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ORIGINAL ARTICLE

Early Stress History Alters Serum Insulin-Like Growth Factor-1 and Impairs Muscle Mitochondrial Function in Adult Male Rats

S. Ghosh, K. K. Banerjee, V. A. Vaidya[1] and U. Kolthur-Seetharam[1]

Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, Maharashtra, India.

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Early-life adversity is associated with an enhanced risk for adult psychopathology. Psychiatric disorders such as depression exhibit comorbidity for metabolic dysfunction, including obesity and diabetes. However, it is poorly understood whether, besides altering anxiety and depression-like behaviour, early stress also evokes dysregulation of metabolic pathways and enhances vulnerability for metabolic disorders. We used the rodent model of the early stress of maternal separation (ES) to examine the effects of early stress on serum metabolites, insulin-like growth factor (IGF)-1 signalling, and muscle mitochondrial content. Adult ES animals exhibited dyslipidaemia, decreased serum IGF1 levels, increased expression of liver IGF binding proteins, and a decline in the expression of specific metabolic genes in the liver and muscle, including *Pck1*, *Lpl*, *Pdk4* and *Hmox1*. These changes occurred in the absence of alterations in body weight, food intake, glucose tolerance, insulin tolerance or insulin levels. ES animals also exhibited a decline in markers of muscle mitochondrial content, such as mitochondrial DNA levels and expression of TFAM (transcription factor A, mitochondrial). Furthermore, the expression of several genes involved in mitochondrial function, such as *Ppargc1a*, *Nrf1*, *Tfam*, *Cat*, *Sesn3* and *Ucp3*, was reduced in skeletal muscle. Adult-onset chronic unpredictable stress resulted in overlapping and distinct consequences from ES, including increased circulating triglyceride levels, and a decline in the expression of specific metabolic genes in the liver and muscle, with no change in the expression of genes involved in muscle mitochondrial function. Taken together, our results indicate that a history of early adversity can evoke persistent changes in circulating IGF-1 and muscle mitochondrial function and content, which could serve to enhance predisposition for metabolic dysfunction in adulthood.

Key words: maternal separation, mitochondria, muscle, IGF-1, metabolism

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Correspondence to: Ullas Kolthur-Seetharam, Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai, Maharashtra 400005, India (e-mail: ullas@tifr.res.in).

[1]These authors contributed equally to the study.

Environmental experience during critical temporal windows of perinatal development plays an important role in programming adult physiological function. The nature of the early experiences that exert such persistent effects on adult physiology is diverse, including varied early inputs such as stress or dietary perturbations (1). The influences of early experiential perturbations have also been linked via both preclinical and clinical studies to an enhanced risk for the development of disease in adulthood (2,3). Early adversity is strongly implicated as one of the key determinants of risk for the development of psychiatric disorders such as anxiety and depression (4,5) and perinatal nutritional perturbations have independently been demonstrated to contribute to the risk for diseases such as Type II diabetes and obesity (6,7). It is intriguing that several epidemiological studies have reported significant comorbidity between specific psychiatric disorders and metabolic dysfunction (8–10). The clinical literature indicates that stress-associated disorders such as major depression significantly enhance the risk for obesity and Type II diabetes (11,12). Furthermore, chronic stress exposure in human subjects has been linked to the development of the metabolic syndrome (13,14). However, it remains poorly understood whether early-life experiences such as the exposure to stress, which programs vulnerability for adult psychopathology, also enhance risk for metabolic disorders in adulthood. The primary aim of the present study was to investigate whether early-life stress experience, in addition to influencing behavioural outcomes, can also impinge upon metabolic pathways, thus programming the potential risk for the emergence of metabolic dysfunction in adulthood.

Preclinical studies have examined the influence of adult-onset stress on metabolic function, and reports indicate that stressors such as social defeat stress, combined acoustic and restraint stress or chronic variable stress can induce metabolic alterations such as enhanced leptin resistance, perturbed insulin function, increased caloric intake and increased adiposity (15–17). Perturbations of the hypothalamic-pituitary-adrenal (HPA) axis, the major stress-responsive pathway in mammals, have also been demonstrated to enhance body weight, adiposity, insulin and triglyceride levels (18). Relatively fewer studies have investigated the influence of early stress exposure on metabolism. These include studies reporting altered insulin resistance in rats with an early stress history when subjected to dietary perturbations in adulthood (19) and an increased incidence of Type I diabetes in voles subjected to early stress (20). Furthermore, clinical studies indicate that individuals with adverse childhood experiences are at a heightened risk for obesity and hypertension (21,22). Given that early stress exposure is known to evoke long-lasting behavioural and neurological consequences, we hypothesised that such early stress experience may also program changes in metabolic pathways that are observable in adulthood, long after the cessation of the early stress.

In the present study, we examined the influence of the early stress of maternal separation (ES) on metabolic homeostasis in adulthood, assessing circulating serum markers of glucose and lipid metabolism. We also examined the influence of ES on insulin and insulin-like growth factor (IGF)-1 signalling pathways, which play an important role in maintaining glucose and lipid homeostasis (23), in the target tissues (i.e. liver and skeletal muscle). Furthermore, we determined the impact of ES history on markers of mitochondrial content and function within the key target tissue (i.e. skeletal muscle). We find that a history of ES induces a decline in circulating IGF-1 levels and impinges upon pathways that regulate muscle mitochondrial content and function in adult male rats. These changes arise in the absence of perturbations of body weight, food intake, insulin levels and glucose homeostasis, suggestive of a state of metabolic dysfunction and enhanced vulnerability for metabolic disorders after ES. Finally, a comparative analysis with the effects of adult-onset stress revealed that the consequences of ES exhibit both overlapping and distinct effects with respect to those observed after adult stress. Our findings highlight dysregulation of metabolic homeostasis in adult animals as a consequence of early adversity.

Materials and methods

Animals

Adult male Sprague–Dawley rats bred and housed in the Tata Institute of Fundamental Research (TIFR) animal facility were used for all experiments. Animals were group housed at 21 °C and maintained under a 12 : 12 h light/dark cycle with *ad lib.* access to food and water. Animals were fed standard chow (22% wheat, 60% gram powder, 4% casein, 5% milk powder, 4% edible oils, 4% starch, 0.5–1% vitamins). Animal procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the TIFR Institutional Animal Ethics Committee.

Animal treatment paradigms

Animals were subjected to the early stress of maternal separation (ES) from postnatal day (P)2 to P14, as described previously (24). Briefly, litters born to pregnant primiparous dams were assigned randomly to control or ES groups on P1. Litter size ranged from eight to 12 pups and, for each experiment, animals were derived from at least three distinct litters to minimise litter-specific effects. This experimental design treats each individual as n = 1, rather than litter groups being treated as n = 1. We chose this design because our experiments were performed in adulthood and we aimed to reduce the numbers of animals required across multiple experiments. Pups in the ES group were separated as a litter from their mothers for a period of 3 h daily from P2 to P14. The dams from the ES group were first removed from their home cage to a novel cage prior to the removal of the litter. During this period of daily separation, pups were placed in beakers with nesting and bedding material similar to their home cage and the beakers were placed on heating pads to maintain euthermal conditions. Pups were returned to their home cage at the end of the separation period prior to return of the dam. Control litters and dams were left undisturbed in their home cage, except for routine cage cleaning, which resulted in the brief handling of both control and ES groups every 3–4 days. All pups across control and ES groups were weaned from their dams at P28, after which they were housed in same-sex sibling groups of three to four animals per cage. Female rats were removed from the experiment at this point, and only male animals were used for subsequent analysis. The body weight of animals was measured at P10 and in adulthood (4 months). ES animals were killed by decapitation in adulthood at either 4 or 8 months of age with their individual age-matched control groups, and tissue and serum were collected for western blotting (n = 5–12 for Control and ES), quantitative real-time polymerase chain reaction (PCR) analysis (n = 17–20 for Control and ES) and serum measurements (n = 10 for Ctrl and ES). ES animals were assessed for serum parameters, insulin/IGF-1 signalling in the liver and muscle, and mitochondrial content in the muscle. In three distinct experimental cohorts of animals, ES and control animals were also analysed for anxiety-like behaviour on the elevated plus maze (EPM) task (n = 7 for Control; n = 12 for ES), response on an i.p. glucose tolerance test (n = 6 for Control and ES) and response on an i.p. insulin tolerance test (n = 6 for Control and n = 7 for ES). All animals used for the experiment were aged between 4 and 8 months, which is within the phase of sexual maturity and adulthood, and several months away from post-reproductive senescence. For specific readouts, where we had results from ES animals at both 4 and 8 months with their individual age-matched control groups, we combined the results normalised to their own controls to gain a more representative understanding of the effects of ES in adulthood.

To draw a comparison between early and adult-onset stress, we compared specific findings observed with ES animals with those observed with an adult-onset stressor. Adult male rats (3 months) were subjected to a chronic unpredictable stress (CUS) paradigm that consisted of a randomised combination of two distinct stressors daily across ten consecutive days, with the time of stressor exposure also randomly varied across days (n = 10 for Ctrl and CUS). The stressors included cage rocking, forced swim, cold isolation, food and water deprivation overnight, lights on during dark period, lights off during light period, immobilisation and social isolation. Control animals were left undisturbed in their home cages other than regular cage cleaning every 3–4 days. On the 11th day, animals were killed by decapitation and serum and tissue were collected for serum measurements (n = 10 for Control and CUS), western blotting (n = 4 for Control and CUS) and quantitative PCR analysis (n = 9–10 for Control and CUS). CUS animals were assessed for serum parameters, insulin/IGF-1 signalling and mitochondrial content in the muscle.

Behavioural analysis

To assess anxiety-like behaviour, animals were subjected to the EPM test (age = 4 months, n = 7 for Control; n = 12 for ES). Animals were placed in the centre of an EPM, 50 cm in height, with two open and two closed arms (50 × 10 cm) and allowed to explore for a period of 10 min. Movements were recorded using an overhead CCD camera, and scored using an automated tracking system (Noldus Ethovision 3.1; Noldus Information Technology, Wageningen, The Netherlands) to measure time spent and total distance moved in open and closed arms, as well as number of entries and latency to enter open arms.

Body weight and food intake measurements

Body weights were measured at P10 (n = 8 for Control and ES) and in adulthood for ES animals (age = 4 months, n = 11 for Control; n = 10 for ES). CUS animals were weighed immediately prior to being killed, at the age of 3.5 months (n = 10 for Control and CUS). ES animals were also assessed for daily food intake in adulthood. A weighed quantity of food was placed in the cage each day halfway through the light cycle, and the food remaining was weighed after 24 h. Mean food intake over two consecutive days was then used to compare across treatment groups (n = 13 cages for Control and ES).

Serum profile analysis

Animals were killed in adulthood by rapid decapitation and trunk blood was collected for serum measurements. Serum was collected in fresh tubes by centrifuging whole blood samples at 1600 *g* for 10 min after coagulation at room temperature for 30 min, and samples were assayed for blood glucose, total triglycerides and cholesterol levels in a pathological laboratory (Shahbazker's Diagnostic Center, Mumbai, Maharashtra, India) (for early stress, age = 4 months, n = 10 for Control and ES; for adult stress, age = 3.5 months, n = 10 for Control and CUS). Circulating serum insulin and IGF-1 levels were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Rat/Mouse Insulin ELISA kit, Catalogue no. EZRMI-13K, Millipore, Billerica, MA, USA; quantikine IGF-1 immunoassay kit, Catalogue no. MG100, R&D Systems Minneapolis, MN, USA) in accordance with the manufacturer's instructions (age = 8 months, n = 8 for Control and ES).

Glucose tolerance test

Animals were subjected to an i.p. glucose tolerance test. Animals were fasted overnight with fasting blood glucose measured from blood collected through the incision of tail tips. All animals then received glucose (1 g/kg in saline) i.p. and blood was collected from tail-tip incisions at intervals of 15, 30, 60 and 120 min after injection. Blood glucose levels were measured using a one-touch blood glucose monitoring system (Accu-Chek Active meter; Roche, Basel, Switzerland) (age = 8 months, n = 6 for Control and ES).

Insulin tolerance test

Control and maternally separated animals were fasted overnight, and fasting blood glucose was measured from blood collected by tail tip incision. All animals were then injected i.p. with insulin (1.5 U/kg), and blood was collected from tail tip incisions 15, 30, 60 and 120 min after injection. Blood glucose levels were measured using a one-touch blood glucose monitoring system (Accu-Chek Active meter) (age = 4 months, n = 6 for Control, n = 7 for ES).

Quantitative real-time PCR

Animals were killed by decapitation and sections of liver and thigh muscle (vastus lateralis) were immediately excised and snap-frozen in liquid nitrogen. RNA was extracted from these samples using Tri reagent (Sigma-Aldrich, St Louis, MO, USA) and RNA concentration was measured spectrophotometrically using a NanoVue spectrophotometer (GE Healthcare, Little Chalfont, UK). RNA purity was determined by measuring the A_{260}/A_{280} and $A_{260}/_{230}$ ratios. The RNA was then reverse transcribed (ABI first-strand cDNA reverse transcription kit; Applied Biosystems, Foster City, CA, USA) and the synthesised cDNA was subjected to quantitative real-time PCR using primers for the genes of interest (for early stress, age = 4 and 8 months with age-matched controls, n = 20 for Control and ES in liver; n = 18 for Control and ES in muscle; for adult onset stress, age = 3.5 months, n = 10 for Control and CUS in liver; n = 10 for Control and CUS in muscle). The primer pairs used for the assay are detailed in the Supporting information (Table S1). Quantitative real-time PCR was performed using the Bio-Rad CFX96 Cycler (Bio-Rad Laboratories, Hercules, CA, USA), using the protocol: initial denaturation at 95 °C for 2 min, followed by 40 cycles of 15 s at 95 °C for denaturation, 30 s at 60 °C for annealing and 20 s at 72 °C for extension. Melt curve peaks were used to check that a single PCR product was formed. Data were quantified using the $\Delta\Delta C_t$ method, as described previously (25). Actin was used for normalisation of data in the muscle and liver, and actin expression was not altered by the stress paradigms used in the present study.

Western blot analysis

Western blotting was performed as described previously (24). Tissue samples were homogenised in a buffer containing Tris buffer, pH 7.5 (200 mM), ethylenediaminetetraacetic acid (EDTA) (1.5 mM), KCl (40 mM), glycerol (5% v/v), dithiothreitol (0.5 mM), sodium fluoride (10 mM), sodium orthovandante (1 mM) and sodium orthophosphate (1 mM), or in radioimmunoprecipitation assay buffer [10 mM Tris-Cl (pH 8.0), 1 mM EDTA, 0.5 mM EGTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 140 mM NaCl] using a Minlys homogeniser (Precellys; Bertin Technologies, Montigny-le-Bretonneux, France). Commercially available protease or phosphatase inhibitors (Roche and Sigma-Aldrich) were added to the buffers immediately before lysis. Samples were centrifuged at 3300 *g* for 10 min, after which the supernatant was collected. The protein concentration of the samples was estimated using the Quantipro BCA assay kit (Sigma-Aldrich) and protein lysates were resolved using a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Proteins were transferred onto polyvinylidene fluoride membranes and probed with appropriate antibodies in accordance with standard procedures. The antibodies purchased from Cell Signaling Technologies (Cell Signaling Technology, Beverly, MA, USA) were: rabbit anti-AKT (dilution 1 : 500) (Catalogue no. 9272), rabbit anti-phospho-AKT (S473) (dilution 1 : 500) (Catalogue no. 9271), rabbit anti-FOXO1 (dilution 1 : 1000) (Catalogue no. 2880S), rabbit anti-phospho-FOXO1 (dilution 1 : 500) (Catalogue no. 9461S) and rabbit anti-insulin receptor (dilution 1 : 500) (Catalogue no. 3025S), whereas rabbit anti-TFAM (Catalogue no. AV31400) antibody (dilution 1 : 1000) was obtained from Sigma-Aldrich. Bands were visualised using a chemiluminiscence kit (ECL prime; GE Healthcare). α-Tubulin was used to normalise the mean band intensities for TFAM (transcription factor A, mitochondrial), whereas the intensity of bands for pAKT was normalised to AKT and pFOXO1 was normalised to FOXO1. Independently, band intensities for AKT were normalised to actin (liver) and tubulin (muscle) to check for baseline differences in expression in ES animals. The relative density of bands was quantified using the IMAGEJ (NIH, Bethesda, MD, USA) (for pAKT/AKT in early stress, age = 4 and 8 months with age-matched controls, n = 9 for Control and ES in the liver; n = 11 for Control, n = 12 for ES in the muscle; for TFAM in early stress, age = 8 months, n = 5 for Control

and ES in the muscle; for pAKT/AKT in adult onset stress, age = 3.5 months, n = 4 for Control and CUS in the muscle; for insulin receptor (INSR) in early stress, age = 4 months, n = 3 for Control, n = 4 for ES in the liver, n = 4 for Control and ES in the muscle; for pFOXO/FOXO in early stress, age = 4 months, n = 4 for Control and ES in the muscle).

Tissue triglyceride measurements

Frozen liver and muscle tissue samples from control and ES animals were weighed before homogenisation in phosphate-buffered saline using a Minlys homogeniser (Precellys; Bertin Technologies). Samples were centrifuged at 3300 *g* to pellet any debris, and the supernatant was assessed for triglyceride content at a pathological laboratory (Shahbazker's Diagnostic Center) (age = 4 months, n = 6 for Control, n = 6 for ES).

Mitochondrial DNA measurement

Mitochondrial DNA levels were compared between Control and ES animals as described previously (26). Briefly, total genomic DNA was extracted from the muscles of Control and ES animals using a commercially available kit (GeneiPure™ Mammalian Genomic DNA Purification kit; Merck, Darmstadt, Germany). Quantitative real-time PCR for a mitochondrial genome-encoded gene (cytochrome *b*) was then used to compare relative mitochondrial DNA levels between groups. Data were normalised to cytochrome c, a nuclear genome-encoded gene, and quantified by the $\Delta\Delta C_t$ method as described previously (25) (age = 8 months, n = 4 for Control and ES). The primer sequences used are provided in the Supporting information (Table S2).

Statistical analysis

An unpaired, two-tailed Student's t-test was used to compare between groups. $P < 0.05$ was considered statistically significant. Welch corrections were applied to the t-test wherever the SDs were significantly different between groups. Normality of data was tested using the Kolmogorov–Smirnov method prior to t-test analysis (Instat; GraphPad Software Inc., San Diego, CA, USA).

Results

Early stress evokes enhanced anxiety and a perturbed plasma metabolite profile in adulthood

Maternal separation is a model of early stress (ES) known to induce persistent behavioural effects on anxiety-like behaviour, as well as disrupt stress responsivity (27). We first validated our model of ES by assessing the impact of maternal separation on anxiety-like behaviour. We subjected animals with a history of ES, as well as their age-matched controls, to the EPM task in adulthood (n = 7 for Control, n = 12 for ES). ES animals exhibited enhanced anxiety-like behaviour on the EPM, as determined by a significant decrease in both the percentage of time spent (P = 0.0025), as well as the percentage distance traversed (P = 0.0006) in the open arms, a significant decline in open arm entries (P = 0.0226) and a higher latency to enter the open arms (P = 0.0398) compared to controls (Fig. 1C–F). We then assessed whether a history of ES influences metabolic status by measuring body weight, food intake and circulating lipid and glucose levels. Body weight measurements both at P10 and in adulthood revealed no difference between experimental cohorts with a history of ES and their age-matched controls (see Supporting information, Fig. S1A,B) (n = 11 for Control, n = 10 for ES). Furthermore, food intake measurements in adult ES animals also did not differ from the control group (see Supporting information, Fig. S1C) (n = 13 cages for Control and ES). We next aimed to

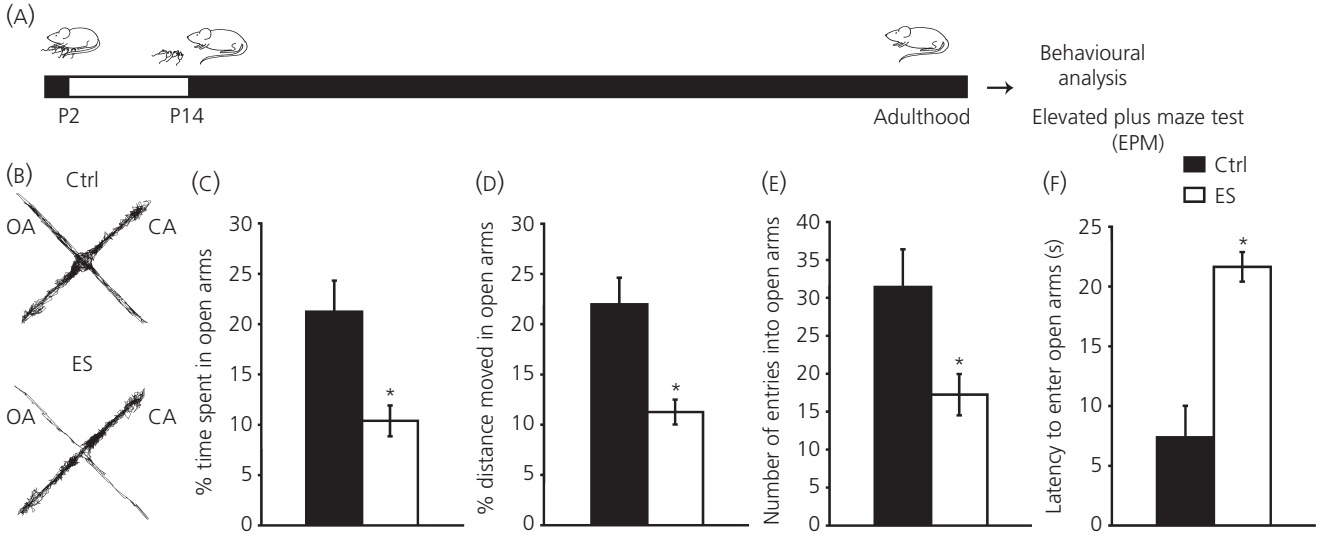

Fig. 1. Early stress (ES) leads to persistent alterations in anxiety-like behaviour. Animals were subjected to the early stress of maternal separation from postnatal day (P)2 to P14 and were assessed for anxiety-like behaviour on the elevated plus maze (EPM). The experimental paradigm is represented in the schematic (A). Shown are representative traces from a control (Ctrl) and an ES animal for the path traversed in the open arms (OA) and closed arms (CA) of the EPM (B). ES animals exhibited enhanced anxiety-like behaviour on the EPM as revealed by a significant decline in the percent of time spent (C) and percentage distance (D) traversed in the open arms, as well as a decline in the number of entries into the open arms (E) and increased latency to enter open arms (F). Results are expressed as the mean ± SEM (age = 4 months, n = 7 for Ctrl; n = 12 for ES), *P < 0.05 compared to age-matched controls (Student's t-test).

determine whether glucose and lipid metabolism, as assessed by circulating levels of glucose, triglycerides and cholesterol, were influenced by early adversity (Table 1). Adult ES animals showed a significant increase in circulating triglyceride levels compared to their respective age-matched controls ($P = 0.0475$). By contrast, circulating blood glucose and cholesterol levels were unchanged in adult ES animals ($n = 10$ for Control and ES). Measurement of triglyceride levels within the target tissues of liver and muscle revealed a trend towards a decrease in liver triglyceride levels ($P = 0.0502$) in ES animals ($n = 6$ for Control, $n = 5$ for ES), whereas muscle triglyceride levels were unaltered between groups ($n = 6$ for Control and ES) (Table 1). Taken together, these results indicate that ES animals in adulthood exhibit anxiety-like behavioural changes accompanied by specific peripheral metabolic changes, namely enhanced circulating triglyceride levels and decreased liver triglyceride levels, suggestive of altered lipid metabolism.

Early stress history does not alter insulin signalling

Lipid and glucose metabolism across multiple tissues is coordinated through the insulin/IGF-1 signalling (IIS) pathway (23). We next examined the influence of ES history on the IIS pathway by measuring circulating insulin levels, as well as assessing downstream signalling pathways within key metabolic target tissues, namely the liver and muscle. We did not detect any change in circulating insulin levels across control and ES groups ($n = 8$ for Control and ES) (Fig. 2B). Although adult ES animals exhibited a significant increase in insulin receptor (*Insr*) mRNA expression in the liver ($P = 0.0008$) ($n = 20$ for Control and ES) and a decline in *Insr* expression in the muscle ($P = 0.0088$) ($n = 17$ for Control and ES) (Fig. 2C,D), these changes were not accompanied by alterations in INSR protein levels in either the liver ($n = 3$ for Control, $n = 4$ for ES) (see Supporting information, Fig. S2A) or the muscle ($n = 4$ for Control and ES) (see Supporting information, Fig. S2B). We then examined pAKT/AKT ratios as a measure of insulin signalling within the target tissues of the liver and muscle to determine whether ES animals exhibit signalling changes ($n = 9$ for Control and ES in the liver; $n = 11$ for Control, $n = 12$ for ES in the muscle). An ES history did not influence either total AKT levels (see Supporting information, Fig. S3A,B) or pAKT/AKT ratios in either the liver or the muscle (Fig. 2F,G).

Although we did not observe molecular perturbations associated with altered insulin signalling, we performed an i.p. glucose tolerance test (GTT) ($n = 6$ for Control and ES) and an i.p. insulin tolerance test (ITT) ($n = 6$ for Control, $n = 7$ for ES) to examine insulin sensitivity at the level of the organism. Our results indicate no perturbation of glucose homeostasis in ES animals as assessed by GTT (Fig. 2E) and normal insulin sensitivity as assessed by ITT (Fig. 2H). Taken together, these results reveal that, although adult ES animals do show changes at the transcript level in insulin receptor expression within metabolic target tissues, this is not accompanied by altered insulin signalling/sensitivity within these target tissues, nor by any change in glucose homeostasis at the organismal level.

Table 1. Serum Metabolites in Control (Ctrl) and Early Stress (ES) Animals.

	Controls	ES	P-value
Blood glucose (mg/dl)	124.63 ± 3.51	123.33 ± 3	0.7867
Triglycerides (mg/dl)	78.5 ± 3.63	93.1 ± 5.82	0.0475*
Total cholesterol (mg/dl)	73 ± 4.61	68.33 ± 4.16	0.8958
HDL (mg/dl)	14.39 ± 0.36	14.72 ± 0.61	0.6503
LDL (mg/dl)	42.04 ± 5.25	35.02 ± 4.27	0.3145
Liver triglyceride (mg/g tissue)	6.70 ± 0.40	5.41 ± 0.21	0.0504#
Muscle triglyceride (mg/g tissue)	12.47 ± 2.53	7.93 ± 2.34	0.2917

ES animals have altered serum and tissue metabolite levels. Shown are serum concentrations of blood glucose, triglycerides, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL). Tissue triglyceride levels were also measured in the liver and muscle (mean ± SEM). ES animals exhibited elevated serum triglyceride levels compared to age-matched controls. A trend towards a decrease in liver tissue triglyceride levels was also noted in ES animals. For measurements of serum metabolites, age = 4 months, $n = 10$ for Control and ES; for liver triglycerides, age = 4 months, $n = 6$ for Control, $n = 5$ for ES; for muscle triglycerides, age = 4 months, $n = 6$ for Control and ES). *$P < 0.05$ compared to controls (Student's t-test). A trend towards significance is indicated: #$P > 0.05$ and < 0.1.

Early stress induces a decline in serum IGF-1

We then aimed to examine the effects of ES history on IGF-1 signalling, which is known to share signalling components with the insulin signalling pathway but has been reported to exert distinct and overlapping effects on cellular physiology in target tissues (28,29). Adult ES animals showed a significant decline in circulating serum IGF-1 levels compared to controls ($P = 0.0043$) (Fig. 3B) ($n = 8$ for Control and ES). The ability of IGF-1 to elicit signalling is tightly regulated by the IGF binding proteins (IGFBPs). We observed significantly enhanced mRNA levels of specific IGFBPs, namely *Igfbp2* ($P = 0.0302$) and *Igfbp5* ($P = 0.0382$), as well as a trend towards an increase in expression of *Igfbp1* that did not reach statistical significance ($P = 0.0593$), in the liver of adult ES animals (Fig. 3C). Muscle expression of the IGFBPs was unaltered in ES animals (Fig. 3E). Transcript levels of the IGF-1 receptor (*IGF-1r*) were not changed in adult animals with an ES history (Fig. 3D,F) ($n = 20$ for Control and ES in liver; $n = 18$ for Control and ES in muscle). These results reveal that ES history is associated with reduced circulating IGF-1 levels.

Given that we observed a decline in circulating IGF-1 levels, using two approaches, we aimed to investigate whether this affected the activity of the Forkhead-box protein transcription factor, FOXO, which is known to be negatively regulated by IGF-1 signalling. The first approach involved measuring the transcript levels of specific metabolic genes that are known to be transcriptionally regulated by FOXO. We observed a significant up-regulation of phosphoenolpyruvate carboxykinase 1 (*Pck1*) ($P = 0.0002$) and lipoprotein lipase (*Lpl*) ($P = 0.0094$) in the liver and enhanced mRNA levels of pyruvate dehydrogenase kinase, isozyme 4 (*Pdk4*) ($P = 0.0042$) and heme oxygenase 1 (*Hmox1*) ($P = 0.0367$) in the muscle (Fig. 3H,I) ($n = 20$ for Control and ES in liver; $n = 18$ for

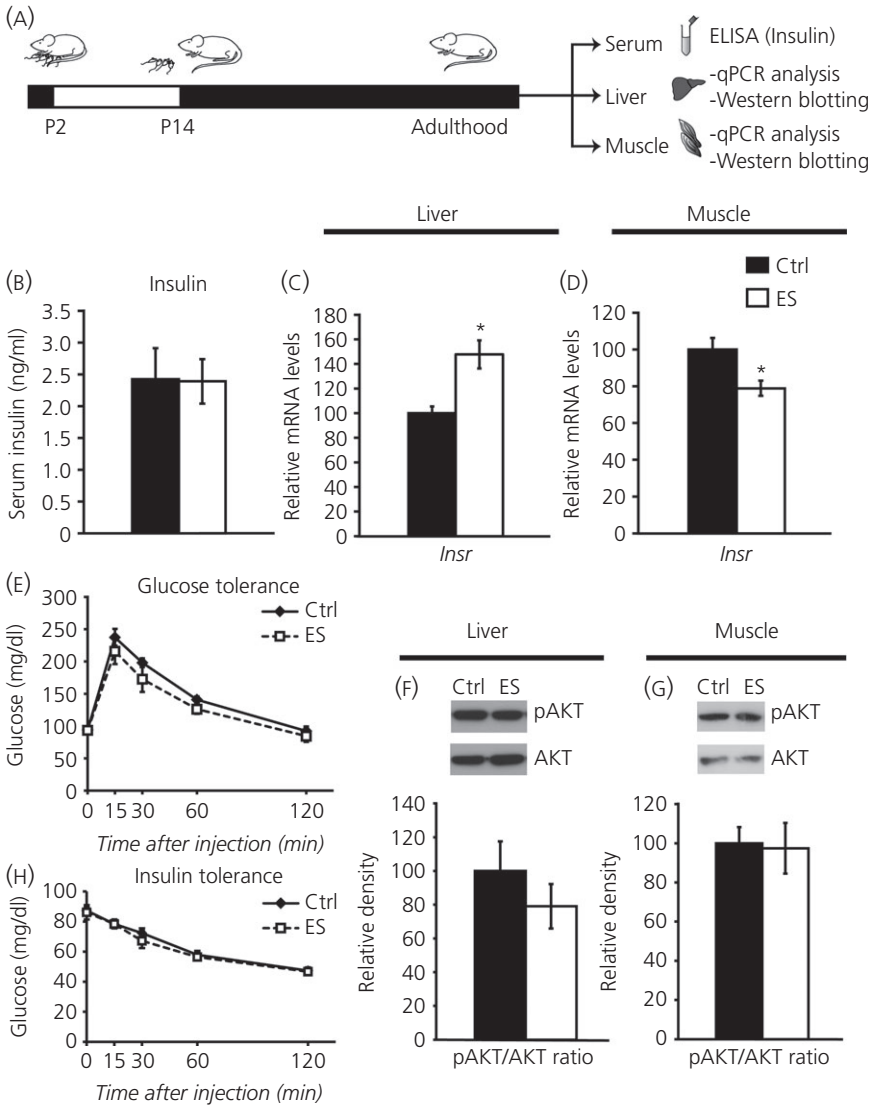

Fig. 2. Early stress (ES) does not alter insulin signalling or influence glucose or insulin tolerance. The experimental paradigm is represented in the schematic (A). Serum insulin levels were found to be unchanged between Control (Ctrl) and ES animals (B). Transcript levels of insulin receptor (*Insr*) revealed an up-regulation in the liver (C) and a decline in skeletal muscle (D). Analysis of blood glucose levels after a glucose tolerance test (GTT) (E) and an insulin tolerance test (ITT) (H) indicated no change between Ctrl and ES animals. Western blotting analysis for pAKT and AKT was performed on lysates from the liver (F) and muscle (G) of Ctrl and ES animals and the ratio of pAKT/AKT was used as an indicator of insulin signalling. There were no differences observed in pAKT/AKT ratios between the Ctrl and ES groups in either the liver or the muscle. Results are expressed as the mean ± SEM [for insulin enzyme-linked immunosorbent assay, age = 8 months, n = 8 for Ctrl and ES; for quantitative polymerase chain reaction (qPCR) in liver, age = 4 and 8 months with age-matched controls, n = 20 for Ctrl and ES; for qPCR in muscle, age = 4 and 8 months with age-matched controls, n = 18 for Ctrl and ES; for GTT, age = 8 months, n = 6 for Ctrl and ES; for ITT, age = 4 months, n = 6 for Ctrl, n = 7 for ES; for western blotting in the liver, age = 4 and 8 months with age-matched controls, n = 9 for Ctrl and ES; for western blotting in the muscle, age = 4 and 8 months with age-matched controls, n = 11 for Ctrl, n = 12 for ES], *$P < 0.05$ compared to controls (Student's t-test). P, postnatal day.

Control and ES in muscle). Second, we determined the phosphorylation levels of FOXO in the muscle by measuring pFOXO/FOXO ratio using western blotting. However, ES animals did not differ from Control animals with respect to the pFOXO/FOXO ratio in the muscle (Fig. 3J). Taken together, these results reveal a decline in IGF-1 levels accompanied by the enhanced transcription of metabolic genes in the target tissues of the liver and muscle, which was not accompanied by changes in FOXO phosphorylation in the muscle.

Early stress is associated with decreased muscle mitochondrial content

Given our findings of enhanced circulating triglyceride levels and decreased IGF-1 signalling, which, in preclinical and clinical studies, have been reported to impinge upon muscle mitochondrial function (30,31), we next aimed to determine whether markers of muscle mitochondrial content and genes regulating mitochondrial function were perturbed in ES animals. We examined relative mitochondrial

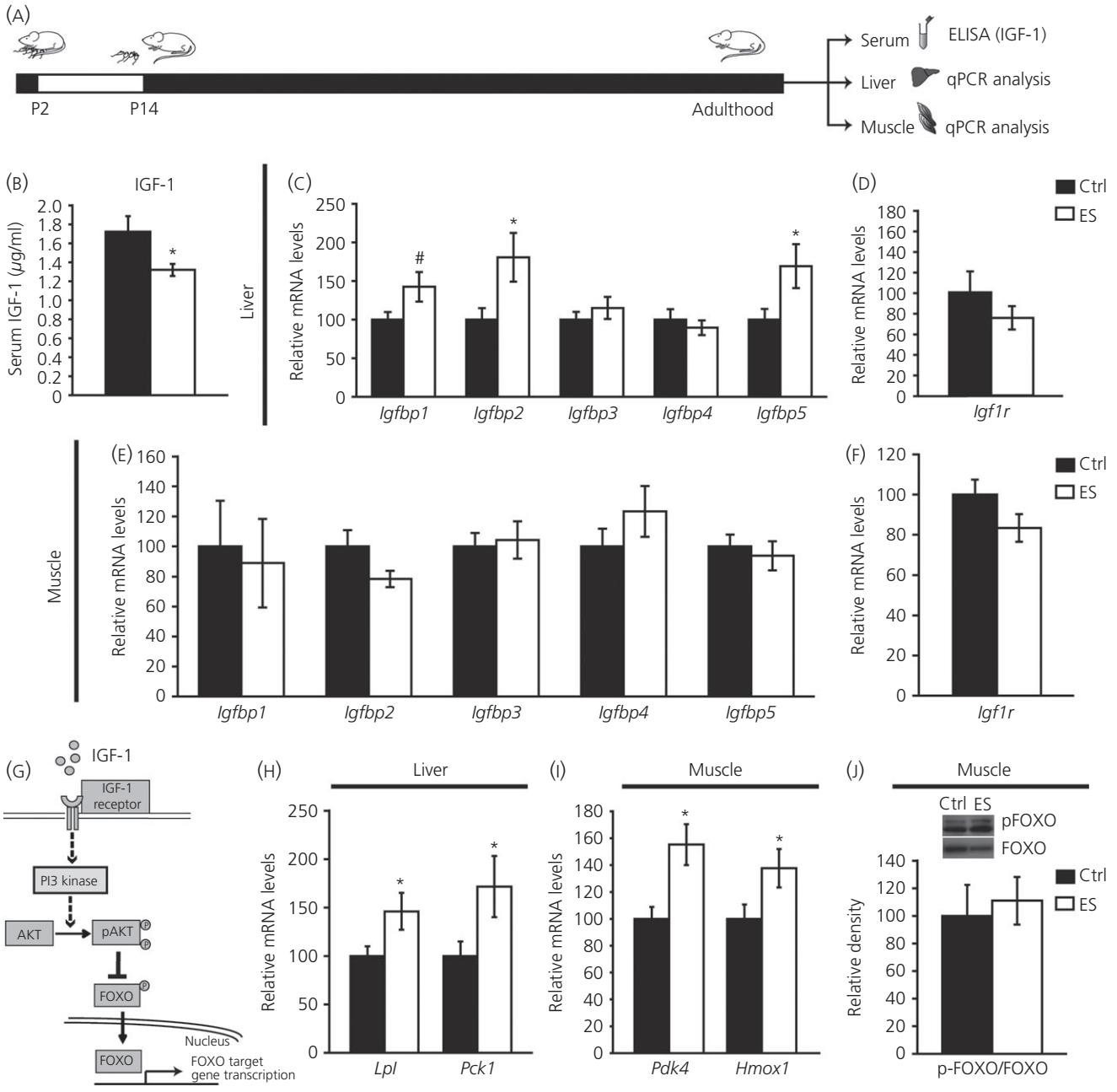

Fig. 3. Early stress (ES) induces a decline in serum insulin-like growth factor (IGF)-1. The experimental paradigm is represented in the schematic (A). Measurement of circulating IGF-1 levels in the serum revealed a significant decline in ES animals (B). Quantitative polymerase chain reaction (qPCR) analysis revealed a significant increase in *Igfbp2* and *Igfbp5*, and a trend towards an increase in *Igfbp1* that did not reach statistical significance (P = 0.0593) in the liver (C) with no change noted in IGF-1 receptor (*Igf1r*) mRNA levels between Control (Ctrl) and ES groups (D). qPCR analysis indicated no change in *Igfbp1-5* I or *Igf1r* (F) expression in muscle derived from Ctrl or ES groups. Shown is a schematic illustrating the regulation of FOXO-based gene transcription by IGF-1 signalling (G). IGF-1 signalling mediates an enhanced phosphorylation of FOXO, thus sequestering FOXO in the cytoplasm and reducing FOXO-mediated gene transcription. qPCR analysis of the metabolic genes phosphoenolpyruvate carboxykinase 1 (*Pck1*) and lipoprotein lipase (*Lpl*) in the liver (H) and pyruvate dehydrogenase kinase, isozyme 4 (*Pdk4*) and heme oxygenase 1(*Hmox1*) in the muscle (I), respectively, revealed increased expression. However, analysis of pFOXO/FOXO ratios in the muscle revealed no differences between Ctrl and ES groups (J). Results are expressed as the mean ± SEM (for IGF-1 enzyme-linked immunosorbent assay, age = 8 months, n = 8 for Ctrl and ES; for qPCR in liver, age = 4 and 8 months with age-matched controls, n = 20 for Ctrl and ES; for qPCR in muscle, age = 4 and 8 months with age-matched controls, n = 18 for Ctrl and ES; for western blotting in the muscle, age = 4 months, n = 4 for Ctrl and ES) *P < 0.05 compared to controls (Student's t-test). A trend towards significance is indicated: #P > 0.05 and < 0.1. P, postnatal day.

DNA (mtDNA) levels, and studied the expression of TFAM, a protein that localises to the mitochondrial matrix, to serve as indicators of mitochondrial content. Interestingly, we observed a significant decline in TFAM protein levels as assessed by western blotting ($P = 0.0035$) (Fig. 4B,C) ($n = 5$ for Control and ES), which was accompanied by a decrease in relative mtDNA levels (Fig. 4D) ($n = 4$ for Control and ES) ($P = 0.0060$) in skeletal muscle derived from adult ES animals compared to age-matched controls. These results are indicative of a decline in muscle mitochondrial mass in adult ES animals. We then assessed the expression of several genes involved in mitochondrial biogenesis, transcription and maintenance in the muscle of ES animals. The genes assessed included peroxisome proliferator-activated receptor γ, coactivator 1α (*Ppargc1a*), a transcription co-activator and a major regulator of mitochondrial biogenesis, nuclear respiratory factor 1 (*Nrf1*), a transcription factor that regulates the expression of several nuclear-encoded mitochondrial genes involved in mitochondrial respiration, transcription and translation, and transcription factor A, mitochondrial (*Tfam*), an activator of mitochondrial transcription and replication (32). Animals with a history of ES exhibited significantly decreased expression of *Ppargc1a* ($P = 0.0017$), *Nrf1* ($P < 0.0001$) and a trend towards a decrease in expression of *Tfam* that did not reach statistical significance ($P = 0.0991$) in the muscle. We also observed a decrease in transcript levels of catalase (*Cat*) ($P = 0.0008$), a protein involved in the scavenging of reactive oxygen species, sestrin 3 (*Sesn3*) ($P = 0.0435$), a stress-responsive protein shown to regulate

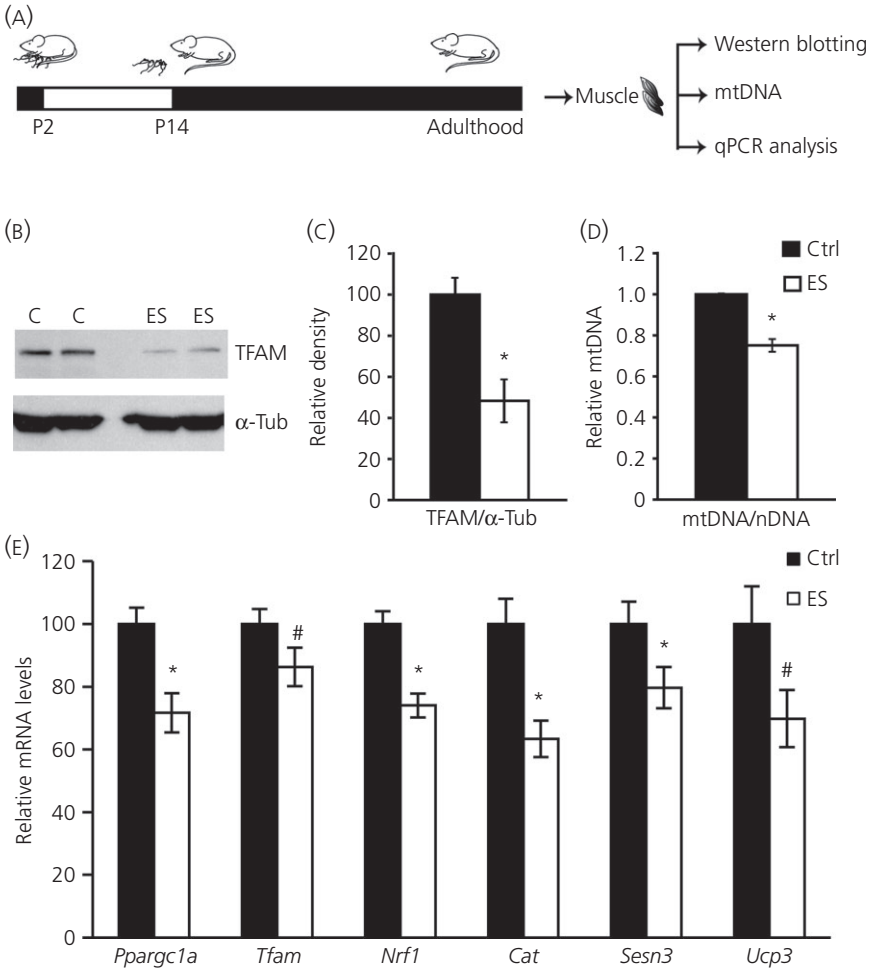

Fig. 4. Early stress (ES) results in a decrease in mitochondrial content in the muscle. The experimental paradigm is represented in the schematic (A). Western blotting analysis for the mitochondrial matrix protein TFAM (transcription factor A, mitochondrial) indicated a significant decrease in TFAM protein levels in ES animals compared to controls. Shown are representative blots for TFAM levels and the loading control α-tubulin (α-Tub) in Control (Ctrl) and ES animals (B) and quantitative densitometric analysis is shown in (C). ES animals showed a significant decline in relative levels of mitochondrial DNA in the muscle (D). Quantitative polymerase chain reaction (qPCR) analysis revealed a decrease in the expression of the following genes known to be involved in the regulation of mitochondrial function: peroxisome proliferator-activated receptor γ, coactivator 1α (*Ppargc1a*), nuclear respiratory factor 1 (*Nrf1*), transcription factor A, mitochondrial (*Tfam*) ($P = 0.0991$), catalase (*Cat*), sestrin 3 (*Sesn3*) and uncoupling protein 3 (*Ucp3*) ($P = 0.0602$) in the muscle of ES animals compared to controls (E). Results are expressed as the mean ± SEM (for western blotting, age = 8 months, $n = 5$ for Ctrl and ES; for mitichondrial DNA, age = 8 months, $n = 4$ for Ctrl and ES; for qPCR, age = 4 and 8 months with age-matched controls, $n = 18$ for Ctrl and ES) *$P < 0.05$ compared to controls (Student's t-test). A trend towards significance is indicated by # where $P > 0.05$ and < 0.1. P, postnatal day.

insulin sensitivity (33), and a trend towards a decrease in expression that did not reach statistical significance for uncoupling protein 3 (*Ucp3*) (P = 0.0602), a mitochondrial uncoupling protein (34) (Fig. 4E) (n = 18 for Control and ES). These results indicate that adult animals with an ES history exhibit a significant reduction in nuclear-encoded genes associated with mitochondrial biogenesis, accompanied by a decline in relative mitochondrial DNA levels and TFAM protein expression. Taken together, these results are suggestive of a decline in mitochondrial content and function as a consequence of ES history.

Adult-onset CUS exerts distinct effects on insulin/IGF-1 signalling and does not alter mitochondrial function

Given our observations of decreased IGF-1 signalling and impaired muscle mitochondrial status in ES animals, we next aimed to investigate whether these metabolic changes were unique to animals with an early stress experience or would also be evoked in response to adult-onset stress exposure. Adult-onset CUS induced an increase in circulating triglyceride levels (P = 0.0369) (Table 2) (n = 10 for Control and CUS) and also showed enhanced insulin receptor (*Insr*) mRNA levels in the liver, similar to the changes noted in animals with an ES history (Fig. 5B) (n = 10 for Control and CUS). However, no change was noted in *Insr* expression in the muscle (Fig. 5D) (n = 10 for Control and CUS). Body weight measurements revealed no differences between control and CUS groups (see Supporting information, Fig. S4A). Signalling through the insulin/IGF-1 pathway was assessed by measuring AKT phosphorylation in the muscle and both total AKT (see Supporting information, Fig. S4B) and the pAKT/AKT ratio (Fig. 5F,G) were found to be unchanged in CUS animals compared to their controls (n = 4 for Control and CUS). By striking contrast to the effects on FOXO target genes observed in ES animals, CUS was associated with a significant decline in expression of the FOXO target gene *Pck1* (P = 0.0265) in the liver and *Pdk4* (P = 0.0078) in the muscle (Fig. 5C,E) (n = 10 for Control and CUS in liver; n = 10 for Control and CUS in muscle). Quantitative PCR analysis of mRNA levels of genes involved in the regulation of mitochondrial function, including *Ppargc1a*, *Sesn3*, *Cat*, *Tfam* and *Nrf1*, showed no change between control and CUS groups (Fig. 5H) (n = 10 for Control and CUS in liver; n = 10 for Control and CUS in muscle).These results clearly show that ES and CUS produce both distinct and overlapping effects on metabolism in key target tissues.

Table 2. Serum Metabolites in Control (Ctrl) and Chronic Unpredictable Stress (CUS) Animals.

	Controls	CUS	P-value
Blood glucose (mg/dl)	155.87 ± 2.64	162.4 ± 3.72	0.1925
Triglycerides (mg/dl)	49.12 ± 5.57	68.7 ± 6.25	0.0369*
Total cholesterol (mg/dl)	51.37 ± 0.94	54.2 ± 1.15	0.086
HDL (mg/dl)	16.32 ± 0.54	16.77 ± 0.4	0.5072
LDL (mg/dl)	25.22 ± 1.24	23.69 ± 1.00	0.3458

CUS animals have altered serum metabolite levels. Shown are concentrations of blood glucose, triglycerides, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) (mean ± SEM) in the serum. CUS animals exhibited elevated serum triglyceride levels compared to age-matched controls (age = 3.5 months, n = 10 for Ctrl and CUS). *P < 0.05 compared to controls (Student's t-test).

Discussion

The major finding of the present study is that ES animals in adulthood demonstrate a dysfunction of metabolic status as revealed by dyslipidaemia, decreased serum IGF-1 levels, perturbed expression of liver IGFBPs and dysregulation of metabolic gene expression in the liver and muscle, accompanied by a decline in mitochondrial content in skeletal muscle (Fig 6). These changes observed in ES animals are noted in the absence of any change in body weight, serum insulin, glucose homeostasis or food intake. These results highlight the importance of early adversity not only for exacerbating the risk for anxiety and depressive behaviour in adulthood, but also for evoking long-lasting perturbations of metabolic homeostasis, thus establishing a putative substratum for enhanced risk towards adult metabolic dysfunction. To the best of our knowledge, this is the first report to indicate the persistent effects of ES on muscle mitochondrial content, as well as circulating IGF-1 levels, which were observable long after the cessation of ES.

The ES of maternal separation has been reported to program behavioural dysfunction with perturbations noted in anxiety and depressive-like behaviour, cognitive performance, and a risk for substance abuse accompanied by persistent endocrine consequences, such as a disruption in HPA axis feedback regulation (24,35,36). Several of the above behavioural and endocrine alterations emerge in adulthood, are persistent, and can be exacerbated further in response to the second hit of adult-onset stress (27). In addition to the behavioural and endocrine dysfunction noted in the maternal separation model, reports also indicate perturbed brain–gut signalling and altered cytokine-linked immune responses (37,38). Thus far, few preclinical studies have investigated whether ES history is also linked to metabolic changes. In this regard, a few clinical studies indicate an association between early adversity and adult metabolic dysfunction, with a longitudinal study reporting a higher risk for the development of obesity in women with a history of childhood sexual abuse (22), as well as reports showing that higher adverse childhood experience scores predict an increased risk for cardiovascular disorders (21). The results of the present study indicate long-lasting alterations in a key metabolic signalling pathway, namely the IGF-1 pathway in animals with a history of ES, accompanied by mitochondrial dysregulation, and suggest that such perturbations may contribute to the links between early adversity and adult metabolic dysfunction.

The insulin/IGF-1 pathway is critical for the maintenance of normal glucose and lipid homeostasis in the body, and genetic/pharmacological disruptions of insulin/IGF-1 signalling lead to severe dysregulation of metabolism in multiple tissues (23,39). Additionally, both insulin and IGF-1 have been shown to cross the blood–brain barrier and affect cognitive, anxiety, depression-like and feeding behaviour in rodents (40,41). In our paradigm, we did not observe

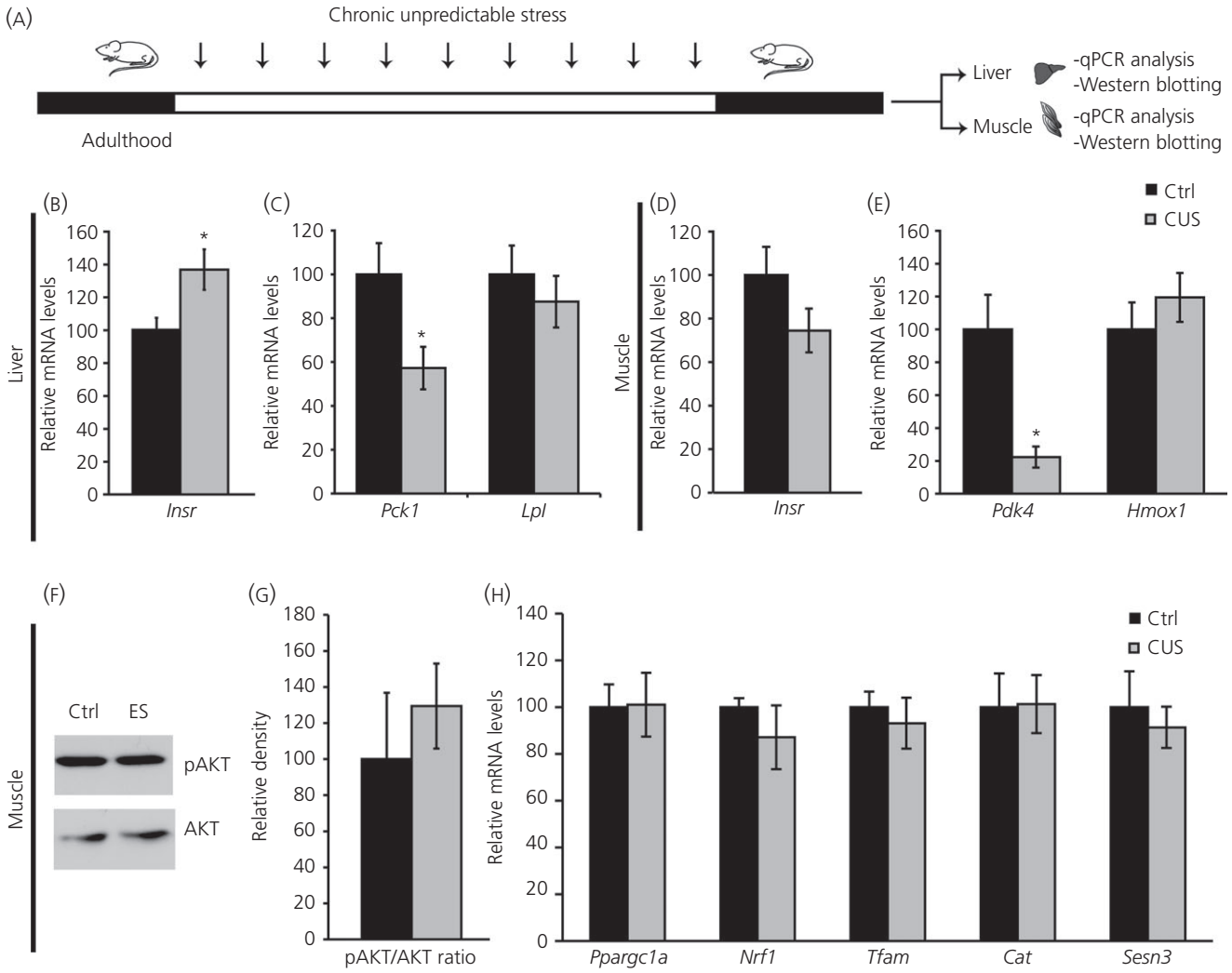

Fig. 5. Adult-onset chronic unpredictable stress influences metabolic gene expression and does not alter mitochondrial function. Shown is a schematic for the stress paradigm after which quantitative polymerase chain reaction (qPCR) and western blot analysis was performed on tissue derived from liver and skeletal muscle (A). Chronic unpredictable stress (CUS) was associated with an increase in mRNA expression of insulin receptor (*Insr*) in the liver (B) accompanied by a decline in expression of *Pck1* and no change in *Lpl* mRNA levels compared to controls (C). CUS animals exhibited no change in *Insr* mRNA expression (D) in skeletal muscle, accompanied by decreased *Pdk4* expression and no change in *Hmox1* mRNA levels (E). Western blotting analysis for pAKT and AKT revealed no change in pAKT/AKT ratios between CUS and Control (Ctrl) groups in the muscle. Shown are representative blots for pAKT and AKT in Ctrl and CUS animals (F) and quantitative densitometric analysis for pAKT/AKT ratios is shown in (G). Muscle mRNA levels of the mitochondrial regulatory genes *Ppargc1a*, *Nrf1*, *Tfam*, *Cat* and *Sesn3* were not significantly altered between Ctrl and CUS groups (H). Results are expressed as the mean ± SEM (for qPCR in liver, age = 3.5 months, n = 10 for Ctrl and CUS; for qPCR in muscle, age = 3.5 months, n = 10 for Ctrl and CUS; for western blotting in the muscle, age = 3.5 months, n = 4 for Ctrl and CUS), *$P < 0.05$ compared to controls (Student's t-test).

any changes in glycaemic state in ES animals, as assessed by fed and fasted serum glucose levels. In the absence of any discernable difference between the two cohorts on a glucose tolerance test or an insulin tolerance test, we conclude that ES does not affect glucose homeostasis, insulin signalling or insulin sensitivity. Furthermore, we did not observe any changes in insulin levels, or downstream signalling in either the muscle or liver, despite a persistent alteration of mRNA levels of the insulin receptor in both tissues in adult ES animals. Indeed, the transcriptional dysregulation of insulin receptor levels was not found to translate into protein level changes, which might explain the absence of any signalling effects. Our findings differ in this regard from previous studies showing an association between chronic stress and the dysregulation of glucose metabolism (16,19). Such a difference in findings may be intrinsically coupled to the nature and duration of the stressor. Maintenance of normal basal insulin signalling and a consequential lack of glycaemic response might reflect the differential ability to buffer diverse stressors. It is also important to note that clinical studies on the effects of early adversity on adult metabolic status are a composite of the effects of early stress, as well as an overlaying of dietary or lifestyle habits. Together, these could serve to exacerbate the metabolic changes evoked by early stress and increase the risk for the emergence of metabolic dysfunction and pathology.

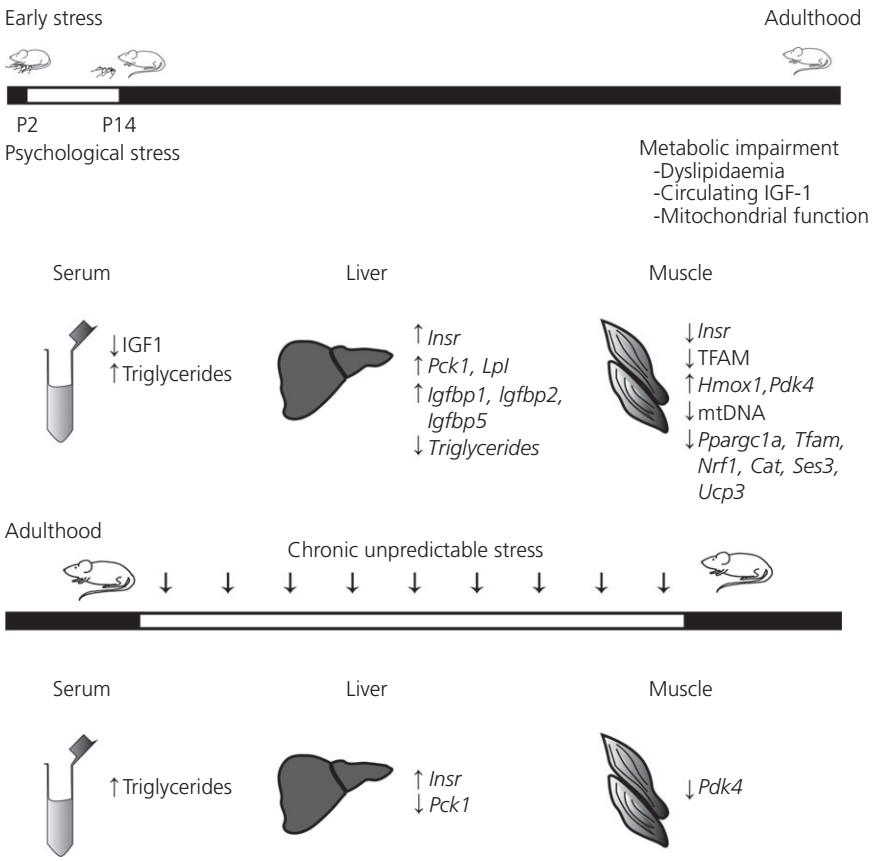

Fig. 6. Summary of the metabolic changes observed in adult animals with a history of the early stress of maternal separation or of adult-onset chronic unpredictable stress (CUS). Early stress (ES) animals in adulthood exhibited metabolic changes as revealed by a decline in circulating serum insulin-like growth factor (IGF)-1 levels and an increase in triglyceride levels. A change in metabolic status in the target tissues of liver and muscle was supported by evidence of an increase in metabolic gene expression, namely *Pck1* and *Lpl* mRNA levels in the liver and *Hmox1* and *Pdk4* mRNA levels in the skeletal muscle. ES history also evoked persistent changes in the muscle mitochondrial status, including a decline in relative mtDNA levels, a decrease in expression levels of the mitochondrial matrix protein TFAM (transcription factor A, mitochondrial), and reduced expression of several genes involved in the regulation of mitochondrial function, namely *Ppargc1a*, *Nrf1*, *Tfam*, *Cat*, *Sesn3* and *Ucp3*. Exposure to adult onset stress (CUS) produced a strikingly different profile of gene expression changes from ES in the liver and muscle, at the same time as displaying a similar increase in triglyceride levels in the serum. These findings suggest that a history of early adversity is associated with long-lasting perturbations of specific metabolic pathways, in particular IGF-1 levels and muscle mitochondrial function, which are effects that are distinct from the effects of adult onset stress. P, postnatal day.

A major highlight of the present study was the observation of a significant decline in circulating IGF-1 levels and an associated increase in liver IGFBP expression in ES animals. IGF-1 in circulation is largely derived from the liver, and its synthesis is regulated by hypothalamic-pituitary inputs via the somatotrophic axis, consisting of growth hormone secretion from the pituitary driven by hypothalamic growth hormone-releasing hormone signalling (42). Altered IGF-1 signalling is known to have significant consequences on insulin sensitivity, bone growth, muscle mass and lipid metabolism (42,43). Despite sharing signalling components, insulin and IGF-1 signalling also have non-overlapping effects on organismal physiology, metabolism and growth, and these differences likely arise from differential effects on gene expression (29). Our observations of reduced IGF-1 levels accompanied by increased liver IGFBP expression are particularly interesting given that the model of maternal separation has been shown to cause a decline in growth hormone levels in the pups (44). We observed a decline in IGF-1 levels in the circulation in ES animals. However, this did not translate into changes in the pAKT/AKT or pFOXO/FOXO ratios in ES animals. Although our results do indicate an altered IGF-1 axis, they do not reveal any change in baseline IGF-1 signalling in target tissues at the time points examined. Despite no change in pFOXO/FOXO ratios, we did note an altered expression of several metabolic genes in target tissues, which are transcriptional targets of multiple transcription factors, including FOXO, glucocorticoid receptor and CREB. This raises the possibility of alternative signalling downstream to IGF-1, which is AKT independent. Alternatively the dysregulated gene expression that we observed could be independent of changes in IGF-1. Collectively, our results indicate a metabolic imprint of early adversity that is maintained long after the cessation of stress (Fig 6). It will be particularly interesting to investigate whether such a shift in metabolic status of ES animals serves to establish vulnerability for metabolic dysfunction under baseline conditions. Alternatively, it could set up a predisposition towards a disease phenotype

when challenged by a 'second hit' of an adult onset stressor or a dietary perturbation.

Amongst the genes that were observed to be upregulated in skeletal muscle derived from adult ES animals was heme oxygenase 1(*Hmox1*). Increased expression of *Hmox1* has previously been shown to reduce mitochondrial function and impinge upon the nuclear transcription of mitochondrial genes via Sirt1 and PGC1α (30). Our results indicate a decrease in mitochondrial biogenesis and overall mitochondrial mass in ES animals, using a number of different markers, including mitochondrial DNA levels and the expression levels of several genes critically involved in regulating mitochondrial function. Further studies are required to investigate whether these molecular changes encode a decline in muscular performance or vulnerability towards muscle wasting disorders. Of significant interest is the possibility that the changes observed in the skeletal muscle may have non-autonomous effects on the central nervous system, as suggested by a recent study in which skeletal muscle PGC1α was shown to regulate stress-associated depression-like behaviour via the modulation of an inflammatory pathway (45). This also raises the question of whether interventions aimed at restoring the peripheral metabolic phenotype, such as exercise, would serve to counteract the behavioural effects of ES that emerge in adulthood.

In our comparative analysis of the effects of ES and adult-onset CUS (Fig 6), we found a significant increase in circulating triglyceride levels in both models. Interestingly, in addition to an increase in circulating triglycerides, we also observed a strong trend towards a decline in stored triglyceride levels in the liver. Similar observations have been reported previously in preclinical models that show impairments in liver triglyceride synthesis or storage, concomitant with a defect in triglyceride clearance (46,47). Our observations highlight the possible contributory effects of psychological stress on the regulation of peripheral metabolism and corroborate data obtained from clinical studies on human subjects showing that an exposure to acute psychological stress is associated with a robust increase in triglyceride levels and an impairment in triglyceride clearance (48). There are contradictory reports in the literature regarding the effects of early stress on triglyceride levels in preclinical models, with studies showing increased (49) or no change (19) in triglyceride levels. However, broad dyslipidaemia, as well as alterations in fat mass and adipose tissue gene expression, have been reported previously with both adult and early stress models (15,19). We propose that dysregulation in lipid metabolism may be a general consequence of exposure to stress events, with the nature and degree of manifestation dependent upon the nature and type of stressor being administered. Our findings with the ES and CUS models differed in their effects on liver and muscle gene expression and in the regulation of muscle mitochondrial status. These findings indicate that the timing and nature of the stressor are likely to regulate the specific type of metabolic consequences evoked, and provide impetus for future studies that aim to uncover whether there exist critical periods in which the impact of stress can exert a permanent effect on metabolic status.

When interpreting the consequences of ES history on metabolic parameters, it is important to note that one of the caveats of the present study is that it is restricted to assessing the consequence of ES in only male animals. A previous study investigating the effects of a history of adverse experience in early life specifically in female rats found reduced insulin resistance markers, decreased *Ppargc1a* expression in the adipose tissue and an altered response to an obesogenic diet in maternally separated rats (19). A comparison of these findings with our results suggests the possibility that there may exist significant sexual dimorphism in the metabolic response to adverse early-life experience. These studies provide impetus for future investigations systematically investigating the effects of ES history in both males and females across the life-span.

In conclusion, the results of the present study demonstrate a persistent and robust dysregulation of circulating IGF-1 levels, as well as muscle mitochondrial content, in adult rats with a history of early adversity. It is possible that this encodes an enhanced vulnerability to metabolic disorders, which may be unmasked by subsequent exposure to a metabolic or psychological challenge, or a natural process such as ageing. In a clinical context, this might indicate a heightened risk towards metabolic disorders in patients who have suffered childhood trauma and abuse. Our results support a strong causative link between psychosocial risk factors and the establishment of vulnerability to metabolic syndromes. Finally, we consider that our results will motivate further studies on investigating mechanistic links between psychological stress and the disruption of metabolic homeostasis, which could uncover potential therapeutic interventions that can counter both behavioural and metabolic dysfunction.

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Conflict of interests

The authors of the manuscript have no conflicts of interest to declare.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Early stress (ES) does not alter body weight or food intake during maternal separation or in adulthood.

Fig. S2. Early stress (ES) does not alter insulin receptor protein expression in the liver or muscle.

Fig. S3. Early stress (ES) does not alter total AKT expression in the liver or muscle.

Fig. S4. Chronic unpredictable stress (CUS) does not lead to any changes in body weight of stressed animals compared to their age-matched controls (A). Total AKT expression in the muscle is not different between Control (Ctrl) and CUS animals (B) (age = 3.5 months, for body weight, n = 10 for Ctrl and CUS; for western blotting, n = 4 for Ctrl and CUS.

Table S1. Primer sequences used for quantitative polmerase chain reaction analysis.

Table S2. Primer sequences used for relative mitochondrial DNA analysis.