Supplemental Data

Comparison of The Effects of High Fat Diet on Energy Flux in Mice Using Two Multiplexed Metabolic Phenotyping Systems

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Running head: Multiplexed Phenotyping & High Fat Diet

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Supplemental Table S1. Heat production (kcal/hr, estimated using the Weir equation) during 2920x Chow feeding, weeks 10/11, corrected for relevant co-variates.

Variables controlled in model	Factor	Mean difference (95% CI)	p-value	AICc
Food ingested, alone	Sex	0.047 (0.030 to 0.064)	0.0000	-264.95
	Instrument	0.014 (0.001 to 0.027)	0.0507	
	Food ingested	0.066 (-0.015 to 0.147)	0.1209	
X+Y physical activity, alone	Sex	0.047 (0.030 to 0.063)	0.0000	-264.67
	Instrument	-0.007 (-0.028 to 0.013)	0.4822	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
Total body mass, alone	Sex	-0.032 (-0.067 to 0.004)	0.0910	-279.30
	Instrument	0.009 (-0.002 to 0.019)	0.1075	
	Total body mass	0.012 (0.007 to 0.018)	0.0001	
Lean body mass, alone	**Sex**	**-0.043 (-0.072 to -0.014)**	**0.0071**	**-289.03 †**
	Instrument	**0.006 (-0.004 to 0.017)**	**0.2321**	
	Lean body mass	**0.016 (0.011 to 0.021)**	**0.0000**	
Total body mass + X+Y physical activity	Sex	-0.030 (-0.066 to 0.007)	0.1262	-276.91
	Instrument	0.005 (-0.016 to 0.025)	0.6560	
	Total body mass	0.012 (0.007 to 0.018)	0.0002	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
Total body mass + Food ingested	Sex	-0.029 (-0.066 to 0.007)	0.1293	-277.12
	Instrument	0.011 (-0.002 to 0.024)	0.1032	
	Total body mass	0.012 (0.007 to 0.018)	0.0002	
	Food ingested	0.024 (-0.053 to 0.102)	0.5472	
Total body mass + X+Y physical activity + Food ingested	Sex	-0.027 (-0.065 to 0.011)	0.1752	-274.69
	Instrument	0.007 (-0.015 to 0.029)	0.5335	
	Total body mass	0.012 (0.006 to 0.017)	0.0003	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
	Food ingested	0.026 (-0.046 to 0.097)	0.4876	
Lean body mass + X+Y physical activity	Sex	-0.030 (-0.066 to 0.007)	0.01262	-276.91
	Instrument	0.005 (-0.016 to 0.025)	0.6560	
	Lean body mass	0.012 (0.007 to 0.018)	0.0002	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
Lean body mass + Food ingested	Sex	-0.042 (-0.072 to -0.013)	0.0094	-286.55
	Instrument	0.007 (-0.005 to 0.020)	0.2700	
	Lean body mass	0.016 (0.011 to 0.021)	0.0000	
	Food ingested	0.009 (-0.062 to 0.081)	0.7990	
Lean body mass + X+Y physical activity + Food ingested	Sex	-0.043 (-0.073 to -0.012)	0.0109	-283.91
	Instrument	0.008 (-0.013 to 0.029)	0.4751	
	Lean body mass	0.016 (0.011 to 0.021)	0.0000	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
	Food ingested	0.009 (-0.056 to 0.074)	0.7837	

*AICc: Akaike information criterion, corrected. * Note that effect of instrument is not significant, regardless of model. † Note AICc value was minimized in model corrected for lean body mass alone*

Supplemental Table S2. Heat production (kcal/hr, estimated using the Lusk equation) during 2920x Chow feeding, weeks 10/11, corrected for relevant co-variates.

Variables controlled in model	Factor	Mean difference (95% CI)	p-value	AICc
Food ingested, alone	Sex	0.047 (0.030 to 0.064)	0.0000	-265.20
	Instrument	0.014 (0.000 to 0.027)	0.0538	
	Food ingested	0.066 (-0.014 to 0.147)	0.1173	
X+Y physical activity, alone	Sex	0.046 (0.030 to 0.063)	0.0000	-264.93
	Instrument	-0.008 (-0.028 to 0.013)	0.4560	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
Total body mass, alone	Sex	-0.032 (-0.067 to 0.003)	0.0876	-279.60
	Instrument	0.008 (-0.002 to 0.018)	0.1184	
	Total body mass	0.012 (0.007 to 0.018)	0.0001	
Lean body mass, alone	**Sex**	**-0.043 (-0.072 to -0.014)**	**0.0066**	**-289.41 †**
	Instrument	**0.006 (-0.004 to 0.016)**	**0.2524**	
	Lean body mass	**0.016 (0.011 to 0.021)**	**0.0000**	
Total body mass + X+Y physical activity	Sex	-0.030 (-0.066 to 0.007)	0.1232	-279.60
	Instrument	0.004 (-0.016 to 0.024)	0.6869	
	Total body mass	0.012 (0.007 to 0.018)	0.0002	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
Total body mass + Food ingested	Sex	-0.029 (-0.066 to 0.007)	0.1257	-277.44
	Instrument	0.011 (-0.002 to 0.024)	0.1092	
	Total body mass	0.012 (0.007 to 0.018)	0.0001	
	Food ingested	0.025 (-0.053 to 0.102)	0.5380	
Total body mass + X+Y physical activity + Food ingested	Sex	-0.027 (-0.065 to 0.011)	0.1726	-275.03
	Instrument	0.007 (-0.015 to 0.028)	0.5558	
	Total body mass	0.012 (0.006 to 0.017)	0.0003	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
	Food ingested	0.026 (-0.045 to 0.097)	0.4767	
Lean body mass + X+Y physical activity	Sex	-0.030 (-0.066 to 0.007)	0.1232	-277.23
	Instrument	0.004 (-0.016 to 0.024)	0.6869	
	Lean body mass	0.012 (0.007 to 0.018)	0.0002	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
Lean body mass + Food ingested	Sex	-0.043 (-0.072 to -0.013)	0.0088	-286.94
	Instrument	0.007 (-0.006 to 0.020)	0.2845	
	Lean body mass	0.016 (0.011 to 0.021)	0.0000	
	Food ingested	0.010 (-0.061 to 0.081)	0.7906	
Lean body mass + X+Y physical activity + Food ingested	Sex	-0.043 (-0.073 to -0.012)	0.0104	-284.30
	Instrument	0.007 (-0.014 to 0.028)	0.4962	
	Lean body mass	0.016 (0.011 to 0.021)	0.0000	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
	Food ingested	0.010 (-0.055 to 0.074)	0.7730	

AICc: Akaike information criterion, corrected. Note that effect of instrument is not significant, regardless of model. † Note AICc value was minimized in model corrected for lean body mass alone.

Supplemental Table S3. Heat production (kcal/hr, estimated using the Weir equation) after 4 weeks of HFD feeding, week 16, corrected for relevant co-variates.

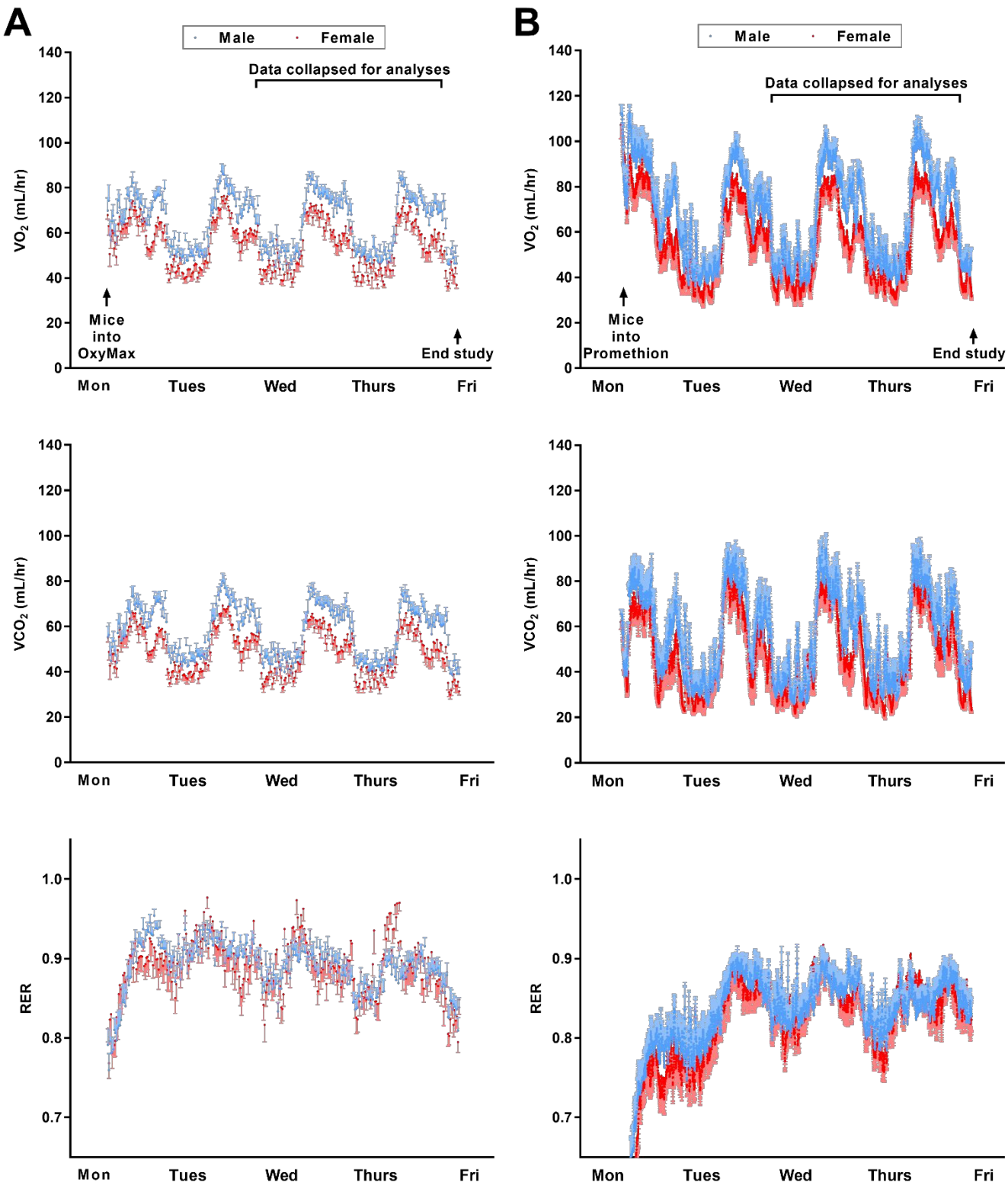
Variables controlled in model	Factor	Mean difference (95% CI)	p-value	AICc
Food ingested, alone	Sex	0.050 (0.030 to 0.071)	0.0000	-119.90
	Instrument	0.031 (0.010 to 0.051)	0.0063 *	
	Food ingested	0.049 (-0.046 to 0.143)	0.3217	
X+Y physical activity, alone	Sex	0.049 (0.028 to 0.070)	0.0001	-118.90
	Instrument	0.030 (-0.002 to 0.062)	0.0780	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
Total body mass, alone	**Sex**	**0.013 (-0.020 to 0.046)**	**0.4315**	**-124.81 †**
	Instrument	**0.026 (0.007 to 0.045)**	**0.0125 ***	
	Total body mass	**0.004 (0.001 to 0.007)**	**0.0166**	
Lean body mass, alone	Sex	-0.037 (-0.093 to 0.018)	0.1987	-122.54
	Instrument	0.022 (0.003 to 0.041)	0.0286 *	
	Lean body mass	0.015 (0.006 to 0.024)	0.003	
Total body mass + X+Y physical activity	Sex	0.011 (-0.023 to 0.044)	0.5366	-122.53
	Instrument	0.036 (0.007 to 0.066)	0.0225 *	
	Total body mass	0.005 (0.001 to 0.008)	0.0109	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.000	
Total body mass + Food ingested	Sex	0.015 (-0.018 to 0.047)	0.3764	-122.94
	Instrument	0.027 (0.008 to 0.046)	0.0085 *	
	Total body mass	0.004 (0.001 to 0.007)	0.0149	
	Food ingested	0.049 (-0.037 to 0.135)	0.2736	
Total body mass + X+Y physical activity + Food ingested	Sex	0.011 (-0.021 to 0.042)	0.5146	-121.85
	Instrument	0.046 (0.016 to 0.077)	0.0062 *	
	Total body mass	0.005 (0.002 to 0.005)	0.0052	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
	Food ingested	0.077 (-0.013 to 0.167)	0.1061	
Lean body mass + X+Y physical activity	Sex	-0.051 (-0.108 to 0.006)	0.0902	-121.32
	Instrument	0.038 (0.010 to 0.066)	0.0144 *	
	Lean body mass	0.017 (0.008 to 0.027)	0.0013	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
Lean body mass + Food ingested	Sex	-0.034 (-0.092 to 0.023)	0.2554	-119.51
	Instrument	0.023 (0.004 to 0.042)	0.0275 *	
	Lean body mass	0.015 (0.005 to 0.024)	0.0054	
	Food ingested	0.015 (-0.072 to 0.103)	0.7345	
Lean body mass + X+Y physical activity + Food ingested	Sex	-0.047 (-0.104 to 0.011)	0.1243	-118.53
	Instrument	0.042 (0.012 to 0.072)	0.0106 *	
	Lean body mass	0.017 (0.008 to 0.026)	0.0018	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
	Food ingested	0.036 (-0.052 to 0.124)	0.4269	

*AICc: Akaike information criterion, corrected. * Note p<0.05 for instrument in almost all models, except X+Y activity alone. † Note AICc value was minimized in model corrected for total body mass alone.*

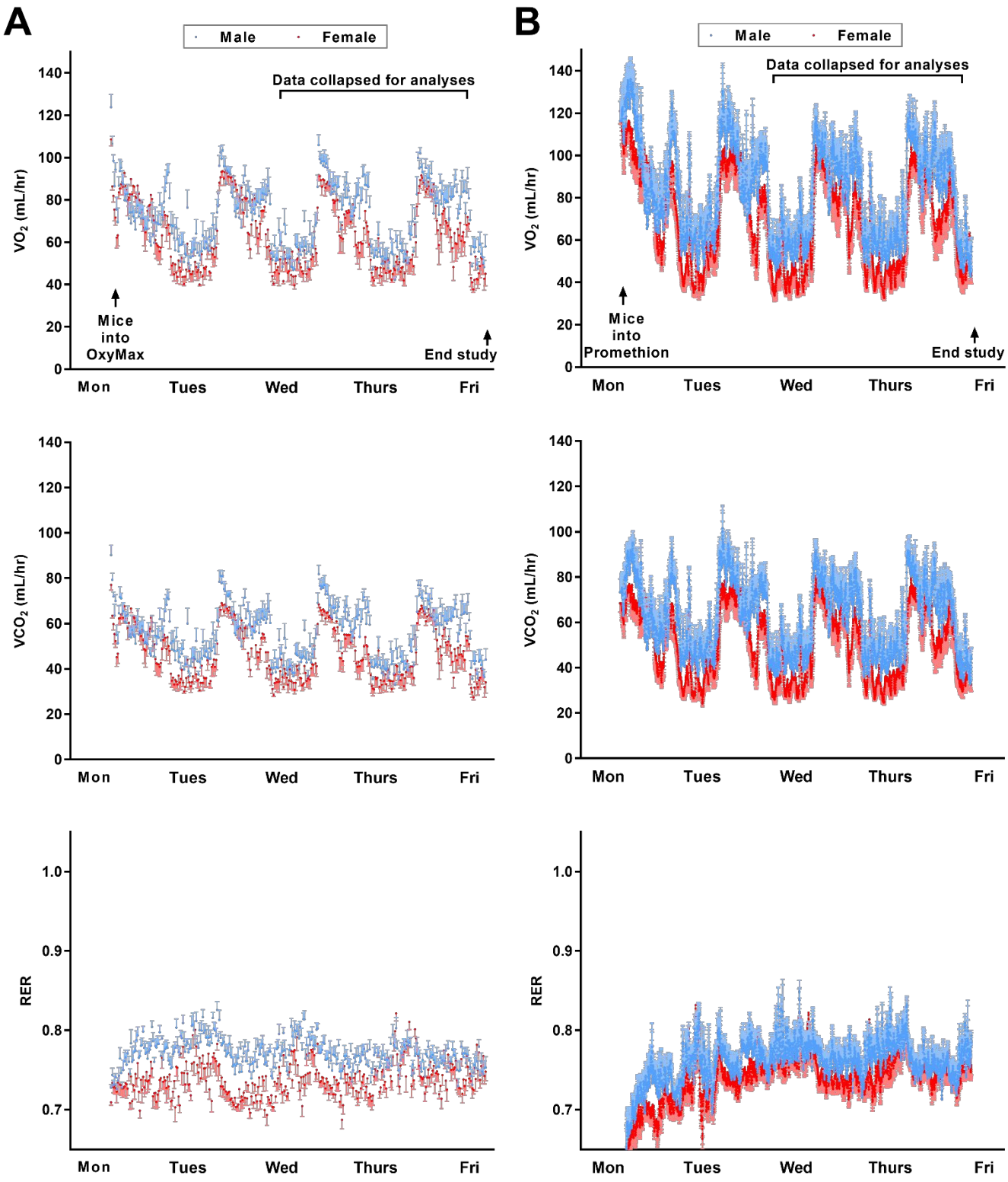
Supplemental Table S4. Heat production (kcal/hr, estimated using the Lusk equation) after 4 weeks of HFD feeding, week 16, corrected for relevant co-variates.

Variables controlled in model	Factor	Mean difference (95% CI)	p-value	AICc
Food ingested, alone	Sex	0.050 (0.030 to 00.070)	0.0000	-120.42
	Instrument	0.031 (0.010 to 0.051)	0.0062 *	
	Food ingested	0.049 (-0.045 to 0.143)	0.3172	
X+Y physical activity, alone	Sex	0.049 (0.028 to 0.069)	0.0001	-119.41
	Instrument	0.030 (-0.002 to 0.062)	0.0777	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
Total body mass, alone	**Sex**	**0.014 (-0.019 to 0.046)**	**0.4222**	**-125.32 †**
	Instrument	**0.026 (0.007 to 0.044)**	**0.0123 ***	
	Total body mass	**0.004 (0.001 to 0.007)**	**0.0166**	
Lean body mass, alone	Sex	-0.037 (-0.092 to 0.018)	0.2036	-123.00
	Instrument	0.022 (0.003 to 0.041)	0.0282 *	
	Lean body mass	0.015 (0.006 to 0.024)	0.0034	
Total body mass + X+Y physical activity	Sex	0.011 (-0.022 to 0.044)	0.5257	-123.03
	Instrument	0.036 (0.007 to 0.065)	0.0224 *	
	Total body mass	0.005 (0.001 to 0.008)	0.0109	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
Total body mass + Food ingested	Sex	0.015 (-0.017 to 0.047)	0.3671	-123.47
	Instrument	0.027 (0.008 to 0.045)	0.0083 *	
	Total body mass	0.004 (0.001 to 0.007)	0.0149	
	Food ingested	0.049 (-0.036 to 0.134)	0.2691	
Total body mass + X+Y physical activity + Food ingested	Sex	0.011 (-0.021 to 0.042)	0.5033	-122.39
	Instrument	0.046 (0.016 to 0.076)	0.0061 *	
	Total body mass	0.005 (0.002 to 0.008)	0.0052	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
	Food ingested	0.077 (-0.012 to 0.166)	0.1040	
Lean body mass + X+Y physical activity	Sex	-0.050 (-0.107 to 0.006)	0.0930	-121.77
	Instrument	0.037 (0.010 to 0.065)	0.0144 *	
	Lean body mass	0.017 (0.008 to 0.027)	0.0013	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
Lean body mass + Food ingested	Sex	-0.034 (-0.091 to 0.024)	0.2624	-119.97
	Instrument	0.023 (0.004 to 0.042)	0.0269 *	
	Lean body mass	0.014 (0.005 to 0.024)	0.0055	
	Food ingested	0.016 (-0.071 to 0.103)	0.7261	
Lean body mass + X+Y physical activity + Food ingested	Sex	-0.046 (-0.103 to 0.011)	0.1283	-119.00
	Instrument	0.042 (0.012 to 0.071)	0.0105 *	
	Lean body mass	0.017 (0.007 to 0.026)	0.0018	
	X+Y physical activity	0.000 (0.000 to 0.000)	0.0000	
	Food ingested	0.036 (-0.051 to 0.124)	0.4208	

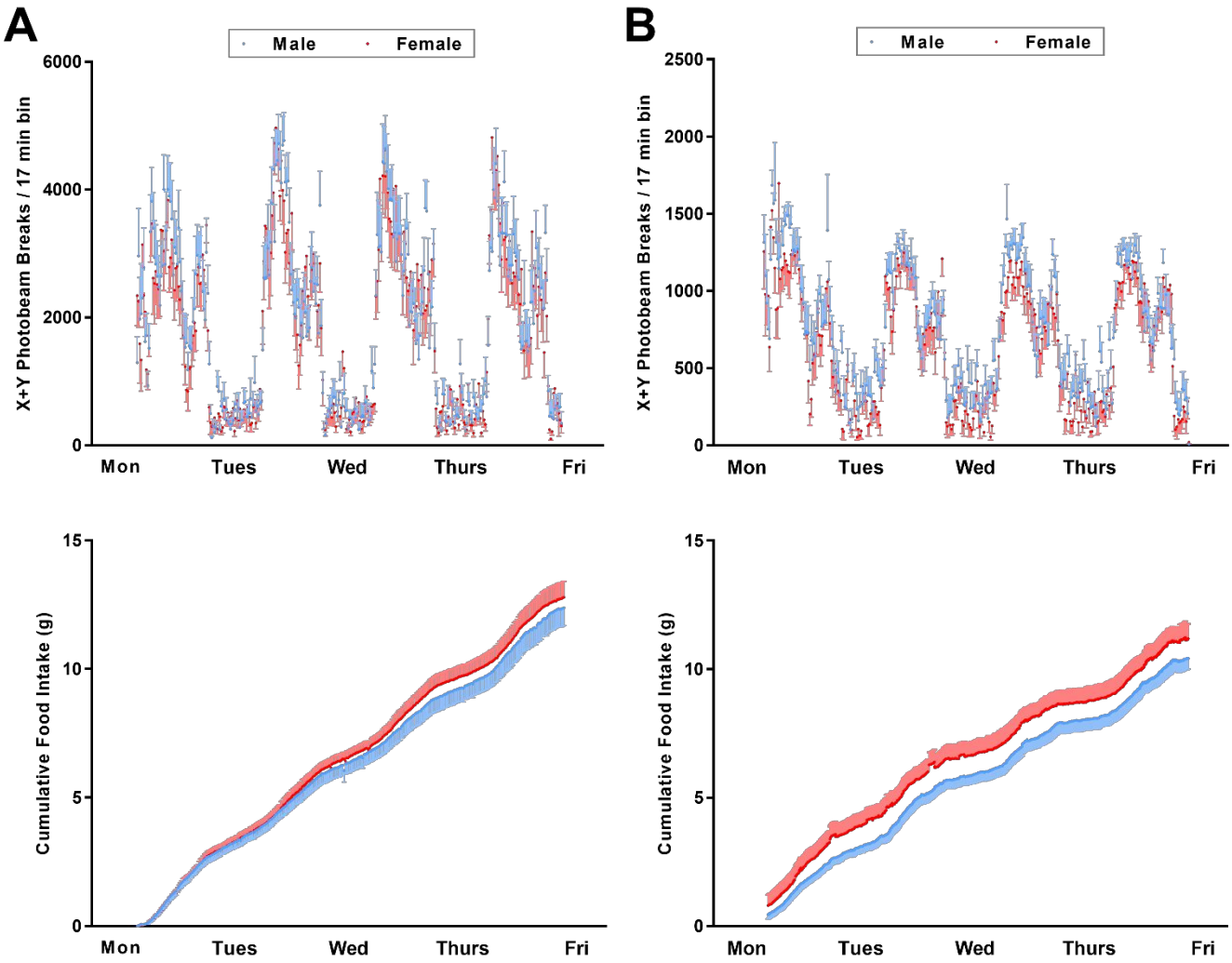
*AICc: Akaike information criterion, corrected. * Note p<0.05 for instrument in almost all models, except X+Y physical activity alone. † Note AICc value was minimized in model corrected for total body mass alone.*



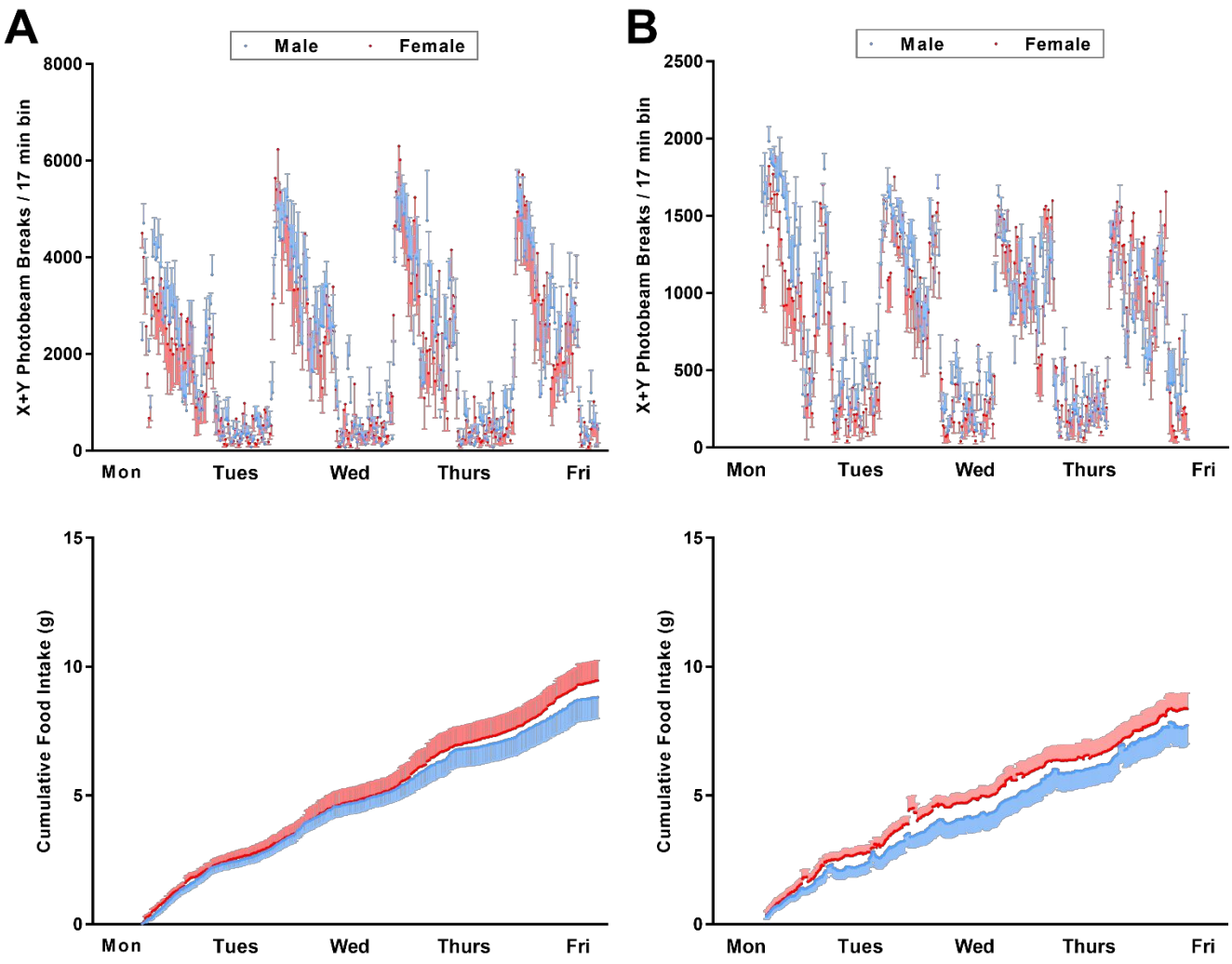
<u>Supplemental Figure S1</u>. Gas analyzer outputs versus time for entire acclimation plus analysis periods, for C57BL/6J mice maintained on 2920x chow, at 10-11 weeks of age. (A) VO_2, VCO_2 and RER values obtained from OxyMax system. (B) VO_2, VCO_2, and RER values obtained from Promethion system. For all studies, n=16 males + 15 females. Data are presented as mean ± SEM (only showing error bars in a single direction, for clarity). Males are illustrated in blue, and females in red.



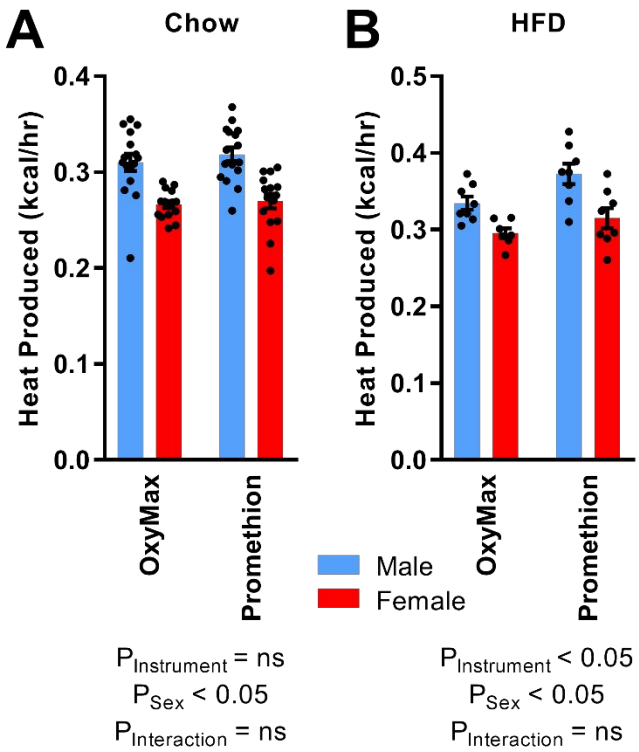
Supplemental Figure S2. Gas analyzer outputs versus time for entire acclimation plus analysis periods, for C57BL/6J mice fed 60% HFD for 5 weeks, at 16 weeks of age. (A) VO_2, VCO_2 and RER values obtained from OxyMax system. (B) VO_2, VCO_2, and RER values obtained from Promethion system. For all studies, n=8 males in OxyMax and 8 in Promethion + 7 females in OxyMax and 8 in Promethion. Data are presented as mean ± SEM (only showing error bars in a single direction, for clarity). Males are illustrated in blue, and females in red.



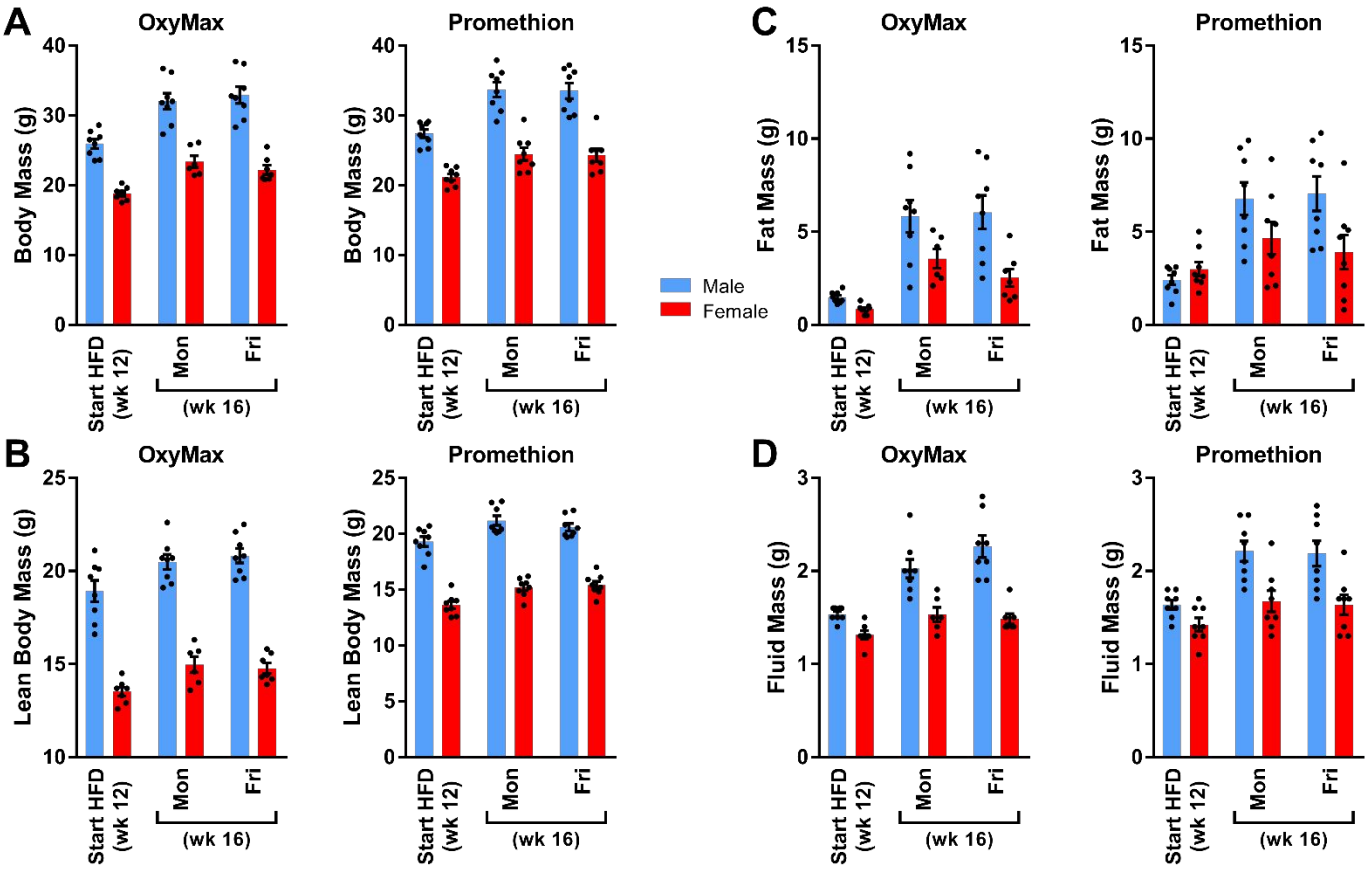
Supplemental Figure S3. Physical activity and food intake versus time for entire acclimation plus analysis periods, for C57BL/6J mice maintained on 2920x chow, at 10-11 weeks of age. (A) Photoelectric beam break counts per 17 minute bin, and cumulative food intake data obtained from OxyMax system. (B) Photoelectric beam break counts (per 17 minute bin, to match sampling rate of OxyMax), and cumulative food intake data obtained from Promethion system. For food intake graphs, only positive error bars for females and negative error bars for males are plotted. For all studies, n=16 males + 15 females. Data are presented as mean ± SEM (only showing error bars in a single direction, for clarity). Males are illustrated in blue, and females in red.



<u>Supplemental Figure S4</u>. Physical activity and food intake versus time for entire acclimation plus analysis periods, for C57BL/6J mice fed 60% HFD for 5 weeks, at 16 weeks of age. A) Photoelectric beam break counts per 17 minute bin, and cumulative food intake data obtained from OxyMax system. (B) Photoelectric beam break counts (per 17 minute bin, to match sampling rate of OxyMax), and cumulative food intake data obtained from Promethion system. For food intake graphs, only positive error bars for females and negative error bars for males are plotted. For all studies, n=8 males in OxyMax and 8 in Promethion + 7 females in OxyMax and 8 in Promethion. Data are presented as mean ± SEM (only showing error bars in a single direction, for clarity). Males are illustrated in blue, and females in red.



Supplemental Figure S5. Estimation of heat production rates using the Lusk equation. (A) Heat production as estimated using the Lusk equation, for mice maintained on 2920x chow, at 10-11 weeks of age. n=16 males + 15 females. (B) Heat production as estimated using the Lusk equation, for mice fed 60% HFD for 5 weeks, at 16 weeks of age. n=8 males in OxyMax and 8 in Promethion + 7 females in OxyMax and 8 in Promethion. Summary data are presented as mean ± SEM.



Supplemental Figure S6. Body composition analyses by time domain nuclear magnetic resonance before and after 4 weeks of 60% HFD. (A) Total body mass. (B) Lean body mass. (C) Fat mass. (D) Fluid mass. For all endpoints, n=8 males in OxyMax vs 8 in Promethion + 7 females in OxyMax and 8 in Promethion. Comparisons performed by three-way ANOVA with $p<0.05$ considered significant. Sex $p<0.05$ and Time $p<0.05$ for all endpoints, and Instrument $p<0.05$ for total and fat masses but p=ns for lean and fluid masses. No significant interactions were observed between [Instrument] and [Sex and/or Time]. Summary data are presented as mean ± SEM.