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Continuous Subcutaneous Insulin Infusion in Patients With Type 2 Diabetes: A Cohort Study to Establish The Relationship Between Glucose Control and Plasma Oxidized Low Density Lipoprotein

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Abbreviations: (BMI) body mass index, (CGM) continuous glucose monitoring, (CSII) – continuous subcutaneous glucose infusion, (MDI) multiple daily injections, (NO) nitric oxide (OAD) oral antidiabetic drugs, (Ox-LDL) oxidized low density lipoprotein, (ROS) reactive oxygen species

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2 Tables, 2 Figures

ABSTRACT

Background

Oxidative stress is a detrimental feature of diabetes implicated in the progression of the disease and its complications. The relationship between insulin therapy and oxidative stress is complex. This study tested the hypothesis that improved glucose control, rather than insulin dose, is central to reduced oxidative stress in patients with type 2 diabetes following continuous subcutaneous insulin infusion (CSII).

Methods

In this 16-week, multicentre study, 54 CSII-naïve patients with type 2 diabetes (age 57±10y, HbA1C 69 ±15 mmol/mol [8.5±1.4%], diabetes duration 13±6y) treated with either oral antidiabetic agents (OAD) alone (n=17), basal insulin ±OAD (n=17) or multiple daily injections (MDI) ±OAD (n=20) were the evaluable group. Diabetes medications except metformin were discontinued, and 16 weeks of CSII was initiated. Insulin dose was titrated to achieve optimal glycemic control. A plasma marker of oxidative stress relevant to cardiovascular disease (oxidized low density lipoprotein [ox-LDL]) was assessed at baseline and week 16.

Results

CSII improved glycemic control (HbA_{1C} -13±2 mmol/mol [-1.2±0.2%]; fasting glucose -36.6±8.4 mg/dL; mean glucose excursion -23.2±6.5 mg/dL, mean±SE; all $P<0.001$) and reduced ox-LDL (-10.5%; $P<0.05$). The antioxidant effect was cohort-independent ($P>0.05$), but was significantly more pronounced in patients on statins ($P=0.019$). The effect of CSII was more

closely correlated to improvements in glucose excursion (P=0.013) than to insulin dose (P>0.05) or reduction in HbA_{1C} (P>0.05).

Conclusions

CSII induces depression of plasma ox-LDL associated with change in glucose control, rather than with change in insulin dose. The effect is augmented in patients receiving statins.

INTRODUCTION

The incidence of type 2 diabetes is increasing rapidly and, with it, the requirement for insulin therapy in advanced disease.[1] Poor glycemic control, as measured by glycated haemoglobin (HbA_{1C}), has long been linked to mortality associated with diabetes[2] and improvement in control is associated with reduced complications.[3] However, intensive therapy to achieve near-normal HbA_{1C} levels in patients with type 2 diabetes has not been shown to reduce cardiovascular events[3, 4] and is associated with a rise in all-cause mortality.[4] The link between diabetes and vascular disease is complex, but oxidative stress, mediated by increased prevalence of harmful reactive oxygen species (ROS), is associated with type 2 diabetes and has been implicated both in its progression via β-cell dysfunction and with macro- and microvascular diabetes complications.[5-7] There is a close relationship between plasma-borne markers of oxidative stress (e.g. oxidized low density lipoprotein [ox-LDL]) and risk of coronary artery disease and stroke.[8, 9] The origins of oxidative stress associated with type 2 diabetes are complex,[10] with mitochondrial dysfunction induced by hyperglycemia and/or excessive glycemic excursions[11], together with advanced glycated end products (AGEs)[12] and inflammation, all contributing to the effect. It follows that improved glycemic control would be expected to reduce oxidative stress, a feature that might play an important role in reducing the rate of progression of β-cell dysfunction as well as improving cardiovascular outcomes. Basal or basal-bolus continuous subcutaneous insulin infusion (CSII; <0.04 unit/kg/day) has been shown to have a significant antioxidant effect in patients with type 2 diabetes, although the beneficial effect was lost in patients treated with higher insulin doses (>0.4 unit/kg/day)[13] – the complexity

of the association prompted the authors of that study to indicate the need for further study.

The benefits of CSII are well established in patients with type 1 diabetes.[14] In type 2 diabetes, studies assessing CSII versus multiple daily injection therapy have generally demonstrated similar improvements in overall glycemic control (as measured by HbA_{1C}),[15, 16] with some studies showing superior postprandial glucose control with CSII.[16, 17]

In previously published findings of the present16-week study, CSII in patients with type 2 diabetes was found to be preferred to patients' previous treatment regimens[18] and to significantly improve both HbA_{1C} and self-monitored 7-point glucose profiles.[19, 20] The aim of the present post-hoc analysis of samples from the same study was to test the hypothesis that these benefits in glycemic control were reflected in depression of markers of oxidative stress.

METHODS

Sample size

A sample size of 20 patients in each cohort was estimated to produce a 90% confidence interval equal to the sample mean with a precision of 0.44 with an estimated standard deviation of 1.2, with respect to HbA_{1C}.

Patients

Fifty-eight patients with type 2 diabetes treated with oral antidiabetic agents (OAD), with or without insulin enrolled in the study at 6 US study sites

between March & December 2008. Approval was obtained from local ethics committees prior to commencement of the study. Written consent was received from all patients taking part in the study, which complied with the Declaration of Helsinki and its amendments.

Enrolled patients were men and women 18-75 years old with type 2 diabetes (HbA_{1C} 53-91 mmol/mol [7.0-10.5%]) and with undetectable anti-glutamic acid decarboxylase antibodies. Exclusion criteria included prior CSII, women who were pregnant, lactating or planning pregnancy, as well as patients with evidence of cardiovascular disease within the last year, including myocardial infarction, stroke, arterial revascularization and/or angina with ischemic changes on ECG at rest, changes on graded exercise test, or positive cardiac imaging test results. Subjects with a past history of cardiovascular events (>1 year from screening) were enrolled if the subject had been stable for at least 6 months and, in the investigator's opinion, the history of cardiovascular disease would not affect successful completion of the study and/or personal well-being.

Eligible patients were assigned to one of three cohorts defined by their therapeutic regimen at baseline: stable regimen of two or more OAD agents (OAD cohort, n=18); basal insulin with or without OAD (basal cohort, n=18) or basal-bolus insulin therapy by multiple daily injections (MDI) of insulin with or without OAD (MDI cohort; n=22). One patient (MDI cohort) withdrew consent, one patient (OAD cohort) did not complete the study (adverse event of severe coronary artery disease), one patient (MDI cohort) was withdrawn from

the study and one patient (MDI) was lost to follow up; the data for these individuals have been excluded from the analysis throughout, leaving a total evaluable population of 54 patients, of which samples were available for 53 patients for oxidative stress measures. Complete sample sets were available for 50 patients for glucose excursion measures, 51 patients for mean glucose measures and 52 patients for HbA_{1C} and fasting plasma glucose measures.

Protocol

Details of the study protocol have been previously reported.[19] In brief, patients entering the study were withdrawn from all antidiabetic medications except metformin for the duration of the study. Sixteen weeks of CSII (Animas® 2020 insulin pump, Animas Corp, West Chester, PA, USA) was initiated using insulin glulisine (Sanofi Aventis, Bridgewater, New Jersey, USA) with one daily basal rate (50% of total daily dose) and insulin boluses (50% of total daily dose) split across each major meal. The initial recommended total daily insulin dose was 0.5 U/kg body weight. Investigators assessed insulin dosing at frequent study site visits, with a target of safely achieving fasting plasma glucose concentrations between 70-130 mg/dL and 1.5-2 h postprandial glucose values below 180 mg/dL.

Measurements

HbA_{1C} was measured in fresh blood samples at baseline and weeks 4, 8, 12 and 16 (Covance Laboratory, Indianapolis, IN, USA). The data relating to these measures have been reported elsewhere.[18-21]

Continuous glucose monitoring (CGM) was conducted using the DexCom seven system. CGM was implemented for 5-7 days prior to Day 1 of CSII (baseline), and for 5-7 days prior to Visit 11 (Week 16). Glucose values from the CGMs were downloaded via USB cable at the study site using the DexCom DM2 Software application (monitor-specific software). The mean percent of CGM blood glucose measurements within the following glucose value ranges was summarized: <70 mg/dL, ≥70 mg/dL to ≤140 mg/dL, >140 mg/dL, ≥70 mg/dL to ≤180 mg/dL, and >180 mg/dL. Data are shown as % time spent with blood glucose between 70mg/dL and 140 mg/dL, as an indicator of tight glucose control and absence of substantial excursion in either direction.

Fasted venous blood samples were drawn from the antecubital fossa (~60 ml) into EDTA tubes for separation of plasma, and blank tubes for separation of serum, at baseline and week 16. Samples were immediately centrifuged; aspirated plasma and serum was frozen and stored below -70^{o}C for subsequent analyses.

Laboratory analyses

Plasma oxidized low density lipoprotein (ox-LDL) was selected for this study on the basis that it is not only a marker of oxidative stress, but is also implicit

in cardiovascular disease development. Ox-LDL was measured using a commercially available kit (Oxidized LDL competitive ELISA, Mercodia; Salem, NC, USA). Measurements were conducted on samples from baseline and week 16.

Statistical analysis

Data were compared using Student's *t*-tests, one way and two-way ANOVA with Bonferroni post-tests, and using Pearson's correlations. $P<0.05$ was considered statistically significant, except for correlation data, where $P<0.0167$ was considered significant to correct for multiple comparisons.

Underlying research materials can be requested from the corresponding author.

RESULTS

Patient characteristics

Baseline patient demographics and characteristics are shown in table 1. There were no significant differences between the cohorts. Approximately 90% of patients were treated with metformin at baseline – this treatment was continued throughout the study.

Impact of CSII on glucose control and ox-LDL: collated data from all three cohorts

HBA_{1C}, fasting glucose, mean glucose and mean postprandial glucose excursion all declined significantly from baseline after 16 weeks of CSII (Table 2). Similarly, plasma ox-LDL was significantly depressed after 16 weeks of CSII compared to baseline (Table 2).

Analysis of data by study cohort

There was no significant difference in effect of 16 weeks of CSII on HbA_{1C} (P=0.51), mean plasma glucose (P=0.98), fasting plasma glucose (P=0.80) or mean glucose excursion (P=0.70; all statistics are one-way ANOVAs between OAD, basal and MDI cohorts). There was no significant difference in plasma concentrations of ox-LDL between the cohorts at baseline (P=0.86); the extent of effect of CSII on ox-LDL was not significantly different between the three cohorts (Fig 1A; P=0.50).

Effect of statin therapy on ox-LDL: collated data from all three cohorts

Sub-group analysis of the data with respect to statin therapy indicated that there was no difference between ox-LDL at baseline in those patients receiving statins (n=22) compared to those not receiving statins (n=31; P=0.21, data not shown). However, 16 weeks of CSII induced a significantly greater reduction in ox-LDL in patients treated with statin therapy compared with those not receiving statin therapy (*P*=0.019; paired Student's *t*-test; Figure 1B).

Association between ox-LDL and changes in insulin dose, HBA_{1C} or tight glucose control

There was no correlation between change in ox-LDL and either change in insulin dose (Figure 2A) or change in HbA_{1C} (Fig 2B) over the 16 week CSII period. However, there was a significant negative correlation between change in ox-LDL and change in the time spent between 70-140 mg/dL glucose, as measured by CGM in week 1 and week 15 (Fig 2C).

DISCUSSION

This study, involving 16 week CSII in patients with type 2 diabetes, shows the therapy to reduce HbA_{1C} and to improve glycemic control, irrespective of whether patients were treated with OAD, basal insulin or MDI prior to starting CSII; there was no difference in any of the plasma markers between the patient cohorts at baseline. CSII significantly reduced plasma ox-LDL concentrations; the depression in plasma ox-LDL correlated with improved glucose control (reduced glucose excursions), but not with insulin dose.

Baseline characteristics of the patients showed remarkable equivalence across the OAD, basal insulin and MDI cohorts. Of the parameters measured, only total insulin dose showed a predictable difference between the cohorts (nil in OAD and highest in MDI). Importantly, plasma ox-LDL was not different at baseline in the three cohorts. This result varies from a previous study comparing oxidative stress in patient groups under different treatment regimens, [13] but a direct comparison between the studies is not appropriate because there were considerable differences in the patient populations in the two studies: those in the current study were generally younger, had diabetes for a shorter period, had a lower BMI, did not have clinical symptoms of cardiovascular disease in the past year and had lower HbA_{1C} at the outset of the study. Furthermore, the current study defined the insulin-treated cohorts by insulin regimen (basal v MDI), as opposed to total daily insulin dose, and measured a plasma marker of oxidative stress (ox-LDL), instead of urinary isoprostanes. Therefore, the current findings do not challenge the results or

conclusions of the previous report; indeed, they serve to endorse the conclusion that the relationship between insulin therapy and oxidative stress is highly complex, depending not only on insulin dose and mode of delivery, but also on a wide range of patient characteristics.

Transfer of patients to CSII had a significant effect on glycemic control, as determined by HbA_{1C}, mean fasting and postprandial glucose concentrations, and pre- to post-meal glucose excursions. The effect was similar in all three cohorts for each of these measures, which is not surprising, given that insulin dosing was adjusted to achieve similar fasting and postprandial glucose targets.

Given the well-documented role of oxidative stress in driving progression of type 2 diabetes[22] and in mediating the macrovascular complications that are associated with the disease,[5, 7] modulation of oxidative stress represents an important target for therapeutic intervention. Hyperglycemia and glucose excursions are at the core of the oxidative stress process, instigating mitochondrial dysfunction and generation of AGEs - prime sources of harmful ROS.[12] The downstream link to cardiovascular disease is driven by a combination of the essential step of ROS-mediated oxidation of LDL in the atherogenic process and the deleterious effect of ROS on endothelial cell survival, function and ability to generate bioavailable, protective, nitric oxide (NO).[10]

Our findings clearly indicate that CSII causes a substantial depression of plasma ox-LDL, a key mediator and predictor of cardiovascular disease.[23] CSII-mediated depression of this marker might prove to be beneficial in reducing cardiovascular risk in this patient group. The extent of the depression

in ox-LDL seen in the current study is similar to that reported for introduction of a multiple insulin injection regimen to previously insulin naïve patients (-15%). [24]

Cohort sub-analysis of the current data revealed that, whilst CSII induced an overall reduction in ox-LDL, there was no statistical difference between the effect in different cohorts, although those previously on basal insulin showed an apparent increase in this marker of oxidative stress. A previous study[25] had shown that gliclazide reduced a number of markers of oxidative stress compared to glibenclamide, but we excluded this as a potential confounder in our study because no patients were on gliclazide at recruitment and sulphonylureas were withdrawn at the start of the study. Statin therapy represents a possible confounder in the current study because previous work has shown that atorvastatin has a profound effect on plasma ox-LDL (-24%),[26] raising the possibility that those patients on statins might be less exposed to oxidative stress and, therefore, less affected by CSII in this regard. Sub-group analysis of the data from the current study, however, indicated that there was a significantly larger effect of CSII on ox-LDL in those patients receiving statin therapy. This finding raises the intriguing possibility of a synergistic effect between CSII and statins that would need to be confirmed and explored further.

Given the previous finding that insulin dose might be important in driving the extent of oxidative stress,[13] it is important to consider this parameter in our study. However, we found that there was no correlation between total insulin dose and plasma ox-LDL, suggesting that the antioxidant effect of insulin was independent of the insulin dose. Instead, it was clear that there was an association between the extent to which blood glucose control was improved

and the reduction in plasma ox-LDL. This is the principal finding of the study because, coupled with the lack of association between HbA_{1C} and ox-LDL after CSII, it implies that glucose excursion rather than glycemia *per se* is the key to determining the extent of plasma LDL peroxidation. This finding corresponds with those relating to other therapies for glucose control[27, 28] and to the suggestion that there is a link between glucose variability and oxidative stress[29] and glucose variability and atherogenic potential[30, 31] that might be worth considering alongside HbA_{1c} as an indicator of risk.

From a therapeutic perspective, this study indicates that switching from MDI to CSII offers a reduced oxidative stress profile, at least over a 16 week period; a direct parallel group study would need to be conducted to determine whether CSII offers an attractive alternative to MDI on this count. A similar improvement might also be realized in patients on OAD, although it is recognized the concept of transferring patients direct from OAD to CSII is an unlikely scenario in practice. The same benefits might not, however, be achieved for those already receiving basal insulin, a result that resonates with the lack of benefit seen with basal insulin with respect to cardiovascular outcomes.[32] Nevertheless, improved glycemic control during daytime and reduced postprandial excursions in response to basal insulin could have other beneficial effects with respect to outcomes in this patient group.

Study Limitations

The major limitation of this study is the lack of a parallel control group, which would be important for any follow-up study. However, the lack of a control

group does not impact on the between-cohort differences or the correlations found. At present we can only surmise that the effects on ox-LDL are in response to intensive insulin therapy, rather than to CSII *per se*, although the impact seen in the cohort formerly on MDI hints at a unique effect of CSII, but this would need to be tested in a study designed specifically for that purpose. This was a relatively small study but nevertheless had sufficient power to establish an association between tight glucose control and ox-LDL – a larger study is warranted to fully explore the possibility of an association between CSII-induced changes in glucose control and markers of oxidative stress. In addition, the retrospective nature of the study represents a limitation in that only baseline and 16 week samples were available for measurement of ox-LDL; it would be interesting to establish the time course of the reduction and to determine the acute impact of glucose excursion in the postprandial period. Whilst the participants were well-matched across the cohorts, it is likely that those patients already receiving insulin have more advanced disease (higher insulin requirements likely reflecting greater β-cell failure), irrespective of the fact that they have not necessarily been diagnosed with the disease for longer.

Conclusion

Implementation of CSII in type 2 diabetes is a contentious issue, with conflicting evidence from several trials as to its relative merits in this setting compared to MDI regimens.[15, 16, 33] The results from our study suggest that CSII might be a useful tool in reducing the consequences of type 2 diabetes by helping to break the oxidative stress link to advanced disease and

cardiovascular complications. This open-label, uncontrolled study suggests that CSII not only improves glycemic control in patients with type 2 diabetes, irrespective of their pre-pump therapeutic regimen, but also has the additional benefit of reducing ox-LDL, an important player in the atherosclerotic process. This benefit is associated with a reduction in glucose excursion rather than insulin dose or HbA_{1C}. Taken together, the findings suggest that a head-to-head assessment of CSII and MDI with respect to oxidative damage and, ultimately, cardiovascular outcome, is merited in patients with type 2 diabetes.

Trial Registration

This study has clinical trial registration number NCT00922649 at ClinicalTrials.gov.

Author contributions:

HA – design, interpretation and editing

AT –analysis of blood samples for oxidative stress markers

AS – analysis of data and editing

SMacR – interpretation and editing

SS –glucose measurements, interpretation, editing

JPF –design, interpretation and editing

ILM – design and data analysis of oxidative stress elements of project, interpretation, writing, editing

Conflict of Interest Statement

This study was funded by Animas Corp and Lifescan Scotland Ltd, both Johnson & Johnson Companies. Lifescan Scotland Ltd also part-funded the PhD studentship for AS and has funded a number of other UHI Department of Diabetes & Cardiovascular Science initiatives.

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TABLES

Table 1. Baseline demographics, characteristics and drug regimens at recruitment.

	OAD cohort	*Basal cohort*	*MDI cohort*	*All cohorts*
Intention to treat population (n)	18	18	22	58
Evaluable population (n)	17[a]	17[b]	20[c]	54
Male:female (n)	11:6	7:10	9:11	27:27
Age (y)	57±7	55±8	57±13	57±10
Diabetes duration (y)	11±6	14±6	15±6	13±10
Weight (Kg)	105±20	96±16	98±20	99±19
BMI (Kg/m^2)	35±5	34±5	34±5	34±5
Systolic BP (mmHg)	129±14	125±15	129±18	128±16
Diastolic BP (mmHg)	77±9	73±11	75±10	75±10
Triglycerides (mmol/L)	3.2±2.0	2.2±1.2	2.1±1.5	2.5±1.7
LDL (mmol/L)	2.6±0.8	2.7±1.1	2.6±0.9	2.6±0.9
Total daily pre-pump insulin (Units)	N/A	31.5±19.7[†††]	99.2±65.3	85.1±35.1
On-pump total daily insulin at day 1 (Units)	41.7±11.7[##]	36.7±12.0[***]	70.9±36.9	51. ±28.7
On-pump daily insulin at week 16 (Units)	46.4±43.9	30.6±16.3	51.9±48.3	43.5±39.8
Cigarette smokers (n)	1	0	1	2
Long-acting insulin analogue (n)	0	17	19	36
Rapid acting insulin analogue (n)	0	0	20	20
Intermediate acting insulin (n)	0	0	3	3
Regular human insulin (n)	0	0	1	1
Metformin (n)	18	15	16	49
Sulfonylurea (n)	16	6	4	26
Thiazolidinedione (n)	8	4	4	16
Exenatide (n)	3	8	1	12
DPP-4 inhibitor (n)	0	1	0	1
Meglitinide (n)	0	1	0	1
Pramlintide (n)	0	0	1	1
Statin therapy (n)	9	5	8	22

[†††] P<0.001 basal v MDI, one-way ANOVA with Bonferronni post-test; [##]P<0.01 OAD v MDI; ***P<0.001 basal v MDI. There were no other statistical differences between the cohorts with respect to the above parameters. Continuous data in this table are expressed as mean±SD. [a]1 patient did not complete; [b]1 patient withdrawn (investigator decision); [c]1 patient withdrew consent, one patient lost to follow-up. [d]Excludes OAD in all cohort data.

Table 2: Measures of glucose control, oxidative stress (ox-LDL): collated data for all cohorts. Data are expressed as mean±SE.

Measure	n	Baseline	16 weeks	P	Change (value)	Change (% baseline)
HbA_{1C} (mmol/mol) **HbA_{1C} (%)**	52	68±2 8.4±0.18	55±1 7.2±0.13	<0.0001	13±2- 1.2±0.2	-14.2
Fasting plasma glucose (mg/dL)	52	166.9±7.3	130.3±4.8	<0.0001	-36.6±8.4	-21.9
Mean plasma glucose (mg/dL)	51	177.5±5.2	152.2±4.4	0.0003	-25.4±6.5	-14.3
Mean daily postprandial glucose excursion (mg/dL)	50	38.5±5.1	15.3±5.9	0.0008	-23.2±6.5	-60.3
Ox-LDL (U/L)	53	85.1±35.1	76.2±31.7	0.033	-8.9±4.1	-10.5

Figure Legends

Fig 1. Sub-group analysis of CSII-induced change in ox-LDL by (A) cohort and (B) statin therapy. There was no significant difference between the cohorts (P=0.50, one-way ANOVA), but there was a significant difference between the effect in patients receiving statins and those not (P=0.019).

Fig 2: A. Association between (A) change in ox-LDL and change in daily insulin dose and, (B) change in ox-LDL and change in HbA_{1C} and (C) change in ox-LDL and change in % time spent with glucose >70<140 mg/dL (measured during week 15 by continuous glucose monitoring).

Fig 1

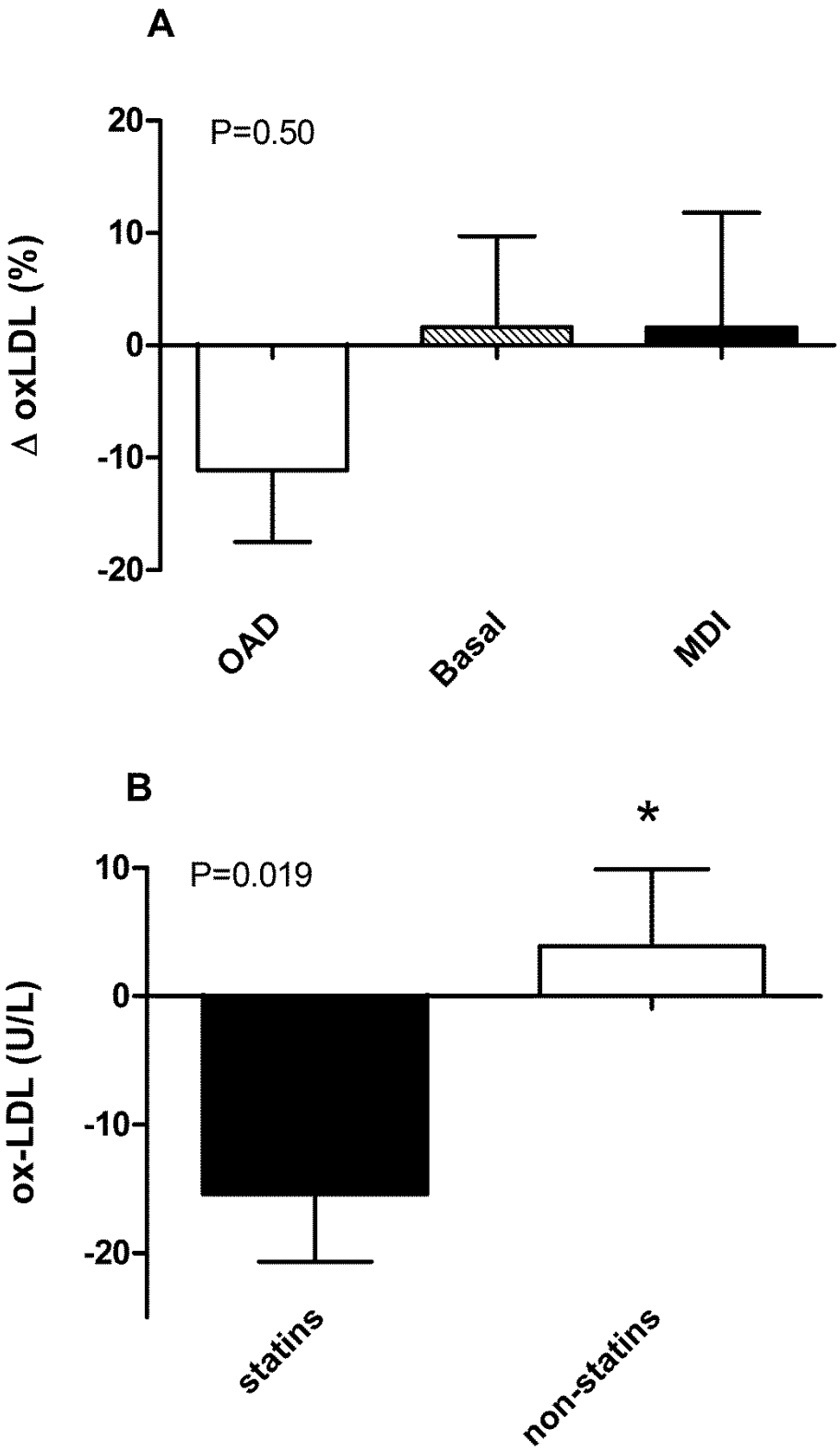
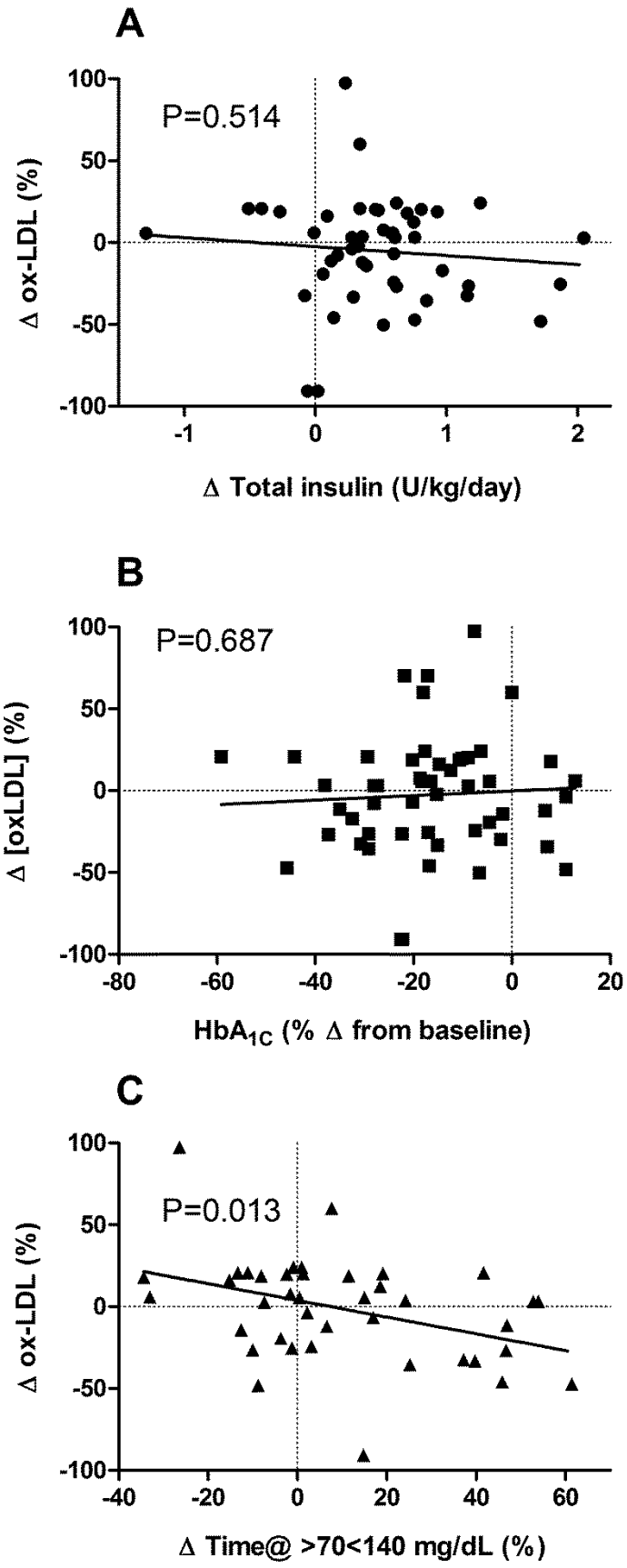

A
P=0.50
20
10
0
-10
-20
Δ oxLDL (%)
OAD
Basal
MDI
B
*
P=0.019
10
0
-10
-20
ox-LDL (U/L)
statins
non-statins

Fig 2


A
P=0.514
Δ ox-LDL (%)
Δ Total insulin (U/kg/day)
B
P=0.687
Δ [oxLDL] (%)
HbA1C (% Δ from baseline)
C
P=0.013
Δ ox-LDL (%)
Δ Time@ >70<140 mg/dL (%)