Supplementary material

Free fatty acids, glicentin and glucose-dependent insulinotropic polypeptide as potential major determinants of fasting substrate oxidation

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Supplementary table 1: Associations with respiratory quotient (RQ) stratified by weight group.
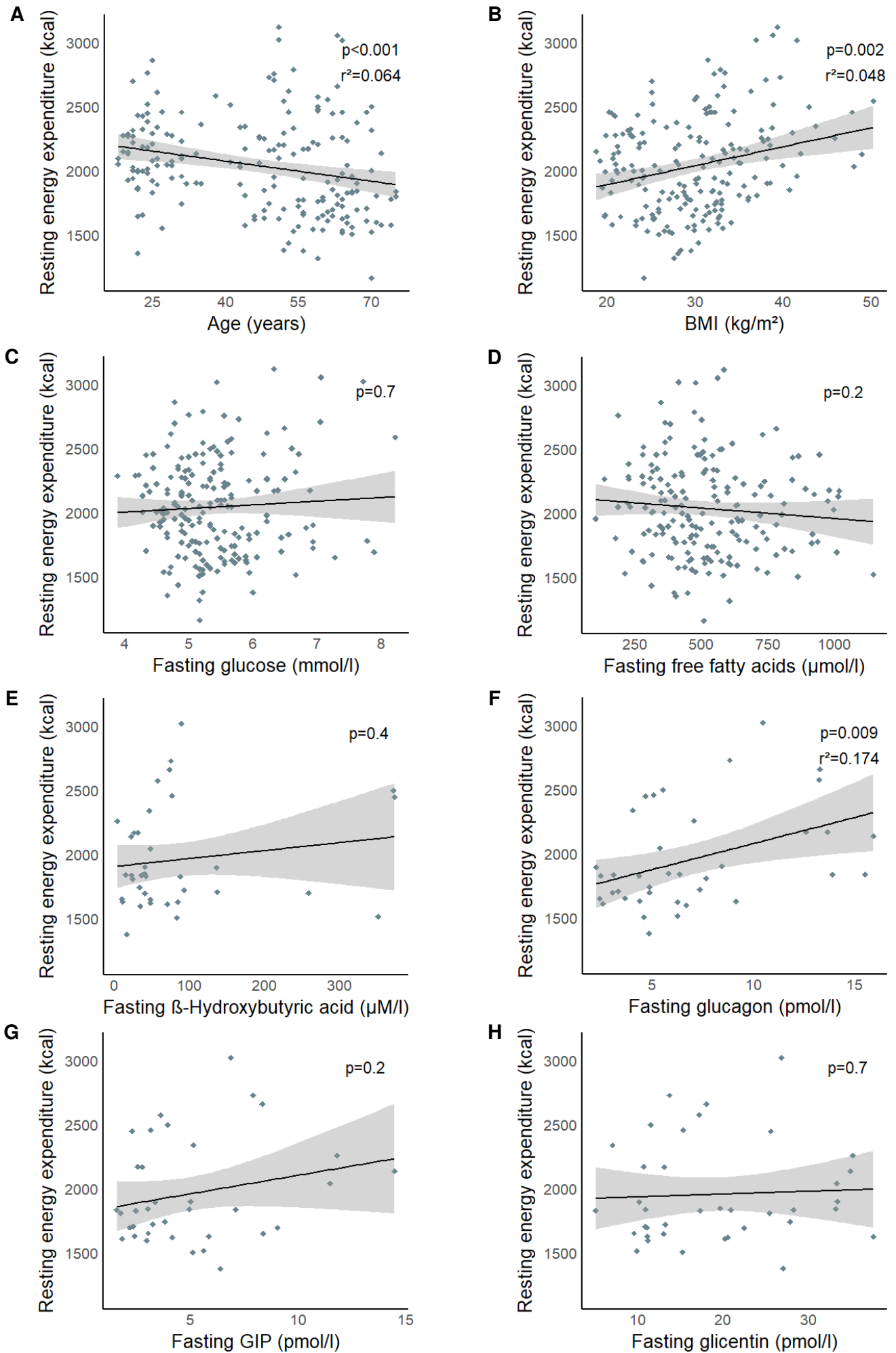
N = 192	Lean/Overweight (BMI < 30 kg/m²) N=95			Obese (BMI ≥ 30 kg/m²) N=97			$p_{interaction}$
	Median (IQR) / n	p value (unadj./adj.*)	Stand. β (standard error)	Median (IQR) / n	p value (unadj./adj.*)	Stand. β (standard error)	
Sex		0.2 / 0.5[#]	-0.078 (0.012)		0.1 / 0.06[#]	-0.191 (0.010)	0.9
Male	65			62			
Female	30			35			
Age (years)	31 (23 - 61)	0.1 / 0.4[##]	-0.113 (0.022)	54 (44 - 62)	**0.03 / 0.02**[##]	-0.245 (0.031)	0.3
Body mass index (kg/m²)	24.6 (21.9 - 27.8)	0.7 / 0.3	0.141 (0.099)	33.6 (32.0 - 37.9)	0.6 / 0.7	0.042 (0.077)	0.5
Blood pressure							
Systolic (mmHg)	132 (125 – 142)[a]	0.2 / 0.1	0.173 (0.101)	140 (133 – 148)	0.07 / 0.1	-0.178 (0.087)	**0.03**
Diastolic (mmHg)	84 (77 – 90)[a]	1.0 / 0.6	0.064 (0.001)	91 (85 – 100)	0.5 / 0.9	-0.016 (0.001)	0.6
Heart rate (bpm)	66 (61 – 75)[a]	0.2 / 0.2	-0.152 (0.059)	72 (65 – 81)	0.7 / 0.7	0.039 (0.060)	0.2
Metabolic syndrome		1.0 / 0.4	-0.096 (0.013)		0.7 / 0.7	0.038 (0.010)	0.8
Yes	19[a]			68[b]			
No	74[a]			26[b]			
Glycemic category		0.7 / 0.2			0.1 / 0.2		0.2
Normal glucose tolerance	66			41			
Prediabetes	28			47			
Diabetes mellitus (newly diagnosed, treatment naïve)	1			9			
Indirect calorimetry							
Fasting respiratory quotient	0.85 (0.79 – 0.90)			0.85 (0.80 – 0.90)			
Resting energy expenditure (kcal)	1925 (1649 – 2211)	0.3 / 1.0	-0.004 (0.073)	2108 (1839 – 2454)[c]	0.9 / **0.03**	-0.310 (0.071)	0.4

Body composition							
Total adipose tissue, MR-derived (l)	23.6 (16.4 – 30.9)[d]	0.8 / 0.9	-0.040 (0.058)	44.5 (41.1 – 55.7)[e]	0.8 / 0.8	0.027 (0.046)	0.7
Subcutaneous adipose tissue lower extremity, MR-derived (l)	9.2 (7.5 – 12.8)[d]	0.9 / 0.9	0.025 (0.057)	16.9 (14.1 – 20.1)[e]	0.9 / 1.0	-0.000 (0.040)	0.9
Visceral adipose tissue, MR-derived (l)	2.1 (1.5 – 3.5)[f]	0.95 / 0.8	0.049 (0.031)	5.1 (4.2 – 7.4)[e]	0.7 / 1.0	-0.003 (0.034)	0.8
Intrahepatic fat, MRS-derived (%)	1.4 (0.8 – 3.1)[g]	0.6 / 0.6	0.075 (0.013)	7.1 (3.4 – 14.2)[h]	0.9 / 0.8	-0.023 (0.011)	0.7
Glycemia							
HbA1c (mmol/mol) / HbA1c (%)	36 (33 – 38)[i] 5.4 (5.2 – 5.7)[i]	0.9 / 0.2	0.160 (0.190)	39 (36 – 42)[j] 5.7 (5.4 – 6)[j]	0.7 / 1.0	-0.000 (0.131)	0.8
Fasting glucose (mmol/l)	5.1 (4.7 – 5.5)[b]	0.8 / 0.3	0.137 (0.093)	5.6 (5.1 – 6.2)[k]	**0.04 / 0.04**	-0.124 (0.072)	0.1
Fasting insulin (pmol/l)	53 (36 – 70)[b]	0.8 / 0.5	0.082 (0.019)	99 (70 – 144)[k]	0.4 / 0.7	0.043 (0.017)	0.7
Fasting C-peptide (pmol/l)	394 (270 – 573)[a]	0.5 / 0.1	0.185 (0.024)	666 (501 – 853)[j]	0.9 / 0.8	-0.025 (0.026)	0.6
Disposition index	1751 (953 – 2735)[j]	0.6 / 0.8	-0.026 (0.014)	1001 (579 – 1470)[l]	0.09 / 0.2	0.146 (0.012)	0.4
Insulin sensitvity index (OGTT-derived)	14.4 (10.2 – 19.2)[m]	0.8 / 0.3	-0.130 (0.021)	6.3 (4.4 – 9.2)[n]	0.7 / 0.9	-0.012 (0.017)	1.0
Adipo-IR (mmol/l*pmol/l)	22.4 (14.5 – 39.5)[a]	0.09 / 0.2	-0.135 (0.014)	49.9 (32.9 – 79.7)[j]	0.3 / 0.3	-0.108 (0.014)	0.7
Plasma lipids							
Free fatty acids (µmol/l)	464 (319 – 654)[a]	**0.005 / 0.01**	-0.269 (0.020)	511 (407 – 632)[j]	**<0.001 / <0.001**	-0.370 (0.028)	0.1
Triglycerides (mg/dl)	73 (54 – 88)[o]	0.9 / 0.6	0.051 (0.025)	108 (82 – 141)[e]	0.6 / 0.7	0.050 (0.022)	0.8
Cholesterol (mg/dl)	172 (147 – 206)[l]	0.6 / 0.5	0.104 (0.000)	210 (177 – 234)[j]	0.9 / 0.7	0.039 (0.000)	0.8
LDL-Cholesterol (mg/dl)	104 (83 – 131)[l]	0.7 / 0.6	0.062 (0.034)	141 (114 – 165)[j]	0.4 / 0.7	-0.051 (0.043)	0.6
HDL-Cholesterol (mg/dl)	55 (44 – 67)[l]	0.7 / 0.7	0.039 (0.042)	49 (43 – 59)[j]	0.5 / 0.1	0.168 (0.045)	0.4

Lipoprotein (a) (mg/dl)	11 (6 – 42)[l]	0.4 / 0.4	-0.096 (0.009)	14 (7 – 32)[j]	0.5 / 0.5	-0.067 (0.010)	1.0
Others							
Thyroid-stimulating hormone (mU/l)	2.0 (1.4 – 3.1)[l]	0.7 / 0.2	-0.14 (0.016)	1.7 (1.1 – 2.4)[j]	0.1 / 0.2	0.137 (0.011)	0.2
C-reactive protein (mg/dl)	0.04 (0.01 – 0.15)[l]	0.6 / 0.2	0.141 (0.007)	0.26 (0.11 – 0.56)[j]	0.8 / 0.9	0.020 (0.008)	0.9
Morning cortisol, serum (nmol/l)	413 (336 – 524)[l]	0.7 / 1.0	0.005 (0.027)	368 (277.8 – 488.8)[j]	0.2 / 0.06	-0.210 (0.026)	0.2

*Adjusted for sex and age; # adjusted for age; ## adjusted for sex. Standardized ß are from multivariate linear regression models. a: n=93; b: n =94; c: n=96; d: n=57; e: n=80, f: n=59; g: n=61; h: n=79; i: n=90; j: n=88; k: n=79; l: n=91; m: n=89; n: n=95; o: n= 71. MR: magnetic resonance; MRS: magnetic resonance spectroscopy; HbA1c: hemoglobin A1c; OGTT: oral glucose tolerance test; Adipo-IR: adipose tissue insulin resistance index; LDL-Cholesterol: low-density lipoprotein cholesterol; HDL-Cholesterol: high-density lipoprotein cholesterol.

Supplementary figure 1

Supplementary figure 1 - Association resting energy expenditure and its´ potential determinants

Resting energy expenditure was negatively associated with age **(A)**, with lower resting energy expenditure with increasing age. There was a positive association of resting energy expenditure and BMI **(B).** Resting energy expenditure was neither significantly correlated with plasma glucose **(C)** nor with free fatty acids **(D)** nor with the ketone body ß-hydroxybutyric acid **(E)**. While glucagon was positively associated with resting energy expenditure **(F)**, this association was not independent of sex (p after adjustment for sex 0.7). Neither GIP **(G)** nor glicentin **(H)** were associated with resting energy expenditure. Data are presented as scatterplots with linear regression lines and 95% CI. P-values were taken from linear regression analyses.

Supplementary table 2: Patient characteristics

	Overall cohort (n=192)	Sub-group with incretin measurements (n=38)
	Median (IQR) / n	
Sex		
Male	100	16
Female	92	22
Age (years)	51 (27 – 62)	62 (56 – 66)
Body mass index (kg/m^2)	30.1 (24.7 – 33.7)	31.5 (28.7 – 33.3)
Total adipose tissue, MR-derived (l)	37.9 (26.5 – 47.3)[a]	39.8 (32.2 – 42.9)
HbA1c (mmol/mol)/ HbA1c (%)	37 (34 – 40)[b] 5.5 (5.3 – 5.8)[b]	39 (37 – 41) 5.7 (5.5 – 5.9)
Fasting glucose (mmol/l)	5.3 (4.9 – 5.8)[c]	5.9 (5.6 – 6.5)
Fasting insulin (pmol/l)	70 (45 – 109)[c]	91 (62 – 147)
Insulin sensitvity index (OGTT-derived)	9.6 (5.6 – 15.2)[d]	5.9 (4.0 – 8.8)
Free fatty acids (µmol/l)	498 (375 – 633)[e]	519 (430 – 651)
Triglycerides (mg/dl)	97 (74 – 137)[f]	98 (73 – 139)[g]
Fasting respiratory quotient	0.85 (0.79 – 0.90)	0.86 (0.80 – 0.92)

Data are presented as median (IQR); a: n=137; b: n =178; c: n=191; d: n=184; e: n=181; f: n=179; g: n=34. MR: magnetic resonance; HbA1c: hemoglobin A1c; OGTT: oral glucose tolerance test.