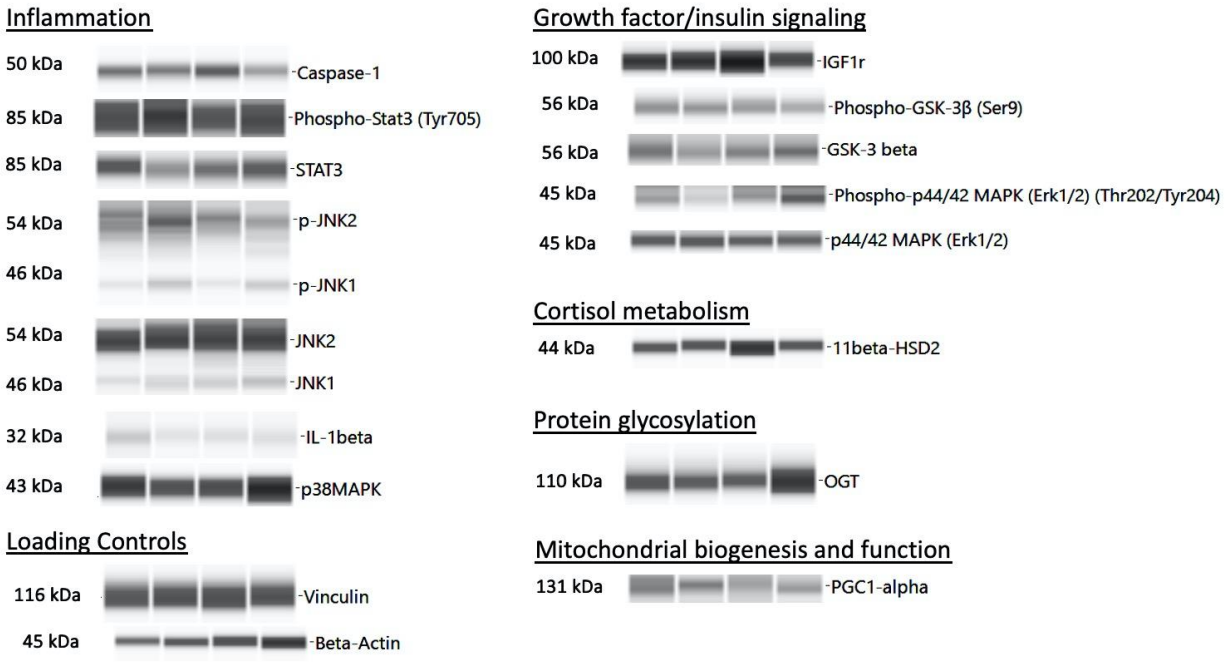
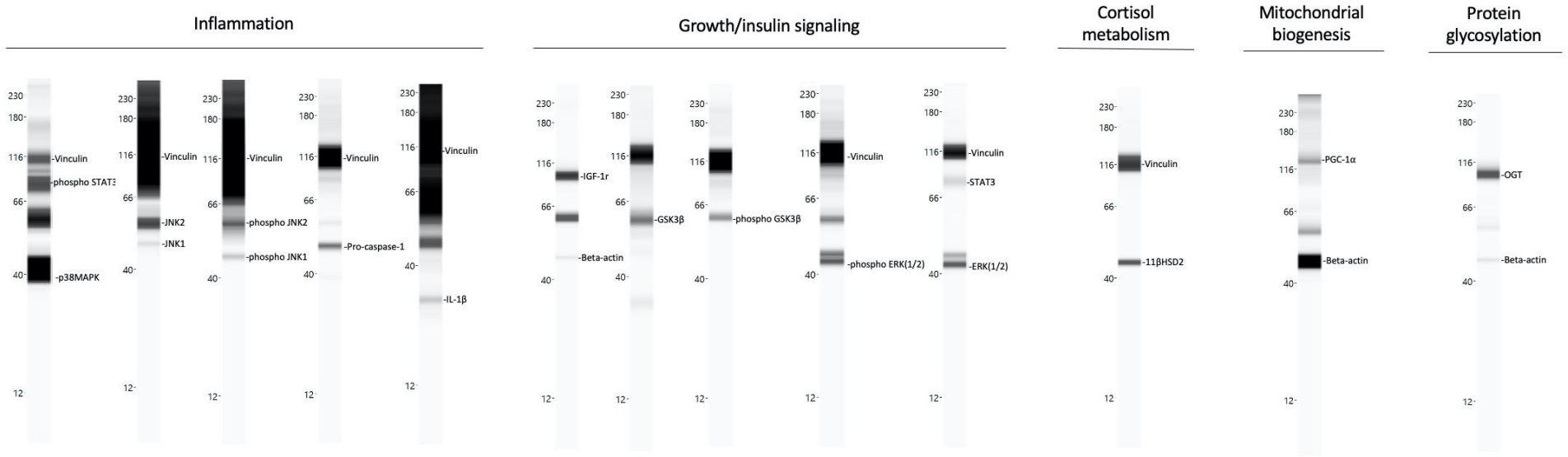
Supplementary Figures and Tables—Keleher et al. 2020

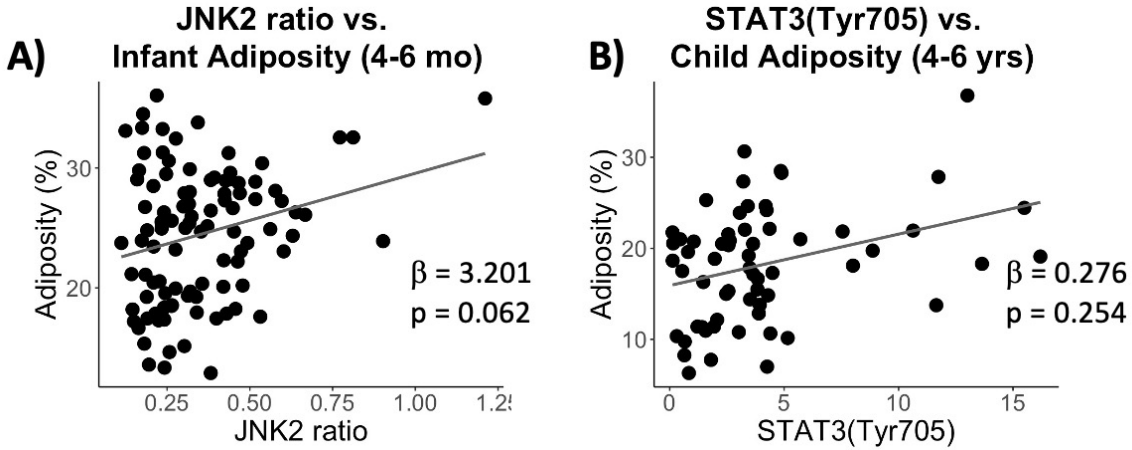
Supplementary Figure S1. Summary of representative images of proteins from WES runs.



Supplementary Figure S2. Full images of proteins from WES runs.



Supplementary Figure S3. Scatter plots showing relationships between proteins in the inflammation pathway and child outcomes. The MANOVA indicated a significant effect of these proteins on child body composition, however the MMR did not reach significance for adiposity or body weight (MMR p-values shown on graphs). A) Correlations showed a positive correlation between JNK2$^{Thr183/Tyr185}$/JNK2 and adiposity (r = 0.253, p = 0.0115) and B) a positive correlation between STAT3^{Tyr705} and adiposity (r = 0.324, p = 0.010).



Supplementary Table S1: Antibodies used.

Pathway	Target	Molecular Weight (kDa)	Supplier	Catalog #	RRID	Dilution
Inflammation	Pro-Caspase 1	50	CST	2225	AB_2243894	1:100
	$STAT3^{Tyr705}$	85	CST	9131	AB_331586	1:10
	Stat3 (124H6)	85	CST	9139	AB_331757	1:50
	$JNK1^{Thr183/Tyr185}$ (81E11)	46	CST	4668	AB_823588	1:100
	$JNK2^{Thr183/Tyr185}$ (81E11)	54	CST	4668	AB_823588	1:100
	JNK1 (SAPK/JNK)	46	CST	9252	AB_2250373	1:100
	JNK2 (SAPK/JNK)	54	CST	9252	AB_2250373	1:100
	IL-1β	32	Abcam	ab9787	AB_308787	1:25
	p38MAPK	43	CST	9212	AB_330713	1:100
Growth Factor/Insulin Signaling	IGF1r	100	Novus Bio	NBP1-77680	AB_11016057	1:100
	$GSK3β^{Ser9}$ (D85E12)	56	CST	5558	AB_10013750	1:50
	GSK3β	56	Novus Bio	NBP1-47470	AB_10010422	1:100
	$ERK(1/2)^{Thr202/Tyr204}$ (D13.14.4E) XP	45/49	CST	4370	AB_2315112	1:200
	p44/42 MAPK (Erk1/2) (137F5)	45/49	CST	4695	AB_390779	1:200
Cortisol Metabolism	11b-HSD2	44	R&D	MAB8630	NA	1:100
Protein Glycosylation	OGT (D1D8Q)	110	CST	23177	AB_2798857	1:100
Mitochondrial Biogenesis	PGC1-α	131	CST	2178	AB_823600	1:25
Loading Controls	Vinculin	116	CST	13901	AB_2728768	1:5000
	beta-actin	45	CST	4970	AB_2223172	1:5000

Supplementary Table S2. Transformations

Pathway	Protein	Lambda value from Box Cox Transformation
Inflammation	Pro-Caspase 1	-0.34
	$STAT3^{Tyr705}$	0.26
	$STAT3^{Tyr705}$/STAT3	0.26
	$JNK1^{Thr183/Tyr185}$	-0.06
	$JNK1^{Thr183/Tyr185}$/JNK1	-0.18
	$JNK2^{Thr183/Tyr185}$	-0.20
	$JNK2^{Thr183/Tyr185}$/JNK2	-0.10
	IL-1β	0.26
	p38MAPK	0.26
Growth Factor/Insulin Signaling	IGF1r	0.14
	$GSK3\beta^{Ser9}$	inverse normal transformation
	$GSK3\beta^{Ser9}$/GSK3	0.06
	$ERK(1/2)^{Thr202/Tyr204}$	0.02
	$ERK(1/2)^{Thr202/Tyr204}$/ERK(1/2)	0.06
Cortisol Metabolism	11b-HSD2	0.14
Protein Glycosylation	OGT	0.50
Mitochondrial Biogenesis and Function	PGC1-α	0.14

Supplementary Table S3. Summary of the statistical models tested.

Model Number	Model
1	Proteins ~ Maternal BMI + Sex + Gestational Age
2	Adiposity (birth) + Body mass (birth) ~ Proteins + Sex + Gestational Age
3	Adiposity (infant) + Body mass (infant) ~ Proteins + Sex + Infant Age
4	ΔAdiposity (infant-birth) + ΔBody mass (infant-birth) ~ Proteins + Sex + Infant Age
5	Adiposity (child) + Body mass (child) ~ Proteins + Sex + Child Age
6	Midthigh + Triceps + Subscapular Skinfolds ~ Proteins + Sex + Child Age
7	Child triglycerides ~ Proteins + Sex + Child Age
8	Child insulin ~ Proteins + Sex + Child Age

Supplementary Table S4. Results of test for the effect of maternal BMI, on the protein pathways (maternal BMI was not significant)

Pathway	Maternal BMI p-value	Sex p-value	Gestational Age p-value	Protein	Maternal BMI p-value	Sex p-value	Gestational Age p-value
Inflammation	0.733	0.509	0.837	Pro-Caspase 1	0.652	0.054	0.273
				$STAT3^{Tyr705}$	0.326	0.160	0.315
				$STAT3^{Tyr705}$/STAT3	0.293	0.275	0.592
				$JNK1^{Thr183/Tyr185}$	0.133	0.683	0.808
				$JNK1^{Thr183/Tyr185}$/JNK1	0.404	0.505	0.865
				$JNK2^{Thr183/Tyr185}$	0.195	0.195	0.779
				$JNK2^{Thr183/Tyr185}$/JNK2	0.322	0.150	0.948
				IL-1β	0.847	0.411	0.774
				p38MAPK	0.437	0.333	0.323
Growth Factor/Insulin Signaling	0.995	0.480	0.948	IGF1r	0.836	0.945	0.530
				$GSK3β^{Ser9}$	0.619	0.377	0.790
				$GSK3β^{Ser9}$/GSK3	0.934	0.160	0.992
				$ERK(1/2)^{Thr202/Tyr204}$	0.573	0.159	0.497
				$ERK(1/2)^{Thr202/Tyr204}$/ERK(1/2)	0.639	0.242	0.465
Cortisol Metabolism	0.803	0.984	0.282	11b-HSD2	0.820	0.936	0.282
Protein Glycosylation	0.290	0.426	0.543	OGT	0.275	0.462	0.543
Mitochondrial Biogenesis and Function	0.425	0.561	0.102	PGC1-α	0.467	0.466	0.102

Supplementary Table S5. Results of MANOVA and MMR tests for the effect of cortisol metabolism on offspring traits (results discussed if the MANOVA was significant)

Pathway	Variable	Birth				Infancy (4-6 months)						Childhood (4-6 years)									
		MANOVA		MMR		MANOVA		MMR		MANOVA		MMR		MANOVA		MMR				multiple regression		multiple regression	
		p-value	F stat	p-value		p-value	F stat	p-value		p-value	F stat	p-value		p-value	F stat	p-value				p-value	t value	p-value	t value
		Body Composition		Adiposity	Body Weight	Body Composition		Adiposity	Body Weight	ΔBody Composition		ΔAdiposity	ΔBody Weight	Body Composition		Adiposity	Body Weight	Skinfolds		Midthigh	Subscapular	Triceps	Insulin		Triglycerides	
cortisol metabolism	11b-HSD2	0.983	0.017			0.592	0.526			0.441	0.825			0.723	0.326			0.159	1.791				0.948	-0.065	0.302	1.047
	Sex	**0.008**	5.048	0.314	**0.017**	**1.43E-07**	18.724	**5.910E-06**	0.408	**0.000**	8.477	**0.000**	0.201	0.199	1.662			0.505	0.789				0.879	-0.153	**0.017**	-2.497
	Age	**5.533E-06**	13.731	**0.005**	**8.800E-07**	**5.40E-08**	20.102	0.362	**2.060E-05**	**5.498E-07**	16.927	0.246	**0.002**	0.229	1.515			**0.025**	3.347	**0.004**	0.695	0.228	0.520	-0.649	0.826	-0.221
	Degrees of Freedom	98				94				93				58				62					39		39	

Supplementary Table S6. Results of MANOVA and MMR tests for the effect of protein glycosylation on offspring traits.

Pathway	Variable	Birth				Infancy (4-6 months)						Childhood (4-6 years)									
		MANOVA		MMR		MANOVA		MMR		MANOVA		MMR		MANOVA		MMR				multiple regression		multiple regression	
		p-value	F stat	p-value		p-value	F stat	p-value		p-value	F stat	p-value		p-value	F stat	p-value				p-value	t value	p-value	t value
		Body Composition		Adiposity	Body Weight	Body Composition		Adiposity	Body Weight	ΔBody Composition		ΔAdiposity	ΔBody Weight	Body Composition		Adiposity	Body Weight	Skinfolds		Midthigh	Subscapular	Triceps	Insulin		Triglycerides	
protein glycosylation	OGT	0.515	0.667			0.372	0.998			0.441	0.826			0.981	0.019			0.898	0.196				0.440	0.780	0.307	1.034
	Sex	**0.009**	4.950	0.286	**0.020**	**1.76E-07**	18.436	**8.380E-06**	0.353	**0.000**	8.276	**0.000**	0.211	0.184	1.743			0.511	0.778				0.982	-0.023	**0.029**	-2.268
	Age	**4.002E-06**	14.147	**0.005**	**6.440E-07**	**3.64E-08**	20.667	0.110	**2.660E-05**	**3.531E-07**	17.536	0.181	**0.002**	0.229	1.513			0.060	2.602				0.455	-0.753	0.766	-0.300
	Degrees of Freedom	98				94				93								60					39		39	

Supplementary Table S7. Results of MANOVA and MMR tests for the effect of the growth/insulin signaling pathway proteins on offspring traits.

Pathway	Variable	Birth				Infancy (4-6 months)						Childhood (4-6 years)									
		MANOVA		MMR		MANOVA		MMR		MANOVA		MMR		MANOVA		MMR				multiple regression		multiple regression	
		p-value	F stat	p-value		p-value	F stat	p-value		p-value	F stat	p-value		p-value	F stat	p-value				p-value	t value	p-value	t value
		Body Composition		Adiposity	Body Weight	Body Composition		Adiposity	Body Weight	ΔBody Composition		ΔAdiposity	ΔBody Weight	Body Composition		Adiposity	Body Weight	Skinfolds		Midthigh	Subscapular	Triceps	Insulin		Triglycerides	
growth factor/insulin signaling	IGF1r	**0.045**	3.217	**0.016**	0.211	0.940	0.062			0.231	1.491			0.936	0.066			0.270	1.343				0.647	-0.462	**0.026**	2.330
	$GSK3\beta^{Ser9}$	0.812	0.208			0.779	0.250			0.625	0.472			0.620	0.482			**0.005**	4.773	0.052	0.936	0.594	0.702	0.386	0.544	-0.612
	$GSK3\beta^{Ser9}$/GSK3	0.378	0.983			0.145	1.972			0.710	0.344			0.548	0.608			0.745	0.413				0.725	-0.355	0.602	0.527
	$ERK(1/2)^{Thr202/Tyr204}$	0.507	0.685			0.491	0.716			0.095	2.416			0.233	1.498			0.171	1.730				0.101	-1.680	0.871	0.163
	$ERK(1/2)^{Thr202/Tyr204}$/ERK(1/2)	0.864	0.146			0.480	0.739			0.842	0.173			0.655	0.426			**0.004**	4.953	**0.011**	0.812	0.106	0.142	1.497	0.882	0.147
	Sex	**0.012**	4.654	0.369	**0.023**	**2.35E-07**	18.172	**1.210E-05**	0.327	**0.001**	8.197	**0.000**	0.247	0.138	2.057			0.748	0.408				0.990	-0.013	**0.016**	-2.537
	Age	**1.11E-05**	12.914	**0.004**	**1.960E-06**	**5.55E-08**	20.232	0.176	**1.930E-05**	**8.665E-07**	16.427	0.234	**0.003**	0.287	1.277			0.082	2.351				0.301	-1.048	0.557	-0.593
	Degrees of Freedom	94				90				89				54				58					39		35	

Supplementary Table S8. Results of MANOVA and MMR tests for the effect of the inflammation pathway proteins on offspring traits.

Pathway	Variable	Birth				Infancy (4-6 months)						Childhood (4-6 years)									
		MANOVA		MMR		MANOVA		MMR		MANOVA		MMR		MANOVA		MMR				multiple regression		multiple regression	
		p-value	F stat	p-value		p-value	F stat	p-value		p-value	F stat	p-value		p-value	F stat	p-value				p-value	t value	p-value	t value
		Body Composition		Adiposity	Body Weight	Body Composition		Adiposity	Body Weight	ΔBody Composition		ΔAdiposity	ΔBody Weight	Body Composition		Adiposity	Body Weight	Skinfolds		Midthigh	Subscapular	Triceps	Insulin		Triglycerides	
Inflammation	Pro-Caspase 1	0.318	1.159			0.323	1.145			0.290	1.258			0.276	1.323			0.374	1.060				0.239	-1.197	0.434	0.793
	$STAT3^{Tyr705}$	0.740	0.302			0.076	2.659			0.383	0.970			**0.047**	3.253	0.254	0.341	0.732	0.431				0.802	-0.253	0.288	1.081
	$STAT3^{Tyr705}$/STAT3	0.245	1.426			0.199	1.647			**0.024**	3.911	0.194	0.216	0.992	0.008			0.617	0.601				0.805	0.249	0.165	-1.421
	$JNK1^{Thr183/Tyr185}$	0.225	1.516			0.441	0.827			0.632	0.461			0.838	0.177			0.479	0.838				0.761	-0.306	0.709	0.376
	$JNK1^{Thr183/Tyr185}$/JNK1	0.918	0.086			0.962	0.039			0.665	0.410			0.081	2.649			0.597	0.634				0.540	0.619	0.237	-1.206
	$JNK2^{Thr183/Tyr185}$	0.789	0.238			0.155	1.903			0.432	0.847			0.666	0.410			0.064	2.571				0.325	0.998	0.433	-0.794
	$JNK2^{Thr183/Tyr185}$/JNK2	0.399	0.929			**0.006**	5.528	0.141	0.755	0.069	2.757			0.129	2.132			**0.037**	3.044	**0.005**	0.446	0.088	0.352	-0.943	0.068	1.894
	IL-1β	0.109	2.269			0.310	1.187			0.108	2.285			0.368	1.021			0.216	1.538				0.968	0.041	0.986	-0.018
	p38MAPK	0.885	0.122			**0.012**	4.614	0.291	0.221	**0.008**	5.148	**0.007**	0.141	0.095	2.472			0.484	0.830				0.881	-0.150	**0.046**	-2.084
	Sex	**0.006**	5.397	0.229	**0.016**	3.38E-06	14.637	**9.580E-05**	0.324	**0.004**	5.802	**0.002**	0.333	0.117	2.245			0.195	1.626				0.685	0.410	**0.034**	-2.219
	Age	**6.43E-05**	13.693	**0.004**	**1.120E-06**	2.55E-07	18.208	0.094	**2.620E-04**	**2.055E-06**	15.367	0.376	**0.002**	0.353	1.064			**0.039**	2.997	**0.004**	0.276	0.119	0.330	-0.988	0.823	0.226
	Degrees of Freedom	90				86				87				50				54					35		31	

Supplementary Table S9. Results of MANOVA and MMR tests for the effect of mitochondria biogenesis on offspring traits.

Pathway	Variable	Birth				Infancy (4-6 months)						Childhood (4-6 years)									
		MANOVA		MMR		MANOVA		MMR		MANOVA		MMR		MANOVA		MMR				multiple regression		multiple regression	
		p-value	F stat	p-value		p-value	F stat	p-value		p-value	F stat	p-value		p-value	F stat	p-value				p-value	t value	p-value	t value
		Body Composition		Adiposity	Body Weight	Body Composition		Adiposity	Body Weight	ΔBody Composition		ΔAdiposity	ΔBody Weight	Body Composition		Adiposity	Body Weight	Skinfolds		Midthigh	Subscapular	Triceps	Insulin		Triglycerides	
mitochondrial biogenesis and function	PGC1-α	0.462	0.778			0.239	1.455			0.457	0.790			0.331	1.128			0.336	1.150				**0.019**	2.443	0.193	1.325
	Sex	**0.011**	4.730	0.403	**0.013**	**2.79E-07**	17.799	**7.710E-06**	0.373	**0.001**	7.791	**0.000**	0.231	0.137	2.061			0.527	0.749				0.965	0.044	**0.027**	-2.291
	Age	**4.090E-06**	14.119	**0.003**	**7.110E-07**	**7.57E-08**	19.621	0.152	**2.810E-05**	**4.984E-07**	17.062	0.248	**0.001**	0.229	1.512			0.067	2.508				0.532	-0.630	0.646	-0.464
	Degrees of Freedom	98				94												60					43		39	