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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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Abstract

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Background: Rising global temperatures have been associated with various health outcomes, including birth defects. Neural tube defects are the second most common type of birth defect in the US, and there has been limited, conflicting research on how maternal exposure to extreme ambient heat in early pregnancy may impact their occurrence. There is an increasing need to understand population health impacts of extreme ambient temperature exposure to guide recommendations and policy.

Methods: We conducted a case-control study using fetal death and birth records in Georgia from 1994-2017. All cases of neural tube defects, specifically anencephaly and spina bifida, were included and matched 4:1 to controls (pregnancies without birth defects) based on maternal county of residence and birth year. Daily county-level ambient temperature data from 2 weeks prior to 6 weeks post-last menstrual period were linked to birth certificate data by county of residence. Four heatwave metrics (2 categorical, 2 dichotomous) were created using different combinations of the duration and intensity of hot days (based on daily apparent temperature exceeding the county-specific 95th percentile). Data were analyzed using conditional logistic regression, adjusting for possible confounders as identified by previous literature.

Results: The study consisted of 673 cases (343 anencephaly, 330 spina bifida) and 2,692 controls. Overall, there was a positive association with maternal exposure to an increasing number of extreme heat days and consecutive extreme heat days during both pre- and post-conception and odds of the offspring developing a neural tube defect. During the defined exposure window, the adjusted odds ratios for neural tube defects were 1.40 (95% CI 1.11, 1.77) for mothers with 13+ days of extreme heat exposure compared to women with no days of extreme heat exposure and 1.36 (1.09, 1.70) for women with 6+ consecutive extreme heat days compared no days of extreme heat exposure.

Conclusion: This study provides epidemiological evidence demonstrating a modest positive association between periconception extreme heat exposure and risk of neural tube defects. Further studies should examine this association, considering individual-level exposures and the role of other possible effect modifiers that were not available in this study, such as folic acid supplement intake.

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Introduction

Neural tube defects (NTDs) are the second most common birth defect in the United States, with about 1 in 1200 live births affected each year.[1] Generally, they occur due to an incomplete closure of the neural tube during the embryonic period. More specifically, this occurs when there are issues with the process of the formation of the central nervous system in an embryo, known as neurulation, between 17- and 28-days post-fertilization.[2] Neural tube defects can cause a variety of symptoms such as paralysis, blindness or deafness, and intellectual disabilities. They vary greatly in severity, with some considered mild and several considered more severe, resulting in early fetal deaths, elective terminations after prenatal diagnosis, or stillbirths.[1] Given their prevalence and potential devastation, much research has been done on exploring potential genetic and environmental risk factors for NTDs, such as maternal race and ethnicity, maternal diabetes, and parental socioeconomic status.[1]

For decades, the association between maternal periconceptional heat exposure and the development of NTDs has been explored. The first documentation of this kind of association occurred when researchers noticed a correlation with influenza pandemics and increased incidence of NTDs.[3] Later, hot tub and sauna exposure became grouped with intrapartum fever as types of acute states of elevated maternal temperature. For example, a large cohort study from New England found that women exposed to hot tubs, saunas, and fevers at least once during pregnancy had an increased risk of having an NTD affected infant. Risk also increased greatly when comparing those exposed to multiple iterations of these heat sources to those only exposed to one, suggesting a synergistic effect.[4] Knowing the potential harm that various extreme heat exposures has on the development of NTDs, it is worthwhile to consider the impact from extreme ambient heat, as well. It is becoming increasingly important to understand the health outcomes that are related to extreme heat and climate change; global temperatures are expected to rise between 2 and 5 degrees Celsius over the course of the century, and building off of that, heat wave prevalence is expected to increase.[5] These changes in climate and heat can have significant impact on pregnant people; research has also shown that due to natural gestational changes in thermoregulation, they may be more vulnerable to extreme heat exposure during this time.[6]

A population-based study of seasonal variation in the birth prevalence of NTDs in Illinois from 1989-2002 found that there was a contrasting difference between affected children conceived between May and November, which indicates a neurulation period during the warmest months of the year, and those conceived during the remainder of the year.[7] This phenomenon was observed again in Jordan in the early 2000's, finding that late summer and early autumn were peak conception periods for babies with NTDs, with August having the highest rate of conception for affected fetuses.[8] While Jordan is not particularly close to the United States, it does share similar latitude, and thus weather patterns. Despite these compelling descriptive data, two population-based case-control studies from the US- one using National Birth Defects Prevention Study data from 1997-2007 (n= 326 NTD cases and 1781 controls) and the other using New York State Congenital Malformations Registry and birth certificate data from 1992–2006 (n=6,422 cases and 59,328 controls) - did not find any significant overall association between weather related extreme heat events during summer embryogenesis periods and NTDs.[9, 10] In contrast, a large (n=877,710) retrospective cohort study in Quebec, Canada from 1988-2012 found a slight association via prevalence ratios between higher temperatures during the fourth week of pregnancy and increased incidence of neural tube defects, diagnosed as either anencephaly or spina bifida.[11] Notable limitations in all of these studies include the lack of individual-level temperature data, as well as restricted abilities to adjust for potential confounders and/or effect modifiers due to limited data availability. Given the limited and conflicting research on the topic, the aim of this study was to evaluate the association between maternal periconceptional exposure to extreme ambient heat and risk of NTDs using a large case-control study of births from the state of Georgia.

Methods

Study Population. Data were collected from all fetal death and birth records in the state of Georgia from 1994 to 2017. Information collected from the fetal death and birth records included date of delivery, estimated gestational age, county of residence, and various maternal demographic factors, such as maternal age, education, race, and ethnicity. Fetal deaths and live births were excluded from the current study if they were part of a multi-fetal gestation, had a gestational age < 20 weeks, or were diagnosed with any known genetic syndromic or chromosomal conditions. NTD cases were defined as any fetal deaths or live births with a diagnosis of anencephaly or with a diagnosis of spina bifida (meningomyelocele). Cases were further classified as “isolated” if no other birth defects collected within the confines of birth records or fetal death records were reported, or “non-isolated” if more than one diagnosis was present.

Study Design. NTD cases were matched 1:4 to controls on county and birth year (grouped into either 1994-1997 and 1998-2017, pre- and post-implementation of mandatory FA fortification in the US, respectively). The temperature exposure window for cases and controls was the 1 month prior to conception and first 4 weeks of pregnancy, known as the periconceptional period. We further broke this window into pre- and post-conception, defined as the first four weeks of the window and last four weeks of the window, respectively. To define these windows, we calculated the date of last menstrual period (LMP) for each mother using both gestational age and reported delivery date and defined the exposure window accordingly.

Meteorologic data. Daily meteorologic data from 1981 to 2017 were obtained from Oak Ridge National Laboratory's Daymet product.[12] Daymet is a gridded meteorology dataset that uses ground-based in situ station observations and a collection of interpolation and regression algorithms to produce 1 km^2 gridded estimates of daily temperature and moisture, among other variables. County-level temperatures were calculated by using the unweighted average of all grid cell estimates within a county. Daily county-level temperature information was then linked to fetal death and birth records based on the reported maternal county and exposure window. An extreme heat day was defined as any day that the mean apparent temperature was above the 30-year (1981-2010) normal, county-specific 95th percentile. Both independent and consecutive total days above the 95th percentile threshold were documented for each birth. A heat wave was, most basically, defined as an occurrence of two or more or three or more consecutive extreme heat days during the periconception window. More prolonged heat waves were also considered.

Statistical Analysis. Demographic characteristics for the birth data were examined via frequencies of their respective variables. A descriptive analysis of the temperature data was also completed via simple statistical analysis of means. Due to the matched case-control design, conditional logistic regression was used to estimate adjusted odds ratios and 95% confidence intervals for all NTDs combined, spina bifida only, and anencephaly only using only isolated cases. We chose to further adjust for maternal age, LMP month, and LMP year; LMP month and year were determined to be a priori confounders and included to account for varying seasonal and/or annual temperatures, which have also been shown by previous literature to influence NTD incidence.[7,8] Maternal age was included to account for known contribution to birth outcomes.

Race and ethnicity were not included within the model, as they were not considered confounders but rather as potential effect modifiers to be explored separately. Analyses were performed for the entire exposure window, as well as the first four weeks alone (preconception) and last four weeks alone (early pregnancy). Sensitivity analyses were performed following the same method to account for the implementation of fortification, looking only at births after 1998. All analyses were conducted in SAS (Version 9.4) and R Studio (Version 2021.09.0).

Results

Between 1994 and 2017, we documented 673 cases of NTDs (343 anencephaly, 330 spina bifida) in Georgia. After matching, we had in 1,372 controls for anencephaly cases, and 1,320 controls for spina bifida cases (2,692 combined controls in total). Overall, most demographic characteristics were similar among cases and controls with a few exceptions (**Table 1**). Neural tube defects case mothers were slightly more likely than control mothers to be White (68.4% vs. 65.3% for anencephaly and 70.6% vs 66.1% for spina bifida) and Hispanic (18.4% vs. 15.3% for anencephaly and 16.4%, vs. 12.1% for spina bifida). There was also a higher proportion of neural tube defects cases that had a calculated LMP in summer months (June, July, August) compared to controls (29.7% vs. 23.8% for anencephaly and 27.6% vs. 23.3% for spina bifida). Neural tube defects cases were much more likely to have been born pre-term (22.2% vs. 7.9% and for anencephaly and 21.5% vs. 8.0% for spina bifida) compared to controls. Anencephaly case mothers were more likely to be married (63.9% vs. 60.6%) and less likely to be nulliparous (33.8% vs. 39.4%) compared to control mothers.

The mean daily apparent temperature in Georgia from 1994-2017 was 18.2 ± 8.7 °C. The mean 95th percentile for apparent temperature across all counties in Georgia was 29.7 ± 1.2 °C (Range: 25.2 to 31.6 °C). In a given year, the average number of days (across all counties) that exceeded the 95th percentile threshold was 23.2 ± 1.8. Given our relatively long (e.g. 8 week) exposure window of interest, it was not uncommon for women to experience an extreme heat event. Overall, 33.3% of cases and 30.1% of controls experienced at least one day >95th percentile in this window and 25.5% of cases and 21.5% of controls experienced at least 3 consecutive days >95th percentile.

As the definition of exposure to extreme heat became more stringent, there were higher odds of NTDs across both the entire exposure period (-2 to 6 weeks post-LMP) as well as during pre-conception (2- to 2 weeks post-LMP) and early pregnancy (3 to 6 weeks post-LMP) (**Table 2**). When looking at the entire exposure period, the adjusted odds ratios for neural tube defects were 1.12 (95% CI 1.04, 1.21), 1.25 (95% CI 1.07, 1.46), and 1.40 (95% CI 1.11, 1.77) for mothers with 1-4, 5-12, and 13+ days of extreme heat exposure, respectively, compared to women with no days of extreme heat exposure. The same phenomenon was also observed with an increasing number of consecutive days exposed to extreme heat. Women with 1-2, 3-5, and ≥6 consecutive days of extreme heat had 1.11 (95% CI 1.03, 1.19), 1.23 (95% CI 1.06, 1.43), and 1.36 (1.09, 1.70) times the odds (adjusted) of neural tube defects, respectively, compared to women with no days of extreme heat exposure. Using a standard definition of heatwave as experiencing 2 or 3 consecutive days of extreme heat, women in our study were estimated to have 1.32 (95% CI 1.10, 1.59) and 1.30 (95% CI 1.07, 1.58) times the odds (adjusted) of neural tube defects compared to women not experiencing heatwaves.

The association between total days and total consecutive days of extreme heat exposure and odds of neural tube defects was slightly stronger during the preconception period as compared to the early pregnancy period. For example, women with ≥6 consecutive days of extreme heat in the preconception window had 1.52 (95% CI 1.17, 1.97) times the odds (adjusted) of neural tube defects compared to women with no extreme heat exposure, while women with ≥6 consecutive days of extreme heat in the early pregnancy window had 1.37 (95% CI 1.03, 1.61) times the odds (adjusted) of neural tube defects compared to women with no extreme heat exposure.

When evaluating the association between extreme heat exposure and odds of anencephaly and spina bifida/meningomyelocele separately, the results were largely similar to those described above, with elevated odds of both outcomes observed with exposure to an increasing number of total and consecutive days of extreme heat. The one exception, however, was exposure to extreme heat in the early pregnancy window, where the association between extreme heat and NTDs was much stronger among spina bifida/meningomyelocele cases as compared to anencephaly cases. The adjusted odds ratio of spina bifida/meningomyelocele was 1.73 (1.18, 2.53) for women with 13+ days total days of extreme heat exposure and 1.58 (95% CI 1.10, 2.26) for women with ≥6 consecutive days of extreme heat exposure in early pregnancy as compared to women with no exposure to extreme heat in early pregnancy. The corresponding adjusted odds ratios for anencephaly were 1.06 (95% CI 0.70, 1.62) and 1.13 (95% CI 0.78, 1.64), respectively. When cases were restricted to only those that occurred between 1998 and 2017 (e.g. the post-folic acid fortification period), the results were very similar to the full analysis (**Table 3**).

Discussion

Our population-based case-control study, using individual-level heat exposure data, showed an increased odds of NTDs in the offspring with higher maternal exposure to extreme ambient heat during preconception and early pregnancy, which increased as the number of days exposed increased. The association was also significant for both NTD sub-types examined in our study, with a stronger association for spina bifida noted.

It is important to consider these results within the context of the two previous studies on this topic. Our results are consistent with the patterns found by Auger et al. (2016), who conducted a retrospective cohort study using the years 1988-2012 in 887,710 fetuses in Quebec, Canada (n=297 NTD cases) and found that exposure to extreme heat (defined as exposure to maximum weekly temperatures > 30°C, relative to 20°C) during week 4 of pregnancy was associated with 1.56 (95% CI 1.04, 2.35) times the prevalence of NTDs compared to mothers with no extreme heat exposure in this period.[11] However, our results are discordant with another study by Soim et al. (2017) which used data from the National Birth Defects Prevention Study in the US (n=326 NTDs).[9] Soim et al. focused on case and control pregnancies delivered between 1997-2007 that were conceived during the summer months and concluded that there was no association overall between extreme heat exposure and NTDs.[9] Of note, the researchers did find elevated, but not statistically significant, estimates by region, including the Southeast region, which contains Georgia. It is important to also note the different recruitment methods between our study and Soim et al.; while our study used records that were not prone to selection bias by election to participate, the National Birth Defects Prevention Study recruits subjects to participate, which may introduce selection bias. We believe that there are two key differences between our study and the Soim et al. study that likely explain our discrepant results: the exposure windows and the definition of extreme heat. While our 95th percentile apparent temperature was defined using the 30-year normal for each county, Soim et al used two similar iterations of maximum temperatures (90th and 95th percentile UATmax). However, they considered extreme temperature relative to the season, year, and temperature station location, allowing for season- and time-specific exposure thresholds. They also further restricted their analysis to only consider pregnancies with the critical exposure window occurring in the summer months. Compared to Soim et al., our definition of extreme heat days was perhaps more liberal, resulting in more days being classified as extreme, however it also allowed for consideration of extreme heat days across the entire year. It may be worthwhile to perform a sensitivity analysis with our study data restricting to cases and controls only with the exposure window occurring in the summer, as results may match more closely with those found by Soim et al. Second, while our current study considered a relatively large (8 week) exposure window, Soim et al. only focused on weeks 3 and 4 post conception. Another component that may influence results is geographic location. North America has various climates with dynamical change, and our current study focused on an area that was not a main focus by either Soim et al. or Auger et al. Given the geography and size of Georgia, it may be that focusing specifically on this state influenced our results, as well.

When exploring the potential mechanism(s) behind the associations between heat exposure and NTDs, it is important to consider the timeline of NTD development. Neurulation is the process in which the formation of the central nervous system in an embryo occurs and takes place between 17- and 28-days post-fertilization. While the precise timing and sequencing of NTDs remains up for debate, it is generally understood that one of the main processes in neurulation is the closing of the neural tube, and issues in this process may result in the formation of a NTD.[2] NTDs can be classified as open or closed, with open referring to an improper closing of the neural tube and exposure of neural tissue in primary neurulation, and closed resulting from a malformation after the neural tube is closed in secondary neurulation. Anencephaly is considered an open NTD, meningocele is a closed NTD, and there are both open and closed forms of spina bifida. This variation, as well as prior understanding of NTD timing, can help explain why our results showed an association over the entire exposure period. The emphasis on the later early pregnancy window being important for spina bifida cases may hint at the fact that it may be a closed NTD, occurring toward the later end of the neurulation period.

When comparing the strictly descriptive demographic characteristics of the mothers and their offspring in this particular study, the differences and similarities in distribution were to be expected. Firstly, research has shown that in the US, the Hispanic population have a higher prevalence of NTDs than any other ethnic group.[13] Our study showed a 3-4% increase in Hispanic-identifying mothers of cases over controls. Similarly, there was a higher (3-4%) proportion NTD cases with White mothers than other races, which is also consistent with previous literature. Due to this, it may be worthwhile to consider a sensitivity analysis allowing for effect modification by race and ethnicity to account for these previously known demographic differences. Additionally, there was a stark difference in pre-term births of cases over controls; approximately 15% more cases were born pre-term than controls. While this is alarming, it is again expected given previous research; it has been shown that babies born prematurely are five times more likely to have birth defects than babies born at term.[14] Another interesting difference was noted in calculated seasons of LMP. There were 3-6% more cases with a summer LMP, which also generally corresponds to neurulation in this season. This heightened prevalence of summer LMPs can connect to two previous studies on neural tube defects and seasonal heat (Fornoff et al., Obeidat et al.) which both showed a positive association between neurulation in warmers months and NTDs.[7, 8] While our analyses were adjusted for LMP month, it is interesting nonetheless to note these crude demographic differences that can reflect previous findings. Additionally, it is important to note that since this study did not include cases who miscarried or were electively terminated, their demographic characteristics are not reflected.

Something interesting we found was that our sensitivity analysis showed virtually no difference in association after the introduction of the mandatory policy on folic acid fortification in the US in 1998, which brings up the question of whether folic acid fortification has a large impact on heat related NTD incidence. Recent reports have shown that widespread fortification efforts have provided somewhere between a 25 and 50% decrease in new cases in the US and Canada.[15] Further, post-fortification prevalence of NTDs in the US remains at about 0.6 per 1000 births.[16] Given the realm of successful folic acid fortification policy in the US, it has become necessary to understand and address other potential causes of NTDs, as possible. An early but large cohort study in New England found that even after controlling for folic acid supplementation (the study was prior to 1998) and familial history, exposure to hot tubs, saunas, and fevers were associated with an increased risk of NTD development.[4] Our results are consistent with this previous finding and suggest that environmental heat related NTDs may be a part of this group of NTDs that are, in fact, folate resistant. For NTDs described as folate resistant, myo-inositol has been shown to have a promising protective effect, posing a potential new intervention method to further reduce NTD incidence.[4] Additional research on this topic is warranted.

It is important that any perinatal outcome research is considers how and why pregnant people may be a particularly vulnerable group when it comes to heat exposure. It is theorized that increased fat deposition and decreased body surface area to mass ratio (due to natural weight gain), cause a reduced ability to lose heat to the environment, thus altering physiologic thermoregulation and causing the person to instead hold onto it.[6] Overall, it is understood that hot ambient conditions and associated heat stress can increase adverse pregnancy outcomes; when looking at pregnant people, they are unique in that their exposures are transferred to their offspring, and this previous research has shown that some additional protections may be beneficial during pregnancy in order to reduce adverse outcomes.[17] Our study fits in this narrative, highlighting how imperative it is to understand these risks and protect them from exposures that may not or cannot inherently change, like extreme heat.

Strengths of our study were the large cohort size, with relatively robust and complete data. We were able to define a clear exposure window for every birth, then match county-level exposure data. This gave us the ability to use every possible case in our analysis. The few existing studies on this topic have all noted the importance of additional research on this topic, with the most recent noting the importance of precise timing of exposure and temperature measurement methods. [7, 8, 9, 10, 11] Additionally, congenital abnormalities, and particularly NTDs, have been labeled as an under evaluated risk of climate change.[18] Thus, a further strength of this study is being able to contribute to the existing literature and research on the association of extreme heat and NTDs, especially within the context of the state of Georgia.

Limitations of our study include using a 30-year window to compute the average normal temperature data on a county level, resulting in percentile markers that may not be reflective of each season. Summer months, for example, had prolonged periods of extreme heat, which may have changed if seasonally adjusted percentile thresholds were used. Additionally, this study included only live births and fetal deaths, which may have missed a proportion of fetuses with neural tube defects who miscarried or were electively terminated, hence findings are only generalizable to the pregnancy outcomes assessed. Further, though we calculated LMP for the purposes of this study, there may still be an issue with imprecise exposure timeline, given the small window of neurulation. Our expanded exposure window hoped to account for this, but there is always the possibility of measurement error. Additionally, it is known that there may be geographical movement from first to third trimester.[19] There could be misclassification of exposure, due to this and the fact that we do not know for certain where the mothers were geographically located during this specific time in pregnancy and relying on birth county as an estimate. Similarly, though county level temperature data is robust, counties can be quite large and county-level data is not as specific as individual-level data would be in this situation. Finally, due to the small number of exposed cases for some particular exposure definitions or demographic groups, some associations were not able to be explored, particularly associations allowing for effect modification by maternal race and ethnicity. Given the known disparities, these associations may be important to look at and consider in evaluating the effect of extreme heat on NTDs.

Conclusion

Our study demonstrated a moderate association between extreme heat exposure during the preconception and early pregnancy period and increased odds of NTDs in the state of Georgia for live and stillbirths recorded in the vital statistics databases from 1994-2017, capturing the folic acid post-fortification era. Further research considering more states, as well as other definitions of extreme heat, is needed to further explore this association. Given the urgency of climate change and nature of these results, it is imperative to continue evaluating the impacts of climate, particularly extreme heat, on human health. Focusing specifically on health outcomes through a maternal and child health lens lays the groundwork for a better understanding of how climate-related exposures, in this case being extreme heat exposure, may impact the health and resiliency of future generations, and what birth outcomes and potential birth defects may be particularly vulnerable to rising temperatures. This information can, in turn, inform policies and recommendations to ensure the health of future generations.

Table 1. Distribution of demographic characteristics between infants with any neural tube defect, anencephaly or spina bifida and matched controls in Georgia, USA (1994–2017).[1]

	Full Analysis		**Anencephaly Analysis**		**Spina Bifida Analysis**	
Characteristics	**All Neural Tube Defect Cases (N=673)**	**All Controls (N=2692)**	**Anencephaly Cases (N=343)**	**Controls (Matched 1:4 to cases) (N=1372)**	**Spina Bifida Cases (N=330)**	**Controls (Matched 1:4 to cases) (N=1320)**
Maternal Age, years						
<20	75 (11.1)	330 (12.3)	34 (9.9)	165 (12.0)	41 (12.4)	165 (12.5)
20-34	515 (76.5)	2,045 (76.0)	266 (77.6)	1040 (75.8)	249 (75.5)	1005 (76.1)
≥35	83 (12.3)	317 (11.7)	43 (12.5)	167 (12.2)	40 (12.1)	150 (11.4)
Maternal Race[2]						
White	467 (69.4)	1,769 (65.7)	234 (68.4)	896 (65.3)	233 (70.6)	873 (66.1)
Black	178 (26.4)	776 (28.8)	94 (27.5)	395 (28.8)	84 (25.5)	381 (28.9)
Other/Mixed	27 (4.2)	132 (5.0)	14 (4.1)	72 (5.2)	13 (3.9)	60 (4.5)
Maternal Ethnicity[2]						
Hispanic	117 (17.4)	370 (13.7)	63 (18.4)	210 (15.3)	54 (16.4)	160 (12.1)
Non-Hispanic	549 (81.6)	2,282 (84.8)	278 (81.0)	1139 (83.0)	271 (82.1)	1143 (86.6)
Maternal Education[2]						
<12 years	38 (5.6)	134 (5.0)	19 (5.5)	70 (5.1)	19 (5.8)	64 (4.8)
≥12 years	603 (89.6)	2,491 (92.5)	304 (88.6)	1267 (92.3)	299 (90.6)	1224 (92.7)
Maternal Marital Status[2]						
Married	435 (64.6)	1,639 (60.9)	219 (63.9)	831 (60.6)	216 (65.5)	808 (61.2)
Unmarried	238 (35.4)	1,051 (39.0)	124 (36.1)	541 (39.4)	114 (34.5)	510 (38.6)
Parity						
0	250 (37.1)	1,123 (41.7)	116 (33.8)	541 (39.4)	134 (40.6)	582 (44.1)
1	226 (33.6)	833 (30.9)	119 (34.7)	447 (32.6)	107 (32.4)	386 (29.2)
≥2	197 (29.3)	736 (27.4)	108 (31.5)	384 (28.0)	89 (27.0)	352 (26.7)
Tobacco Use During Pregnancy[2]						
No	618 (91.8)	2,463 (91.5)	323 (94.2)	1262 (92.0)	295 (89.4)	1201 (91.0)
Yes	53 (7.9)	202 (7.5)	20 (5.8)	96 (7.0)	33 (9.7)	106 (8.0)

Alcohol Use During Pregnancy[2]						
No	552 (82.0)	1,925 (71.5)	295 (86.0)	997 (72.7)	257 (77.9)	928 (70.3)
Yes	7 (1.0)	13 (0.5)	5 (1.5)	7 (0.5)	2 (0.6)	6 (0.5)
Birth Year						
1994-1997	128 (19.0)	512 (19.0)	76 (22.2)	304 (22.2)	52 (15.8)	208 (15.8)
1998-2017	545 (81.0)	2,180 (81.0)	267 (77.8)	1068 (77.8)	278 (84.2)	1112 (84.2)
Season of LMP[3]						
Winter (Dec, Jan, Feb)	162 (24.1)	691 (25.7)	81 (23.6)	335 (24.4)	81 (24.6)	356 (27.0)
Spring (Mar, Apr, May)	157 (23.3)	670 (24.9)	80 (23.3)	349 (25.5)	77 (23.3)	321 (24.3)
Summer (Jun, Jul, Aug)	193 (28.7)	635 (23.6)	102 (29.7)	327 (23.8)	91 (27.6)	308 (23.3)
Fall (Sep, Oct, Nov)	161 (23.9)	696 (25.8)	80 (23.3)	361 (26.3)	81 (24.5)	335 (25.4)
Gestational age[4]						
Preterm	147 (21.8)	214 (8.0)	76 (22.2)	109 (7.9)	71 (21.5)	105 (8.0)
Term birth	526 (78.2)	2,478 (92.0)	267 (77.8)	1263 (92.1)	259 (78.5)	1215 (92.0)
Medical Insurance						
Medicaid	221 (32.8)	1,062 (39.5)	115 (33.5)	536 (39.1)	106 (32.1)	526 (39.8)
Private	59 (8.8)	372 (13.8)	16 (4.7)	177 (12.9)	43 (13.0)	195 (14.8)
Other/Unknown	393 (58.4)	1,258 (46.7)	212 (61.8)	659 (48.0)	181 (54.9)	599 (45.4)

[1] Spina Bifida diagnosis includes either Spina Bifida or Meningomyelocele

[2] These variables had missing values, thus totals may not add to 100%

[3] LMP refers to Last Menstrual Period

[4] Term is defined as 37 weeks for this study

Table 2. Association of exposure to extreme heat of varying durations during preconception and post conception to risk of neural tube defects (combined and by specific type) in Georgia, USA (1994-2017).[1]

	All Neural Tube Defects (n=673 cases, 2692 controls)		**Anencephaly Only (n=343 cases, 1372 [1:4 matched] controls)**		**Spina Bifida Only (n=330 cases, 1320 [1:4 matched] controls)**	
	N of Exposed Cases/Controls	**aOR[1] (95% CI)**	**N of Exposed Cases/Controls**	**aOR[1] (95% CI)**	**N of Exposed Cases/Controls**	**aOR[1] (95% CI)**
Periconception (-2 to 6 weeks post-LMP)						
Total days >95th T_{app}						
0	448/1890	1.0 (REF)	222/960	1.0 (REF)	226/930	1.0 (REF)
1-4	60/271	1.12 (1.04, 1.21)	37/137	1.10 (0.99, 1.23)	23/134	1.13 (1.01, 1.26)
5-12	71/252	1.25 (1.07, 1.46)	42/128	1.22 (0.98, 1.52)	29/124	1.27 (1.02, 1.58)
≥13	94/279	1.40 (1.11, 1.77)	42/147	1.35 (0.96, 1.88)	52/132	1.43 (1.02, 1.98)
Total Consecutive Days >95th T_{app}						
0	448/1890	1.0 (REF)	222/960	1.0 (REF)	226/930	1.0 (REF)
1-2	52/230	1.11 (1.03, 1.19)	36/118	1.10 (0.99, 1.22)	17/113	1.11 (1.00, 1.23)
3-5	66/246	1.23 (1.06, 1.43)	35/126	1.21 (0.97, 1.50)	31/118	1.23 (0.99, 1.51)
≥6	107/326	1.36 (1.09, 1.70)	50/168	1.33 (0.96, 1.83)	56/159	1.36 (0.99, 1.86)
≥2 consecutive days >95th T_{app}						
No	473/2037	1.0 (REF)	239/1037	1.0 (REF)	234/1001	1.0 (REF)
Yes	200/655	1.32 (1.10, 1.59)	104/335	1.34 (1.03, 1.76)	96/319	1.29 (0.99, 1.67)
≥3 consecutive days >95th T_{app}						
No	500/2120	1.0 (REF)	258/1078	1.0 (REF)	243/1043	1.0 (REF)
Yes	173/572	1.30 (1.07, 1.58)	85/294	1.24 (0.93, 1.65)	87/277	1.35 (1.02, 1.77)
Preconception (-2 to 2 weeks post-LMP)						

Total days >95th percentile T_{app}						
0	492/2100	1.0 (REF)	245/1064	1.0 (REF)	247/1036	1.0 (REF)
1-4	77/265	1.16 (1.06, 1.28)	47/145	1.15 (1.00, 1.32)	30/120	1.16 (1.01, 1.33)
5-12	60/203	1.36 (1.12, 1.65)	33/199	1.33 (1.00, 1.75)	27/104	1.35 (1.03, 1.77)
≥13	44/124	1.58 (1.18, 2.11)	18/64	1.53 (1.01, 2.32)	26/60	1.56 (1.04, 2.35)
Total Consecutive Days >95th T_{app}						
0	492/2100	1.0 (REF)	245/1064	1.0 (REF)	247/1036	1.0 (REF)
1-2	63/213	1.15 (1.05, 1.25)	41/122	1.15 (1.01, 1.30)	22/92	1.15 (1.01, 1.30)
3-5	50/195	1.32 (1.11, 1.57)	23/86	1.32 (1.02, 1.69)	26/109	1.31 (1.03, 1.68)
≥6	68/184	1.52 (1.17, 1.97)	34/100	1.51 (1.04, 2.20)	35/83	1.51 (1.04, 2.18)
≥2 consecutive days >95th T_{app}						
No	527/2229	1.0 (REF)	269/1138	1.0 (REF)	258/1092	1.0 (REF)
Yes	146/463	1.33 (1.08, 1.64)	74/234	1.29 (0.95, 1.73)	72/228	1.34 (0.99, 1.79)
≥3 consecutive days >95th T_{app}						
No	555/2313	1.0 (REF)	286/1186	1.0 (REF)	269/1128	1.0 (REF)
Yes	118/379	1.33 (1.06, 1.66)	57/186	1.29 (0.93, 1.80)	61/192	1.34 (0.98, 1.83)
Post-Conception (3 to 6 weeks post-LMP)						
Total days >95th percentile T_{app}						
0	500/2100	1.0 (REF)	257/1067	1.0 (REF)	243/1033	1.0 (REF)
1-4	65/233	1.12 (1.02, 1.23)	39/114	1.02 (0.89, 1.17)	26/119	1.20 (1.06, 1.36)
5-12	65/219	1.24 (1.03, 1.50)	34/120	1.04 (0.79, 1.38)	31/99	1.44 (1.11, 1.86)

≥13	43/140	1.39 (1.05, 1.84)	13/71	1.06 (0.70, 1.62)	30/69	1.73 (1.18, 2.53)
Total Consecutive Days >95th T_{app}						
0	500/2100	1.0 (REF)	257/1067	1.0 (REF)	243/1033	1.0 (REF)
1-2	52/192	1.10 (1.01, 1.20)	30/94	1.04 (0.92, 1.18)	20/99	1.16 (1.03, 1.31)
3-5	59/180	1.21 (1.02, 1.44)	30/97	1.09 (0.85, 1.39)	31/83	1.35 (1.07, 1.72)
≥6	62/220	1.33 (1.03, 1.72)	26/114	1.13 (0.78, 1.64)	36/105	1.58 (1.10, 2.26)
≥2 consecutive days >95th T_{app}						
No	521/2216	1.0 (REF)	269/1122	1.0 (REF)	252/1094	1.0 (REF)
Yes	152/476	1.37 (1.12, 1.69)	74/269	1.24 (0.93, 1.67)	78/226	1.51 (1.13, 2.02)
≥3 consecutive days >95th T_{app}						
No	552/2292	1.0 (REF)	287/1161	1.0 (REF)	263/1132	1.0 (REF)
Yes	121/400	1.28 (1.03, 1.61)	56/211	1.10 (0.79, 1.52)	67/188	1.57 (1.15, 2.13)

[1] All models adjusted for maternal age and last menstrual period month and year. Cases were matched 1:4 to controls based on pre/post folic acid fortification time window (1994-1997 or 1998-2017) and county. T_{app} signifies apparent temperature.

Table 3. Association of exposure to extreme heat of varying durations during preconception and post conception to neural tube defects (combined and by specific type) in Georgia, USA solely following the mandatory folic acid fortification policy of staple grain products (1998-2017).[1]

	All Neural Tube Defects (n=545 cases, 2180 controls)		**Anencephaly Only (n=267 cases, 1068 [1:4 matched] controls)**		**Spina Bifida Only (n=278 cases, 1112 [1:4 matched] controls)**	
	N of Exposed Cases/Controls	**aOR[1] (95% CI)**	**N of Exposed Cases/Controls**	**aOR[1] (95% CI)**	**N of Exposed Cases/Controls**	**aOR[1] (95% CI)**
Periconception (-2 to 6 weeks post-LMP)						
Total days >95th T_{app}						
0	354/1494	1.0 (REF)	168/723	1.0 (REF)	186/771	1.0 (REF)
1-4	50/231	1.12 (1.03, 1.22)	31/114	1.09 (0.96, 1.23)	19/117	1.15 (1.02, 1.29)
5-12	61/218	1.26 (1.06, 1.50)	36/108	1.18 (0.92, 1.52)	25/110	1.32 (1.04, 1.67)
≥13	80/237	1.42 (1.10, 1.83)	32/123	1.28 (0.88, 1.87)	48/114	1.52 (1.07, 2.15)
Total Consecutive Days >95th T_{app}						
0	354/1494	1.0 (REF)	168/723	1.0 (REF)	186/771	1.0 (REF)
1-2	45/197	1.11 (1.02, 1.20)	31/97	1.08 (0.96, 1.22)	14/100	1.12 (1.00, 1.25)
3-5	57/209	1.22 (1.04, 1.44)	29/109	1.17 (0.92, 1.49)	28/100	1.26 (1.00, 1.57)
≥6	89/280	1.35 (1.06, 1.73)	39/139	1.26 (0.88, 1.82)	50/141	1.41 (1.01, 1.98)
≥2 consecutive days >95th T_{app}						
No	374/1616	1.0 (REF)	182/784	1.0 (REF)	192/833	1.0 (REF)
Yes	171/564	1.32 (1.08, 1.62)	85/284	1.29 (0.96, 1.74)	86/279	1.34 (1.01. 1.77)
≥3 consecutive days >95th T_{app}						
No	399/1691	1.0 (REF)	199/820	1.0 (REF)	200/871	1.0 (REF)

Yes	146/489	1.29 (1.04, 1.60)	68/248	1.17 (0.85, 1.61)	78/241	1.41 (1.05, 1.88)
Preconception (-2 to 2 weeks post-LMP)						
Total days >95th percentile T_{app}						
0	392/1671	1.0 (REF)	187/806	1.0 (REF)	205/865	1.0 (REF)
1-4	69/230	1.15 (1.04, 1.28)	42/126	1.11 (0.95, 1.31)	27/104	1.17 (1.02, 1.35)
5-12	47/175	1.33 (1.08, 1.65)	25/86	1.24 (0.90, 1.71)	22/89	1.38 (1.03, 1.84)
≥13	37/104	1.54 (1.12, 2.12)	13/50	1.39 (0.86, 2.24)	24/54	1.61 (1.05, 2.49)
Total Consecutive Days >95th T_{app}						
0	392/1671	1.0 (REF)	187/806	1.0 (REF)	205/865	1.0 (REF)
1-2	55/185	1.14 (1.04, 1.26)	35/107	1.12 (0.97, 1.29)	20/78	1.15 (1.01, 1.31)
3-5	42/168	1.30 (1.07, 1.58)	20/74	1.25 (0.94, 1.66)	22/95	1.32 (1.02, 1.72)
≥6	56/156	1.48 (1.11, 1.98)	25/81	1.40 (0.92, 2.14)	31/74	1.52 (1.02, 2.26)
≥2 consecutive days >95th T_{app}						
No	422/1780	1.0 (REF)	207/869	1.0 (REF)	215/912	1.0 (REF)
Yes	123/400	1.29 (1.03, 1.62)	60/199	1.21 (0.87, 1.68)	63/200	1.34 (0.98, 1.84)
≥3 consecutive days >95th T_{app}						
No	447/1856	1.0 (REF)	222/913	1.0 (REF)	225/943	1.0 (REF)
Yes	98/324	1.29 (1.01, 1.65)	45/155	1.22 (0.84, 1.76)	53/169	1.33 (0.95, 1.86)
Post-Conception (3 to 6 weeks post-LMP)						
Total days >95th percentile T_{app}						
0	394/1672	1.0 (REF)	195/811	1.0 (REF)	199/861	1.0 (REF)

1-4	54/204	1.15 (1.04, 1.27)	33/96	1.01 (0.87, 1.18)	21/108	1.26 (1.10, 1.44)
5-12	57/180	1.31 (1.07, 1.61)	29/96	1.03 (0.75, 1.40)	28/84	1.59 (1.22, 2.08)
≥13	40/124	1.51 (1.11, 2.04)	10/65	1.04 (0.66, 1.66)	30/59	2.01 (1.34, 3.00)
Total Consecutive Days >95th T_{app}						
0	394/1672	1.0 (REF)	195/811	1.0 (REF)	199/861	1.0 (REF)
1-2	43/168	1.13 (1.03, 1.24)	27/78	1.03 (0.90, 1.19)	16/90	1.21 (1.07, 1.37)
3-5	54/155	1.27 (1.05, 1.53)	25/85	1.07 (0.81, 1.42)	29/70	1.46 (1.14, 1.88)
≥6	54/185	1.43 (1.08, 1.89)	20/94	1.11 (0.73, 1.68)	34/91	1.77 (1.21, 2.59)
≥2 consecutive days >95th T_{app}						
No	411/1771	1.0 (REF)	205/855	1.0 (REF)	206/916	1.0 (REF)
Yes	134/409	1.44 (1.15, 1.80)	62/213	1.24 (0.90, 1.72)	72/196	1.65 (1.21, 2.25)
≥3 consecutive days >95th T_{app}						
No	437/1840	1.0 (REF)	222/889	1.0 (REF)	215/951	1.0 (REF)
Yes	108/340	1.38 (1.08, 1.75)	45/179	1.04 (0.72, 1.50)	63/161	1.77 (1.27, 2.45)

[1] All models adjusted for maternal age and last menstrual period month and year. Cases were matched 1:4 to controls based on pre/post folic acid fortification time window (1994-1997 or 1998-2017) and county. T_{app} signifies apparent temperature.

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