SUPPLEMENTARY MATERIAL

Supplementary methods:

Glycome analysis – isolation of IgG. Plasma samples from both MusculRA and SWET-RA studies were randomly distributed across a 96-well plate containing samples, nine standards, and one blank. IgG was isolated from 25 µL of thawed plasma using the CIM® r-Protein G LLD 0.05 mL Monolithic 96-well Plate (2 µm channels) (BIA Separations, Slovenia, Cat. No. 120.1012–2), following previously published protocols,(1, 2) with modifications to use one-quarter of the reagent volumes specified in the original procedure. Elution of IgG was carried out with 0.1 M formic acid (pH 2.5), and eluates were collected in a 96-deep-well plate before neutralization with 1 M ammonium bicarbonate (Acros Organics, USA). After isolation, the monolithic plate was stored at 4 °C in 20% (v/v) ethanol prepared in 20 mM TRIS with 0.1 M NaCl, pH 7.4. Subsequently, 20 µL of purified IgG was aliquoted into a PCR plate (Thermo Scientific, UK) and dried in a vacuum centrifuge. For downstream IgG N-glycan analysis by capillary gel electrophoresis with laser-induced fluorescence (CGE-LIF), deglycosylation, labeling of released N-glycans, and clean-up were performed following modified protocols reported previously.(3, 4)

IgG glycan release, labeling, and clean-up. IgG samples were dried in a vacuum concentrator, resuspended in 3 µL of 1.66× PBS (w/v) and denatured by adding 4 µL of 0.5% SDS (w/v) (Sigma-Aldrich, USA), followed by incubation at 65 °C for 10 min. After denaturation, 2 µL of 4% Igepal-CA630 (Sigma-Aldrich, USA) was introduced, and the mixture was placed on a shaker for 5 min. To release glycans, 1 µL of enzyme solution—consisting of 1.2 U PNGase F (Promega, USA) in 1 µL 5× PBS—was added to each sample and incubated at 37 °C for 3 h. Following enzymatic digestion, the deglycosylation mixture was dried in a vacuum concentrator

for 1 h, reconstituted in 2 µL of ultrapure water, and incubated on a shaker for 5 min to prepare for labeling of the released N-glycans.

The labeling solution was freshly prepared for each sample by combining 2 µL of 30 mM 8-aminopyrene-1,3,6-trisulfonic acid trisodium salt (APTS) (Synchem, DE) in 3.6 M citric acid (Sigma-Aldrich, USA) with 2 µL of 1.2 M 2-picoline borane dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA). Samples were vortexed several times to ensure mixing and then incubated at 37 °C for 16 h. The reaction was terminated by adding 100 µL of cold 80% acetonitrile (ACN) (Carlo Erba, Spain) before proceeding to hydrophilic interaction chromatography solid-phase extraction (HILIC-SPE) for clean-up.

For the clean-up step, 200 µL of Bio-Gel P-10 slurry was applied to each well of a 0.2 µm wwPTFE AcroPrep filter plate (Pall Corporation, USA) and served as the stationary phase. Prior to sample loading, the wells were washed three times with 200 µL of ultrapure water and 200 µL of 80% cold ACN (v/v) (Carlo Erba, Spain). Samples were then loaded and incubated on a shaker for 5 min, after which the wells were washed with 5 × 200 µL of 80% ACN containing 100 mM TEA, pH 8.5 (Carlo Erba, Spain/MilliporeSigma, USA), followed by 3 × 200 µL of 80% ACN (Carlo Erba, Spain), with 2 min incubation at room temperature for each wash. IgG N-glycans were eluted with a total of 500 µL of ultrapure water, collected after 5 min incubation at room temperature, and pooled eluates were stored at −20 °C until further use.

CGE-LIP analysis and data processing. IgG N-glycoprofiling by capillary gel electrophoresis with laser-induced fluorescence (CGE-LIF) was carried out on an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, USA) fitted with an 8-capillary array, each 50 cm in length (Thermo Fisher Scientific, USA). Separation within the capillaries was achieved using

POP-7 polymer (Thermo Fisher Scientific, USA) as the matrix. For sample preparation, 2 μL of APTS-labeled N-glycans were mixed with 8 μL of HiDi formamide in a MicroAmp Optical 96-well reaction plate, ensuring complete and homogeneous resuspension. The composition corresponding to each peak had been determined previously.(5)

Plasma glycan release, labelling, and clean-up. The entire procedure was performed in a 96-well plate format as previously described,(6) using ultrapure water at every step. Briefly, plasma samples (10 μL) were denatured by adding 20 μL of 2% (w/v) SDS (Invitrogen, USA) and incubating at 65 °C for 10 min. Following a 30-min cooling period at room temperature, 10 μL of 4% (v/v) Igepal-CA630 (Sigma-Aldrich, USA) was added, and the mixture was shaken for 15 min on a plate shaker (GFL, Germany). Release of N-glycans was achieved by adding 1.2 U of PNGase F (Promega, USA) prepared in 10 μL of 5× PBS, followed by incubation at 37 °C for 18 h.

After deglycosylation, samples were fluorescently labeled by adding 25 μL of a freshly prepared labeling solution consisting of 2-aminobenzamide (2-AB; Sigma-Aldrich) and 30% glacial acetic acid (Merck, Germany) in dimethyl sulfoxide (Sigma-Aldrich), and incubating for 2 h at 65 °C. Excess reagents were removed by purifying the 2-AB–labeled N-glycans using hydrophilic interaction liquid chromatography solid-phase extraction (HILIC-SPE) with 0.2 μm wwPTFE 96-well membrane filter plates (Pall, New York, NY, USA). During clean-up, samples were repeatedly washed with freshly prepared 96% acetonitrile (ACN), and the labeled glycans were subsequently eluted with 2 × 90 μL of ultrapure water. Purified eluates were stored at −20 °C until further analysis.

HILIC-UHPLC-FLR (Hydrophilic Interaction Chromatography—Ultra-High-Performance Liquid Chromatography with Fluorescence Detection) plasma N-glycan analysis. The analysis of 2-AB–labeled plasma protein N-glycans was carried out on Waters Acquity UPLC H-class instruments. Separation of labeled N-glycans was achieved with Waters UPLC Glycan BEH Amide columns (130 Å, 1.7 μm BEH particles, 2.1 × 10 mm). Chromatograms obtained from the runs were manually integrated and resolved into 39 distinct glycan peaks (GP1–GP39).

Supplementary References:

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6. Akmacic IT, Ugrina I, Stambuk J, Gudelj I, Vuckovic F, Lauc G, et al. High-throughput glycomics: optimization of sample preparation. Biochemistry (Mosc). 2015;80(7):934-42.

Supplemental Table 1: Linear regression associations between IgG di-galactosylated glycans with clinical and physiological variables in patients with RA (n=29)

Independent variable (units)	Dependent variable	Covariates	p value for covariate: age	p value for covariate: sex	Parameter estimate of overall model	p value of overall model
RA disease duration (months)	IgG-G2	Age, Sex	0.002	0.68	0.005	0.35
RA disease activity (DAS-28)	IgG-G2	Age, Sex	0.0003	0.8	- 0.63	0.18
Swollen Joints (#)	IgG-G2	Age, Sex	0.001	0.48	- 0.06	0.72
Tender Joints (#)	IgG-G2	Age, Sex	0.002	0.42	0.10	0.50
Patient Global Health Score (mm)	IgG-G2	Age, Sex	0.0007	0.53	- 0.007	0.78
ESR (mm/hour)	IgG-G2	Age, Sex	0.0001	0.68	- 0.06	0.02
Fat Mass (kg)	IgG-G2	Age, Sex	0.0007	0.60	0.06	0.41
Fat-free mass (kg)	IgG-G2	Age, Sex	0.01	0.008	17.89	0.008
Absolute $\dot{V}O_2$ peak (L/min)	IgG-G2	Age, Sex	0.10	0.68	3.51	0.06
Relative $\dot{V}O_2$ peak (ml/kg/min)	IgG-G2	Age, Sex	0.25	0.25	0.24	0.30
Grip Strength (kg)	IgG-G2	Age, Sex	0.0001	0.05	0.17	0.04
Knee extension strength (Nm)	IgG-G2	Age, Sex	0.0007	0.95	-0.01	0.49

RA rheumatoid arthritis, IgG immunoglobulin G, G2 di-galactosylated glycans, DAS-28 disease activity score 28 joints, ESR erythrocyte sedimentation rate, VO_2 Absolute cardiorespiratory fitness at peak exercise

Supplemental Table 2: Plasma glycome characteristics

Variables	Healthy Control (n=10) Mean (SD)	Early RA (n=10) Mean (SD)	Established RA (n=19) Mean (SD)
Plasma-LB (relative units)	78.1 (2.3)	77.2 (1.4)	76.8 (2.1)
Plasma-HB (relative units)	18.7 (2.5)	19.9 (1.5)	20.0 (2.3)
Plasma-G0 (relative units)	5.4 (2.2)	6.9 (3.0)	6.6 (1.8)
Plasma-G1 (relative units)	9.3 (1.8)	9.8 (1.8)	9.0 (2.2)
Plasma-G2 (relative units)	63.3 (2.2)	60.5 (4.4)	61.1 (3.3)
Plasma-G3 (relative units)	13.5 (2.1)	13.2 (1.9)	13.6 (2.3)
Plasma-G4 (relative units)	**5.3 (0.9)**	**6.7 (1.0)***	6.4 (1.3)
Plasma-S0 (relative units)	19.4 (3.6)	20.6 (4.4)	19.2 (4.2)
Plasma-S1 (relative units)	**22.5 (1.1)**	**21.5 (1.4)***	21.8 (2.7)
Plasma-S2 (relative units)	40.3 (2.7)	38.9 (3.3)	39.8 (3.7)
Plasma-S3 (relative units)	**11.9 (1.6)**	**12.9 (1.3)***	13.1 (1.7)
Plasma-S4 (relative units)	2.8 (0.4)	3.1 (0.4)	2.9 (0.4)
Plasma-Bisecting (relative units)	9.0 (1.4)	9.2 (1.4)	10.0 (3.2)
Plasma-OligoMan (relative units)	**3.2 (0.3)**	**2.9 (0.4)***	3.3 (0.5)
Plasma-CoreF (relative units)	32.7 (5.1)	33.1 (4.3)	32.7 (6.1)
Plasma-AntF (relative units)	**2.4 (0.5)**	**2.9 (0.3)***	3.1 (0.6)

All values are reported as mean (SD) unless noted otherwise. SD standard deviation, RA rheumatoid arthritis, LB low-branched glycans, HB high-branched glycans, G0 non-galactosylated glycans, G1 monogalactosylated glycans, G2 di-galactosylated glycans, G3 tri-galactosylated glycans, G4 tetra-galactosylated glycans, S0 non-sialylated glycans, S1 mono-silaylated glycans, S2 di-sialylated glycans, S3 tri-sialylated glycans, S4 tetra-sialylated glycans, Bisecting glycans with bisecting N-acetylglucosamine, OligoMan Oligo-mannose glycans, CoreF core-fucosylated glycans, AntF antennary fucosylated glycans

p < 0.05 for healthy control versus early RA comparisons

Supplemental Table 3: Percent change in glycome factors following SWET/CHAT lifestyle interventions in older participants with RA and overweight/obesity from the SWET-RA study (n=20)

Variables (% change)	Mean	SD	Median	Minimum	Maximum	p-value
Glycan age	1.73	7.87	0	-10.96	31.25	0.34
IgG-G0	1.51	4.70	1.30	-5.61	16.52	0.17
IgG-G1	-0.05	1.54	-0.22	-3.10	2.32	0.88
IgG-G2	-0.99	5.52	-1.19	-10.48	13.58	0.43
IgG-G	-0.35	1.80	-0.40	-4.25	2.46	0.39
IgG-S0	0.21	1.20	0.02	-1.75	2.84	0.43
IgG-S1	-0.87	4.62	-0.26	-12.58	7.25	0.41
IgG-S2	-1.11	11.61	0.69	-24.44	32.78	0.67
IgG-S	-1.01	5.43	0.12	-15.40	6.92	0.42
IgG-F	-0.002	0.36	-0.07	-0.87	0.66	0.98
Plasma-LB	0.15	1.81	-0.21	-2.99	4.22	0.47
Plasma-HB	-0.41	7.73	-0.13	-16.42	14.36	0.72
Plasma-G0	8.52	29.58	0.90	-11.18	130.45	0.82
Plasma-G1	5.74	21.52	0.12	-9.98	93.91	0.21
Plasma-G2	-0.69	2.67	-0.54	-10.84	1.94	0.25
Plasma-G3	-1.83	8.39	-2.39	-18.54	12.73	0.26
Plasma-G4	3.39	10.72	0.42	-11.69	28.74	0.34
Plasma-S0	6.08	21.38	-0.08	-10.43	93.29	0.17
Plasma-S1	-0.01	2.34	-0.41	-3.18	4.83	0.22
Plasma-S2	-1.39	4.11	-0.85	-17.67	2.39	0.91
Plasma-S3	-0.78	7.5	-0.89	-16.20	14.61	0.15
Plasma-S4	4.83	12.98	0.35	-13.50	28.68	0.64
Plasma-Bisecting	1.86	5.95	0.48	-6.59	17.81	0.11
Plasma-OligoMan	-0.43	6.24	-0.03	-14.19	16.01	0.18
Plasma-CoreF	2.71	10.36	-0.11	-8.45	40.81	0.76

All values in Table are reported as percent (%) change. IgG immunoglobulin G, G0 non-galactosylated glycans, G1 monogalactosylated glycans, G2 di-galactosylated glycans, G all galactosylated glycans, S0 non-sialylated glycans, S1 mono-silaylated glycans, S2 di-sialylated glycans, S all sialylated glycans, F all fucosylated glycans, LB low-branched glycans, HB high-branched glycans, G3 tri-galactosylated glycans, G4 tetra-galactosylated glycans, S3 tri-sialylated glycans, S4 tetra-sialylated glycans, Bisecting glycans with bisecting N-acetylglucosamine, OligoMan Oligo-mannose glycans, CoreF core-fucosylated glycans, AntF antennary fucosylated glycans