Prediction of cardiometabolic health through changes in plasma proteins with intentional weight loss in the DiRECT and DIADEM-I randomised clinical trials of type 2 diabetes remission

Short running title: proteomic changes in diabetes remission trials

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Intentional weight loss in type 2 diabetes rapidly induces changes in protein-based risk models consistent with widespread cardiometabolic improvements, especially in those that lost 10kg or more.

Abstract

Objective: To determine to what extent changes in plasma proteins, previously predictive of cardiometabolic outcomes, predict changes in two diabetes remission trials.

Research Design and Methods: SomaSignal® predictive tests (each derived from ~5000 plasma proteins measurements using aptamer-based proteomics assay) were assessed in baseline and 1-year samples in trials (DiRECT n=118, DIADEM-I n=66) and control (DiRECT n=144, DIADEM-I n=76) participants.

Results: Mean weight losses in DiRECT (UK) and DIADEM-I (Qatar) were 10.2 (SD 7.4) kg and 12.1 (SD 9.5) kg, respectively, versus 1.0 (3.7) kg and 4.0 (SD 5.4) kg in control groups. Cardiometabolic SomaSignal tests improved significantly (Bonferroni-adjusted $p< 0.05$) in DiRECT and DIADEM-I (expressed as relative difference in intervention minus control) as follows, respectively: liver fat (-26.4%, -37.3%), glucose tolerance (-36.6%, -37.4%), body fat percentage (-8.6%, -8.7%), resting metabolic rate (-8.0%, -5.1%), visceral fat (-34.3%, -26.1%) and cardiorespiratory fitness (+9.5%, +10.3%). Cardiovascular risk (measured by SomaSignal tests) also improved in interventions groups relative to control but significant only in DiRECT (DiRECT: -44.2%, DIADEM-I: -9.2%). However, weight loss >10kg predicted significant reductions in CV risk of -19.1% (CI -33.4 to -4.91) in DiRECT and -33.4% (CI -57.3, -9.6) in DIADEM-I. DIADEM-I also demonstrated rapid emergence of metabolic improvements at 3 months.

Conclusion: Intentional weight loss in recent onset type 2 diabetes rapidly induces changes in protein-based risk models consistent with widespread cardiometabolic improvements, including cardiorespiratory fitness. Protein changes with larger (>10kg)

weight loss also predicted lower cardiovascular risk, providing a positive outlook for relevant ongoing trials.

Article Highlights (99 words)

- The DiRECT and DIADEM-I trials have demonstrated excess adiposity is a key driver for type 2 diabetes, and healthy weight loss can lead to remission. Here, we determined whether previously validated protein-based models would predict cardiometabolic improvements in response to weight loss intervention.
- We demonstrated rapid and widespread improvements in protein-based models, some consistent with what we noted by direct measurement in the trial.
- Protein changes also predicted improvements in cardiorespiratory fitness and in cardiovascular risk, more so when greater weight loss occurred.
- These data add more support for an intentional weight loss impact on multiple important cardiometabolic outcomes.

Introduction

The DiRECT trial in the UK primary care population demonstrated, for people with type 2 diabetes of <6 years duration, remission occurred in 46% at 1 year, with a mean weight loss of 10.2 kg.(1) A maintained weight loss >10 kg led to remission for about 70% of intervention group participants at both 12 and 24 months.(2) The DIADEM-I remission trial included younger participants from 13 different countries in the Middle East and North Africa (MENA) region and shorter diabetes duration (≤ 3 years). It showed that a mean weight loss of 11.98 kg at 12 months resulted in 61% remission.(3) These trials have placed remission as an option for people with early type 2 diabetes, now translated into routine patient care across some countries, e.g. England where a successful pilot has led to wider adoption by the NHS. Furthermore, DIRECT showed weight loss was accompanied by substantial reductions in liver fat, and hepatic triglyceride export, as well as improvements in pancreatic function and morphology.(4, 5)

Observational evidence in people with type 2 diabetes have suggested an improved survival with intentional weight loss in a clinic-based study of newly diagnosed patients(6) and in insurance data.(7) In the Look AHEAD trial, while there was no overall cardiovascular benefit from an intervention designed to improve cardiovascular fitness with intensive lifestyle intervention over 4 years(8), however in post hoc analysis, a 10kg weight loss was by year one associated with a 21% lower risk (95% CI 2 to 36%) of the primary composite cardiovascular outcome.(9) Ample observational data also suggest

significant weight loss with bariatric surgery is associated with reduced cardiovascular risk, including in those with type 2 diabetes.(10)

In this post hoc analysis of proteomic data from DiRECT and DIADEM-I, we examined a broader set of clinically relevant outcomes beyond those available from routine clinical data. Our group recently established circulating protein-based algorithms which predict several cardiometabolic outcomes in a combined analyses of seven prospective cohorts (the UK Fenland, the Norwegian HUNT3, the Swiss BASEL VIII, the international EXSCEL trial and the US Covance, ARIC, and HERITAGE).(11, 12) In that proof of concept study, we demonstrated these protein-based tests can provide individualized health assessment across multiple conditions simultaneously from a single blood sample. Our hypothesis was that plasma protein changes linked to ≥ 10 kg weight loss in DiRECT and DIADEM-I would predict cardiovascular benefits and improvements in cardiovascular fitness. We also wished to demonstrate the dynamic properties of these tests, further supporting their utility as tools for precision medicine. These are important questions to inform patients embarking on intentional weight loss as well as healthcare services.

Research Design and Methods

Study design

The Diabetes Remission Clinical Trial (DiRECT) was designed to target type 2 diabetes and cardiovascular risk factors through weight loss.(13) The DiRECT study is a large-scale randomised trial that demonstrated dietary and lifestyle intervention in a primary

care setting could achieve and maintain remission of type 2 diabetes (defined as HbA1c <6.5% after at least 2 months off all diabetes medications) and improve blood pressure control.(1, 13) The intervention group was taken off all glucose-lowering medications, and diuretic and antihypertensive medications, and put on a total diet replacement using formula-based meal replacement products (825–853 kcal/day formula) for 3–5 months, followed by a stepped food reintroduction (2–8 weeks), and structured support for long-term weight loss maintenance.(1, 13) Protocols based on national guidelines were used to reintroduce medications if blood glucose or blood pressure exceeded treatment thresholds. The DiRECT dataset used for these analyses included all participants (n = 262) with analysable baseline and 1-year follow-up biological samples (n = 524 samples). More than half (50.9%) of the patients in the intervention arm analysed in this data subset showed remission of type 2 diabetes. EDTA plasma samples were collected from fasting individuals and spun at 2000g at 4°C for 15min within 4 hours of collection. Samples were decanted within 30 min of centrifugation and stored at -80°C.

The Diabetes Intervention Accentuating Diet and Enhancing Metabolism (DIADEM-I) study was a randomised controlled clinical trial designed using a similar dietary intervention to DiRECT, delivered in a primary care and community setting.(14) Participants recruited originated from the MENA region and were younger and had a shorter diabetes duration than DiRECT participants. The dietary intervention included total diet replacement using formula-based meal replacement products (800–820 kcal/day) for 3 months, followed by a stepped food introduction over the subsequent 3 months followed by regular food intake. In addition to the dietary intervention, DIADEM-I

participants received regular unsupervised support for physical activity. Diabetes medications were stopped at the outset in those undergoing the intervention. Blood pressure medication management was through clinical evaluation following national and international guidelines. The measured outcomes were weight loss and diabetes remission (defined as HbA1c <6.5% after at least 3 months off all diabetes medications). The DIADEM-I dataset used for these analyses included all participants (n=142) with analysable baseline and 1-year follow-up samples (n=284 samples), and longitudinal analysis on baseline plus 3-monthly follow-up biological samples up to 1 year (n=597 samples). EDTA plasma samples were collected from fasting individuals and spun at 1300g at 4°C for 10min approximately 2-3 hours after collection. Samples were decanted within 30 min of centrifugation and stored at -80°C. Both trials received ethical approval by their respective Institutional review boards.

Sensitivity to change in response to the DiRECT and DIADEM-I interventions was assessed for 10 SomaSignal tests; results for 8 of these SomaSignal tests (described below) were included in the analyses. Two SomaSignal tests: Alcohol Impact(11) and Primary Cardiovascular Risk – 4 years(11) were run for exploratory purposes (Alcohol Impact test was included as a negative control and Primary Cardiovascular Risk test is not appropriate for participants with elevated cardiovascular disease (CVD) risk factors, such as type 2 diabetes) and were accounted for in Bonferroni-adjustments for multiple testing but are not included in the results.

SomaSignal Tests

The SomaSignal tests applied to the DiRECT and DIADEM datasets were previously developed and validated.(11, 12) The Liver Fat test was developed and validated in over 10,000 participants from the Fenland cohort with liver ultrasound scores and reports results as: ‘no excess fat’ or ‘some excess fat’ based on standard ultrasound scoring (score ≤4: normal and score ≥5: mild/moderate/severe grades of fat).(11) The body composition tests: Lean Body Mass, Body Fat Percentage, and Visceral Fat were developed and validated in over 11,000 participants from the Fenland cohort with dual-energy X-ray absorption (DEXA) measurements and report results as continuous measurements of body fat (%), lean body mass (kg) and visceral fat (kg).(11) The Cardiorespiratory Fitness – VO2 Max test predicts maximal oxygen uptake on a cycle ergometer as a continuous measurement (ml/kg/min) and was developed and validated on over 700 participants from the Heritage exercise intervention study.(11)

The Cardiovascular Risk – 4 years test predicts the likelihood of a cardiovascular (CV) event (myocardial infarction, stroke, trans-ischemic attack, hospitalization for heart failure or all-cause death) in individuals ≥40 years with one or more known causes of elevated cardiovascular risk, including individuals presenting with: stable cardiovascular disease (defined as a history of a myocardial infarction or stroke (>6 months prior), history of heart failure, revascularization, abnormal stress test or imaging suggesting coronary heart disease including coronary artery stenosis >50% by angiogram or elevated coronary calcium score), type 2 diabetes, symptoms consistent with chronic obstructive coronary artery disease, or over 65 years old without known elevated cardiovascular risk.(12) The test was developed in 813 participants from the HUNT3

and ARIC cohorts and independently validated in 11,609 participants from HUNT3, ARIC, BASEL, and EXSCEL cohorts and reported results as a risk category and percentage range.(12)

The resting metabolic rate test was developed and validated in over 9,000 participants from the Fenland cohort with resting energy expenditure measured by indirect calorimetry and reports results as a continuous measure (integer) in kcal/day. The glucose tolerance test was developed and validated in over 11,700 participants from the Fenland cohort with 2-hour oral glucose tolerance test (OGTT) results and reports results as: ‘normal tolerance’ (<7.8 mmol/L) or ‘impaired tolerance’ (≥7.8 mmol/L) based on standard OGTT cut-offs. For all SomaSignal tests statistical analysis plans and minimum performance criteria were predefined and documented in a regulatory document vault (Arena Solutions).

Proteomic platform

The modified aptamer binding reagents,(15) SomaScan® assay(16) and its performance characteristics(17) have been previously described. The SomaScan Assay uses ~5000 individual DNA-based slow off-rate modified aptamers (SOMAmer® reagents), which complex with proteins in the diluted plasma. After various steps to eliminate non-specific binding, the DNA-based reagents are quantified by hybridisation on an Agilent array as described in the **Supplement**.

Statistical analyses on sensitivity to change

SomaSignal tests were assessed on both baseline and 1-year follow-up samples to look for favourable changes in the model outputs in the intervention and control arms, as well as comparing difference in changes between the two arms. Within the intervention arm these tests were also assessed on participants stratified by weight loss using a 10kg cut-off (i.e., participants that lost 10kg or more vs. those that did not). Six of the SomaSignal tests associated with cardiometabolic health (Cardiovascular Risk – 4 years, Liver Fat, Body Fat Percentage, Visceral Fat, Resting Energy Rate and Glucose Tolerance) were hypothesised a-priori to improve in the intervention group in both the DiRECT and DIADEM-I studies. Additionally, the Cardiorespiratory Fitness – VO2 Max, and Lean Body Mass were hypothesised to improve in the intervention group in the DIADEM-I study, which included specific physical activity support as part of the structured weight loss protocol. For tests with a continuous output (the Cardiovascular Risk – 4 years, Body Fat Percentage, Visceral Fat, Cardiorespiratory Fitness – VO2 Max, Lean Body Mass and Resting Energy Rate tests) paired t-tests were used to determine if the means of the predictions are significantly lower (at alpha=0.05, Bonferroni-adjusted for multiple testing) after the intervention. Tests with binary outputs (Liver Fat and Glucose Tolerance) used a one-tailed binomial test of proportions to determine if the proportion of individuals predicted as impaired (probability > 0.5 for predictions of some increased liver fat or impaired glucose tolerance, respectively) decreases significantly after the intervention. One-tailed tests were used to maximize power to detect cardiometabolic changes hypothesized a-priori to be associated with

weight loss. Two-tailed paired t-tests were used to determine if there was a significant overall change due to the intervention in SomaSignal tests that were not hypothesized to change in response to treatment. This same approach was used to assess the SomaSignal tests in the control arms in both studies. Model outputs were not expected to change in the control arms and two-tailed paired t-tests were used for all SomaSignal tests to determine if predictions change significantly (alpha = 0.05) in the control group. Finally, groupwise changes (mean of continuous predictions, or proportion for binary outputs) were compared with t-tests to determine if the changes in the control and intervention arms were significantly different (at alpha = 0.05).

Longitudinal analyses of the DIADEM-I samples were assessed for change in each SomaSignal test across timepoints: baseline, 3-months, 6-months, 9-months, and 1-year. Repeated measures ANOVA was used to determine if the means of the predictions are significantly different between each time point (alpha = 0.05), using time and treatment arm as fixed effects and subject ID as the random effect.

In supplementary analyses, we combined data from both trials and tested the effect of intervention on cardiometabolic SomaSignal tests (in the entire trial) using a mixed effects linear regression model account for study, sex, age, subject ID as a random effect and including an interaction term of timepoint x treatment arm.

Results

Demographics for the DiRECT and DIADEM-I participants included in the analyses are described in **Table 1**. Participants in DiRECT were about a decade older than DIADEM-I participants, and more had existing cardiovascular disease and hypertension. The majority of DiRECT participants were White European while in DIADEM-I, the majority of participants were from the MENA region. DIADEM-I included a larger number of males. Weight loss was comparable between the two trials.

SomaSignal tests related to cardiometabolic health were assessed for change in response to the DiRECT and DIADEM-I weight loss interventions. These tests include measures of future cardiometabolic risk: Cardiovascular Risk – 4 years, and current health state: Liver Fat, Cardiorespiratory Fitness – VO2 Max, Lean Body Mass, Body Fat Percentage, Visceral Fat, Glucose Tolerance, and Resting Energy Rate. Six of the SomaSignal tests were hypothesized to change in concordance with the DiRECT intervention and reversal of type 2 diabetes and eight of the SomaSignal tests were hypothesized to change in concordance with the DIADEM-I intervention (a-priori hypotheses included in the methods section). Assessing the change in proteins within treatment groups, six of the SomaSignal tests improved in the DiRECT intervention group, and seven improved in the DIADEM-I intervention group. In the control arms, SomaSignal test results remained unchanged or improved to a lesser extent. Key performance indicators for paired testing of intervention and control groups for the SomaSignal tests are shown in **Supplementary Tables 1 and 2**.

In addition, the magnitude of the mean change between the control and intervention groups (control vs. intervention) for the SomaSignal tests were assessed. These changes were assessed using the same hypotheses for directionality (described above). When comparing changes in proteins between the DiRECT intervention and control arms (control vs. intervention), all six of the SomaSignal tests that significantly improved in the intervention arm also exhibited significantly higher improvements in the intervention vs. control group, with results adjusted for multiple testing; in addition, the differences in CVD risk were significant (see **Table 2)**. Similarly, in DIADEM-I, six of the seven SomaSignal tests that improved in the intervention arm alone had significantly stronger improvements in the intervention group vs. control group when controlled for multiple testing. However, while directionally consistent with DIRECT, the effect on cardiovascular risk in DIADEM-I was not statistically significant. The mean change between the control and intervention groups for the SomaSignal tests is illustrated in **Supplementary Figure 1.**

Since remission of type 2 diabetes and its maintenance at 2 years, were closely related to sustaining >10kg weight loss in the DiRECT trial,(1, 2) the SomaSignal tests were also assessed on participants within the intervention arms stratified by weight loss using a 10 kg cut-off (i.e., participants with ≥10 kg vs. <10kg weight loss). The DiRECT and DIADEM-I results mirrored the paired testing done in the unstratified intervention group (**Table 2**) with the same SomaSignal tests improving with intervention. However, the effect size was generally greater in those who lost 10kg of weight or more compared to those who lost less than 10 kg of weight (see **Supplementary Table 3 and Figure 1**).

Figure 1 also illustrates that relative predicted risks were reduced most in participants in the intervention group with ≥10kg loss, with particularly strong (>50%) predicted reductions for glycaemia and ectopic fat depot SomaSignal tests, and more modest, ~20-30% relative risk reductions for the CVD SomaSignal test in DIRECT and DIADEM-I. In nearly all cases, a dose response effect was evident: those in the intervention arm who lost >10kg had the greatest magnitude of change, followed by those with <10kg loss (typically intermediate changes), and then the control arm with the smallest or no change from baseline.

Supplementary analyses combining data from both trials mirrored the results seen in the separate DiRECT and DIADEM-I analyses (Supplementary Table 4). Change in cardiovascular risk was the only proteomic test that changed significantly in the combined analyses but did not in both trials independently. This combined result was driven mainly by the larger DiRECT study which showed a significant effect on the mixed effects linear regression model, and had higher baseline cardiovascular risk scores compared to DIADEM-I (**Supplementary Figure 1**), and a larger increase in cardiovascular risk in the control arm from baseline to 1-year.

Finally, with the availability of serial samples in DIADEM-I, temporal effects were identified whereby rapid (within 3 months) changes were seen in protein predicted metabolic /adiposity parameters as well as in cardiorespiratory fitness and resting energy expenditure, whereas changes in cardiovascular risk appeared to take longer (**Figures 2 and Supplementary Figure 2**).

Conclusions

Our results show weight loss, which led 46% of people with recently diagnosed type 2 diabetes to be in remission after 1 year in DiRECT, alters protein levels in a validated algorithm commensurate with future reductions in a range of cardiometabolic outcomes, including cardiovascular outcomes. Although a post-hoc analysis, the data stem from a well characterised randomised trial of typical people living in the UK with early type 2 diabetes, and add to the evidence base on the effects of large scale weight loss on cardiovascular outcomes.(18) The SomaSignal protein tests indicate meaningful reductions in cardiovascular risk and also suggest that the 1-year weight loss of 10kg or more leads to around 10% reduction in resting energy expenditure and a comparable ~13% rise in cardiorespiratory fitness. Finally, larger predicted improvements in glycaemia, liver fat and body composition measures, all of which improved rapidly as measured by gold standard measurements in trial, lend strong internal validity(4). Such findings concur with our recent concept paper on the timing of differing cardiometabolic benefits post weight loss.(19) We saw broadly similar results in DIADEM-I, a trial conducted in the MENA region, which also showed fast improvements in metabolic parameters, whereas any cardiovascular benefit would take longer to emerge. Indeed, body composition and cardiometabolic fitness significantly improved within the first 6 months before plateauing during the weight loss maintenance period. Lean body mass and resting energy expenditure decreased significantly within the first 3-months but then slowly increased from 3-months to 1-year, potentially consistent with an introduction of exercise regimen plus a modest regain in weight. Collectively, the work adds further

evidence for rapid multifactorial metabolic and predicted longer-term cardiovascular benefits of *intentional* weight loss in the context of type 2 diabetes.

Diabetes is a major public health problem across the world, and cardiovascular disease remains the leading cause of death and disability.(20) The lack of overall benefit in hard cardiovascular outcomes in the Look AHEAD trial(8) fuelled a perception in some quarters that there is little vascular benefit to be gained from lifestyle improvements in people with type 2 diabetes. Our results, albeit using a surrogate protein-based index of cardiovascular risk, but one recently shown to correctly predict the results of multiple intervention trials,(12) challenge this concept. In doing so, they add support to the post hoc results of Look AHEAD in whom people who had lost at least 10% of their bodyweight in the first year of the study had a 21% lower risk (95% CI 2 to 36%) of cardiovascular outcomes.(9) This risk reduction level is close to the ~20-30% reductions in CVD SomaSignal test predictions we report for those in the intervention arm who lost 10kg of weight (~10%) by 1-year in DiRECT and DIADEM-I (**Supplementary Table 3, Figure 1**), even though the cardiovascular outcome included in our analyses was somewhat different to those in the Look AHEAD analysis. It will be of interest to see if SELECT(21) (non-diabetes, prevalent ASCVD; semaglutide versus placebo) and SURPASS CVOT(22) (prevalent diabetes, and cardiovascular disease; tirzepatide versus dulaglutide), both of which anticipate substantial weight loss, report MACE reduction. If so, this may add further credence to the notion that institutions with intervention development pipelines may look to proteomics-based approaches to gain

insights into potential outcomes, to help inform on decisions about the best weight loss candidates for phase 3 trials.

The SomaSignal tests were developed and validated on large cohorts(11) but it is worth noting liver fat algorithms were developed using less sensitive ultrasound, not MRI, and the glucose tolerance SomaSignal was developed using oral glucose tolerance test data. Even so, the significant >50% relative reductions in these measures in both trials in those who lost >10kg of weight over the first year is strongly consistent with findings with liver fat (via MRI) and glycaemia changes (via HbA1c) we reported previously in the DIRECT trial.(4, 13)

The predicted reduction in the resting energy expenditure in both trials accords with expectations and with other controlled weight loss studies which saw a 5.6%-14.6% reduction in REE with similar weight loss.(23–25) Lighter people must burn less calories unless more active and accelerometer data from DiRECT showed minimal change in activity.(26). Notably, the improvement in cardiorespiratory fitness appeared to be rapid in DIADEM-I.

Together these new data provide further evidence that patterns of plasma proteins can meaningfully approximate a range of important metabolic outcomes or risk parameters (some of which require imaging or dynamic testing) as well as capture, the often rapid, changes in such risk parameters following dietary-driven intentional weight loss. These findings thereby extend the notion that protein-based tests could not only

simultaneously provide individualized health guidance across multiple conditions from a single blood sample, but that they could also track treatment-induced improvements. Most importantly, they highlight a potential cardiovascular benefit of intentional weight loss, especially when such weight loss is >10kg in people with type 2 diabetes, a topic of extreme interest given availability of newer therapies that can now help people with type 2 diabetes lose an average of >10kg in weight e.g. Tirzepatide.(27)

We accept several limitations. First, we had blood samples only for people in the intervention arm who remained in the trial up to 1 year in DIRECT. Lifestyle trials tend to have larger dropout rates than drug trials but even so, near 80% of participants in the active arm provided biomarker samples, with 97% of the control participants providing such samples. We did not control for drug changes but, notably, patients in the intervention group were on *less* oral diabetes agents (mostly metformin) and blood pressure medications at year 1, a pattern that may be predicted to minimise group differences in CV risk, rather than to exaggerate them. We also accept predicted cardiovascular benefits were not nominally significant in DIADEM-I but notably, this cohort was of lower baseline cardiovascular risk, being more than a decade younger and far fewer having hypertension and only one patient having established cardiovascular disease. This means that there may have been less scope and statistical power to identify a potentially beneficial effect on the proteins corresponding to changes in CVD risk. Finally, we accept SomaSignal protein predicted outcomes are not substitutes for hard outcomes but, even so, it is notable that large weight loss due to surgery in a 10 year follow up to a trial in patients with diabetes showed large reductions

in vascular outcomes (both macro and microvascular events, though numbers were small) compared to much lower weight loss in a medical arm.(28) Whilst other surgery-based information also suggests benefits of substantial weight loss on hard outcomes, such information comes from observational data rather than randomized clinical trials. The totality of evidence from DiRECT and DIADEM-I biomarkers reported here, together with post hoc Look Ahead data,(9) and surgical data including recently in people with type 2 diabetes,(10) all suggest *intentional* (as opposed to unintentional) weight loss is likely to lessen a range of cardiometabolic outcomes, including, over time, atherosclerotic cardiovascular disease. Forthcoming trials will test this hypothesis, with SURPASS CVOT, a trial in patients with diabetes, being particularly relevant.

In summary, analyses of changes in plasma proteins from the DiRECT and DIADEM-I randomized trial predicted significant improvements in many domains beyond glycaemia and adiposity to include future cardiovascular risk, resting energy expenditure, and cardiorespiratory fitness by intention-to-treat intervention data and, especially for those achieving >10% weight loss. These findings lend confidence that intentional weight loss early during type 2 diabetes is advantageous for multiple metabolic traits and if weight loss is sustained, could reduce future cardiovascular endpoints and that such benefits could be captured or monitored by proteomic analyses of plasma.

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Conflict of Interests

NS has consulted for and/or received speaker fees from Abbott Laboratories, Amgen, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Hanmi Pharmaceuticals, Janssen, Merck Sharp & Dohme, Novartis, Novo Nordisk, Pfizer, Roche Diagnostics, and Sanofi; and received grant support paid to his University from AstraZeneca, Boehringer Ingelheim,

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Author Contributions

NS conceived the idea wrote the first draft of the manuscript.

JC conceived the presented idea and planned the analyses, contributed to the interpretation of the results, and aided in the writing of the manuscript.

MAH planned and carried out the analyses, contributed to the interpretation of the results, and aided in the writing of the manuscript.

DPA planned and carried out the analyses, contributed to the interpretation of the results, and aided in the writing of the manuscript.

SW conceived the presented idea and planned the analyses and contributed to the interpretation of the results.

EVT contributed to the interpretation of the results and aided in the writing of the manuscript.

ST led the DIADEMI trial and conceived idea and contributed to writing of the paper.

ML and RT co-led DIRECT and contributed to writing of the paper.

MVH, PW, HZ OD all contributed to the writing of the paper.

NS and JC are the guarantors and accept full responsibility for the work and the decision to submit the manuscript for publication.

Prior Presentation

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Table 1.

Study		DiRECT		DIADEM-I	
Covariate	**Measure**	**Weight Loss Intervention**	**Control**	**Weight Loss Intervention**	**Control**
Sample size	Number (n)	118	144	66	76
Type 2 Diabetes Reversal at 1 year	Yes (%)	60 (50.9%)	6 (4.2%)	36 (78.3%)	7 (12.3%)
Weight Change, Baseline to Follow-up (kg)	Mean (SD)	-10.22 (7.37)	-1.014 (3.72)	-12.2 (9.53)	-3.99 (5.39)
	Median	-9.30	-1.05	-10.5	-2.75
	Range	-31.6 – 13.7	-13.5 – 13.3	-51.8 – -0.9	-23.5 – +6.7
Age (years)	Mean (SD)	53.96 (7.08)	56.15 (6.93)	41.8 (5.52)	42.4 (5.83)
	Median	54.98	57.39	43	44
	Range	38.1 – 65.4	35.6 – 65.9	27 – 50	29 – 50
Sex	Male (%)	67 (56.8%)	90 (62.5%)	48 (72.7%)	57 (75%)
Race/Ethnicity	White (%)	115 (97.5%)	142 (98.6%)	-	-
	Black (%)	2 (1.7%)	-	-	-
	Asian (%)	-	-	-	-
	MENA (%)	-	-	66 (100%)	76 (100%)
	Other (%)	1 (0.9%)	2 (1.4%)	-	-
BMI (kg/m^2)	Mean (SD)	34.8 (5.77)	34.7 (5.06)	35.0 (4.6)	34.3 (4.3)
	Median	34.1	34.2	34.0	34.0
	Range	26.3 – 54.9	27 – 51.6	27.3 – 44.9	27.5 – 44.7
Duration of Type 2 Diabetes (years)	Mean (SD)	3.2 (1.6)	3.0 (1.8)	1.8 (0.9)	1.7 (1.1)
	Median	3.2	2.7	2	1.8
	Range	0 – 6.0	0.2-6.0	0.1 – 3	0 – 3.4
History of Hypertension	Yes (%)	64 (54.2%)	86 (59.7%)	21 (31.8%)	22 (28.9%)
History of CVD	Yes (%)	10 (8.5%)	23 (16.0%)	1 (1.5%)	0 (0%)

Baseline Demographics of DiRECT and DIADEM-I Participants Studied with available samples at one year in the intervention and control groups.

Table 2:

Test	Within-Group Mean Change (From Baseline to Follow-up)		Difference in Mean Predicted Change Between Groups (95 % CI) (% Difference Intervention vs. Control)	p-value (**Bonferroni** - ~~un~~adjusted)	Within-Group Mean Change (From Baseline to Follow-up)		Difference in Mean Predicted Change Between Groups (95 % CI) (% Difference Intervention vs. Control)	p-value (**Bonferroni** - ~~un~~adjusted)
	DIRECT Control (n=144) **(%)**	DIRECT Intervention (n=118) **(%)**			DIADEM-I Control (n=76) **(%)**	DIADEM-I Intervention (n=66) **(%)**		
Cardiovascular Risk	**0.0488 (31.9 %)**	**-0.0182 (-12.3%)**	**-0.0671 (-0.0861, -0.0480) (-44.2%)**	**1.63e-10**	-0.0192 (-21.2%)	-0.0349 (-30.4%)	-0.0156 (-0.0481, 0.0169) (-9.2%)	0.170
Liver Fat (proportion predicted some excess fat)	**-0.00694 (-0.69%)**	**-0.271 (-27.1%)**	**-0.264 (-35.3%, -17.5%) (-26.4%)**	**2.39e-9**	**-0.105 (-10.5%)**	**-0.478 (-47.8%)**	**-0.373 (-0.558, -0.189) (-37.3%)**	**3.07e-05**
Lean Body Mass (g)	479 (0.86%)	-383 (-0.68%)	-862 (-1703, -20.8) (-1.54%)	0.446	-701 (-1.2%)	-479 (-0.8%)	223 (-971, 1420) (0.4%)	0.644
Glucose Tolerance	**-0.00694 (-0.69%)**	**-0.373 (-37.3%)**	**-0.366 (-46.2%, -**	**1.01e-13**	**-0.0175 (-1.8%)**	**-0.391 (-39.1%)**	**-0.374 (-0.539, -**	**2.05e-06**

(proportion predicted glucose intolerant)			**28.4%) (-36.6%)**				**0.209) (-37.4%)**	
Body Fat Percentage	**-0.00409 (-1.0%)**	**-0.0379 (-9.6%)**	**-0.0338 (-0.408, -0.0268) (-8.6%)**	**1.15e-16**	**-0.0164 (-3.9%)**	**-0.0470 (-12.6%)**	**-0.0306 (-0.0456, -0.0155) (-8.7%)**	**5.60e-05**
Resting Energy Rate (kCal/day)	**17.8 (0.87%)**	**-148 (-7.1%)**	**-166 (-203, -129) (-8.0%)**	**1.82e-15**	**-32.2 (-1.5%)**	**-143 (-6.6%)**	**-111 (-178, -42.6) (-5.1%)**	**8.45e-04**
Visceral Fat (g)	**-99.7 (-4.1%)**	**-914 (-38.4%)**	**-814 (-996, -632) (-34.3%)**	**1.26e-15**	**-337 (-12.8%)**	**-968 (-38.9%)**	**-631 (-939, -323) (-26.1%)**	**4.88e-05**
VO2max (ml/kg/min)	**-0.281 (-1.1%)**	**2.07 (8.4%)**	**2.35 (1.87, 2.82) (9.5%)**	**1.39e-17**	**0.968 (4.5%)**	**3.49 (14.8%)**	**2.53 (1.60, 3.45) (10.3%)**	**2.27e-07**

Results for testing of mean changes between control and intervention groups in DIRECT and DIADEM-I.

Significant results at Bonferroni adjusted alpha=0.05 are in bold. Tests with continuous or risk probability outputs report absolute mean change in prediction in units particular to each test, whereas tests with classification outputs report the proportion that changed. Significance of results are shown for respective two-tailed or pre-specified one-tailed hypothesis tests as described earlier; two-tailed 95% confidence intervals are shown for group estimates regardless of hypothesis tests. Statistical testing was applied to the group mean changes in respective units for each test; relative (percentage) summary changes are also shown.

Figure Legends

Figure 1. Relative predicted changes over one year during DiRECT trial (left) and DIADEM-I (right) of 8 SomaSignal proteomic tests, stratified by control (no dietary intervention); dietary intervention with up to 10kg weight loss observed; and dietary intervention with 10kg or more weight loss observed. Point estimates are relative percent change for predictive results with continuous outcomes, or change in proportion for binary status tests, with horizontal bars showing 95% confidence interval of estimates. Glucose Tolerance and Liver Fat tests show the change in the percent of participants predicted as glucose intolerant and having some excess liver fat, respectively.

Figure 2. Longitudinal changes from baseline for each of the SomaSignal tests in DIADEM-I. The thin lines are the individual trajectories for each subject, the thick lines are the fits from a repeated measures model, and the ribbons are the 95% confidence interval from the model fit. The control arm is colored purple, and the treatment arm is colored teal.