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Mechanical-tactile stimulation (MTS) during neonatal stress prevents hyperinsulinemia despite stress-induced adiposity in weanling rat pups

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Abstract

Stress in early life negatively influences growth quality through perturbations in body composition including increased fat mass. At term (40 weeks) preterm infants have greater fat mass and abdominal visceral adipose tissue than term-born infants. Mechanical-tactile stimulation (MTS) attenuates the stress response in preterm infants and rodents. We tested the hypothesis that MTS, administered during an established model of neonatal stress, would decrease stress-driven adiposity and prevent associated metabolic imbalances in rat pups. Pups received one of three treatments from postnatal days 5 to P9: Neonatal Stress (Stress; n = 20) = painful stimulus and hypoxic/hyperoxic challenge during 60 min of maternal separation; MTS (n = 20) = neonatal stress + 10 min of MTS; or Control (n = 20). Body weight, DXA whole body fat mass (g), MRI subcutaneous and visceral adipose tissue, and fasting adiponectin, leptin, glucose, insulin, and corticosterone were measured at weaning (P21). Stress and MTS weight gain (g/d) were accelerated following neonatal stress with greater fat mass, abdominal subcutaneous adipose tissue, serum adiponectin, leptin, and fasting glucose at weaning (P21). Male Stress and MTS pups had greater visceral adipose tissue depot. Male and female Stress pups were hyperinsulinemic. In summary, neonatal stress compromised body composition by increasing fat mass and abdominal subcutaneous adipose tissue depot, and in males, visceral adipose tissue depot. Importantly, MTS prevented hyperinsulinemia despite of stress-induced adiposity. We conclude that MTS during neonatal stress has the potential to minimize metabolic consequences associated with stress-driven perturbations in fat mass and abdominal adipose depots.

Keywords

Mechanical-tactile stimulation; Body composition; Subcutaneous adipose tissue; Visceral adipose tissue; Hyperinsulinemia

1. Introduction

Physiologic stressors in early postnatal life are associated with perturbations in growth quality specifically increased abdominal adipose tissue deposition, the development of adult obesity, and metabolic consequences [1–3]. This association has focused concern on prematurely born infants (<37 weeks gestation) who may be exposed to repeated stressful events including maternal separation, hypoxic/hyperoxic episodes, and painful procedures such as injections or intravenous catheter insertion [4,5]. Although lighter and shorter, preterm infants at term gestation (40 weeks) have greater total fat mass [6,7] with increased abdominal visceral (VAT) adipose tissue depots [8] than term-born infants. Greater fat mass, in association with insulin resistance and glucose intolerance, has also been documented in prematurely born young adults [9].

Abdominal fat is comprised of two distinct, functionally different adipose tissue depots: subcutaneous (SAT) and VAT [10]. SAT is protective while VAT is detrimental to the development of metabolic disease [11]. Both SAT and VAT depots produce the adipocyte-specific hormones, adiponectin and leptin [12]. Stress increases circulating glucocorticoid (GC) which alters leptin to hypothalamic-pituitary-adrenal (HPA) axis feedback and in turn greater SAT deposition and VAT lipolytic activity [13]. Similar to leptin, insulin also regulates body fat mass via the hypothalamus [14]. Evidence suggests that disruption of the leptin- or insulin-HPA axis feedback loop leads to resistance to their actions and increased peripheral and/or central body fat mass [13,14]. Neonatal stress may also alter insulin-HPA axis feedback [15,16]. Disruption of leptin- or insulin-HPA axis feedback, therefore, may be responsible, in part, for the adipose tissue deposition perturbation observed in preterm infants.

Infant massage consisting of mechanical-tactile stimulation (MTS) is promoted as effective intervention for stress reduction and enhancement of weight gain in premature infants [17]. In animals, stressors such as maternal separation, hypoxia, or exogenous glucocorticoid (GC) exposure in early neonatal life may permanently change stress response [15,16,18,19], body structure [20], and metabolism [21] increasing the incidence of adult chronic diseases [15,16,21,23]. In rats, MTS during early postnatal life increases weight gain [24] and decreases neuroendocrine response to stress in adult animals [25,26]. Therefore, a rat model may provide a good model to study mechanisms underlying MTS intervention to modulate neonatal stress and thereby stress-driven adipose tissue deposition and metabolism.

This study determined the effect of MTS, designed to mimic infant massage therapy, on stress-driven adipose tissue deposition in rat pups. Our investigation, which used an animal model of neonatal stress known to alter neuroendocrine response, examined weight gain, fat mass and its deposition, and serum metabolic hormones. We hypothesized that MTS would decrease neonatal stress-mediated adipose tissue deposition and in turn, adipose tissue directed metabolism of weanling rat pups.

2. Methods

2.1. Animals

Timed pregnant, 3-month old Sprague–Dawley rats (n = 6) were allowed to deliver spontaneously at term (E21) and litters culled to 10 pups (5 M and 5 F). All dams were fed standard rat chow and housed in the same environment. Litters were randomly assigned to one of three treatment groups: Stress (S = 60 min of maternal separation with hypoxia/hyperoxia and a saline injection, n = 20); MTS (10 min of MTS at the end of the stress intervention, n = 20), or Control (n = 20). All treatments started on P5 and were completed on P9 of life. Control pups handling was minimal and limited to daily cross-fostering (P9–

P20) or weekly weights and cage cleaning. All procedures were approved by the University of Utah Animal Care Committee and are in accordance with the American Physiological Society's guiding principles [27].

2.2. Intervention

Our intervention was based upon well established models of neonatal stress that range from severe physiological stress (hypoxia followed by hyperoxia and painful procedures) to mild stress (physical separation from the dam) [20–23]. We used a similar model that consisted of a 60 min period of maternal separation that included 1) needle puncture, 2) 8 min of hypoxia, and 3) 4 min of hyperoxia (Fig. 1). The needle puncture, to simulate a painful procedure such as insertion of an IV catheter line or blood draw, was given immediately prior to the hypoxia challenge. Hypoxia was achieved by placing the pups in a 22 liter closed container kept at 37 °C in a humidified gas mixture (100% N₂) for 8 min; at the end of the hypoxic challenge, the chamber was flushed with 100% O₂ for 4 min (hyperoxic condition), followed by room air (21% O₂) as described by McPherson et al. [23]. MTS pups received mechanical/tactile stimulation for 10 min following the stress exposure and within the 60 min maternal separation period. The MTS intervention consisted of 5 min of range-of-motion movements to the fore- and hindlimbs to achieve mechanical stimulation while tactile stimulation was provided by stroking with a soft camel hair brush to the ventral and dorsal body for 5 min. MTS was provided by one of three research technicians following a strict rotation schedule to assure equal distribution between the timing (0, 10, or 20 min post-neonatal stress) and research technicians among the MTS pups.

2.3. Body morphometry and composition

Body weight was obtained on an electronic scale (APX-203, Denver Instrument) to the nearest 0.01 g on P0, P5–P9, and P21. Whole body absolute and relative fat mass (FM, g and %) were determined by DXA (pDXA, Norland, Fort Atkinson, WI) on P21. DXA measurements were performed after the animal was sedated with Avertin (150 mg/100 g). The inter- and intra-coefficients of variation for fat mass by DXA in our laboratory were 2% and <1%, respectively.

To determine adipose tissue distribution, a subset of animals (4 M/4 F per treatment, n = 24) was randomly selected for MRI measurement. Both the MRI (Bruker Biospec 70/30, Billerica, MA) and image analysis (ImageJ software version 1.410) have been described previously in detail [28]. In brief, a measurement was taken of the total area of adipose tissue depots within the abdominal cavity. After manually defining the boundary of the peritoneal cavity, the area of the subcutaneous (SAT) and visceral (VAT) adipose tissue depots was measured and expressed per g weight.

2.4. Serum metabolic hormones

Fasting blood glucose and serum adiponectin, leptin, glucose, insulin, and corticosterone (GC) were measured. All pups were fasted for 12 h prior to blood collection. Fasting blood glucose was determined at the time of collection by a glucometer (Accu-Chek®, Roche, Pompano Beach, FL). The remaining blood was centrifuged at 2000 × g for 15 min and serum collected and stored at –80 °C until analysis. Serum corticosterone, insulin, adiponectin, and leptin were measured in duplicate by ELISA assay as per manufacturer guidelines (Rat corticosterone EIA, DSL, Inc, Webster, TX; rat insulin adiponectin, and leptin ELISA, R & D Systems, Minneapolis, MN).

2.5. Statistical analysis

Data are presented as the mean $\pm$ SD. Post-hoc calculations were performed to determine weight gain (g/d) during and following neonatal stress. ANCOVA determined differences between treatments with gender and P21 body weight as co-factors. SPSS software (v17.0, SPSS, Inc, Chicago, IL) was used for all analyses with $p \leq 0.05$ considered significant.

3. Results

3.1. Body weight gain

The impact of neonatal stress on growth was determined by body weight during and after treatment. Body weight was similar between treatments at birth (5.1 ± 0.5 g Control; 5.0 ± 0.5 g Stress; 5.1 ± 0.5 g MTS) and weaning (48.1 ± 2.3 g Control; 47.1 ± 2.6 g Stress; 47.2 ± 2.5 g MTS). Weight gain (g/d) during neonatal stress (P5–P9) was not significantly different across treatments (1.9 ± 0.2 Control; 1.8 ± 0.2 Stress; 1.8 ± 0.2 MTS) (Fig. 2). Stress and MTS pups, however, experienced accelerated weight gain trajectory following neonatal stress (P10–P21) (1.9 ± 0.2 g/d Control; 2.1 ± 0.2 g/d Stress; 2.1 ± 0.02 g/d MTS; $p < 0.001$) (Fig. 2). Body weight at weaning was higher in male pups compared to female pups independent of treatment (48.8 ± 2.5 g male versus 46.1 ± 2.4 g female; $p < 0.01$).

3.2. DXA whole body fat mass

Whole body measures of FM (g) at weaning (P21) were determined by DXA. The percent FM was significantly higher in both Stress ($6.7 \pm 1.4\%$) and MTS ($7.4 \pm 1.5\%$) pups compared to Control ($2.9 \pm 2.2\%$; $p < 0.001$) (Fig. 3). MTS female pups had greater FM than Stress female pups ($8.1 \pm 1.4\%$ MTS versus $6.2 \pm 1.4\%$ Stress; $p = 0.03$).

3.3. MRI SAT and VAT depots

The impact of postnatal stress on adipose tissue depots was measured by MRI at P21. Similar to whole body fat, abdominal SAT depot (per g body weight) was two- to three-fold higher in Stress and MTS pups compared to Controls (80.9 ± 6.3 /g weight Stress; 89.2 ± 6.4 /g weight MTS; 26.1 ± 2.4 /g weight Control; $p < 0.001$) (Fig. 4A). VAT depot was significantly greater in Stress and MTS male pups (6.0 ± 1.2 /g weight Stress and 5.7 ± 1.0 /g weight MTS) compared to Control males (4.1 ± 0.9 /g weight) and Control and Stress females (both 5.1 ± 1.0 /g weight; $p < 0.01$) (Fig. 4B).

3.4. Serum metabolic hormones

The effect of neonatal stress and MTS on metabolic hormones was evaluated by examining fasting levels of adiponectin, leptin, glucose insulin, and GC (Table 1). Serum adiponectin and leptin levels were higher in Stress and MTS ($p < 0.01$). MTS female pups had significantly higher serum leptin compared to MTS males ($p = 0.02$). Hyperglycemia was present in Stress and MTS ($p < 0.001$) while only Stress pups had hyperinsulinemia ($p < 0.05$). At P21 Stress and MTS pups had lower serum GC levels compared to Control ($p < 0.001$).

4. Discussion

To our knowledge we are the first to document increased fat mass and serum adiponectin, leptin, glucose, and insulin levels induced by neonatal stress in weanling rats. More striking, MTS pups did not experience hyperinsulinemia despite hyperglycemia and a two-to-threefold increase in body fat mass. These findings are important as attenuation of neonatal stress by MTS may decrease the risk of adipose tissue-induced metabolic consequences in later life. Further we demonstrated the feasibility an animal model for translational

investigations of the biology of neonatal stress, MTS, body composition, and metabolism designed to complement massage therapy studies in preterm infants.

Weight gain was accelerated following neonatal stress in both Stress and MTS animals. Body mass is comprised of lean and fat tissue as well as extracellular and intracellular fluid compartment. Weight gain or loss reflects changes in body composition in any of these four areas [29]. Thus body weight or weight gain trajectory alone is an insufficient indicator of growth quality.

Our earlier work found greater lean tissue mass in weanling rat pups that were given tactile stimulation during 10 min of daily maternal separation from P6 to P10 [24]. Although these results appear contradictory, it is important to remember that the neonatal stress model used in the current study exposed rat pups to a variety of stressors and for a longer duration, i.e. 60 versus 10 min/day. Acute and/or chronic stress increases circulating glucocorticoid levels which may induce transient or persistent changes to HPA axis feedback [15,16,18,21]. These changes to glucocorticoid-HPA axis signaling pathway decrease insulin-mediated glucose uptake and lipolysis resulting in increased fat mass [12]. Rat pups that experience accelerated growth following postnatal caloric restriction have adipocyte hypertrophy and become more obese as adults [28,30–32]. However, we found lower circulating GC levels in either Stress or MTS pups at weaning. This finding is in agreement with other investigations of neonatal stress in rodents [15,16]. The ‘blunted’ GC response is attributed to HPA axis hypersensitization as a result of neonatal stress [15]. We did not track circulating GC levels during neonatal stress although a cumulative effect of sequential periods of neonatal stress on basal GC levels in rat pups has been reported by other investigators [31]. Serial monitoring of GC levels during period of neonatal stress is needed to understand 1) the cumulative effects of and 2) the ability of interventions such as MTS to modulate the response to neonatal stress. Despite this limitation, our model provides compelling evidence of the negative impact neonatal stress has on body composition. We speculate that neonatal stress initiated a cascade of events that altered glucocorticoid-HPA axis feedback which in turn, facilitated the greater fat mass deposition.

Increased VAT was only noted in male Stress and MTS compared to male Control pups. Female VAT depot did not differ between treatments, was similar to male Stress and MTS, and greater than male Control pups. These sex-specific differences are puzzling, as in general, VAT depot is higher in males and postmenopausal females compared to premenopausal females. This sex-specific difference in VAT depot is attributed to the female sex steroid estrogen [10]. Estrogen regulates VAT deposition by direct interaction with the leptin signaling pathway. Specifically, estrogen activates estrogen receptors in VAT to directly decrease lipogenesis or indirectly by enhancing leptin-induced activation of the sympathetic nervous system which decreases fat accrual in the VAT depot [10,11]. While we do not have an explanation for the female results, the higher VAT depot in male Stress and MTS pups demonstrates a sex-specific difference for adipose tissue deposition following neonatal stress.

The elevated serum adiponectin and leptin levels in Stress and MTS pups are consistent with the positive relationship between these adipocytokines, whole body fat mass, and SAT depot reported by others [11,13,14]. Elevated adiponectin levels in neonatal rats exposed to chronic hypoxia from birth to P7 have been attributed to decreased insulin sensitivity [12]. Adiponectin improves insulin sensitivity by enhancing muscle tissue metabolism of insulin-stimulated glucose disposal [11]. In humans and rodents, serum leptin values reflect body fat mass and are higher in females [10,11]. A sex-specific difference in serum leptin was noted in MTS, but not Stress pups despite greater adiposity in both genders. Further evaluation of

the interplay between adiponectin, leptin, and muscle metabolism is needed in order to elucidate potential modulating affects of MTS during neonatal stress.

Basal hyperglycemia was observed in neonatal stress independent of treatment or gender, however, hyperinsulinemia was only found in Stress pups. Stress stimulates lipolytic activity which, in turn, decreases hepatic insulin extraction. These events cause systemic hyperinsulinemia as well as suppression of inhibition of hepatic glucose production by insulin. In addition, lipolysis accelerates gluconeogenesis and decreases insulin-stimulated peripheral glucose disposal, which under normal conditions, increases peripheral hyperinsulinemia [10]. McPherson et al. [23] report hyperglycemia and insulin resistance in adult rats following neonatal stress exposure. Thus, the presence of hyperinsulinemia concurrent with elevated adiponectin observed in Stress pups provides evidence of a persistent change in adipose tissue directed metabolism. If true, we speculate that the absence of hyperinsulinemia in MTS pups suggests improved insulin-adiponectin signaling. However, the use of single measures of fasting glucose and insulin is limited in their ability to detect insulin resistance [33]. Future work will need to incorporate euglycemic-insulin clamp studies to confirm benefits of MTS during neonatal stress on adipose tissue directed insulin sensitivity.

In summary, our findings confirm the negative impact of neonatal stress on body composition in terms of body fat mass and depot phenotype of weanling rat pups. Both human and animal models demonstrate the lifelong negative effects of early postnatal stress on body fat and metabolism. Further we provide novel evidence that MTS during neonatal stress prevents hyperinsulinemia in the presence of increased adiposity. Our results demonstrate the need for translational studies of neonatal stress, MTS, body composition, and metabolism that complement clinical studies in order to answer questions directed at the immediate and long-term benefits of massage therapy to preterm infants.

Abbreviations

FM	fat mass (g)
MTS	mechanical-tactile stimulation
SAT	subcutaneous adipose tissue
VAT	visceral adipose tissue

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STRESS	Needle puncture	Hypoxia 8 min	Hyperoxia 4 min	Continuation of maternal separation in isolated cup	
		60 min of maternal separation			
MTS	Needle puncture	Hypoxia 8 min	Hyperoxia 4 min	MTS 10 min	Continuation of maternal separation in isolated cup
		60 min of maternal separation			
CONTROL		Dam raised, minimal handling with cage cleaning and body weight measurements only			

Fig. 1.

Time sequence for stressors during neonatal stress protocol for Stress and MTS pups.

Stressors were administered daily starting at P5 and ended on P9 and in the following order:

1) needle puncture, 2) 8 minute hypoxic challenge, and 3) 4 minute hyperoxic challenge during 60 min of maternal separation. All pups were placed in an individual cup during the 60 min of maternal separation to maintain isolation from littermates. MTS pups received 10 min of mechanical-tactile stimulation immediately following the completion of the hyperoxic challenge. Handling of Control pups was limited to body weight on P5, P9, P16, and P21 and routine cage cleaning.

A) Males

B) Females

Fig. 2.

Weight gain (g/d) during (P5–P9) and following (P10–P21) neonatal stress for each treatment for (A) male and (B) female pups. Both Stress (□) and MTS (■) pups experienced accelerated weight gain after cessation of daily neonatal stress compared to Control (Δ) ($p < 0.001$). Male weight gain was greater than female for all treatments. Data analyzed by repeated measures MANCOVA with gender as cofactor and presented as the mean $\pm$ SD. ^aControl, $p < 0.001$.

Fig. 3.

DXA whole body fat mass (g) at weaning (p21) by treatment for (A) male and (B) female pups. Both Stress and MTS had significantly greater fat mass at weaning than Control. In females, MTS had greater fat mass than Stress. Data tested by MANCOVA with gender and body weight as cofactors and presented as the mean $\pm$ SD. ^aControl, $p < 0.001$; ^bStress, $p < 0.05$.

Fig. 4.

A. MRI abdominal SAT/g weight at weaning (P21) by treatment for (A) male and (B) female pups. Both Stress and MTS had significantly greater SAT at weaning than Control. Data tested by MANCOVA with gender and body weight as cofactors and presented as the mean $\pm$ SD. ^aControl, $p < 0.001$. B. MRI abdominal VAT/g weight at weaning (P21) by treatment for (A) male and (B) female pups. In males, Stress and MTS had significantly greater VAT at weaning than Control. No differences were found for females. Data tested by MANCOVA with gender and body weight as cofactors and presented as the mean $\pm$ SD. ^aControl, $p < 0.001$.

Table 1

Fasting adiponectin, leptin, glucose, insulin, and corticosterone at weaning (P21) by treatment and gender (mean $\pm$ SD).

	Stress	MTS	Control
Adiponectin (pg/ml)			
Male (n = 10)	5432.8 $\pm$ 1947.6 ^a	4780.2 $\pm$ 2048.4 ^a	2261.7 $\pm$ 626.2
Female (n = 10)	4219.3 $\pm$ 840.9 ^a	4580.0 $\pm$ 2139.3 ^a	2829.9 $\pm$ 1329.7
Leptin (pg/ml)			
Male (n = 10)	1505.5 $\pm$ 151.8 ^a	1280.9 $\pm$ 464.2 ^a	498.1 $\pm$ 106.1
Female (n = 10)	1509.7 $\pm$ 696.7 ^a	2021.9 $\pm$ 659.9 ^{a,b}	632.7 $\pm$ 432.2
Glucose (mg/dl)			
Male (n = 10)	213.0 $\pm$ 15.8 ^a	204.8 $\pm$ 18.8 ^a	148.6 $\pm$ 31.2
Female (n = 10)	219.6 $\pm$ 14.9 ^a	208.6 $\pm$ 16.1 ^a	135.0 $\pm$ 19.4
Insulin (pg/ml)			
Male (n = 10)	2108.3 $\pm$ 1845.8 ^{a,c}	989.1 $\pm$ 737.3	845.5 $\pm$ 312.4
Female (n = 10)	1923.2 $\pm$ 1596.4 ^{a,c}	1119.1 $\pm$ 701.4	907.3 $\pm$ 419.4
Corticosterone (ng/ml)			
Male (n = 8)	13.9 $\pm$ 4.3	11.0 $\pm$ 4.2	21.7 $\pm$ 8.8 ^{c,d}
Female (n = 8)	10.1 $\pm$ 4.6	8.1 $\pm$ 2.5	18.7 $\pm$ 14.6 ^{c,d}

ANCOVA with sex and P21 body weight as co-factors.

^a Control.

^b Males.

^c MTS.

^d Stress, $p < 0.01$.