieve II after 3 months of treatment. Follow-up until 18 months post-SI was completed by all subjects. The primary outcome was the re-establishment of II following SI with etanercept/daclizumab induction and prolonged exenatide treatment.

To see if SI outcomes were comparable with initial islet infusion outcomes, metabolic data at 3 months post-supplemental and initial islet infusions (completion) were compared.

Subjects with GDF were commenced on subcutaneous EXN (Byetta®, Amylin Pharmaceuticals, Inc. San Diego, CA and Eli Lilly and Company, Indianapolis, IN), as described (15). Median dose was 17.5 µg (10–25 µg), for a median time of 171 days (155–197) prior to SI. One-hour before SI, a single exenatide dose was administered subcutaneously (median 5, range 5–10 µg). After SI, each subject continued exenatide at pre-SI levels (Table 1), and subsequently doses were adjusted as necessary as dictated by clinical condition.

Islets were processed as described (22,23). Initial islet transplants were performed as previously described (2,4), the main difference being fresh (ITN) versus cultured islets.

In SI-control group, for SI, induction consisted of daclizumab (Zenapax, Roche, Nutley, NJ) 1mg/kg biweekly for 5 doses starting at transplant. This was continued monthly in first year and bimonthly in second year. Maintenance immunosuppressive drugs consisted of tacrolimus (Prograf, Astellas Pharma US, Inc., Deerfield, IL) and sirolimus (Rapamune, Wyeth Pharmaceuticals, Inc., Madison, NJ) (4,24).

In SI-EXN subjects, induction and maintenance immunosuppression was the same as SI-control subjects, except for administration of EXN and etanercept (Enbrel, Immunex Corporation, Thousand Oaks, CA). Etanercept dosage was 50 µg IV one hour pre-transplant then 25 µg subcutaneously twice a week for two weeks. Daclizumab induction consisted only of initial five doses.

Mixed meal tolerance test (MMTT, 5hr) and intravenous glucose tolerance test (IVGTT) (15, 21) without exenatide injection were performed during follow-up visits every 3 months to assess graft function. In SI-EXN group, additional MMTT with exenatide was performed to evaluate its acute effects. Exenatide was administered 1 hr before MMTT on the first day and then discontinued entirely until MMTT without EXN, typically performed on the third day of the visit.

To evaluate if acute administration of exenatide could improve first phase insulin release (FPIR), IVGTT with and without exenatide administration before the tests were performed at the 15-month follow-up visit.

Statistical Analysis

Baseline characteristics were compared between SI-EXN and SI-Control groups with two-tailed t-tests. Kaplan-Meier survival curves were used to assess II duration in the two groups. II duration at 12 and 18 months post-SI was compared with an exact two sample test of proportions. For each metabolic and clinical outcome measure, a linear mixed model regression was fit to the data to estimate and compare mean response values corresponding to all acute and chronic assessments of interest. This method generalizes linear regression techniques allowing for repeated observations by taking into account correlation within observations from the same subject to more appropriately estimate variances used in tests of significance. Therefore, we can simultaneously estimate and compare inter- and intra-group differences over time while appropriately accounting for within subject correlation. Similarly, we can estimate differences between MMTT performed with and without exenatide at 3–12 and 18 months of follow-up and between IVGTT performed with and without exenatide at 15 month follow-up. Post-SI IVGTT and MMTT results were compared with 3 months post-islet completion period. Results are expressed as means $\pm$ SE, a p -value <0.05 was considered statistically significant.

RESULTS

Subject Characteristics

SI-Control and SI-EXN groups had similar baseline characteristics except for II duration after initial islet infusion(s), 221 ± 58 vs 648 ± 34 days respectively, $p < 0.001$ and GDF duration before SI, 326 ± 93 vs. 664 ± 83 days, $p = 0.03$ (Table 1). All subjects in both groups had negative T-cell crossmatch pre-SI and negative PRA pre and post-SI. The mean HLA class I and II mismatch/HLA repetition among donors were similar. The SI-Control and SI-EXN groups received mean of 8713 ± 2123 and 5613 ± 485 IEQ/kg, respectively ($p = 0.25$). There were no significant differences in donor characteristics, cold ischemia time or perfluorocarbon use. Islet isolation techniques were comparable. Main difference was that for SI-control group, DNAase (0.625 mL/liter, Pulmozyme [dornase alfa] recombinant Genentech, Inc., San Francisco, CA) was used throughout the isolation procedure including digestion, dilution, and wash. For SI-EXN group, DNAase was only used during the digestion inside Ricordi chamber, whereas heparin (10 units/mL, Heparin, Sodium, American Pharmaceutical Partners, Schaumburg, IL) was used throughout isolation procedure. Digestion was performed with Liberase enzyme blend (Roche Pharmaceuticals, Basel, Switzerland) in both groups. Islets were cultured in both groups. Islet viability and stimulation index were not different in both groups. IV heparin was given in the infusion bag in both groups (4). There were no differences in peri-transplant insulin regimen. No group received IV insulin therapy. Immunosuppressive drug levels were comparable during the study.

Baseline metabolic function was similar between the two groups before SI. Pre-SI mixed meal tolerance testing showed comparable AUC_{Glucose} (53489 ± 6084 vs 45714 ± 4407 mg·min·dl⁻¹; $p = 0.36$) and $AUC_{\text{C-peptide}}$ (596 ± 48 vs 619 ± 139 ng·min·ml⁻¹; $p = 0.86$) in SI-

Control and SI-EXN groups respectively. SI-EXN data is that obtained 48-hours after being off exenatide injections.

Insulin Independence and Clinical Outcomes

II was achieved in 3/5(60%) SI-Control and 4/4(100%) SI-EXN subjects. By 18 months only 1 out of 4 (20%) of SI-control subjects were II compared with 100% in SI-EXN group ($P=0.04$). Comparison of the survival curves illustrating II for these groups suggests significantly longer duration of II over the 18-months follow-up post-SI in the EXN group, compared to SI-Control ($p=0.025$) (Figure 1).

With development of GDF post-SI, HbA1c in SI-Control group increased with time and was no longer different to pre-SI at 12 and 18 months (Table 1). In contrast, improvement in HbA1c in SI-EXN group was sustained throughout follow-up (HbA1c decrement 0.48–1.2%, $p<0.05$).

SI-EXN subjects experienced nausea of varying degrees at the beginning of exenatide administration (pre-SI) (15). After SI, no side effects related to exenatide or etanercept were reported. Subjects were tolerating exenatide well. Body weight was stable throughout study period.

Metabolic and Hormonal Responses to Stimulation Testing

IVGTT—Up to 9 months post-SI, acute insulin response to glucose (AIRg) in SI-EXN group was similar to that observed at 3 months post-completion of the initial islet transplant (20.8 ± 0.9 vs 19 ± 2.9 $\mu\text{U/mL}$, respectively, $P=0.55$). Over time (Table 2), AIRg values in the absence of exenatide prior to the test were 10.2 ± 3.2 , 9.6 ± 2.3 and 11.7 ± 1.7 $\mu\text{U/mL}$ at 12, 15, and 18 months, respectively.

MMTT—A significant and sustained increase in peak and $\text{AUC}_{\text{C-peptide}}$ responses to MMTT in SI-EXN group was observed at all time points ($p<0.002$) (Figure 2) while $\text{AUC}_{\text{C-peptide}}$ in SI-Control (680 ± 86 $\text{ng}\cdot\text{min}\cdot\text{mL}^{-1}$) was not different from pre-SI (596 ± 43 $\text{ng}\cdot\text{min}\cdot\text{mL}^{-1}$). SI-EXN subjects demonstrated higher $\text{AUC}_{\text{C-peptide}}$ at 3, 9, and 15 months than SI-control ($P<0.01$). $\text{AUC}_{\text{C-peptide}}$ in SI-EXN 3-months post-SI (1024 ± 75 $\text{ng}\cdot\text{min}\cdot\text{mL}^{-1}$) was higher than 3-months post-islet transplant completion (708 ± 48 $\text{ng}\cdot\text{min}\cdot\text{mL}^{-1}$ $p<0.001$) and pre-SI (620 ± 121 $\text{ng}\cdot\text{min}\cdot\text{mL}^{-1}$, $p<0.001$). To correct for glucose levels, the ratio of the $\text{AUC}_{\text{C-peptide}}$ to- $\text{AUC}_{\text{Glucose}}$, namely mixed meal stimulation index (MMSI) (25) was calculated. Significant and sustained increase in MMSI was observed in SI-EXN group throughout time ($P<0.01$) (Table 2). In SI-Control group, an increase in MMSI was only observed at 3–9 months as compared to pre-SI ($p<0.03$). Amylin levels in SI-EXN group followed the same pattern as C-peptide values (Figure 3). The abnormal glucagon secretion observed pre-SI was ameliorated by chronic exenatide administration. $\text{AUC}_{\text{Glucagon}}$ at 6 and 15 months were 15407 ± 2988 and 16929 ± 1627 , respectively vs 21812 ± 888 $\text{pg}\cdot\text{min}\cdot\text{mL}^{-1}$ pre-SI ($p<0.03$).

Fasting plasma glucose post-SI in both groups was not different from pre-SI. $\text{AUC}_{\text{Glucose}}$ was significantly decreased at 3–18 months in SI-Control ($p<0.026$) and 3–15 months in SI-Exn ($p<0.029$). $\text{AUC}_{\text{Glucose}}$ was not significantly different between groups (Figure 2). Note that the comparison was made with mixed meal tests without exenatide and off for 48-hours.

Effects of acute exenatide administration: We observed improvement of first and second phase insulin release in the SI-EXN group, when exenatide was administered 1-hour before the **IVGTT** at 15 months (AIRg 20.6 ± 4.2 vs 9.6 ± 2.3 $\mu\text{U/mL}$, $p=0.02$; $\text{AUC}_{\text{insulin}}$ at 10 to 60 min 1117 ± 189 vs 584 ± 74 $\mu\text{U}\cdot\text{min}\cdot\text{mL}^{-1}$, $p=0.036$ with and without EXN, respectively) (Figure 3). This was accompanied by faster glucose disappearance rate (K_{30} -1.65 vs -0.95 $\%/min$, $p=0.03$, with and without exenatide, respectively).

Acute EXN administration resulted in suppression of the abnormal glucagon response during **MMTT** (Figure 3). The post-prandial glucose rise was also prevented. C-peptide and amylin responses were delayed and diminished and followed glucose. These changes were maintained up to 18 months post-SI.

DISCUSSION

SI is a potential solution for GDF provided that further islet loss can be prevented. At our center, SI were performed in five subjects in an 'Edmonton-like' protocol with some success (4). The rationale then was to see if restoring islet mass to greater than marginal levels can result in longer duration of II. While II could be restored in many subjects, it deteriorated over time similar to that seen with initial islet infusions. With the emergence of exenatide and etanercept better islet survival was potentially possible and since subjects in these protocols were already immunosuppressed they were considered a good group to test these agents.

Administration of these two agents at time of SI resulted in significantly improved short and long term outcomes, notably all subjects were II by 18 months in SI-EXN group, compared to only one in SI-Control group.

Limitations of this study are the small sample size and the non-randomized sequential comparison of SI-EXN with historical controls which may lead to differences between the two groups that affect outcomes. Indeed, SI-EXN subjects had longer duration of both II and GDF following initial infusions however the groups were equal in their metabolic testing suggesting functional β -cell mass should be comparable.

Similarly, evaluation of the islet product in both groups demonstrated minor differences in islet preparation techniques, however IEQ/kg, viability, and stimulation index were not significantly different in the two groups.

Metabolic testing in the SI-EXN groups post-SI demonstrated that not only was transplant function restored to the best it had ever been, it was even better with superior responses to both mixed meal and intravenous glucose stimuli (i.e, significantly higher and sustained C-peptide responses were observed after MMTT; and up to 9 months, insulin secretion in response to intravenous glucose was sustained). In addition, at 15 months, acute exenatide administration was able to restore first and second phase insulin release. These shows improved β -cell secretory capacity similar to that observed after exenatide administration in patients with type 2 diabetes (26,27).

In contrast, exenatide treatment of ITx recipients with existing GDF (14,15,19) or marginal mass islet transplantation did not restore FPIR (14,19) suggesting that FPIR is dependent on certain number of functional islets. Treatment with exenatide should be more effective if administered at time of initial islet infusions and continued chronically. The greater achievement of II in SI-EXN group may be due to prevention of apoptosis, protection from immunosuppressive-mediated toxic effects (8,9,28) at time of islet infusion, thereby improving engraftment. These same effects of exenatide, plus its ability to improve glycemic control through glucagon secretion suppression and reduced gastric emptying may explain the long-term functional improvement.

Finally, the possibility of increased islet regeneration induced by GLP-1 analogues (10-12) has been demonstrated in animal models, but remains to be proven in human studies and until islet mass can be accurately measured will likely remain elusive. The current preparation of exenatide with its side effects may limit the use in some patients.

These data do not allow differentiation between the effects of etanercept and exenatide. Etanercept administration at peri-transplant and early post-transplant period may have contributed to better islet engraftment resulting from its anti-inflammatory effects (18,19), a synergistic effect with exenatide cannot be excluded. It is unlikely that etanercept had a significant impact in the long term effects observed, except for an increased islet mass at outset.

Abnormal postprandial hyperglucagonemia has been documented in subjects with diabetes (29-31). We confirmed post-prandial hyperglucagonemia in this ITx population also. Acute EXN administration resulted in significant suppression in glucose and glucagon elevation during MMTT. These effects were sustained throughout the period of follow-up. C-peptide and amylin were also suppressed, likely due to near normal glucose levels, secondary to EXN related glucagon suppression and decreased gastric emptying (32).

As mentioned above, there are important limitations to this study; these observations will be evaluated in a prospective randomized controlled fashion in the imminent Clinical Islet Transplant Consortium trial.

Based on the 18-month data collected so far, it appears that the effects of exenatide plus etanercept treatment on SI grafts are highly beneficial in sustaining long-term islet survival and function thereby improving the outcomes of islet transplantation. Further benefits could be gained from the use of EXN at all stages of islet preparation, transplantation, and follow-up. This could result in even greater effects on engraftment and long-term islet allograft function and may even allow for more consistent achievement of sustained II after single donor ITx.

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ABBREVIATIONS

AUC, Area under the curve
 GDF, Graft dysfunction
 GLP-1, Glucagon-like peptide 1
 EXN, Exenatide
 FPIR, first phase insulin release
 IEQ, Islet equivalents
 II, Insulin independence
 ITx, Islet transplant/Islet transplantation
 IVGTT, Intravenous glucose tolerance test
 MMTT, Mixed meal tolerance test
 SI, Supplemental islet infusion
 TNF- α , Tumor necrosis factor alfa

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FIGURE 1. Insulin-independence probability

Kaplan-Meier survival curves illustrating insulin independence (accounted from time of achievement of insulin independence to reintroduction of insulin) in supplemental islet infusions-exenatide (SI-EXN) (discontinuous line) and SI-control group (continuous line) ($P=0.025$).

Figure 2

FIGURE 2. Metabolic profile after supplemental islet infusion in the control (A & B) and SI-EXN group (C & D)

Glucose (A) and C-peptide (B) responses to a 5-hr mixed meal tolerance test at pre-supplemental islet infusion (Pre-SI; white triangle), 3 (3 mo post-SI; white squares) and 18 months (18 mo post-SI, black squares) post supplemental islet infusion in SI-control group. Glucose (C) and C-peptide (D) responses to a 5-hr mixed meal tolerance test at pre-supplemental islet infusion (Pre-SI; white triangle), 3 (3 mo post-SI; white squares) and 18 months (18 mo post-SI, black squares) post supplemental islet infusion in SI-EXN group. Responses at 3 and 18 months follow-up are those from MMTTs without exenatide administration before the test (Exn-). Three months post initial islet transplant completion (3 mo Post-C; grey circle) responses are depicted in SI-Control and SI-EXN figures for comparison.

Figure 3

FIGURE 3. Metabolic and hormonal effects of acute exenatide administration (IVGTT, A&B; MMTT, C, D & E)

Glucose (A) and insulin (B) responses to IVGTT at 15 months post supplemental islet infusion in SI-EXN group, with (Exn+; black squares) and without (Exn-; white squares) exenatide administration before the test. There is a significant improvement of first and second phase insulin release in supplemental islet infusions under exenatide administration:

Glucose (C), C-peptide (D), glucagon (E) responses to a 5-hr mixed meal tolerance test at 18 months post supplemental islet infusion in SI-EXN group, with (Exn+; black squares) and without (Exn-, white squares) exenatide administration before the test. A suppression of glucose, C-peptide and glucagon responses is observed after acute exenatide administration.

Subcutaneous exenatide administration was given at -60 min in the Exn+ test.

Table 1

Demographic characteristics of SI-Control and SI-EXN groups, and follow-up at 3, 12 and 18 months post-supplemental islet infusion.

	Supplemental Infusion (SI)	
	SI-Control (SI-C)	SI-Exenatide (SI-EXN)
n	5	4
Age (years)	45.6±4.09 (38–61)	45.8±4.13 (38–54)
Diabetes Duration (years)	33±6.03 (12–48)	29.2±6.7 (22–49)
Weight (kg)	68.0±5.2	67.7±8.8
IND period before SI (days)	221±58	648±34 **
GDF to SI (days)	326±93	664±83 **
Total IEQ received	557960±99148	364384±15282
IEQ/kg of SI	8713±2123	5613±485
% islet viability	82±11	96±1
Stimulation index	1.5±0.2	2.3±0.8
No. of HLA class I and II MM		
1 st infusion	5	5
2 nd infusion	5	5
SI	4	5
No. of HLA class I and II repetition	3	2
Time on EXN pre SI	NA	171 (155–197)
EXN dose (µg)		
Pre-SI	NA	17.5 (10–25)
3 months	NA	13.75 (12.5–30)
12 months	NA	11.9 (1.25–30)
18 months	NA	13.75 (0–30)
Time to IND after SI (days)	39 (0–81)	55 (38–233)
IND after SI		
3 months	3/5 (60%)	3/4 (75%)
12 months	2/5 (40%)	4/4 (100%)
18 months	1/5 (20%)	4/4 (100%) **
Insulin dose (IU/Kg/day)		
Pre-SI	0.12± 0.05	0.15±0.01
3 months	0.058±0.03	0.022±0.02
12 months	0.1±0.06	0
18 months	0.21±0.13	0
HbA1c (%)		
Pre-SI	6.58±0.08	6.68±0.12
3 months	6.02±0.18 *	5.48±0.07 *
12 months	6.18±0.36	6.07±0.18 *
18 months	7.05±0.55	6.2±0.08 *

Values expressed as mean±SE or median (range)

SI: supplemental islet infusion, EXN: Exenatide, IND: Insulin Independence, GDF: Graft

Dysfunction, IEQ: Islet equivalent number. MM: mismatch

*
p<0.05 compared to pre-SI

**
p<0.05 compared to control group

Table 2
Metabolic variables (AIRg and MMSI) observed before and at different time points after supplemental infusion in each patient of the SI-Control and SI-EXN groups.

Variable	Time point	SI-Control					SI-EXN			
		1	2	3	4	5	1	2	3	4
AIRg	pre-SI	3.64	0.76	ND	1.13	17.05	0.56	1.01	0.00	2.05
	3mo	1.39	7.91	28.81	20.21	18.36	24.11	14.56	14.71	5.65
	9mo	3.70	0.63	ND	22.11	21.23	21.96	17.65	22.95	20.55
	15mo	0.00	1.36	29.11	19.36	16.90	4.43	7.23	9.96	16.73
	18mo	0.41	0.15	ND	34.39	18.35	5.86	13.28	12.66	14.83
MMSI	pre-SI	0.32	0.21	0.41	0.42	0.62	0.16	0.65	0.41	0.67
	3mo	0.35	ND	1.04	0.67	0.68	0.72	1.39	0.78	0.99
	9mo	0.15	0.57	0.82	0.78	0.84	0.73	1.14	0.91	1.27
	15mo	0.10	0.46	0.65	0.82	0.48	0.47	0.88	0.94	1.06
	18 mo	ND	0.24	ND	0.84	0.75	0.54	0.74	0.55	1.38

Numbers marked in italics represent subjects on insulin at the time of the metabolic test.
ND=Not done