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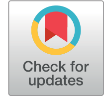

Maternal Exercise and Paternal Exercise Induce Distinct Metabolite Signatures in Offspring Tissues

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METABOLISM

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That maternal and paternal exercise improve the metabolic health of adult offspring is well established. Tissue and serum metabolites play a fundamental role in the health of an organism, but how parental exercise affects offspring tissue and serum metabolites has not yet been investigated. Here, male and female breeders were fed a high-fat diet and housed with or without running wheels before breeding (males) and before and during gestation (females). Offspring were sedentary and chow fed, with parents as follows: sedentary (Sed), maternal exercise (MatEx), paternal exercise (PatEx), or maternal+paternal exercise (Mat+PatEx). Adult offspring from all parental exercise groups had similar improvement in glucose tolerance and hepatic glucose production. Targeted metabolomics was performed in offspring serum, liver, and triceps muscle. Offspring from MatEx, PatEx, and Mat+PatEx each had a unique tissue metabolite signature, but Mat+PatEx offspring had an additive phenotype relative to MatEx or PatEx alone in a subset of liver and muscle metabolites. Tissue metabolites consistently indicated that the metabolites altered with parental exercise contribute to enhanced fatty acid oxidation. These data identify distinct tissue-specific adaptations and mechanisms for parental exercise–induced improvement in offspring metabolic health. Further mining of this data set could aid the development of novel therapeutic targets to combat metabolic diseases.

Regular exercise is an important lifestyle intervention to combat obesity and promote metabolic adaptations that decrease risk of chronic disease development (1). Animal studies have shown that both maternal exercise (2–11) and paternal exercise (2–9,11) are effective to improve metabolic health in adult offspring. Maternal exercise confers these beneficial effects to offspring through modifications to the offspring liver (9,11) and improved mitochondrial biogenesis in skeletal muscle (12). Paternal exercise results in improved glucose tolerance and enhanced insulin-stimulated glucose uptake in skeletal muscle of male offspring (2,3) and improved whole-body insulin sensitivity and decreased adiposity in female offspring (2,13). Importantly, maternal and paternal exercise can negate the detrimental effects of a parental high-fat diet on offspring metabolic health (2,6,8,9,11).

The effects of parental exercise on offspring tissue metabolites have not been investigated. In adults, intra- and intertissue metabolites serve to connect and synchronize metabolic requirements among tissues, which has been hypothesized to mediate whole-body metabolic homeostasis (14), and comprehensive metabolite profiling has led to the discovery of several disease markers (15–18). Thus, alteration of metabolites in the offspring of exercise-trained parents may contribute to improved metabolic homeostasis of offspring.

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Here, we investigated the beneficial effects of parental exercise training in the presence of high-fat feeding on offspring metabolic health. We used targeted metabolomics to determine the impact of maternal exercise, paternal exercise, and the combination of maternal and paternal exercise on the metabolite profile in offspring liver, skeletal muscle, and serum. All forms of parental exercise improved whole-body glucose metabolism in offspring as adults, and metabolomics profiling of offspring serum, muscle, and liver reveal that parental exercise results in extensive effects across all classes of metabolites in all of these offspring tissues. However, these data reveal that each parental exercise paradigm induced a distinctly different tissue metabolite signature in adult offspring, suggesting different underlying mechanisms mediating the beneficial effects of parental exercise on offspring systemic glucose homeostasis.

RESEARCH DESIGN AND METHODS

Mice and Training Paradigm

All procedures were followed as approved by the Institutional Animal Care and Use Committee at the Joslin Diabetes Center. Seven-week-old virgin male C57BL/6 mice were placed on a high-fat diet (60% kcal from fat) for 3 weeks prior to conception and then further subdivided into two groups: sedentary (placed in a static cage) or exercise trained (mice housed with running wheels for 3 weeks). Six-week-old virgin female C57BL/6 mice were placed on a high-fat diet (60% kcal from fat) for 2 weeks preconception, during gestation, and until pup weaning. Female mice were subdivided into two groups: sedentary (placed in a static cage) or exercise trained (mice housed with running wheels for 2 weeks prior to conception and during gestation). Male and female mice were set up as breeders in four different cohorts: sedentary (Sed) (both parents sedentary); MatEx (exercise-trained mothers and sedentary fathers), PatEx (exercise-trained fathers and sedentary mothers), and Mat+PatEx (both parents exercise trained). Litters were culled to six pups per litter, and offspring were chow fed and housed in static cages (sedentary) from birth onward (Fig. 1*A*). One male or female animal was used per litter for each experiment; n represents the number of litters (e.g., for the metabolomics data, $n = 8$ means that one male mouse from eight different litters was used in each group).

In Vivo Metabolic and Physiological Assessments

For intraperitoneal glucose tolerance tests (GTT), mice were fasted for 11 h (2200–900 h) with free access to drinking water. A baseline blood sample was collected from the tails of fully conscious mice, followed by injection of glucose (2 g glucose/kg body wt i.p.), and blood was taken from the tails for glucose measurements at 0, 15, 30, 60, and 120 min.

Glucose Uptake In Vivo

Glucose uptake in vivo was measured as previously described (19). Briefly, mice were fasted overnight (2200–900 h) and then anesthetized with sodium pentobarbital (85–100 mg/kg mouse body wt i.p.). After 30 min, blood was taken from the tail to assess basal glucose concentrations and background radioactivity levels. Mice were injected with either saline or 1 mg glucose in combination with 0.33 μCi [^{3}H]2-deoxyglucose/g mouse body wt administered via the retro-orbital sinus, and blood samples were taken 5, 10, 15, 25, 35, and 45 min later for the determination of glucose and [^{3}H]2-deoxyglucose levels. After the last blood draw, animals were sacrificed by cervical dislocation and tibialis anterior, gastrocnemius, soleus, extensor digitorum longus (EDL), perigonadal white adipose tissue (WAT), subcutaneous WAT, brown adipose tissue (BAT), and heart were harvested and immediately frozen in liquid nitrogen. Accumulation of [^{3}H]2-deoxyglucose was assessed in tissues with a perchloric acid/$Ba(OH)_2$/$ZnSO_4$ precipitation procedure modified from previous work (20).

Glucose Production in Isolated Hepatocytes

Glucose production was measured in primary hepatocytes as previously described (21). Briefly, primary hepatocytes were isolated by liver perfusion with type II collagenase, plated on collagen-coated plates, and subjected to glucose production assays (22–24). Glucose in culture medium was measured and normalized to total protein levels, and the normalized values were used as an index to estimate glucose production.

Metabolite Profiling

Panels of amino acids and acylcarnitines (AC) were measured with targeted tandem mass spectrometry, organic acids (OA) with targeted gas chromatography/mass spectrometry, and nucleotides with targeted liquid chromatography–tandem mass spectrometry as previously described (25–29). Absolute levels of metabolites are reported, calculated relative to stable isotope-labeled internal standards added to serum or extracts of liver and triceps skeletal muscle samples.

Statistical Analysis

All data assumptions for normality (Shapiro-Wilk test), homogeneity of variances (Levene test), and independence were met. Data are presented as mean ± SEM and significance is defined as $P \leq 0.05$ and determined by one-way or two-way ANOVA with a Tukey post hoc test or Bonferroni correction (https://www.sciencedirect.com/topics/medicine-and-dentistry/post-hoc-analysis). Specific statistical analysis used for each experiment is identified in the figure legends. SAS JMP platform and GraphPad Prism

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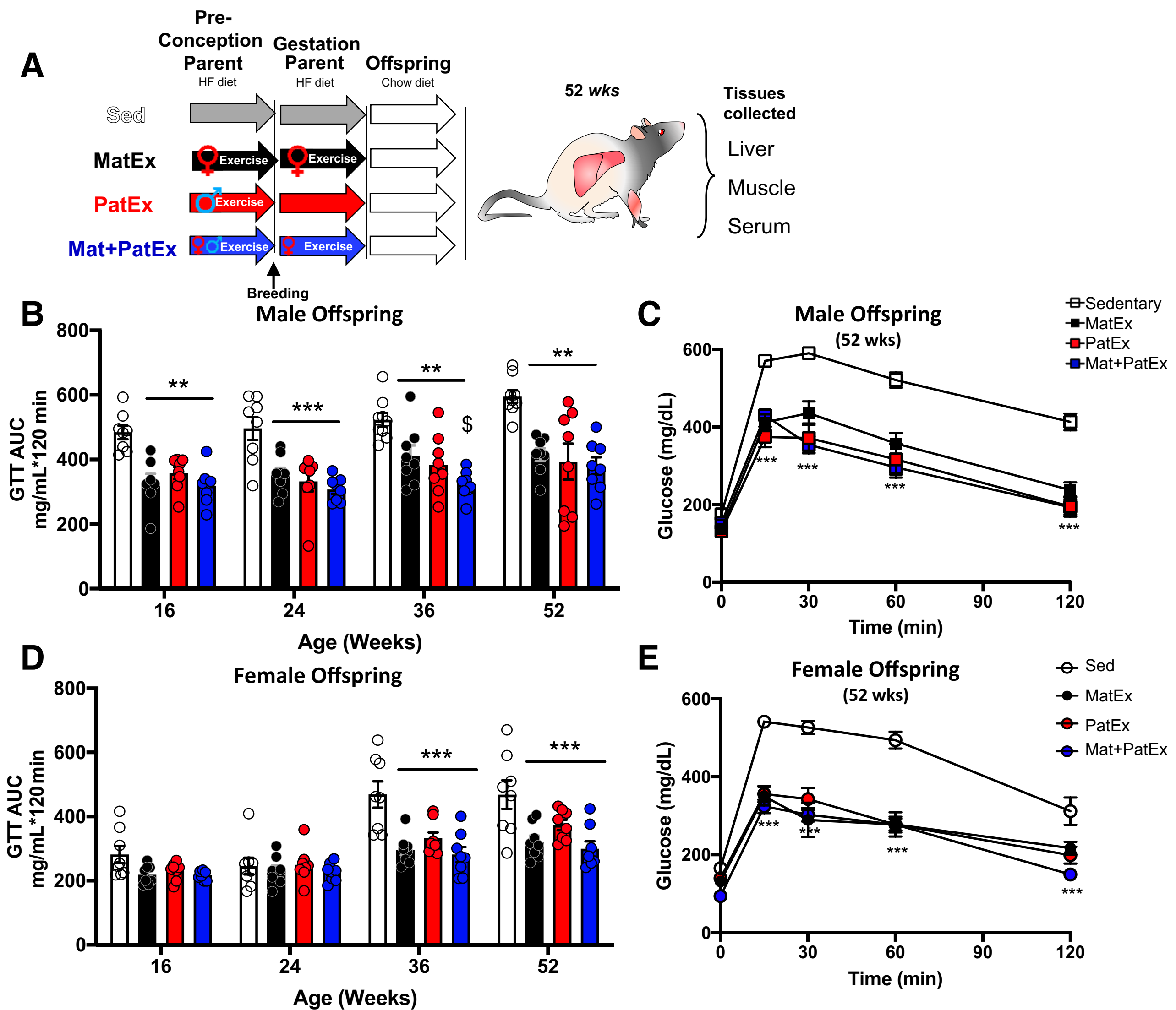

Figure 1—Glucose tolerance is improved in MatEx, PatEx, and Mat+PatEx offspring. (*A*) Experimental design with parental conditions prior to conception shown: Sed, MatEx, PatEx, Mat+PatEx. Glucose tolerance was measured in offspring from Sed, MatEx, PatEx, and Mat+PatEx over a 52 week period, beginning at 16 weeks of age. For GTTs, mice were injected with 2 g glucose/kg body wt i.p. GTT area under the curve (AUC) (*B*) and glucose excursion curve (*C*) at 52 weeks of age in male offspring and GTT area under the curve (*D*) and glucose excursion curve (*E*) at 52 weeks of age in female offspring. Data are presented as calculated area under the curve. Data are means ± SEM (n = 8/group). Asterisks represent differences in comparisons with Sed offspring (**$P < 0.01$; ***$P < 0.001$). HF, high-fat; wks, weeks; \$$P < 0.05$, MatEx vs. Mat+PatEx.

software was used. Heat map analysis was done with a two-way hierarchical clustering using euclidean measure with MetaboAnalyst. Since the parent was treated, the "n" was determined by litter.

Data and Resource Availability

All data sets and resources are available on reasonable request.

RESULTS

Characterization of Dams and Sires

The pregnant dams ran an average of 6 km/day (range 4.2–9.1) during the prepregnancy period and 4 km/day (range 2.2–6.1) during gestation, similar to observations in our previous studies (6,8,11) (Supplementary Table 1). There was no difference in glucose tolerance in sedentary or exercise-trained dams fed a high-fat diet, but body weight, fasting glucose, and fasting insulin were reduced with exercise, similar to what we have previously observed (8,11) (Supplementary Fig. 1).

Exercise training in males significantly reduced body weight. The exercised sires had significantly lower fasting blood glucose and insulin concentrations, as well as improved glucose and insulin tolerance, similar to findings in our previous studies (2,8) (Supplementary Table 2). These data demonstrate that voluntary wheel running improved the metabolic health of sires.

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Maternal, Paternal, and Combined Maternal + Paternal Exercise Training Improves Glucose Tolerance in Male and Female Offspring

In our previous study we identified improved glucose tolerance in MatEx, PatEx, and Mat+PatEx offspring compared with Sed, lower body weights in male offspring from Mat+PatEx compared with all other groups, and no change in food intake or fasting insulin at 52 weeks of age (8). Here, we performed GTTs of offspring born to Sed, MatEx, PatEx, or Mat+PatEx parents at 16, 24, 36, and 52 weeks of age (Fig. 1). Glucose tolerance was improved in male offspring from MatEx, PatEx, and Mat+PatEx compared with offspring from Sed starting at 16 weeks and continuing through 52 weeks of age (Fig. 1*B* and *C*). Female offspring from MatEx, PatEx, or Mat+PatEx also had improved glucose tolerance compared with offspring from Sed parents at 36 and 52 weeks of age (Fig. 1*D* and *E*). The effects of parental exercise on offspring glucose tolerance occurring at a later age in the female offspring (36 weeks) compared with the male offspring (16 weeks) may be due to a more pronounced effect of aging to impair glucose tolerance in male mice (Fig. 1*B* and *C*). These data show that all forms of parental exercise have pronounced effects on glucose tolerance in male and female offspring as they age.

Hepatocyte Function Is Improved in Offspring From Exercise-Trained Parents

Impaired liver glucose metabolism affects whole-body glucose homeostasis (30,31). Previous studies in both rodents and humans demonstrated that maternal or paternal consumption of a high-fat diet impairs liver function in offspring (9,11,12,32–35). We previously determined that maternal exercise improves hepatocyte insulin sensitivity in female offspring (11). To determine whether paternal or combined maternal and paternal exercise also affects hepatocytes, we measured glucose production in primary hepatocytes isolated from all groups of male and female offspring at 6, 12, and 24 weeks of age. Basal rates of hepatocyte glucose production were lower in offspring from exercise-trained parents (MatEx, PatEx, and Mat+PatEx) compared with offspring from Sed parents at 6, 12, and 24 weeks of age (Fig. 2*A*). Hepatocytes isolated from offspring from Sed parents had increased glucose production in the presence of insulin compared with offspring from MatEx, PatEx, and Mat+PatEx as early as 6 weeks of age (Fig. 2*B*). Isolated hepatocytes from offspring from Sed parents also exhibited significantly higher rates of glucagon-stimulated hepatic glucose production at 6, 12, and 24 weeks of age compared with MatEx, PatEx, and Mat+PatEx offspring (Fig. 2*C*). These data indicate that parental exercise elicits a tempering effect on glucose production in concert with increased insulin sensitivity in isolated hepatocytes. This improvement in hepatocyte glucose production at an early age in offspring from exercised parents precedes the improvements in offspring glucose tolerance, indicating that adaptations to the liver could be responsible for altered glucose metabolism as the offspring age.

Paternal Exercise Increases Glucose Uptake in Skeletal Muscle, Heart, and Adipose Tissue

To determine whether additional tissues are involved in the improvements in glucose tolerance in offspring, we assessed in vivo glucose uptake in 52-week-old male offspring. Mice were injected with [^{3}H]2-deoxyglucose in saline (basal) or a 20% glucose solution (glucose) that results in a physiological insulin release (36). At 45 minutes after saline or glucose injection, multiple tissues were removed and glucose uptake was determined. There was no difference in basal glucose uptake among groups in any tissue (Fig. 3). Glucose-stimulated glucose uptake into the EDL muscle was significantly higher in offspring from PatEx compared with all other groups (Fig. 3*A*), and glucose uptake in soleus muscle was significantly higher in offspring from PatEx compared with offspring from Sed and MatEx (Fig. 3*B*). Glucose-stimulated glucose uptake in the

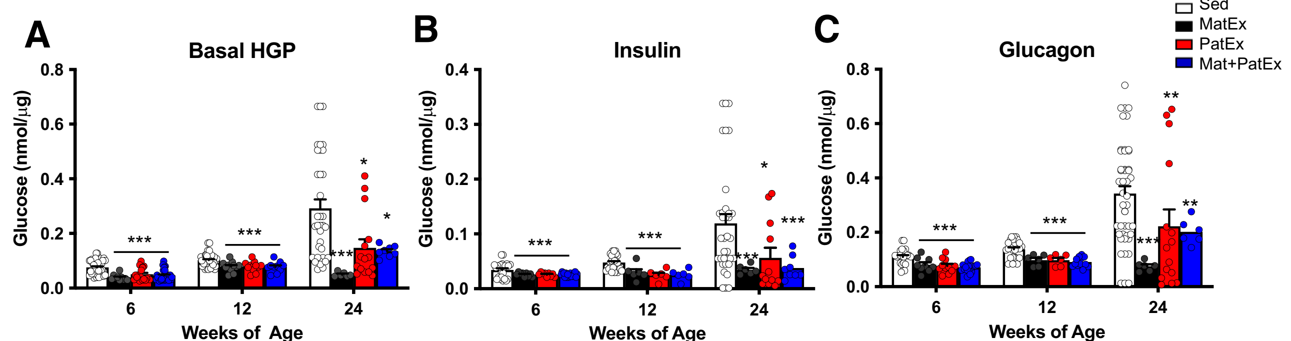

Figure 2—Parental exercise improves hepatic function in isolated hepatocytes. Hepatic glucose production (HGP) was measured in isolated hepatocytes after 4 h in the basal state (*A*), after incubation with insulin (*B*), or after stimulation with glucagon (*C*). Data are expressed as mean ± SEM (*n* = 8/group). Asterisks represent differences in comparisons with Sed offspring (**P* < 0.05; ***P* < 0.01; ****P* < 0.001).

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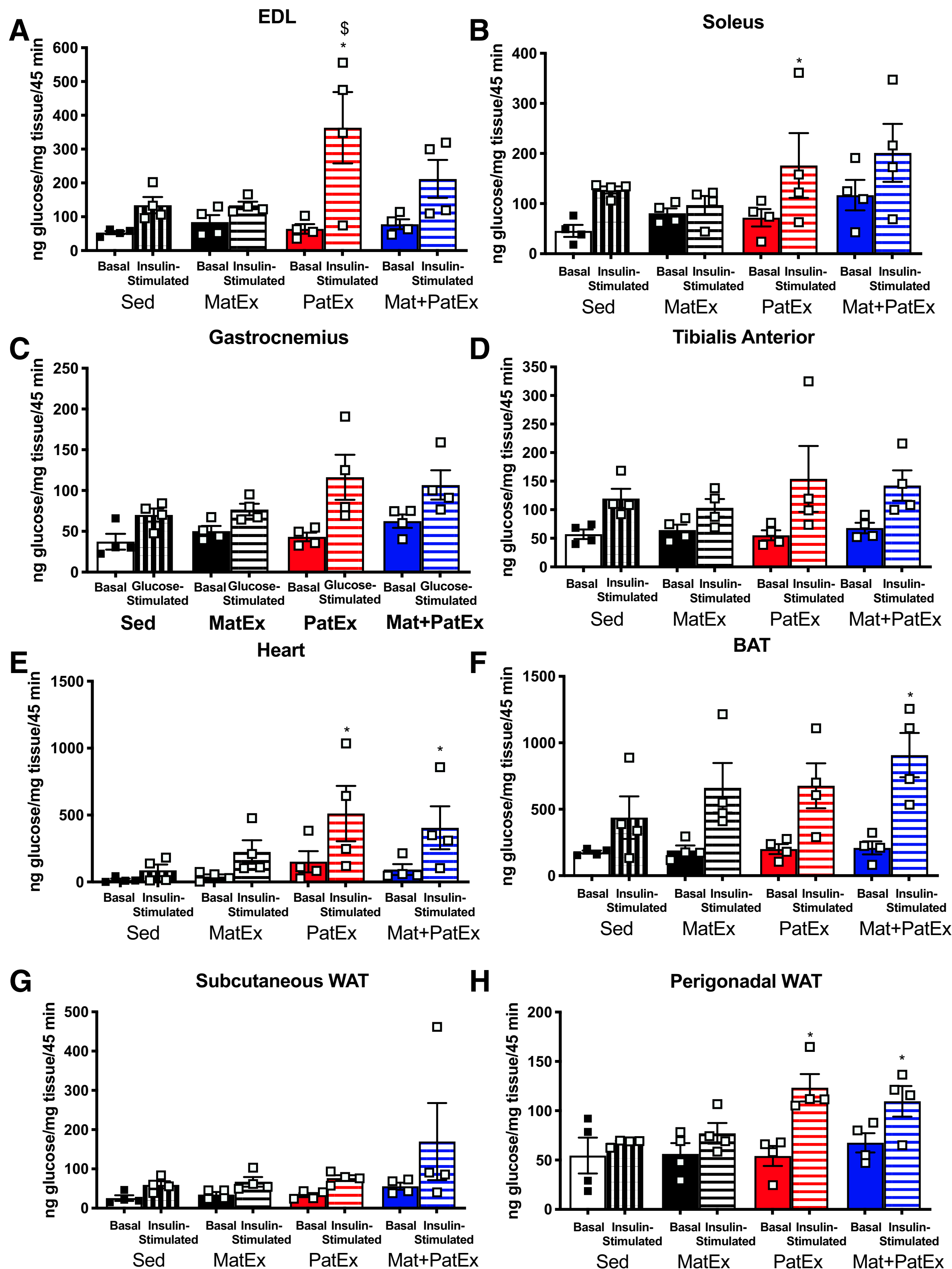

Figure 3—PatEx improves glucose-stimulated glucose uptake in skeletal muscle. Male offspring were administered [^{3}H]2-deoxyglucose in the presence of saline (basal) or 1 mg/kg body wt glucose (glucose-stimulated). Glucose uptake was measured in EDL (*A*), soleus (*B*), gastrocnemius (*C*) and tibialis anterior (*D*) muscle, heart (*E*), BAT (*F*), subcutaneous WAT (*G*), and perigonadal WAT (*H*). Data are means ± SEM (*n* = 4/group). Asterisks represent differences in comparisons with Sed offspring (**P* < 0.05 and ***P* < 0.01). $*P* < 0.05, MatEx vs. PatEx.

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gastrocnemius (Fig. 3C) or tibialis anterior muscles (Fig. 3D) was not different among groups. In the heart, glucose-stimulated glucose uptake was increased in PatEx and Mat+PatEx offspring compared with Sed offspring (Fig. 3E). In BAT, Mat+PatEx offspring had a significant increase in glucose-stimulated glucose uptake compared with Sed offspring (Fig. 3F), whereas in perigonadal WAT PatEx and Mat+PatEx offspring had a significant increase in glucose-stimulated glucose uptake compared with Sed offspring (Fig. 3H). These data suggest that the mechanism by which parental exercise improves offspring glucose homeostasis involves an improvement in glucose uptake in specific skeletal muscle depots, heart, BAT, and perigonadal WAT.

Parental Exercise Affects Tissue Metabolite Concentrations

Abnormal intracellular metabolite concentrations are a hallmark of metabolic disease (37), and comprehensive metabolite profiling could shed light on intra- and inter-tissue metabolite processing that may predict or contribute to development of a disease state (16,17). Since parental exercise significantly impacts glucose metabolism in adult offspring, metabolomics analyses were performed on the serum, liver, and triceps skeletal muscle of 52-week-old male offspring from Sed, MatEx, PatEx, and Mat+PatEx. Targeted mass spectrometry–based methods were used to measure the concentrations of 15 amino acids (Supplementary Fig. 1, Tables 1–3, and Supplementary Tables 3–5), 35 short AC from C2 to C14 (Supplementary Fig. 2, Tables 1–3, and Supplementary Tables 3–5), 32 long AC from C16 to C22 (Supplementary Fig. 2, Tables 1–3, and Supplementary Tables 3–5), 7 OA (Supplementary Fig. 3, Tables 1–3, and Supplementary Tables 3–5), and 24 nucleotide species (Supplementary Fig. 4, Tables 1–3, and Supplementary Tables 3–5).

Heat maps are shown depicting relative average levels of the metabolites summarized in liver, skeletal muscle, and serum across the four experimental groups of offspring: Sed, MatEx, PatEx, and Mat+PatEx (Fig. 4A–C). Two-way hierarchical clustering was performed with mean values scaled for each individual metabolite, revealing differences in metabolite levels among experimental groups. To probe possible exercise-induced changes in individual

Table 1—Exercise effects in liver metabolites

Metabolite class	Metabolite	Sed	MatEx	PatEx	Mat+PatEx
Amino acids	Serine$	42.20 ± 3.21	56.31 ± 3.62*	49.68 ± 2.74	55.56 ± 2.01*
	Proline$	3.83 ± 0.38	7.07 ± 1.14	5.86 ± 0.89	7.46 ± 1.06*
	Leucine/isoleucine$	10.22 ± 0.61	13.66 ± 1.86	12.59 ± 0.96	15.11 ± 1.03*
	Histidine$	17.48 ± 1.15	22.94 ± 2.06	21.37 ± 1.46	24.50 ± 0.94*
	Phenylalanine$	3.21 ± 0.15	4.38 ± 0.53	3.67 ± 0.23	4.64 ± 0.32*
	Tyrosine$	2.80 ± 0.13	4.36 ± 0.52*	3.29 ± 0.18	4.25 ± 0.39*
	Ornithine$	4.99 ± 0.31	8.91 ± 1.78	7.75 ± 1.00	9.70 ± 1.23*
	Arginine$	0.77 ± 0.04	1.12 ± 0.08*	0.96 ± 0.11	1.12 ± 0.07*
	Citrulline$	1.03 ± 0.09	1.51 ± 0.16*	1.07 ± 0.10	1.14 ± 0.10
OA	Pyruvate$	2.11 ± 0.23	1.22 ± 0.12	1.95 ± 0.24*	1.26 ± 0.16*
	α-Ketoglutarate$	0.38 ± 0.05	0.25 ± 0.01	0.31 ± 0.05	0.21 ± 0.01*
Nucleotides	ATP#	1,097.86 ± 126.35	772.56 ± 60.16	877.29 ± 61.85	711.38 ± 73.38*
	NMN#	231.66 ± 18.37	307.05 ± 40.18	290.66 ± 35.59	373.98 ± 37.58*
	UDP-galactose#	530.72 ± 37.77	445.18 ± 29.06	382.55 ± 47.51*	309.98 ± 23.27*
	GDP-mannose#	21.00 ± 0.80	18.51 ± 1.20	17.72 ± 1.21	16.14 ± 1.14*
	GMP#	283.69 ± 12.63	256.48 ± 15.64	202.19 ± 4.8*	208.12 ± 13.33*
	AMP#	1,409.96 ± 121.85	1,227.10 ± 52.83	998.55 ± 79.24*	1,152.04 ± 97.57
	ADP#	639.77 ± 25.31	564.68 ± 42.10	537.94 ± 28.52	492.39 ± 38.94*
Short AC	C4-OH$	0.0631 ± 0.0065	0.0676 ± 0.0059	0.0572 ± 0.0056	0.0851 ± 0.0059*
	C8$	0.0402 ± 0.0026	0.0739 ± 0.0126*	0.0415 ± 0.0052	0.0539 ± 0.0087
	C10:1$	0.0083 ± 0.0005	0.0061 ± 0.0007	0.0055 ± 0.0006*	0.0065 ± 0.0005
	C12$	0.0236 ± 0.0023	0.0199 ± 0.0010	0.0222 ± 0.00016	0.0158 ± 0.0013*
	C14:2$	0.0085 ± 0.0007	0.0064 ± 0.0005	0.0068 ± 0.0007	0.0054 ± 0.0006*
	C14:1$	0.0307 ± 0.0058	0.0197 ± 0.0026	0.0356 ± 0.0054	0.0147 ± 0.0031*
	C14$	0.0489 ± 0.0064	0.0346 ± 0.0033	0.0477 ± 0.0064	0.0235 ± 0.0040*
	C14:3-OH/C12:3-DC$	0.0042 ± 0.0006	0.0019 ± 0.0004*	0.0028 ± 0.0004	0.0026 ± 0.0003
Long AC	C16:1$	0.0925 ± 0.0192	0.0581 ± 0.0101	0.0960 ± 0.0156	0.0371 ± 0.0081*
	C16:2-OH/C14:2-DC$	0.0064 ± 0.0008	0.0036 ± 0.0004*	0.0048 ± 0.0005	0.0038 ± 0.0003*
	C22:3$	0.0015 ± 0.0003	0.0012 ± 0.0002	0.0015 ± 0.0001	0.0026 ± 0.0005*

Data are means ± SEM. Only metabolites with statistically significant effects are shown. GMP, guanosine monophosphate; NMN, nicotinamide mononucleotide; UDP, uridine diphosphate. *$P < 0.05$ vs. Sed offspring with one-way ANOVA and Tukey post hoc test. $Metabolite concentrations are expressed as micromoles per liter. #Metabolite concentrations are expressed as picomoles per milligram.

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Table 2—Summary of significant exercise effects in muscle metabolites

Metabolite class	Metabolite	Sed	MatEx	PatEx	Mat+PatEx
Amino acids	Serine$	28.85 ± 1.16	33.74 ± 1.48*	32.75 ± 1.04	34.38 ± 1.27*
	Arginine$	8.25 ± 0.25	9.87 ± 0.83	6.25 ± 0.61*	8.15 ± 0.50
OA	Lactate$	389.37 ± 28.26	276.96 ± 27.33*	367.02 ± 14.67	295.74 ± 10.47*
	Succinate$	15.53 ± 1.09	18.66 ± 1.38	19.94 ± 1.06	21.12 ± 1.33*
	Malate$	8.70 ± 0.41	6.37 ± 0.39*	6.52 ± 0.40*	6.59 ± 0.21*
	Citrate$	7.24 ± 1.11	4.31 ± 0.28*	4.56 ± 0.64*	4.12 ± 0.43*
Nucleotides	ATP#	2,567.55 ± 135.13	3,127.72 ± 139.32*	3,198.61 ± 118.65*	3,144.16 ± 134.06*
	NAD^+#	371.47 ± 30.31	483.54 ± 16.32*	416.70 ± 21.76	449.59 ± 15.15
	UDP-galactose#	5.89 ± 0.98	8.44 ± 0.68*	5.39 ± 0.30	5.66 ± 0.32
	IMP#	313.26 ± 59.23	103.38 ± 16.19*	198.23 ± 35.47	109.29 ± 22.06*
	ADP-ribose#	90.65 ± 12.01	31.95 ± 5.72*	37.53 ± 5.69*	28.63 ± 4.63*
	AMP#	202.82 ± 21.82	153.55 ± 19.41	157.06 ± 17.16	124.75 ± 21.12
	NADP#	8.64 ± 0.62	11.52 ± 0.74*	9.23 ± 0.54	8.65 ± 0.33
	ADP#	254.24 ± 61.26	62.34 ± 14.45*	120.09 ± 25.96	97.87 ± 21.93*
	GTP#	29.57 ± 2.27	37.64 ± 1.40*	32.93 ± 2.14	32.08 ± 1.30
	s-AMP#	7.67 ± 1.54	2.66 ± 0.54*	2.82 ± 0.52*	2.72 ± 0.79*
Short AC	C5:1$	0.0491 ± 0.0060	0.0538 ± 0.0063	0.0499 ± 0.0021	0.0722 ± 0.0050*
	C8$	0.0177 ± 0.0019	0.0111 ± 0.0012*	0.0129 ± 0.0008	0.0145 ± 0.0013
	C10:1$	0.0047 ± 0.0009	0.0030 ± 0.0002	0.0025 ± 0.0002	0.0021 ± 0.0004*
	C10$	0.0196 ± 0.0018	0.0134 ± 0.0012*	0.0140 ± 0.0012*	0.0121 ± 0.0013 *
	C10-OH/C8-DC$	0.0054 ± 0.0006	0.0033 ± 0.0004*	0.0037 ± 0.0002	0.0041 ± 0.0004
	C12:1$	0.0112 ± 0.0010	0.0074 ± 0.0011*	0.0072 ± 0.0007*	0.0068 ± 0.0009*
	C14:1$	0.0694 ± 0.0067	0.0391 ± 0.0069*	0.0503 ± 0.0043	0.044 ± 0.0054*
	C14:2-OH/C12:2-DC$	0.0041 ± 0.0003	0.0025 ± 0.0002*	0.0026 ± 0.0003*	0.0029 ± 0.0005
	C14:1-OH/C12:1-DC$	0.0125 ± 0.0009	0.0082 ± 0.0014*	0.0086 ± 0.0006	0.0083 ± 0.0009*
Long AC	C20:4$	0.0295 ± 0.0044	0.0161 ± 0.0034*	0.0169 ± 0.0024	0.0179 ± 0.0033
	C22:4$	0.0100 ± 0.0015	0.0046 ± 0.0009*	0.0053 ± 0.0006*	0.0048 ± 0.0008*
	C22:3$	0.0032 ± 0.0003	0.0016 ± 0.0002*	0.0021 ± 0.0006	0.0019 ± 0.0002

Data are means ± SEM. UDP, uridine diphosphate. *$P < 0.05$ vs. Sed offspring with one-way ANOVA and Tukey post hoc test. $Metabolite concentrations are expressed as micromoles per liter. #Metabolite concentrations are expressed as picomoles per milligram.

metabolites in offspring tissues more precisely, we performed volcano plot analysis and focused on analytes that changed on a Log2-fold scale with $P < 0.05$, when comparing each of the exercise interventions with the sedentary control across the three sampled tissues (Fig. 4*D*–*F*). In viewing analyses like this, some uniformity and additivity of responses begin to emerge. For example, in liver of offspring, the levels of several amino acids, including His, Glx, Cit, and Tyr, were increased by MatEx. Tyr and His trended to increase in response to PatEx, but the

Table 3—Summary of significant exercise effects in serum metabolites

Metabolite class	Metabolite	Sed	MatEx	PatEx	Mat+PatEx
Amino acids	Valine$	301.81 ± 20.46	241.02 ± 11.29*	240.51 ± 6.92*	248.20 ± 13.86
	Histidine$	70.62 ± 3.55	76.31 ± 2.38	86.18 ± 3.76*	80.31 ± 3.05
	Aspartate/asparagine$	38.74 ± 1.99	45.90 ± 2.32	53.44 ± 4.64*	54.43 ± 3.70*
	Glutamate/glutamine$	192.19 ± 8.92	238.35 ± 11.35*	227.67 ± 14.94	218.54 ± 10.95
Short AC	C3$	0.83 ± 0.08	1.34 ± 0.11*	1.04 ± 0.06	1.34 ± 0.13*
	C4-OH$	0.2060 ± 0.0154	0.2563 ± 0.0143	0.2945 ± 0.0208*	0.3054 ± 0.0275*
	C4-DC/Ci4-DC$	0.0899 ± 0.0057	0.1416 ± 0.0138*	0.1414 ± 0.0174*	0.1113 ± 0.0079
	C8:1-OH/C6:1-DC$	0.0049 ± 0.0005	0.0087 ± 0.0010*	0.0087 ± 0.0008*	0.0077 ± 0.0009
	C14:1$	0.0858 ± 0.0108	0.0496 ± 0.0041*	0.0632 ± 0.0048	0.0584 ± 0.0055*
Long AC	C16$	0.1983 ± 0.0107	0.1510 ± 0.0108*	0.1579 ± 0.0076*	0.1498 ± 0.0109*
	C16:1-OH/C14:1-DC$	0.0161 ± 0.0014	0.0110 ± 0.0007*	0.0173 ± 0.0010	0.0147 ± 0.0019
	C18:1$	0.2694 ± 0.0297	0.1756 ± 0.0168*	0.1960 ± 0.0158	0.1869 ± 0.0183*
	C18$	0.0672 ± 0.0052	0.0421 ± 0.0072*	0.0535 ± 0.0019	0.0583 ± 0.0034
	C18:1-OH/C16:1-DC$	0.0211 ± 0.0026	0.0126 ± 0.0015*	0.0199 ± 0.0019	0.0166 ± 0.0019
	C20:4$	0.0252 ± 0.0026	0.0182 ± 0.0010*	0.0191 ± 0.0010	0.0151 ± 0.0012*

Data are means ± SEM. *$P < 0.05$ vs. Sed offspring with one-way ANOVA and Tukey post hoc test. $Metabolite concentrations are expressed as micromoles per liter.

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Figure 4—Global metabolite profile reveals differential metabolite handling effect by parental exercise in male offspring. Tissue-specific average of metabolite heat maps in liver (*A*), tricep muscle (*B*), and serum (*C*). Two-way hierarchical clustering using log-transformed and autoscaled mean values within each individual metabolite. Experimental groups are shown at the bottom of each heat map. Volcano plots of tissue metabolites in male offspring at 52 weeks in liver (*D*), tricep muscle (*E*), or serum (*F*) with comparison of the fold induction in male offspring from MatEx (black dots), PatEx (red dots), or Mat+PatEx (blue), compared with Sed offspring. Cutoff-adjusted *P* value = 0.05. *n* = 8/group. Adj. *P* value, cutoff adjusted *P* value.

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combination of Mat+PatEx resulted in significant increases in a larger group of amino acids, including Phe, Tyr, Arg, Leu/Ile, Pro, Orn, and Glx. Similarly, MatEx or PatEx each caused a significant decrease in two to three AC, whereas combined Mat+PatEx caused a decrease in a larger array of AC, including several of the prominent species derived from fatty acid oxidation such as C18:1, C16:0, and C14:0. Mat+PatEx, but not the individual interventions, also caused an increase in hepatic C4-OH carnitine (β-hydroxybutyrylcarnitine), a marker of increased fatty acid oxidation and ketone production, which could suggest that the decreases in fatty acid–derived acylcarnitine species occurred as a result of increased fatty acid oxidation. Consistent with this idea, C4-OH carnitine was also increased in serum of both the PatEx and Mat+PatEx offspring. Finally, MatEx caused a decrease in liver Pyr and α-ketoglutarate levels, and PatEx caused a decrease in AMP, ADP, and guanosine diphosphate (GDP)-mannose, whereas combined Mat+PatEx resulted in clear decreases in hepatic levels of Pyr and α-ketoglutarate, combined with decreases in multiple nucleotide metabolites (guanosine monophosphate, ADP, ATP, guanosine, GDP-mannose). Overall, results of this analysis reveal an additive effect of Mat+PatEx on levels of a subset of metabolites in offspring liver samples.

In skeletal muscle from offspring, both MatEx and PatEx caused a decrease in ADP-ribose and adenolylsuccinate (s-AMP). These analytes were also decreased in muscle of the Mat+PatEx group, now accompanied by decreases in additional nucleotide biosynthetic precursors ADP and inosine monophosphate (IMP). MatEx also increased end products of the nucleotide synthesis pathway in muscle, including ATP, NADP, guanosine-5′-triphosphate (GTP), and NAD. In muscle from offspring in the Mat+PatEx group, all of the nucleotide pathway precursor analytes decreased, including IMP, AMP, ADP, and s-AMP, whereas NAD and NADH levels increased. Similarly, both MatEx and PatEx caused decreases in three to five fatty acid–derived acylcarnitine species in muscle, whereas a larger group of seven lipid-derived acylcarnitine species was decreased in response to Mat+PatEx.

Taken together, the liver, muscle, and serum profiles are consistent in suggesting that parental exercise resulted in enhanced fatty acid oxidation in offspring of exercised parents, especially for combined Mat+PatEx. Combined exercise also caused a number of nucleotide synthesis precursors to decrease in both liver and muscle, accompanied in muscle by increased levels of nucleotide pathway end products (e.g., ATP, NAD, NADH). These changes could suggest increased energetic demand and an attendant increase in fatty acid oxidation in muscle tissue of offspring from exercised parents, an effect that seems to be additive with the combination of maternal and paternal exercise. The increase in multiple amino acids in liver of offspring of exercised parents could reflect a decrease in amino acid oxidation in the face of increased fatty acid catabolism, a decrease in gluconeogenesis, or some combination of these events. Metabolic flux studies will be needed in the future for proper testing of these ideas.

DISCUSSION

The beneficial effects of parental exercise on offspring metabolic health have been well established in rodents (2–11), but the role of parental exercise on offspring metabolite profiles has not been investigated. In the current study, we determined the effects of MatEx, PatEx, and Mat+PatEx on offspring whole-body glucose metabolism, tissue glucose metabolism, and tissue metabolomics. Similar to findings of previous studies (2,3,6–8,11,13,38–40), we found that parental exercise improves glucose tolerance in male and female offspring, an effect associated with improved hepatic function and increased glucose-stimulated glucose uptake in multiple tissues. Importantly, while there were similar improvements in whole-body glucose metabolism in offspring from MatEx, PatEx, and Mat+PatEx, the effects of MatEx, PatEx, and Mat+PatEx on offspring tissue metabolite concentrations were distinct, which may contribute to the offspring's ability to conditionally adapt to metabolic challenges.

The different parental exercise interventions resulted in distinct metabolite profiles in offspring liver, muscle, and serum samples, and volcano plots revealed a discrete set of significantly altered analytes spread among the targeted amino acid, acylcarnitine, and nucleotide modules used in our analysis. Mat+PatEx offspring tended to have a higher number of analytes changed within an analyte module (amino acids, AC, nucleotides) relative to MatEx or PatEx alone. There were no changes in liver and muscle metabolites from PatEx offspring relative to Sed offspring, while multiple analytes were increased in MatEx and Mat+PatEx offspring. In contrast, offspring from MatEx, PatEx, and Mat+PatEx all had significant decreases in select liver and muscle metabolites relative to the Sed offspring.

These commonalities and differences in tissue metabolic profiles in MatEx, PatEx, and Mat+PatEx offspring are difficult to align with effects of different forms of parental exercise on indices of glucose metabolism. Male and female offspring from MatEx, PatEx, and Mat+PatEx exhibited an essentially equal improvement in glucose tolerance relative to Sed offspring at all time points measured. Similarly, measurements of glucose production from hepatocytes isolated from the four experimental groups showed significant decreases in basal, insulin-suppressed, and glucagon-stimulated glucose production in MatEx, PatEx, and Mat+PatEx offspring relative to sedentary controls, albeit with a more pronounced reduction in basal- and glucagon-stimulated glucose output in the MatEx group compared with PatEx or Mat+PatEx. Finally, glucose-stimulated glucose uptake varied among experimental groups and tissues assayed; PatEx offspring had improved glucose-stimulated glucose uptake in EDL, soleus, heart,

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and perigonadal WAT, whereas Mat+PatEx enhanced glucose uptake in heart, BAT, and perigonadal WAT, and MatEx had no significant effects on glucose uptake in any tissue assayed.

These considerations complicate attempts to associate metabolic changes with enhancements in glucose handling and homeostasis among the offspring groups, but there are elements of the metabolite profiles that may contribute to the improved glucose homeostasis in offspring in response to parental exercise. For example, alterations in urine and serum metabolites are seen in patients with type 2 diabetes, including a decrease in OA such as citrate, lactate, and succinate (41–43). We did not measure OAs in serum but found that parental exercise decreases the concentration of the OA metabolites succinate and lactate in muscle, suggesting that muscle metabolism of these OAs may be increased. Thus, profiling of the metabolites pattern within metabolic tissues and serum could potentially serve as a strong biomarker for disease onset.

Our results show that MatEx and Mat+PatEx decrease long and hydroxylated AC in male offspring. This could be important for the metabolic phenotype of offspring given that a high-fat diet has been shown to impair AC metabolism (44) and create a mitochondrial bottleneck that leads to the escape of AC from mitochondrial β-oxidation (37). Elevation of longer and hydroxylated AC are associated with mitochondrial overload and inadequate fatty acid oxidation (18,45,46). Accretion of AC, specifically long and hydroxylated species in skeletal muscle, is related to consumption of a high-fat diet, and the increased presence of β-oxidation products precedes the insulin resistance state (46), ultimately leading to lipid-induced mitochondrial overload and dysfunction. Thus, the decrease in long and hydroxylated AC in muscle and liver may contribute to the offspring's improved insulin sensitivity.

Nucleotide metabolism revolves around the intracellular cycling of purine and pyrimidine nucleotides and regulates, among other things, the pool of adenylate and guanylate, which is an indicator of the absolute concentration of ATP and GTP (47,48), and inadequate regulation of these energetic mediators is related to metabolic disease. Analysis of rodent models of type 2 diabetes and humans with type 2 diabetes shows similar dysregulation of methylamine and nucleotide metabolism, including an increase in *N*-methyl nicotinamide and *N*-methyl-2-pyridone-5-carboxamide in urine (49). Thus, the metabolomics analysis of nucleotide metabolism could provide biomarkers of dysfunctional cellular energetics. Our data indicate that parental exercise increases nicotinamide mononucleotide in the liver of offspring from combined maternal and paternal exercise, which might contribute to improved metabolic health and energy production.

While these data are very informative and set the stage for multiple future analyses, there are some limitations. The metabolite analyses were performed only on male mice, and although we have found that male and female offspring have a similar response in terms of glucose tolerance (6,8,11) (Fig. 1), it is possible that the metabolite response in female offspring is slightly different. In addition, here we only investigated the effects of parental exercise and did not consider whether parental enrichment (i.e., exposure to a wheel cage) could contribute to the beneficial effects in offspring. Our pilot data generated to test this indicate that the effect is exercise specific, but this has not been rigorously investigated. Another limitation is that the effects of parental exercise on parental stress hormones were not measured in this study; it is possible that some of the beneficial effects of exercise are conferred by meditating the levels of stress hormones in the parents, and this should be accounted for in future studies. Finally, these studies set the stage for future analyses of metabolic flux involving stable isotope–labeled substrates (e.g., ^{13}C-labeled fatty acids or amino acids) and investigation of the transcriptome or DNA methylome to provide a complete picture of how parental exercise reprograms offspring tissues and metabolism.

In conclusion, we provide the first evidence that parental exercise regulates tissue metabolites in offspring. Surprisingly, we found that while the improvements in offspring glucose metabolism in response to maternal, paternal, and the combination of maternal and paternal exercise are similar, they induced distinctly different metabolomics phenotypes. The underlying molecular mechanisms for these effects will require further investigation, but the current data will provide a comprehensive resource for future hypothesis-driven investigations of the mechanisms through which parental exercise affect the metabolic health of offspring. These data may reveal novel pathways for maternal, paternal, and the combination of maternal and paternal exercise to regulate whole-body metabolic health of offspring and potentially provide new therapeutic targets to treat metabolic disease.

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Author Contributions. D.H.-S. analyzed metabolomics data and wrote and edited the manuscript. C.M., L.A.B., and J.E.H. analyzed metabolomics data. T.H. performed experiments with isolated hepatocytes. M.F.H. performed in vivo glucose uptake experiments. O.I. performed the metabolomics experiments and helped with the analysis. C.B.N. provided oversight for all metabolomics experiments and wrote and edited the manuscript. K.I.S. designed experiments, performed experiments, analyzed data, and wrote and edited the manuscript. L.J.G. designed experiments, analyzed data, and wrote and edited the manuscript. K.I.S. and L.J.G. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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