

## ESM: Ceramide stearic to palmitic acid ratio predicts incident diabetes

### ESM Tables

**ESM Table 1.** Baseline characteristics of the FINRISK 2002 study population.

Characteristic	No diabetes	Incident diabetes	Prevalent diabetes
N	7007	593	445
Sex (male)	46 %	58 %	48 %
Current smoker	26 %	27 %	20 %
Lipid treatment	6 %	14 %	28 %
Eastern Finland	68 %	66 %	73 %
Age (years)	47 (36-57)	55 (47-61)	58 (50-64)
BMI	25.9 (23.4-28.7)	30.4 (27.7-33.7)	29.8 (26.5-33.5)
Waist (cm)	87 (78-96)	102 (94-110)	100 (90-109)
Systolic blood pressure (mmHg)	131 (120-145)	143 (130-158)	140 (126-155)
TC (mmol/l)	5.5 (4.8-6.2)	5.8 (5.1-6.6)	5.4 (4.8-6.1)
LDL-C (mmol/l)	3.3 (2.7-3.9)	3.6 (3.0-4.2)	3.2 (2.7-3.7)
HDL-C (mmol/l)	1.5 (1.2-1.8)	1.2 (1.1-1.5)	1.3 (1.1-1.6)
TG (mmol/l)	1.1 (0.8-1.6)	1.7 (1.2-2.4)	1.6 (1.1-2.4)
CRP (mg/l)	1.0 (0.5-2.3)	2.0 (1.0-4.3)	1.8 (0.9-3.7)
Cer(d18:1/18:0)/Cer(d18:1/16:0)	0.398 (0.337-0.470)	0.490 (0.420-0.558)	0.459 (0.389-0.554)
Cer(d18:1/16:0)/Cer(d18:1/24:0)	0.126 (0.111-0.144)	0.119 (0.104-0.136)	0.122 (0.109-0.143)
Cer(d18:1/18:0)/Cer(d18:1/24:0)	0.050 (0.041-0.062)	0.059 (0.049-0.070)	0.057 (0.047-0.069)
Cer(d18:1/24:1)/Cer(d18:1/24:0)	0.674 (0.593-0.762)	0.688 (0.613-0.779)	0.688 (0.607-0.787)
Cer(d18:1/16:0) (μmol/l)	0.283 (0.241-0.334)	0.297 (0.257-0.351)	0.296 (0.250-0.351)
Cer(d18:1/18:0) (μmol/l)	0.112 (0.087-0.146)	0.145 (0.115-0.184)	0.136 (0.104-0.177)
Cer(d18:1/24:0) (μmol/l)	2.251 (1.838-2.738)	2.486 (2.043-3.036)	2.420 (1.951-2.948)
Cer(d18:1/24:1) (μmol/l)	1.504 (1.235-1.831)	1.683 (1.418-2.067)	1.651 (1.350-2.019)

*Data are median (interquartile range) or % from total. The data of all variables were available for all individuals. LDL-C, LDL cholesterol; TC, total cholesterol.*

**ESM Table 2.** Hazard ratios of cardiovascular risk associated ceramides and ceramide ratios for incident diabetes in FINRISK 2002.

FINRISK 2002	HR (95% CI) <sup>a</sup>	<i>p</i> value <sup>a</sup>	HR (95% CI) <sup>b</sup>	<i>p</i> value <sup>b</sup>
Cer(d18:1/16:0)/Cer(d18:1/24:0)	0.83 (0.77, 0.90)	1.2×10 <sup>-5</sup>	0.85 (0.78, 0.93)	2.4×10 <sup>-4</sup>
Cer(d18:1/18:0)/Cer(d18:1/24:0)	1.72 (1.59, 1.87)	<2.2×10 <sup>-16</sup>	1.19 (1.09, 1.31)	2.4×10 <sup>-4</sup>
Cer(d18:1/24:1)/Cer(d18:1/24:0)	1.19 (1.09, 1.29)	5.6×10 <sup>-5</sup>	1.02 (0.94, 1.11)	0.629
Cer(d18:1/16:0)	1.25 (1.15, 1.35)	6.8×10 <sup>-8</sup>	1.04 (0.95, 1.13)	0.425
Cer(d18:1/18:0)	1.95 (1.80, 2.12)	<2.2×10 <sup>-16</sup>	1.32 (1.20, 1.45)	1.7×10 <sup>-8</sup>
Cer(d18:1/24:0)	1.37 (1.26, 1.49)	1.2×10 <sup>-13</sup>	1.16 (1.06, 1.26)	0.002
Cer(d18:1/24:1)	1.53 (1.41, 1.66)	<2.2×10 <sup>-16</sup>	1.17 (1.07, 1.28)	3.8×10 <sup>-4</sup>
FINRISK 2002 substudy				
Cer(d18:1/16:0)/Cer(d18:1/24:0)	0.90 (0.79, 1.04)	0.147	1.02 (0.88, 1.18)	0.794
Cer(d18:1/18:0)/Cer(d18:1/24:0)	1.64 (1.43, 1.87)	3.6×10 <sup>-13</sup>	1.25 (1.08, 1.46)	0.004
Cer(d18:1/24:1)/Cer(d18:1/24:0)	1.14 (1.00, 1.31)	0.056	1.15 (1.00, 1.32)	0.052
Cer(d18:1/16:0)	1.06 (0.93, 1.21)	0.395	1.07 (0.93, 1.24)	0.320
Cer(d18:1/18:0)	1.66 (1.45, 1.90)	1.7×10 <sup>-13</sup>	1.27 (1.09, 1.48)	0.002
Cer(d18:1/24:0)	1.13 (0.98, 1.29)	0.086	1.05 (0.91, 1.22)	0.506
Cer(d18:1/24:1)	1.24 (1.09, 1.42)	0.002	1.17 (1.01, 1.34)	0.032

HRs are expressed per 1 SD.

<sup>a</sup>Unadjusted models, stratified by sex.

<sup>b</sup>The models were stratified by sex and adjusted for age at baseline, BMI, waist circumference, eastern location in Finland, HDL-C, CRP, systolic blood pressure (15 mmHg added for those on antihypertensive medication), lipid lowering treatment, current smoking, family history of diabetes, physical activity and educational level, and in the substudy additionally for fasting glucose and HbA<sub>1c</sub>.

**ESM Table 3.** Baseline characteristics of the FINRISK 2002 substudy with fasting glucose and HbA<sub>1c</sub> data available.

Characteristic	No diabetes	Incident diabetes	Prevalent diabetes
N	1752	217	150
Sex (male)	48 %	54 %	49 %
Current smoker	19 %	14 %	14 %
Lipid treatment	12 %	19 %	33 %
Eastern Finland	70 %	70 %	71 %
Age (years)	59 (53-67)	60 (54-67)	62 (54-67)
BMI	26.7 (24.3-29.5)	30.1 (27.9-33.3)	30.2 (27.6-33.1)
Waist (cm)	90 (82-98)	100 (91-110)	100 (92-108)
Glucose (mmol/l)	5.6 (5.3-6.0)	6.4 (5.9-6.9)	7.0 (6.1-8.5)
HbA <sub>1c</sub> (mmol/mol)	37 (34-39)	40 (38-43)	44 (40-56)
HbA <sub>1c</sub> (%)	5.5 (5.3-5.7)	5.8 (5.6-6.1)	6.2 (5.8-7.3)
Systolic blood pressure (mmHg)	140 (127-155)	148 (135-163)	142 (130-158)
TC (mmol/l)	5.7 (5.1-6.4)	5.8 (5.1-6.7)	5.6 (4.9-6.3)
LDL-C (mmol/l)	3.5 (2.9-4.0)	3.6 (3.0-4.1)	3.3 (2.8-3.8)
HDL-C (mmol/l)	1.5 (1.2-1.8)	1.3 (1.1-1.5)	1.3 (1.1-1.5)
TG (mmol/l)	1.2 (0.9-1.7)	1.7 (1.2-2.2)	1.6 (1.1-2.4)
CRP (mg/l)	1.2 (0.6-2.5)	1.9 (1.0-3.8)	1.9 (0.9-3.8)
Cer(d18:1/18:0)/Cer(d18:1/16:0)	0.415 (0.351-0.482)	0.498 (0.423-0.561)	0.466 (0.397-0.563)
Cer(d18:1/16:0)/Cer(d18:1/24:0)	0.127 (0.112-0.144)	0.123 (0.108-0.142)	0.119 (0.106-0.139)
Cer(d18:1/18:0)/Cer(d18:1/24:0)	0.052 (0.043-0.064)	0.060 (0.051-0.075)	0.057 (0.047-0.072)
Cer(d18:1/24:1)/Cer(d18:1/24:0)	0.688 (0.609-0.787)	0.701 (0.632-0.795)	0.681 (0.612-0.762)
Cer(d18:1/16:0) (μmol/l)	0.304 (0.260-0.350)	0.298 (0.261-0.361)	0.302 (0.249-0.366)
Cer(d18:1/18:0) (μmol/l)	0.123 (0.099-0.158)	0.155 (0.115-0.188)	0.138 (0.106-0.187)
Cer(d18:1/24:0) (μmol/l)	2.380 (1.980-2.867)	2.437 (1.998-2.991)	2.458 (2.086-3.085)
Cer(d18:1/24:1) (μmol/l)	1.645 (1.358-1.950)	1.694 (1.425-2.102)	1.678 (1.382-2.083)

Data are median (interquartile range) or % from total. The data of all variables were available for all individuals. LDL-C, LDL cholesterol; TC, total cholesterol.

**ESM Table 4.** Hazard ratios of clinical laboratory measurements and systolic blood pressure for incident diabetes in the FINRISK 2002 substudy.

	HR (95% CI) <sup>a</sup>	<i>p</i> value <sup>a</sup>	HR (95% CI) <sup>b</sup>	<i>p</i> value <sup>b</sup>
Glucose	2.02 (1.89, 2.16)	<2.2×10 <sup>-16</sup>	1.67 (1.49, 1.87)	<2.2×10 <sup>-16</sup>
HbA <sub>1c</sub>	2.44 (2.14, 2.78)	<2.2×10 <sup>-16</sup>	1.59 (1.36, 1.87)	6.3×10 <sup>-9</sup>
CRP	1.51 (1.33, 1.71)	8.7×10 <sup>-11</sup>	1.10 (0.94, 1.29)	0.252
TC	1.08 (0.95, 1.24)	0.234	1.07 (0.93, 1.23)	0.355
LDL-C	1.13 (0.98, 1.29)	0.087	1.00 (0.86, 1.17)	0.963
HDL-C	0.63 (0.55, 0.73)	3.3×10 <sup>-10</sup>	0.79 (0.67, 0.94)	0.006
TG	1.66 (1.47, 1.86)	1.1×10 <sup>-16</sup>	1.26 (1.08, 1.47)	0.003
Systolic blood pressure	1.58 (1.38, 1.81)	4.3×10 <sup>-11</sup>	1.28 (1.09, 1.49)	0.002

HRs are expressed per 1 SD. LDL-C, LDL cholesterol; TC, total cholesterol.

<sup>a</sup>Unadjusted models, stratified by sex.

<sup>b</sup>The models were stratified by sex and adjusted for age at baseline, BMI, waist circumference, eastern location in Finland, HDL-C, CRP, systolic blood pressure (15 mmHg added for those on antihypertensive medication), lipid lowering treatment, current smoking, family history of diabetes, physical activity and educational level, fasting glucose and HbA<sub>1c</sub>.

**ESM Table 5.** Baseline characteristics of the WECAC study.

Characteristic	WECAC		WECAC (HbA <sub>1c</sub> < 47.5 mmol/mol)	
	No diabetes	Incident diabetes	No diabetes	Incident diabetes
N	3190	154	2258	94
Sex (male)	71 %	69 %	72 %	76 %
Current smoker	32 %	34 %	32 %	33 %
Statin treatment	71 %	71 %	71 %	74 %
Age (years)	61 (54-69)	62 (57-71)	61 (54-69)	65 (58-72)
BMI	26.0 (24.0-28.0)	29.0 (26.0-31.0)	26.0 (24.0-28.0)	29.0 (26.0-31.0)
Glucose (mmol/l)	5.4 (5.0-6.0)	6.4 (5.7-7.6)	5.4 (5.0-6.0)	6.2 (5.6-7.5)
HbA <sub>1c</sub> (mmol/mol)	41 (34-49)	44 (37-53)	38 (31-42)	40 (33-43)
HbA <sub>1c</sub> (%)	5.9 (5.3-6.6)	6.2 (5.6-7.0)	5.6 (5.0-6.0)	5.8 (5.2-6.1)
Systolic blood pressure (mmHg)	140 (125-152)	140 (126-155)	139 (125-152)	144 (130-155)
TC (mmol/l)	5.0 (4.3-5.8)	5.0 (4.3-5.7)	5.0 (4.3-5.8)	5.0 (4.2-5.6)
LDL-C (mmol/l)	3.0 (2.4-3.8)	3.0 (2.4-3.6)	3.0 (2.4-3.8)	3.0 (2.4-3.6)
HDL-C (mmol/l)	1.3 (1.1-1.5)	1.1 (1.0-1.3)	1.3 (1.1-1.5)	1.1 (1.0-1.3)
TG (mmol/l)	1.4 (1.1-2.0)	1.8 (1.3-2.6)	1.4 (1.1-2.0)	1.7 (1.2-2.4)
CRP (mg/l)	1.7 (0.8-3.3)	2.5 (1.2-5.0)	1.6 (0.8-3.3)	2.2 (1.1-4.2)
Cer(d18:1/18:0)/Cer(d18:1/16:0)	0.387 (0.326-0.450)	0.449 (0.385-0.507)	0.382 (0.324-0.446)	0.429 (0.364-0.497)
Cer(d18:1/16:0)/Cer(d18:1/24:0)	0.099 (0.085-0.117)	0.096 (0.083-0.117)	0.100 (0.085-0.118)	0.098 (0.089-0.123)
Cer(d18:1/18:0)/Cer(d18:1/24:0)	0.038 (0.031-0.048)	0.044 (0.036-0.053)	0.038 (0.031-0.048)	0.045 (0.036-0.054)
Cer(d18:1/24:1)/Cer(d18:1/24:0)	0.415 (0.339-0.511)	0.437 (0.353-0.509)	0.419 (0.342-0.513)	0.448 (0.365-0.566)
Cer(d18:1/16:0) (μmol/l)	0.254 (0.216-0.301)	0.257 (0.217-0.310)	0.256 (0.217-0.303)	0.250 (0.216-0.298)
Cer(d18:1/18:0) (μmol/l)	0.098 (0.077-0.123)	0.113 (0.091-0.145)	0.097 (0.077-0.123)	0.107 (0.088-0.139)
Cer(d18:1/24:0) (μmol/l)	2.605 (2.082-3.180)	2.602 (2.188-3.239)	2.607 (2.082-3.180)	2.575 (2.063-2.975)
Cer(d18:1/24:1) (μmol/l)	1.065 (0.881-1.272)	1.153 (0.969-1.396)	1.072 (0.885-1.278)	1.147 (0.969-1.368)

Data are median (interquartile range) or % from total. HbA<sub>1c</sub> measurements were available for 99% of the all WECAC population, all other data were available for all individuals of the study. LDL-C, LDL cholesterol; TC, total cholesterol.

**ESM Table 6.** Hazard ratios of clinical laboratory measurements for incident diabetes in the WECAC study.

WECAC	HR (95% CI) <sup>a</sup>	p value <sup>a</sup>	HR (95% CI) <sup>b</sup>	p value <sup>b</sup>	HR (95% CI) <sup>c</sup>	p value <sup>c</sup>
Glucose	2.18 (1.92, 2.49)	<2.2×10 <sup>-16</sup>	1.96 (1.71, 2.24)	<2.2×10 <sup>-16</sup>	1.93 (1.68, 2.21)	<2.2×10 <sup>-16</sup>
HbA <sub>1c</sub>	1.46 (1.21, 1.76)	6.3×10 <sup>-5</sup>	1.37 (1.13, 1.66)	0.001	1.25 (1.04, 1.51)	0.018
CRP	1.41 (1.21, 1.64)	6.9×10 <sup>-6</sup>	1.20 (1.02, 1.43)	0.033	1.17 (0.99, 1.39)	0.064
TC	0.98 (0.83, 1.15)	0.798	1.05 (0.88, 1.27)	0.567	1.05 (0.87, 1.27)	0.605
LDL-C	0.99 (0.85, 1.16)	0.923	1.01 (0.84, 1.21)	0.907	1.05 (0.87, 1.26)	0.614
HDL-C	0.65 (0.56, 0.77)	2.8×10 <sup>-7</sup>	0.67 (0.56, 0.80)	1.1×10 <sup>-5</sup>	0.72 (0.60, 0.87)	5.9×10 <sup>-4</sup>
TG	1.54 (1.33, 1.78)	5.3×10 <sup>-9</sup>	1.32 (1.10, 1.58)	0.003	1.15 (0.95, 1.38)	0.143
WECAC (HbA <sub>1c</sub> < 47.5 mmol/mol)						
Glucose	2.03 (1.73, 2.40)	<2.2×10 <sup>-16</sup>	1.86 (1.56, 2.21)	2.3×10 <sup>-12</sup>	1.85 (1.55, 2.20)	5.4×10 <sup>-12</sup>
HbA <sub>1c</sub>	1.36 (1.04, 1.77)	0.026	1.24 (0.95, 1.63)	0.111	1.17 (0.90, 1.53)	0.234
CRP	1.30 (1.07, 1.58)	0.009	1.09 (0.88, 1.36)	0.428	1.11 (0.89, 1.39)	0.346
TC	0.89 (0.73, 1.10)	0.288	0.97 (0.76, 1.23)	0.787	0.94 (0.74, 1.20)	0.634
LDL-C	0.99 (0.81, 1.21)	0.919	1.03 (0.81, 1.31)	0.806	1.03 (0.81, 1.31)	0.796
HDL-C	0.65 (0.52, 0.80)	7.4×10 <sup>-5</sup>	0.66 (0.52, 0.84)	6.4×10 <sup>-4</sup>	0.69 (0.54, 0.88)	0.003
TG	1.34 (1.10, 1.62)	0.003	1.11 (0.88, 1.40)	0.386	1.00 (0.79, 1.27)	0.988

HRs are expressed per 1 SD. LDL-C, LDL cholesterol; TC, total cholesterol.

<sup>a</sup>Unadjusted models, stratified by vitamin B intervention groups.

<sup>b</sup>The models are stratified by vitamin B intervention groups and adjusted for age at baseline, sex, BMI, HDL-C, CRP, systolic blood pressure, lipid lowering treatment and current smoking and <sup>c</sup>additionally for fasting glucose and HbA<sub>1c</sub>.

**ESM Table 7.** Performance of predictive models in the WECAC substudy with HbA<sub>1c</sub> < 47.5 mmol/mol.

Variables	C statistic	HL <i>p</i> value	Δ C statistic	Δ <i>p</i> value
Age + BMI + Sex + TG + Ceramide ratio	0.719	0.084	0.011	0.312
Age + BMI + Sex + TG	0.708	0.385		
Age + BMI + Sex + HDL-C + Ceramide ratio	0.731	0.123	0.012	0.343
Age + BMI + Sex + HDL-C	0.719	0.129		
Age + BMI + Sex + CRP + Ceramide ratio	0.719	0.603	0.016	0.228
Age + BMI + Sex + CRP	0.703	0.547		
Age + BMI + Sex + Systolic bp + Ceramide ratio	0.712	0.299	0.019	0.124
Age + BMI + Sex + Systolic bp	0.692	0.387		
Age + BMI + Sex + Ceramide ratio	0.717	0.494	0.018	0.188
Age + BMI + Sex	0.699	0.508		
Age + Sex + Ceramide ratio	0.658	0.260	0.086	0.007
Age + Sex	0.572	0.127		

In addition to C statistics, the table shows Hosmer-Lemeshow (HL) test *p* value for model calibration, and difference of C statistics together with *p* values for models with and without the Cer(d18:1/18:0)/Cer(d18:1/16:0) ceramide ratio.

<sup>a</sup>DM2 (type 2 diabetes) risk factors = family history, physical activity, waist circumference, systolic blood pressure with 15 mmHg added for those on antihypertensive medication

**ESM Table 8.** Baseline characteristics of the PrevMetSyn study.

Characteristic	Baseline
N	371
Sex (male)	51 %
Age (years)	49 (41-54)
BMI	30.0 (28.6-31.8)
Glucose (mmol/L)	5.5 (5.2-5.8)
HbA <sub>1c</sub> (mmol/mol)	38 (36-41)
HbA <sub>1c</sub> (%)	5.6 (5.4-5.9)
Systolic blood pressure (mmHg)	130 (119-141)
TC (mmol/L)	5.3 (4.7-6.0)
LDL-C (mmol/L)	3.6 (3.0-4.2)
HDL-C (mmol/L)	1.4 (1.2-1.7)
TG (mmol/L)	1.2 (0.9-1.7)
CRP (mg/L)	1.2 (0.4-3.1)
Cer(d18:1/18:0)/Cer(d18:1/16:0)	0.494 (0.416-0.577)
Cer(d18:1/16:0)/Cer(d18:1/24:0)	0.081 (0.072-0.095)
Cer(d18:1/18:0)/Cer(d18:1/24:0)	0.040 (0.033-0.049)
Cer(d18:1/24:1)/Cer(d18:1/24:0)	0.493 (0.425-0.556)
Cer(d18:1/16:0) (μmol/l)	0.231 (0.201-0.269)
Cer(d18:1/18:0) (μmol/l)	0.117 (0.093-0.146)
Cer(d18:1/24:0) (μmol/l)	2.844 (2.393-3.443)
Cer(d18:1/24:1) (μmol/l)	1.380 (1.191-1.617)

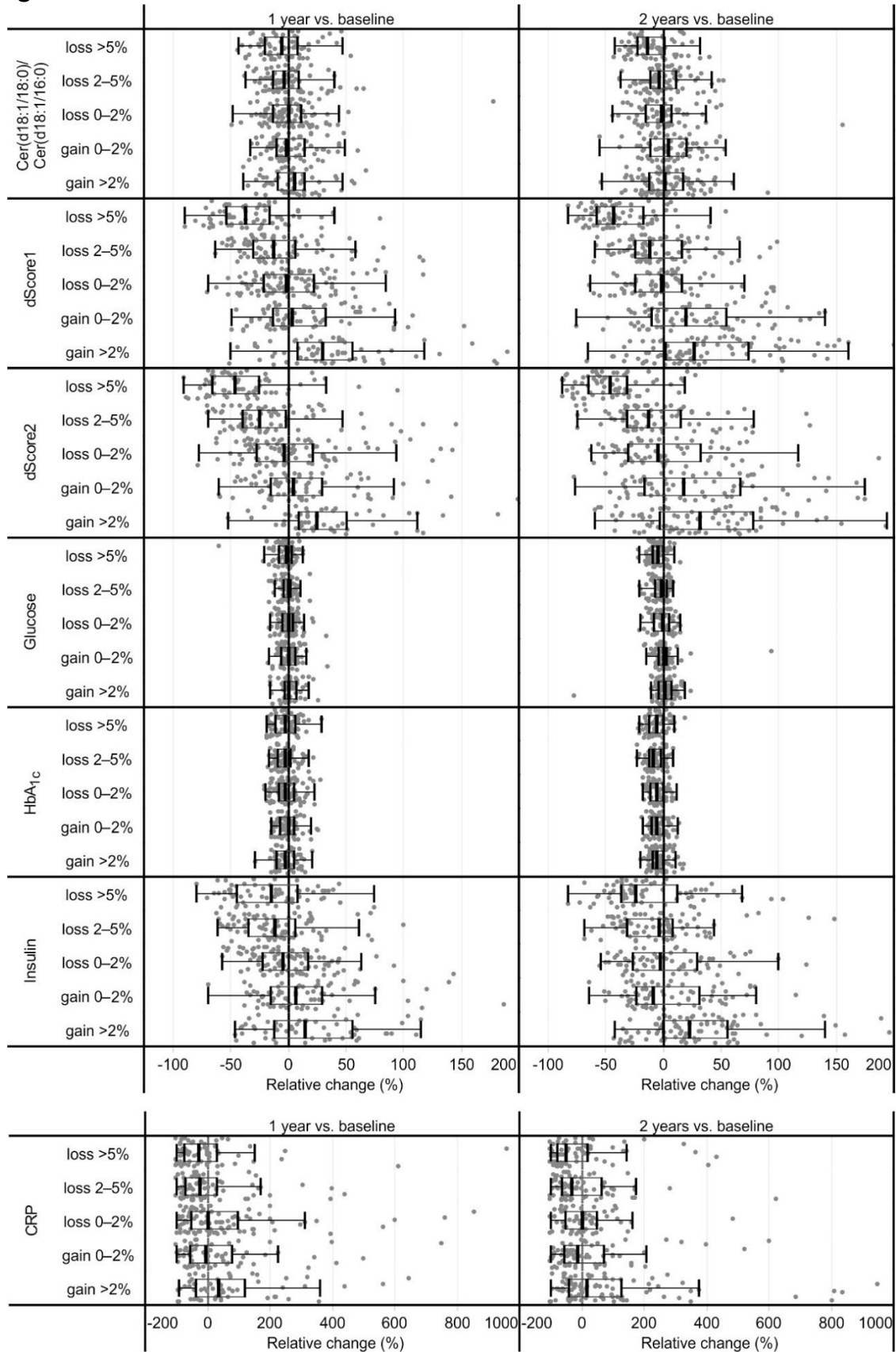
*Data are median (interquartile range) or % from total.*

*Data for all variables were available for all participants.*

*LDL-C, LDL cholesterol; TC, total cholesterol.*



## ESM Figures



**ESM Fig. 1.** Change of ceramide ratio and other variables shown for all participants with different weight change after 1 or 2 years due to dietary intervention. 7 outlier observations have been excluded from dScore1, dScore2 and CRP variables to improve readability of the figure. Whiskers in boxplots represent data within 1.5 times the interquartile range.