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

Supplemental Materials

SomaScan® assay description

The SomaScan Assay is based on a 2-catch assay principle, (refer to https://youtu.be/8Jj-7TV7a8U for an animation of the process) as previously described.(1, 2) Briefly, the SomaScan Assay begins as a mix of thousands of SOMAmer® reagents labeled with a 5' fluorophore, photocleavable linker, and biotin are immobilized on streptavidin (SA)-coated beads through biotin-streptavidin interaction and incubated with 55 ml of EDTA plasma.

Cognate and noncognate SOMAmer-protein complexes form on the SA beads (Catch 1). After washing unbound proteins away, captured proteins are labeled with biotin. SOMAmer-protein complexes are released from the beads by photocleavage of the linker with UV light and incubated in a buffer containing a polyanionic competitor which selectively enriches for specific interactions.

SOMAmer-protein complexes are recaptured on a second set of SA-coated beads through biotin-labeled proteins (Catch 2) followed by additional washing steps that facilitate further removal of nonspecifically bound SOMAmer reagents. SOMAmer reagents are then released from the complex in a denaturing buffer.

For readout, SOMAmer reagents are hybridized to complementary sequences on a DNA microarray chip and quantified by fluorescence. Fluorescence intensity is related to the bound reagent amount in the original signal. Using the scanned image capturing the probes' fluorescence intensities, feature extraction is performed. Fluorescence intensity in the SomaScan assay is related to the relative availability of the three-dimensional shape-charge epitope on each protein (the binding site of the SOMAmer reagent) in the original sample. This is a reflection of each protein's abundance and availability of the binding epitope.

To correct for intrinsic assay variation, data are normalized according to the following procedure. Sample data is first normalized to remove hybridization variation within a run. This is followed by median normalization across calibrator samples to remove other assay biases within the run. Overall scaling and calibration is then performed on a per-plate basis to remove overall intensity differences between runs. Finally, median normalization to an external reference standard of normal plasma is performed.

References

1. Williams SA, Kivimaki M, Langenberg C, *et al.* Plasma protein patterns as comprehensive indicators of health. *Nat Med* 2019;25:1851–1857.

2. Rohloff JC, Gelinas AD, Jarvis TC, *et al.* Nucleic acid ligands with protein-like side chains: Modified aptamers and their use as diagnostic and therapeutic agents. *Mol Ther - Nucleic Acids* 2014;3:e201.

Supplementary Table 1: Results for DiRECT paired testing of the Control Group (n=144 pairs) and Intervention Group (n=118 pairs). Significant results at alpha=0.05 are in bold. Tests with continuous outputs report change in prediction whereas tests with classification outputs report the proportion that changed.

Test	Group	Mean Prediction or Proportion		Mean Difference to Follow-up (% Difference to Follow-up)	p-value (raw/unadjusted)	p-value (Bonferroni-adjusted)
		Baseline	Follow-up			
Cardiovascular Risk	**Control**	**0.153**	**0.202**	**0.0488 (31.9%)**	**1.87e-11**	**1.87e-10**
	Intervention	**0.148**	**0.130**	**-0.0182 (-12.3%)**	**0.00508**	**0.0508**
Liver Fat (proportion predicted some excess fat)	Control	0.979	0.972	-0.00700 (-0.70%)	1	1
	Intervention	**0.992**	**0.720**	**-0.271 (-27.1%)**	**4.56e-9**	**4.56e-8**
Lean Body Mass (g)	Control	55,700	56,200	479 (0.86%)	0.0571	0.571
	Intervention	56,300	55,900	-383 (-0.68%)	0.2707	1
Glucose Tolerance (proportion predicted glucose intolerant)	Control	0.931	0.938	0.00690 (0.69%)	1	1
	Intervention	**0.941**	**0.568**	**-0.373 (-37.3%)**	**3.981e-11**	**3.981e-10**
Body Fat Percentage	Control	0.391	0.387	-0.00410 (-1.0%)	0.00783	0.0783
	Intervention	**0.394**	**0.356**	**-0.0379 (-9.6%)**	**7.733e-22**	**7.733e-21**
Resting Energy Rate (kCal/day)	Control	2,050	2,070	17.8 (0.87%)	0.104	1
	Intervention	**2,090**	**1,940**	**-148 (-7.1%)**	**7.62e-17**	**7.62e-16**
Visceral Fat (g)	Control	2,430	2,330	-99.7 (-4.1%)	0.0861	0.861
	Intervention	**2,380**	**1,470**	**-914 (-38.4%)**	**4.919e-24**	**4.919e-23**
VO2max (ml/kg/min)	Control	24.3	24.0	-0.290 (1.2%)	0.0276	0.276
	Intervention	**24.6**	**26.7**	**2.07 (8.4%)**	**1.509e-17**	**1.509e-16**

Supplementary Table 2: Results for DIADEM paired testing of the Control Group (n=76 pairs) and Intervention Group (n=66 pairs). Significant results at alpha=0.05 are in bold. Tests with continuous outputs report change in prediction whereas tests with classification outputs report the proportion that changed.

Test	Group	Mean Prediction or Proportion		Mean Difference to Follow-up (% Difference to Follow-up)	p-value (raw/unadjusted)	p-value (Bonferroni-adjusted)
		Baseline	Follow-up			
Cardiovascular Risk	Control	0.102	0.0827	-0.0192 (21.2%)	0.0807	0.807
	Intervention	**0.0919**	**0.057**	**-0.0349 (-30.4%)**	**1.22e-05**	**1.22e-04**
Liver Fat (proportion predicted some excess fat)	Control	0.982	0.877	-0.105 (-10.5%)	0.0334	0.334
	Intervention	**1**	**0.522**	**-0.478 (-47.8%)**	**1.43e-07**	**1.43e-06**
Lean Body Mass (g)	Control	57,900	57,200	-701 (-1.2%)	0.0411	0.411
	Intervention	58,100	57,600	-479 (-0.8%)	0.148	1
Glucose Tolerance (proportion predicted glucose intolerant)	Control	0.965	0.947	-0.018 (-1.8%)	0.500	1
	Intervention	**0.913**	**0.522**	**-0.391 (-39.1%)**	**4.14e-05**	**4.14e-04**
Body Fat Percentage	**Control**	**0.377**	**0.361**	**-0.0164 (-3.9%)**	**8.06e-04**	**0.00806**
	Intervention	**0.381**	**0.334**	**-0.047 (-12.6%)**	**9.09e-11**	**9.09e-10**
Resting Energy Rate (kCal/day)	Control	2,090	2,060	-32.2 (-1.5%)	0.0495	0.495
	Intervention	**2,090**	**1,950**	**-143 (-6.6%)**	**2.14e-07**	**2.14e-06**
Visceral Fat (g)	**Control**	**2,430**	**2,090**	**-337 (-12.8%)**	**2.05e-05**	**2.05e-04**
	Intervention	**2,460**	**1,490**	**-968 (-38.9%)**	**1.44e-13**	**1.44e-12**
VO2max (ml/kg/min)	Control	24.5	25.4	0.968 (4.5%)	0.00549	0.0549
	Intervention	**23.6**	**27.1**	**3.49 (14.8%)**	**4.89e-16**	**4.89e-15**

Supplementary Table 3. Results for paired testing of Intervention Group, ≥ 10kg weight loss (DiRECT: n = 54 pairs, DIADEM-I: n = 23 pairs).

Test	Mean Prediction or Proportion in DIRECT		Mean Difference (95% CI) to Follow-up (% Difference to Follow-up)	p-value (unadjusted)	Mean Prediction or Proportion in DIADEM-I		Mean Difference (95% CI) to Follow-up (% Difference to Follow-up)	p-value (unadjusted)
	Baseline	Follow-up			Baseline	Follow-up		
Cardiovascular Risk	0.133	0.107	-0.0254 (-0.0443, -0.0065) -19.1% (-33.4%, -4.91%)	0.00468	0.0958	0.0638	-0.0320 (-0.0549, -0.00915) -33.4% (-57.3%, -9.6%)	8.49e-04
Liver Fat (proportion predicted some excess fat)	0.982	0.463	-51.9% (-67.5%, -36.2%)	3.31e-9	1	0.348	-0.652 (-0.890, -0.414) -65.2% (-89.0%, -41.4%)	5.33e-06
Lean Body Mass (g)	57,400	56,200	-1210 (-2390, -33.0) -2.1%, (-4.2%, 0.0%)	0.0441	59,000	58,200	-737 (-2200, 733) -1.2% (-3.7%, 1.2%)	0.155
Glucose Tolerance (proportion predicted glucose intolerant)	0.926	0.296	-63.0% (-78.9%, -47.1%)	3.67e-11	0.913	0.435	-0.4783 (-0.755, -0.202) -52.4% (-82.7%, -22.1%)	8.30e-04
Body Fat Percentage	0.388	0.328	-0.0598 (-0.0698, -0.0499) -15.4% (-18.0%, -12.9%)	4.53e-17	0.383	0.313	-0.0699 (-0.0865, -0.0532) -18.3% (-22.6%, -13.9%)	6.95e-09
Resting Energy Rate (kCal/day)	2,110	1,880	-231 (-276, -186) -10.9%, (-13.1%,-8.8%)	1.27e-14	2,110	1,920	-187.2 (-273, -102) -8.9% (-12.9%, -4.8%)	8.57e-05
Visceral Fat (g)	2,420	1,110	-1310 (-1530, -1090) -54.1% (-63.3%, -44.8%)	1.14e-16	2,450	1,160	-1290 (-1640, -940) -52.6% (-66.9%, -38.3%)	3.08e-10

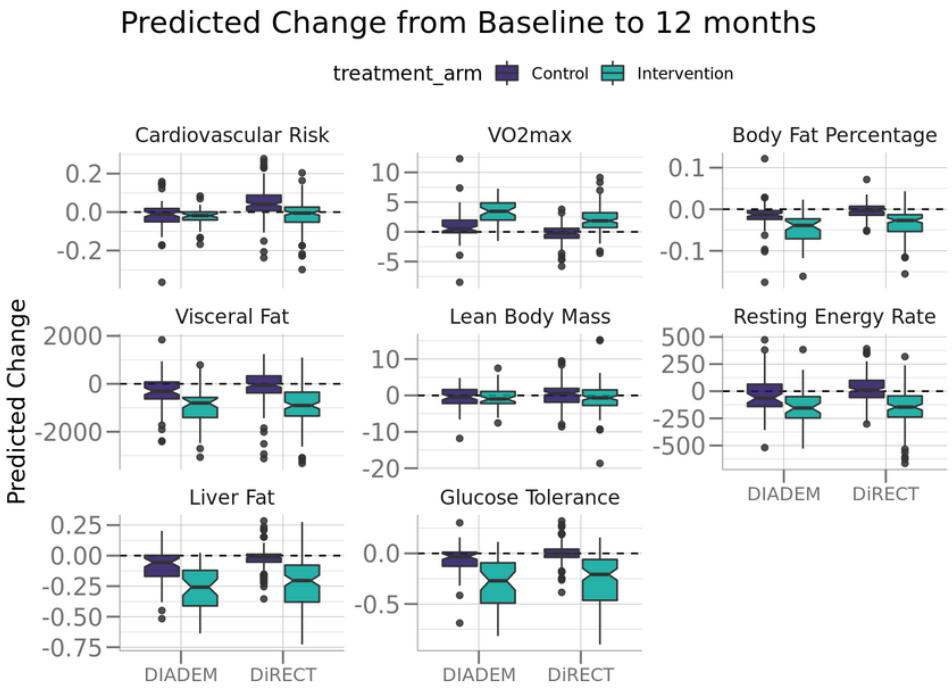
VO2max (ml/kg/min)	**24.9**	**28.2**	**3.26 (2.62, 3.90) 13.1% (10.5%, 15.7%)**	**4.68e-14**	**23.8**	**28.3**	**4.46 (3.63, 5.29) 18.7% (15.3%, 22.2%)**	**8.32e-11**

Significant results at Bonferroni adjusted alpha=0.05 (unadjusted p < 0.005) 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.

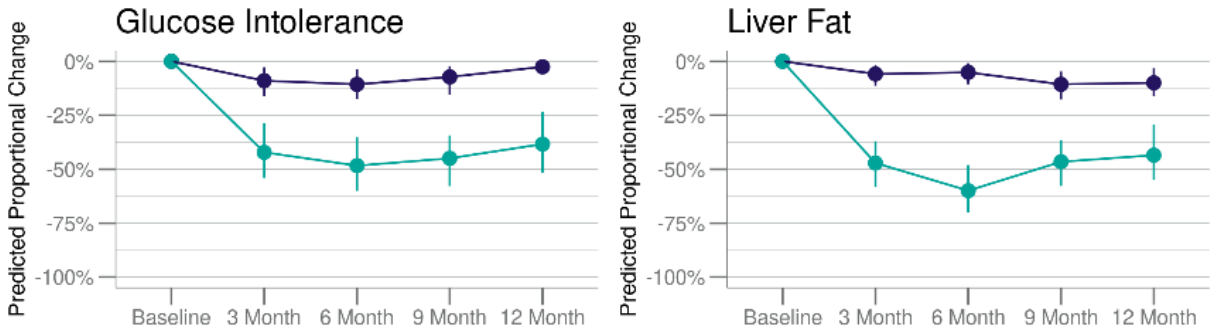
Supplementary Table 4. Results for combined analyses (DiRECT + DIADEM).

SomaSignal Test	Difference in Mean Predicted Change Between Groups	p-value (unadjusted)	Difference in Mean Predicted Change Between Groups in participants that lost ≥ 10kg.	p-value (unadjusted)
Cardiovascular Risk	-0.0503	**1.80e-10**	-0.0254	**0.000601**
Liver Fat (proportion predicted some excess fat)	-0.207	**7.00e-31**	-0.373	**1.28e-30**
Lean Body Mass (g)	-0.519	0.154	-1.03	**0.0474**
Glucose Tolerance (proportion predicted glucose intolerant)	-0.275	**4.15e-34**	-0.431	**7.46e-25**
Body-fat percentage	-0.0320	**4.76e-22**	-0.0599	**1.35e-23**
Resting Energy Rate (kCal/day)	-146	**3.37e-17**	-207	**3.25e-16**
Visceral Fat (g)	-754	**2.88e-24**	-1290	**8.20e-23**
VO2max (ml/kg/min)	2.40	**5.65e-25**	3.61	**9.86e-23**

Supplementary Figure 1. Predicted SomaSignal test change from baseline to 12 months in the DiRECT and DIADEM trials.



Supplementary Figure 2: Longitudinal proportional changes in from baseline for the glucose tolerance and liver fat SomaSignal tests in DIADEM-I.



Supplementary Figure 2. Longitudinal change in proportion of subjects predicted to be either glucose intolerant or have excess liver fat. The 95% confidence intervals, calculated via bootstrapping, are shown as vertical lines. The control arm is colored purple, and the treatment arm is colored teal.