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Maternal Adrenocorticotropin, Cortisol and Thyroid Hormone Responses to All Three Trimester-Equivalent Repeated Binge Alcohol Exposure: Ovine Model

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Abstract

Alcohol-mediated alterations in hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-thyroid (HPT) axis function are two proposed mechanisms by which alcohol causes neurodevelopmental injury to the fetus. We previously reported that third trimester-equivalent only alcohol exposure in sheep results in increases in the maternal and fetal adrenocorticotropin (ACTH) and cortisol levels, and decreases in the fetal thyroid hormones T₃ and T₄ and maternal T₃ levels. In this study, we wished to characterize the maternal HPA and HPT hormone responses to repeated binge alcohol exposure during all three trimester-equivalents of pregnancy in sheep. Pregnant ewes received intravenous infusions of alcohol at doses of 0.75, 1.25 or 1.75 g/kg over 1 hour with mean peak blood alcohol concentrations of 90, 126 or 183 mg/dl respectively on three consecutive days each week beginning on gestation day (GD) 4. Maternal blood samples were collected on GDs 6, 40, 90, and 132. Maternal plasma concentrations of ACTH and cortisol increased in response to the high alcohol dose, and the magnitude of these elevations was not different across gestation. Thyroid hormone levels were not different when comparing among treatment groups at any time point during gestation. However, there was an ontogenetic decrease in the maternal T₃ concentration beginning between GDs 6 and 40 and a decrease in maternal T₄ and free T₄ beginning between GDs 40 and 90. The current findings suggest that: 1) maternal alcohol consumption at any time during gestation stimulates the HPA axis, 2) maternal HPA responsiveness to alcohol does not change across gestation, 3) binge alcohol exposure at these doses lasting all three trimester equivalent of human brain development does not reduce maternal thyroid hormone concentration, 4) alterations in fetal thyroid function in response to alcohol exposure do not occur as a result of diminished maternal thyroid hormone contribution, and 5) there is an ontogenetic decrease in ovine maternal thyroid hormones over gestation.

Keywords

ethanol; teratogen; ACTH; FAS; FASD

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INTRODUCTION

Maternal alcohol abuse can lead to Fetal Alcohol Syndrome (FAS), a condition that is estimated to be the leading preventable cause of mental retardation in the Western world (Abel and Sokol, 1991). The prevalence of alcohol consumption among women of child-bearing age remains essentially unchanged despite considerable efforts to educate women about the harmful consequences of alcohol consumption during pregnancy (Caetano et al., 2006; CDC, 2004; Institute of Medicine, 1996; Maternal and Child Health (MCH) Data Report, 2003; NIAAA, 2000) requiring the need to consider other approaches to reduce the negative impact of prenatal alcohol exposure (Cudd, 2005). Numerous hypotheses have been proposed to account for the teratogenic effects of alcohol of which alcohol induced alterations in hypothalamic-pituitary-adrenal (HPA) axis, and hypothalamus-pituitary-thyroid (HPT) axis function are popular (For review, see Zhang et al., 2005).

Appropriate cortisol concentrations during gestation are essential for normal fetal brain growth (Bohn, 1984). Prenatal alcohol exposure has been demonstrated to result in elevated cortisol levels in infants (Jacobson et al., 1999), neonatal rats (Weinberg, 1989), and in the fetal lamb (Cudd et al., 2001) during the third trimester-equivalent of human brain development (Dobbing and Sands, 1979). These increases in cortisol during development could interfere with neuronal proliferation and differentiation (Bohn, 1984). High levels of glucocorticoids are also known to result in altered hippocampal development and learning ability (Bodnoff et al., 1995). We had previously demonstrated that maternal alcohol exposure limited to the third trimester-equivalent at a dose that results in fetal neuronal loss results in increased maternal and fetal adrenocorticotropin (ACTH) and cortisol levels in a sheep model where all three trimester-equivalents occur *in utero* (Cudd et al., 2001; West et al., 2001).

Cortisol readily crosses the placenta and maternal cortisol is the chief source of cortisol in the ovine fetus until gestational day 121 (term is 147 days), following which 12 to 40% is derived from the mother (Beitins et al., 1970; Hennessy et al., 1982). Further, alterations in fetal cortisol concentrations by alcohol may also impact on fetal thyroid hormone concentrations (Forhead et al., 2006). Cortisol can directly suppress basal thyroid stimulating hormone levels as well as the conversion of T_4 to T_3 (Larsen et al., 2003). In the present study, we hypothesized that alcohol administration at any time during gestation would result in altered function of the maternal HPA axis, and lead to increases in ACTH and cortisol levels in pregnant sheep. We also hypothesized that HPA responsiveness to alcohol would change over the period of all three trimester-equivalent exposure.

We also wished to investigate the effects of alcohol on the HPT axis as alterations in thyroid hormone levels during development may permanently alter thyroidal function and physiological responsiveness in adulthood (Wilcoxon and Redei, 2004). Evidence for the involvement of thyroid hormones in FAS comes from the fact that children born to hypothyroid mothers or those with congenital hypothyroidism can show behavioral and neurological abnormalities similar to those observed in FAS children (Hannigan, 1994; Man and Serunian, 1976), and chronic alcohol exposure has been demonstrated to result in decreases in thyroid hormone concentrations in pregnant rats (Portoles et al., 1988). It has also been shown that adult thyroid function is altered in rats prenatally exposed to alcohol and that administration of T_4 to alcohol-consuming pregnant rats reverse the learning deficits and depressive behavior in their adult offspring (Wilcoxon et al., 2005; Wilcoxon and Redei, 2004). In contrast to these observations in rats, studies conducted in humans report no change in basal thyroid hormone (T_4) levels in children exposed to alcohol prenatally (Hannigan et al., 1995). T_3 , T_4 , and thyroid stimulating hormone (TSH) levels are reported to be in the normal range in all of the 7 children diagnosed with FAS in another clinical study (Castells et al., 1981). Hernandez and coworkers

(1992) also observed no differences in T₄ levels in 31 children born to mothers who abused alcohol with or without other drugs. However, there is no experimental evidence at present to answer whether alcohol alters the human thyroid state during gestation. We, utilizing the sheep model, previously reported that third trimester-equivalent only binge alcohol exposure decreases fetal T₃ and T₄ levels, but maternal basal levels of T₄ are not altered and that T₃ is altered only towards the end of gestation (Cudd et al., 2002). The ovine gestational period is long and all three trimester-equivalents occur *in utero*, in common with humans. The ontogeny of the thyroidal system in sheep is also very similar to that in humans (Cudd et al., 2002), making the sheep an excellent model for studying thyroid function in response to gestational alcohol exposure. In this study we characterized the T₃, T₄ and free T₄ responses in pregnant sheep to alcohol exposure, but extending the duration of alcohol exposure to all three trimester-equivalents of gestation. We employed a “binge” alcohol exposure paradigm, a drinking pattern common in women who use alcohol during pregnancy (Caetano et al., 2006; Cudd et al., 2001; Ebrahim et al., 1999; Gladstone et al., 1996; Maier and West, 2001) to characterize the maternal thyroid hormones, ACTH and cortisol responses to alcohol over the entire period of gestation.

METHODS

Animals and breeding

All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University. Suffolk ewes (n=31) were maintained on Bermuda grass pasture and supplemented with alfalfa. Pregnancies of known conception date were achieved by manipulating the estrous cycle through the use of progesterone impregnated vaginal implants (EAZI-BREED™ CIDR®, Pharmacia & Upjohn Ltd., Auckland, New Zealand). Implants were inserted and then removed 11 days later at which time intramuscular prostaglandin F_{2α} (20 mg LUTALYSE®, Pharmacia & Upjohn, Kalamazoo, MI) was administered. The following day, ewes were placed with a ram fitted with a marking harness for a period of 24 hours. Marked ewes were assessed ultrasonographically on 25, 60 and 90 days to confirm pregnancy. On gestational day (GD) 4, subjects in saline and alcohol groups were moved into individual pens. From these pens, the ewes were able to maintain visual contact at all times with herdmates in adjacent pens. Conditions of constant temperature (22° C) and a fixed light dark cycle (12:12) were maintained. Once confined, the saline and alcohol treatment group subjects received daily 2 kg of complete pellet ration (Sheep and Goat Pellet, Producers Cooperative Association, Bryan, TX). Daily feed consumption was monitored; subjects in the alcohol and saline treatment groups consumed all of the food offered. Subjects in the normal control group remained in pens with herdmates throughout the study and were offered, as a group, an equivalent amount of feed compared to subjects in the saline and alcohol control groups, though individual feed consumption was not monitored.

Alcohol Treatment Protocol

The alcohol treatment groups (0.75 g/kg, 1.25 g/kg, and 1.75 g/kg body weight) and the saline (0.9%) control group received intravenous infusions delivered by peristaltic pump (Masterflex, model 7014-20, Cole Parmer, Niles, IL) through a 0.2 μm bacteriostatic filter. Pumps were calibrated before infusion. Alcohol infusions were 40% w/v in sterile saline administered over one hour. In the alcohol or saline treatment groups, treatments began on GD 4 and were administered on three consecutive days followed by four consecutive days without treatment and this pattern was continued until the final alcohol exposure day on GD 132.

Experimental Protocol

Details of this experimental protocol have been previously described (Ramadoss et al., 2006). In brief, an intravenous catheter (16 gauge, 5.25 inches Angiocath™ Becton Dickinson, Sandy,

UT) was percutaneously placed into the jugular vein on GD 4. On infusion days, the ewes were connected to the infusion pump by 0830 hr and infusions were delivered continuously at the same rate over 1 hr. On GD 42, ewes underwent surgery to chronically implant femoral arterial and venous vascular access ports (V-A-P™, Model CP47P, Access Technologies, Skokie, IL). The ewes were not subjected to surgery and general anesthesia until after the first trimester to avoid early embryonic losses. After catheter implantation, infusions were administered via the venous vascular access port and blood samples were obtained from the arterial port.

Blood was obtained from the jugular vein catheter on GD 6 and GD 40 and from the femoral arterial catheter on GD 90 and GD 132 every 30 min for 2 hrs and at 6 and 24 hrs beginning immediately before the commencement of the infusions for the measurement of blood alcohol concentration (BAC) and plasma hormone concentrations. On GD 133, the sheep were euthanized using an overdose of pentobarbital and fetal organs were collected and weighed.

Assay Methodologies

A 20 µl aliquot of blood was collected into microcapillary tubes and transferred into vials containing 0.6 N perchloric acid and 4 mM of n-propyl alcohol (an internal standard). The vials were tightly capped with a septum sealed lid and were stored at room temperature for at least 24 hrs before being analyzed by headspace gas chromatography (Varian Associates, Model 3900, Palo Alto, CA). The basic chromatographic parameters were similar to those reported by Penton (1985), with the exception of the column (DB-wax, Megabore, J&W Scientific, Folsom, CA) and the carrier gas (helium) as previously reported (West et al., 2001). Blood samples (4 ml) were collected and placed in chilled polystyrene tubes containing 200 µl of 0.5 M ethylenediaminetetraacetic acid every 30 min, beginning immediately before the beginning of infusions, for 2 hrs and then at 6 and 24 hrs. The tubes were maintained in icewater until being centrifuged for 20 min at 2800 x g at 4°C. Plasma was separated and stored in aliquots at -20°C. Plasma ACTH was measured by specific radioimmunoassay (DiaSorin Inc., Stillwater, MN) as previously described (Cudd et al., 1996). Cortisol was measured by specific radioimmunoassay (Cortisol Coat-a-Count™, Diagnostic Products Corp., Los Angeles, CA) as previously described (Cudd et al., 1996). Basal plasma total T₃, total T₄ and free T₄ concentrations were measured by specific radioimmunoassays (Total T₃ Coat-a-Count™, Total T₄ Coat-a-Count™, Free T₄ Coat-a-Count™ respectively, Diagnostic Products Corp., Los Angeles, CA) as previously described (Cudd et al., 2002).

Data analysis

Plasma ACTH and cortisol were measured at all seven time points on each of the four different GDs while thyroid hormones were measured only at time 0 on each of the five different GDs. The ACTH and cortisol data were analyzed using mixed ANOVA with GD and time as within factors and treatment group as between factor. T₃, T₄ and free T₄ data were analyzed using a mixed ANOVA with treatment group as between and GD as within factor. Post-hoc tests were conducted using Student-Newman-Keuls test. Statistical significance was established *a priori* at $p < 0.05$. All data are presented as mean $\pm$ SEM.

RESULTS

Blood Alcohol Concentrations (BACs)

The mean BACs measured on GDs 6, 40, 90, and 132 of gestation for the alcohol groups peaked at 1 hr which coincided with the end of the infusion period. The peak BACs were not different across gestation. Analysis of peak BACs by a mixed ANOVA with treatment group as between factor and GD as within factor yielded no significant two-way interaction or a main effect of GD. The peak values for the 0.75 g/kg, 1.25 g/kg and 1.75 g/kg alcohol groups were 93 ± 5 mg/dl, 126 ± 5 mg/dl and 183 ± 5 mg/dl respectively.

Adrenocorticotrophic hormone (ACTH)

Maternal plasma ACTH analyzed by mixed ANOVA with time and GD as within factors and treatment group as between factor yielded no significant three-way interaction. Similarly, there was no interaction between day and time, day and treatment, and a main effect of day. For instance, the baseline ACTH levels in the 1.75 g/kg group were 54 ± 13 pg/ml, 38 ± 13 pg/ml, 51 ± 18 pg/ml, and 40 ± 22 pg/ml on GDs 6, 40, 90, and 132 respectively. However, there was a significant interaction between treatment and time ($F_{18,574} = 2.088$, $p = 0.005$). A main effect of treatment ($F_{3,574} = 5.803$, $p < 0.001$) and time ($F_{6,574} = 7.911$, $p < 0.001$) was also noted. Post-hoc analysis identified that the plasma ACTH concentration in 1.75 g/kg group was elevated at 2 hours compared with that in all other treatment groups. Values at 1 and 1.5 hours in the 1.75 g/kg dose group were also different than that in all other groups except for the 1.25 g/kg dose group (Figure 1).

Cortisol

As with ACTH, maternal plasma cortisol concentration did not show a significant three-way interaction or a two-way interaction between GD and treatment and GD and time. A significant main effect of GD was also not noted. However, a significant interaction between treatment and time was identified ($F_{18,588} = 6.520$, $p < 0.001$). A main effect of treatment ($F_{3,588} = 44.606$, $p < 0.001$) and time ($F_{6,588} = 12.427$, $p < 0.001$) was also observed. Post-hoc analysis identified that the 1.75 g/kg dose was significantly elevated ($p < 0.001$) at 1, 1.5, and 2 hours after the beginning of the infusion compared to all other treatment groups (Figure 2).

Thyroid hormones

Thyroid hormones were measured from the sample collected immediately before the beginning of the infusion, time 0, on GD 6, 40, 90 and 132. The data were analyzed using mixed ANOVA with GD as within factor and treatment group as between factor. For T_3 , there was no significant interaction between GD and treatment. A significant main effect of treatment was also not noted. For instance, on GD 41, the T_3 levels for the saline control and the 0.75 g/kg, 1.25 g/kg and 1.75 g/kg alcohol groups were 72 ± 4 ng/dl, 69 ± 4 ng/dl, 65 ± 15 ng/dl, and 65 ± 8 ng/dl respectively. In contrast, a significant main effect of GD ($F_{3,82} = 3.72$, $p = 0.0150$) was noted. T_3 was significantly higher ($p < 0.05$) on GD 6 compared to all other GDs (Figure 3). Similarly, for total T_4 , there was a significant effect of GD ($F_{3,82} = 10.11$, $p < 0.001$), but not treatment or an interaction. T_4 was significantly lower ($p < 0.05$) on GDs 90 and 132 compared to 6 and 40. For free T_4 , there was a significant effect of GD ($F_{3,82} = 10.05$, $p < 0.001$), but not treatment or an interaction. Free T_4 was lower ($p < 0.05$) on GDs 90 and 132 compared to GDs 6 and 40.

Fetal whole body and organ weights were not different when comparing among groups (Table 1).

DISCUSSION

The maternal HPA axis is stimulated by alcohol during all three trimester-equivalents

Previously, we had reported that maternal and fetal plasma concentrations of ACTH and cortisol were elevated in response to alcohol exposure (1.75 g/kg) limited to the third trimester-equivalent of human brain development (Cudd et al., 2001). Here, we report increases in maternal plasma concentrations of ACTH and cortisol in response to alcohol (1.75 g/kg) during all three trimester-equivalents. Furthermore, there was no change in maternal HPA axis responsiveness to alcohol exposure as the magnitude of increases in the HPA hormones in response to alcohol were not different across gestation.

Few studies exist in the human literature on the effects of alcohol consumption on maternal ACTH and cortisol concentrations or on the HPA function of alcohol-exposed children (Zhang

et al., 2005). Early human FAS studies on HPA function are based on limited sample size and unreliable self-reporting (Root et al., 1975). A more recent study has reported that infants (1 year of age) born to mothers who consumed alcohol at conception and during pregnancy exhibit higher basal cortisol levels (Jacobson et al., 1999). Animal studies utilizing the rat model have similarly demonstrated maternal elevations in corticosterone in response to gestational alcohol exposure (Weinberg and Bezio, 1987). We had previously demonstrated that alcohol administration limited to the third trimester-equivalent resulting in increases in maternal ACTH and cortisol is accompanied by a similar change in the fetal levels (Cudd et al., 2001). In the present study, the fetuses were not instrumented, and therefore fetal plasma concentrations of ACTH and cortisol were not measured, as it is not possible to chronically instrument fetuses before mid-gestation. However, cortisol in fetal sheep comes almost entirely from the mother before GD 122 (Hennessy et al., 1982), and therefore, maternal elevations in cortisol as observed in this study would result in some degree of cortisol elevation in the fetus. These elevations may permanently alter the development and function of the HPA axis, and thus lead to long-term behavioral, cognitive, and immune deficits (Zhang et al., 2005). The HPA axis is highly vulnerable to altered programming during fetal development and alcohol-induced increases in maternal and fetal cortisol may reprogram the HPA axis leading to altered behavioral and physiological responsiveness and increase the vulnerability to illnesses or disorders later in life (Zhang et al., 2005). Ongoing studies in our laboratory are investigating the effects of repeated elevations in fetal cortisol similar to that produced by alcohol independent of alcohol on fetal the brain.

In this study, we utilized a chronic weekly-weekend binge paradigm throughout gestation. ACTH and cortisol responses were measured on specific days throughout gestation and no differences were found in the magnitude of elevations in ACTH and cortisol across gestation. This finding coupled with our observations in a previous report where we found that a single acute alcohol infusion in adult female sheep results in increases in the levels of ACTH and cortisol (Cudd et al., 1996) provides evidence that every bout of alcohol is accompanied by elevations in glucocorticoid levels. These observations are also true for repeated alcohol exposures restricted to the third trimester-equivalent of gestation (Cudd et al., 2001). In summary, these reports from our laboratory demonstrate that maternal alcohol consumption at any time during gestation stimulates the HPA axis, and that maternal HPA responsiveness to alcohol does not change across gestation.

Maternal thyroid hormone concentrations are not altered by all three trimester-equivalent binge alcohol exposure

All three-trimester equivalent binge alcohol exposure did not alter T_3 , T_4 or free T_4 concentrations in pregnant sheep. Previously, we had reported that third trimester-equivalent only binge alcohol exposure decreased fetal T_3 and T_4 levels, but maternal basal levels of T_4 were not altered and that T_3 was altered only towards the end of gestation on GD 132 and not on GD 118 (Cudd et al., 2002). Studies utilizing the rat model have demonstrated that prenatally alcohol exposed offspring exhibit decreased T_4 levels (Hannigan and Bellisario, 1990; Portoles et al., 1988; Wilcoxon and Redei, 2004). Though there is no experimental evidence at present to answer whether alcohol alters the human fetal thyroidal state during gestation, a number of studies have demonstrated no alteration in the thyroid function in infants exposed to alcohol prenatally. For example, a study conducted by Hannigan and coworkers (1995) demonstrated no change in basal T_4 levels in infants exposed to alcohol prenatally. Another human study reported that T_3 , T_4 , and TSH levels were in the normal range in all of the 7 children diagnosed with FAS (Castells et al., 1981). Similarly, Hernandez and coworkers (1992) also noted no difference in T_4 levels in 31 children whose mothers were exposed to alcohol with or without cocaine and/or marijuana. Taken together, these findings support the conclusion that alcohol alters HPT function in the fetus but not through a diminished maternal thyroid hormone

contribution. This finding is important because of the requirement of maternal contribution of thyroid hormone necessary to support normal fetal development (Man and Serunian, 1976; Matsuura and Konishi, 1990).

A note on the normal ontogeny of ovine maternal thyroid hormone concentrations during pregnancy

We found that there were decreases in the maternal T₄ and free T₄ concentrations between GD 40 and GD 90 and a reduction in the maternal T₃ concentrations between GD 6 and GD 40 in all control and treatment groups. This finding is consistent with the data on the ontogeny of maternal thyroid hormone concentrations in pregnancy in humans. A study conducted using 10 different commercially available kits demonstrated decreased T₃ and T₄ levels in term pregnant women compared with non-pregnant women (Roti et al., 1991). In humans, there is an early increase in T₄ levels due to the thyrotrophic activity of the early transient increase in the human chorionic gonadotropin (HCG), followed by a marked decrease due to significant increases in the hepatic production of plasma T₄-binding globulin (TBG) during pregnancy, increased gestational T₄ requirements, and a reduction in HCG levels (For review, see Burrow et al., 1994), leading to an overall reduction in T₄/TBG ratio, and lower T₃ and T₄ levels (Glinioer et al., 1990).

Conclusion

In summary, we and others have demonstrated that maternal alcohol consumption at any point of time during gestation would result in increases in the HPA hormones, responses that may permanently alter fetal brain development and physiological and behavioral functions in the offspring. We did not identify a change in maternal HPA axis responsiveness to alcohol across gestation. We also found that maternal thyroid hormone secretion is not altered by all three trimester-equivalent alcohol exposure. Therefore, it does not appear that the reported fetal HPT axis changes in response to prenatal alcohol exposure are a result of diminished maternal thyroid hormone contribution. Finally, we have also demonstrated that ovine maternal thyroid hormone concentrations normally decrease over gestation.

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Figure 1.

Maternal plasma ACTH concentrations (pg/ml $\pm$ SEM) were measured from the beginning of the infusion period every 30 min for 2 hours and then at 6 and 24 hours on gestational days (GDs) 6, 40, 90 and 132 and the responses to alcohol were not different when compared between GD. The infusion of 1.75 g/kg of alcohol significantly increased maternal plasma ACTH concentrations at 1 and 1.5 hours compared to all other treatment groups except for the 1.25 g/kg group (#) and compared to all other treatment groups at 2 hours (*).

Figure 2.

Maternal plasma cortisol concentrations (ng/ml $\pm$ SEM) were measured from the beginning of the infusion period every 30 min for 2 hours and then at 6 and 24 hours on GDs 6, 40, 90 and 132 and responses to alcohol were not different when compared between GD. The infusion of 1.75 g/kg of alcohol significantly increased maternal plasma cortisol concentrations at 1, 1.5 and 2 hours compared to all other treatment groups (*).

Figure 3.

Maternal plasma T₃ (ng/dl ± SEM), T₄ (μg/ml ± SEM) and free T₄ concentrations (ng/dl ± SEM) responses to infusions of saline or 0.75, 1.25 and 1.75 g/kg of alcohol on GDs 6, 41, 90 and 132 were measured and no differences were found between treatment groups. T₃ was higher on GD 6 compared to all other GDs (&). Total T₄ and free T₄ were lower on GDs 90 and 132 compared to GDs 6 and 40 (*).

Mean $\pm$ SEM fetal organ weights (grams) measured on day 133 of gestation in the normal control (NN), pair-fed saline control (SC), and alcohol exposed (dosages, E075, 0.75 g/kg; E125, 1.25 g/kg; E175, 1.75 g/kg) fetuses. No significant differences were found among groups.

Table 1

Fetal Body/ Organ Weights	NN	SC	E075	E125	E175
Whole Body	4.72 $\pm$ 0.22	4.30 $\pm$ 0.33	4.69 $\pm$ 0.17	4.55 $\pm$ 0.48	4.63 $\pm$ 0.37
Adrenals	0.42 $\pm$ 0.03	0.38 $\pm$ 0.04	0.40 $\pm$ 0.02	0.39 $\pm$ 0.06	0.39 $\pm$ 0.06
Thymus	5.95 $\pm$ 0.60	5.40 $\pm$ 0.70	5.08 $\pm$ 0.36	4.63 $\pm$ 1.3	5.44 $\pm$ 1.05
Thyroids	0.93 $\pm$ 0.13	0.87 $\pm$ 0.10	0.87 $\pm$ 0.10	0.96 $\pm$ 0.10	1.04 $\pm$ 0.08
Liver	106.73 $\pm$ 7.29	109.74 $\pm$ 10.97	107.84 $\pm$ 7.64	114.28 $\pm$ 13.27	122.98 $\pm$ 14.20
Spleen	5.69 $\pm$ 0.41	5.94 $\pm$ 0.85	5.09 $\pm$ 0.31	5.52 $\pm$ 0.66	5.66 $\pm$ 0.62
Heart	27.29 $\pm$ 2.64	28.44 $\pm$ 1.82	27.09 $\pm$ 1.92	29.07 $\pm$ 3.39	29.37 $\pm$ 2.77
Lungs	130.57 $\pm$ 7.82	134.90 $\pm$ 8.65	119.25 $\pm$ 8.13	117.69 $\pm$ 18.83	122.81 $\pm$ 14.69
Kidneys	21.51 $\pm$ 1.10	22.46 $\pm$ 1.15	22.00 $\pm$ 0.72	22.93 $\pm$ 2.35	21.47 $\pm$ 1.80