SUPPORTING INFORMATION

Adiposity-Independent Effects of Aging on Insulin Action and Clearance in Mice and Humans

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Hyperinsulinemic-euglycemic clamps

Mice on chow diet were analyzed at the University of Massachusetts Medical School MMPC, as described (1). In brief, after priming with human insulin (150 mU/kg), mice were subjected to a continuous infusion of 2.5 mU/kg/min insulin and 20% glucose at variable rates to maintain euglycemia. During clamping, [3-^{3}H]-glucose was infused continuously to assess glucose turnover rates and a bolus of 2-[1-^{14}C]-deoxyglucose ([^{14}C]DG) was used to determine tissue-specific glucose uptake. Infusions were administered through the right jugular vein and blood samples were obtained from the tail vein.

Mice on HF diet were clamped at the Vanderbilt University MMPC. Catheters were implanted into a carotid artery and a jugular vein of mice for sampling and infusions respectively five days before the study, as described (2). Insulin clamps were performed on mice fasted for 5 h using a modification of the method described by Ayala et al. (3). [3-^{3}H]-glucose was primed (1.5 μCi) and continuously infused for a 90 min equilibration and basal sampling periods (0.075 μCi/min). [3-^{3}H]-glucose was mixed with the non-radioactive glucose infusate (infusate specific activity of 0.5 μCi/mg) during the 2 h clamp period. Arterial glucose was clamped using a variable rate of 50% glucose (plus trace [3-^{3}H]-glucose) infusion, which was adjusted based on the measurement of blood glucose at 10 min intervals. By mixing radioactive glucose with the non-radioactive glucose infused during a clamp, deviations in arterial glucose specific activity are minimized and steady state conditions are achieved. The calculation of glucose kinetics is therefore more robust (4). Baseline blood or plasma variables were calculated as the

mean of values obtained in blood samples collected at −15 and −5 min. At time zero, insulin infusion (4 mU/kg of body weight per min) was started and continued for 120 min. Mice received heparinised saline-washed erythrocytes from donors at 5 μl/min to prevent a fall in hematocrit. Blood was taken from 80–120 min for the determination of [3-^{3}H]-glucose. Clamp insulin was determined at t=100 and 120 min. At 120 min, 13μCi of [^{14}C]DG was administered as an intravenous bolus. Blood was taken from 2-25 min for determination of [^{14}C]DG. After the last sample, mice were anesthetized and tissues were freeze-clamped for further analysis. Plasma insulin was determined by RIA at the Vanderbilt Hormone Assay and Analytical Core. Radioactivity of [3-^{3}H]-glucose and [^{14}C]DG in plasma samples, and [^{14}C]2DG-6-phosphate in tissue samples were determined by liquid scintillation counting. Glucose appearance (Ra) and disappearance (Rd) rates were determined using steady-state equations (5). Endogenous glucose appearance (endoRa) was determined by subtracting the GIR from total Ra. The glucose metabolic index (Rg) was calculated as previously described (6).

Assessment of physical activity in human subjects

Physical activity was assessed using the 12-item multiple choice Morgenstern physical activity questionnaire (PAQ-M) (7). Each question was assigned a score associated with its average metabolic equivalents (METs), which is the ratio of energy expenditure in a particular activity to the energy expenditure at rest for an individual (8). Activity, measured by number of hours per week, was codified using midpoints of 8 different response categories: 0 (none), 0.5 (<1), 1.5 (1–2), 4 (3–5), 7.5 (6–9), 14.5 (10–19), 24.5 (20–29), and 35 (≥35) (7). A sum of hours in each category weighted by its

corresponding MET value was used to assess the total physical activity score (PAS) in kcal/kg/week.

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Table S1. Hyperinsulinemic-euglycemic clamp results on chow diet

	Young	Aged	Young vs Aged
	n = 12	n = 11	p
Basal			
Body weight (g)	26.1 ± 0.6	26.9 ± 0.5	0.354
Lean mass (g)*	27.0 ± 0.6	27.6 ± 0.4	0.391
Fat mass (g)*	3.1 ± 0.2	3.3 ± 0.2	0.373
% fat*	9.8 ± 0.6	10.4 ± 0.5	0.464
Glucose (mg/dL)	118 ± 4	115 ± 6	0.698
Insulin (ng/mL)	0.150 [0.095]	0.150 [0.108]	0.902
Hepatic glucose production (mg/kg/min)	12.3 ± 0.8	11.3 ± 0.9	0.405
Clamp			
Glucose (mg/dL)	118 ± 2	121 ± 4	0.493
Insulin (ng/mL)	0.731 [0.272]	0.902 [0.175]	**0.039**
Glucose infusion rate (mg/kg/min)	40.7 ± 2.1	35.0 ± 1.9	0.058
Whole body glucose turnover (mg/kg/min)	29.2 ± 1.5	25.7 ± 1.2	0.077
Hepatic glucose production (mg/kg/min)	-11.5 ± 1.7	-9.4 ± 2.1	0.443
Suppression of hepatic glucose production (%)	98.8 ± 1.2	97.0 ± 3.0	0.582
Gastrocnemius muscle glucose uptake (nmol/g/min)	374 ± 42	331 ± 37	0.465
Gonadal adipose tissue glucose uptake (nmol/g/min)	38.2 ± 4.2	31.9 ± 3.3	0.259
MCRI (mL/min/kg)	122 [46.8]	99.0 [20.2]	**0.045**

Values represent means ± SE or median [IQR]. n, number of animals; MCRI, metabolic clearance rate of insulin.
$p<0.05$ appear in bold. *, measured before surgery.

Table S2. Hyperinsulinemic-euglycemic clamp results on high-fat diet

	Young	Aged	Young vs Aged
	n = 7-8	n = 5-6	p
Basal			
Body weight (g)	42.5 ± 1.9	42.7 ± 1.3	0.934
Lean mass (g)*	22.9 ± 0.5	23.3 ± 0.4	0.874
Fat mass (g)*	46.5 ± 1.4	46.4 ± 1.5	0.962
% fat*	32.8 ± 1.3	32.2 ± 1.4	0.738
Glucose (mg/dL)	151 ± 7	117 ± 3	**0.002**
Insulin (ng/mL)	2.30 [3.07]	3.70 [3.57]	0.268
Hepatic glucose production (mg/kg/min)	13.9 ± 1.1	14.4 ± 0.8	0.751
Clamp			
Glucose (mg/dL)	128 ± 5	129 ± 3	0.608
Insulin (ng/mL)	4.50 [6.95]	12.0 [4.85]	**0.048**
Glucose infusion rate (mg/kg/min)	15.4 ± 3.3	24.7 ± 3.9	0.093
Whole body glucose turnover (mg/kg/min)	21.5 ± 2.4	27.6 ± 3.1	0.139
Hepatic glucose production (mg/kg/min)	7.13 ± 0.82	1.72 ± 0.67	**<0.001**
Suppression of hepatic glucose production (%)	46.1 ± 5.4	87.5 ± 3.5	**<0.001**
Gastrocnemius muscle glucose uptake (nmol/g/min)	108 ± 27.8	101 ± 21.8	0.848
Gonadal adipose tissue glucose uptake (nmol/g/min)	15.1 ± 3.8	20.8 ± 2.9	0.961
MCRI (ml/min/kg)	31.7 [38.3]	11.9 [3.75]	**0.048**

Values represent means ± SE or median [IQR]. n, number of animals; MCRI, metabolic clearance rate of insulin.
p<0.05 appear in bold. *, measured before surgery.

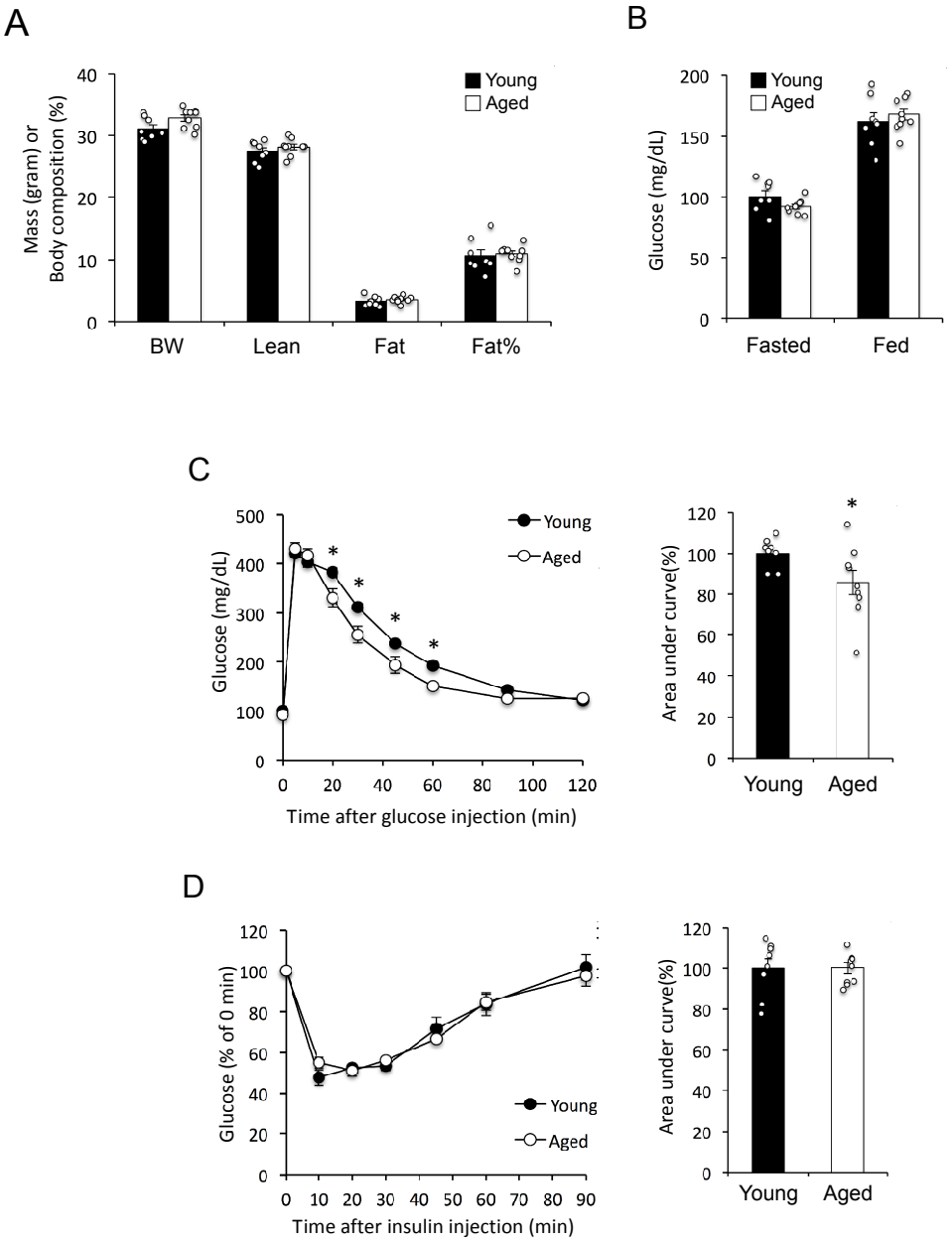

Figure S1 – Aging is associated with improved glucose tolerance in male C57BL/6J mice on a chow diet. *A*: Body weight (BW) and composition of young (6 months) and aged (25 months) mice (n = 8 young + 9 aged mice). Vertical axis shows mass in grams for BW, Lean and Fat, or body composition as percentage of fat for Fat%. *B:* Blood glucose levels in overnight fasted and *ad libitum* fed mice (n = 8 young + 9 aged mice). *C*: Glucose tolerance test (n = 8 young + 9 aged mice). *, $p < 0.05$; comparison by 2-way repeated measures ANOVA (left panel) or Student's t test (right panel). *D*: Insulin tolerance test (n = 8 young + 9 aged mice). Data are presented as mean ± SEM.

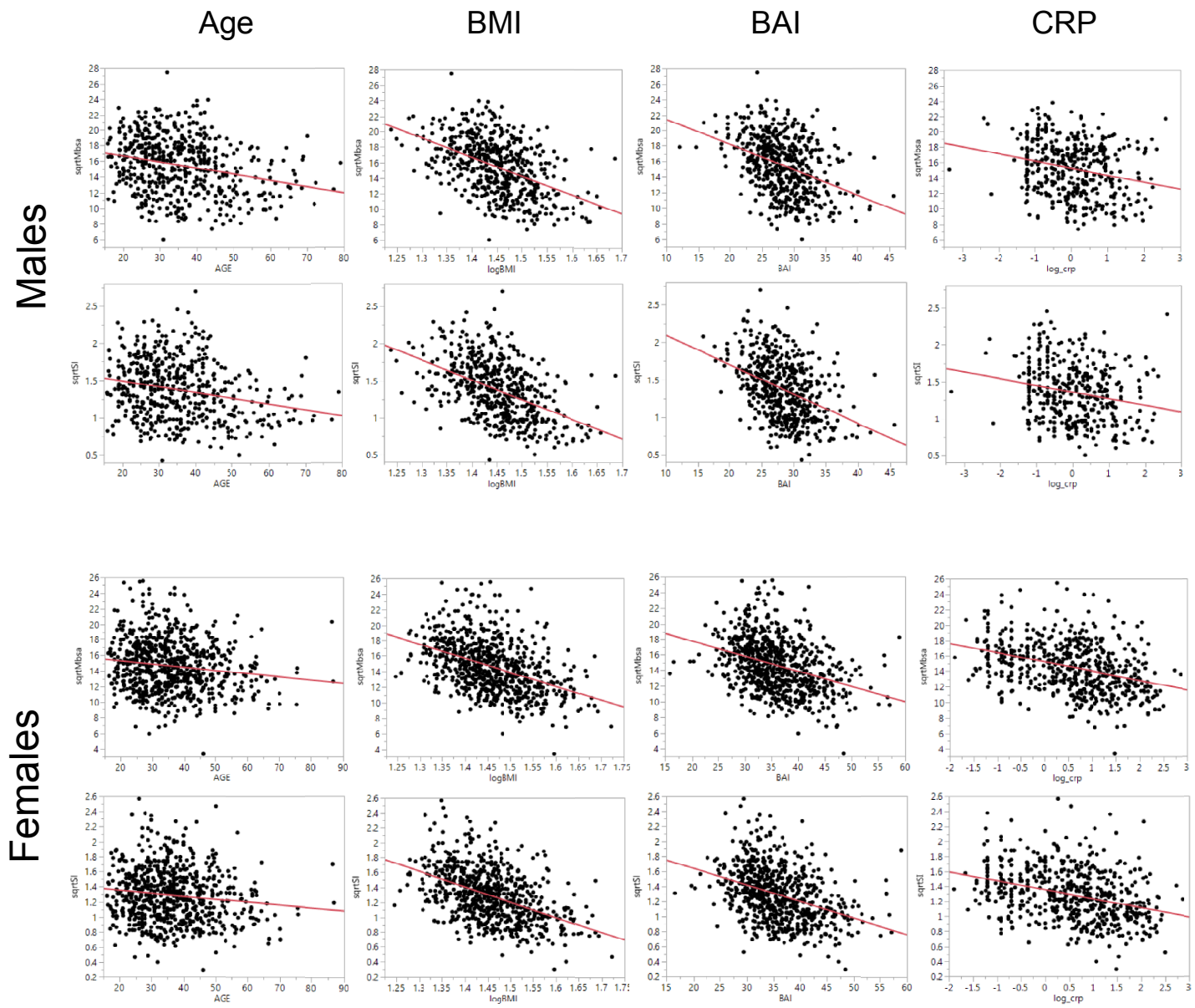

Figure S2 – Correlation of insulin sensitivity with Age, BMI, BAI and CRP levels in Mexican-American males and females. Vertical axes represent M (Mbsa) or M/I (SI), two measures of insulin sensitivity. Horizontal axes show Age, BMI, BAI or CRP. Number of subjects (n), coefficients of correlation (r) and statistical significance (p) for each correlation are presented in Tables 2-4.