



# Neurotoxins during the Renaissance. Bioarcheology of Ferrante II of Aragon (1469–1496) and Isabella of Aragon (1470–1524)

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## ABSTRACT

We show that statistical modeling of analytical results is useful in providing insights into metabolism and disease in bioarcheology. Our results also imply that during the Renaissance in Europe widespread pollution of the biosphere with heavy metals such as mercury and lead affected the Italian nobility at that time. The activity of biologic clocks which control metabolism and autonomic nervous system (ANS) function can be gleaned from the analysis of hair. This provides a means of assessing the health of individuals who lived some six centuries before the present and allows the reconstruction of disease from archived tissues such as hair.

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## 1. Introduction

Heavy metal exposure in Medieval and Renaissance Europe was widespread in many geographic areas because of industrial pollution, dietary exposure and medicinal uses, especially of mercury and lead. The health consequences of this pollution, however, are not well understood (Rasmussen et al., 2008). We hypothesized that the exposure to toxic metals may have had effects on the metabolism of Renaissance individuals especially the nobility who historically had easier access to such neurotoxins.

Here we report analyses of hair samples from Ferdinando II of Aragon (Ferrante), 1469–1496, and Isabella of Aragon (Isabella), 1470–

1524 using hydrogen isotope ratios. The samples were stored at room temperature at the University of Pisa in the Department of Translational Research on New Technologies in Medicine and Surgery, until analysis.

The analyses interrogated 0.3 cm long adjacent and consecutive hair segments. The analyses were done from the root to the tip of the hairs. The orientation of the hair was assessed by microscopy. The hydrogen isotope measurements are described in Supplemental Text S1. The control hair samples were collected from contemporaneous Huancavelica, Peru, inhabitants. This is a mining town in the Andes where mercury has been mined for millennia (Lombardi et al., 2012).

Statistical modeling of our results allowed us to evaluate the role of mercury and lead in the health and early death of both nobles. Our results show that Isabella reached a tipping point (cessation of normal oscillation of her biologic rhythms) in her metabolisms which lead to death due to chronic mercury poisoning. Ferrante, on the other hand,

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had lead intoxication. However, his metabolism was not yet at the tipping point; his death, at a relatively young age, was most likely caused by numerous other ailments such as infections (Fornaciari et al., 2011).

Previous research showed that both individuals were exposed to heavy metals during life which may have been contributing factors to their death (Lanzirotti et al., 2014).

We hypothesized that analyses of their hair using analytical methods and statistical modeling of the results might reveal the importance of the neurotoxic loads in their health and eventual death.

Mercury and lead are especially injurious to the autonomic nervous system (ANS) and their toxicity is manifested by disturbances in metabolic rhythms (Appenzeller and Oribe, 1997). We found evidence for elevated levels of heavy metals in both nobles most likely accumulated in-vivo (Lanzirotti et al., 2014) and deranged biologic rhythms in these historical figures. We evaluated the effects of such toxins on their metabolism some six hundred years after their death. We speculate, using statistical modeling, how these neurotoxins may have compromised their metabolism and potentially accelerated their death at a relatively young age.

## 2. Materials and methods

The samples for this study were derived from autopsies performed on Ferrante and Isabella at the church of San Domenico Maggiore in Naples in 1984. The study was approved by the SOPRINTENDENZA PER I BENI ARTISTICI E STORICI DELLA CAMPANIA, Naples, Italy May 22, 1984 Protocol # 4800. IRB approval for the bioarcheological examination of the remains was obtained from the University of Pisa and for the additional studies performed in Albuquerque from the New Mexico Health Enhancement and Marathon Clinics Research Foundation in Albuquerque NM protocol #2011-18. The authors declare no conflicts of interest.

Samples of hair collected from Ferrante and Isabella were analyzed using hydrogen isotope analysis at the University of New Mexico's Department of Earth & Planetary Sciences using a Finnigan MAT Delta XL Plus mass spectrometer. The hair was removed at the time of the post-mortem examination and provided for analyses by Gino Fornaciari. Strands selected for analysis ranged from 7.5–10 cm in length and each analysis interrogated 0.3 cm long adjacent and consecutive hair segments. Hydrogen isotope ratios on each segment were determined using a continuous flow high temperature reduction technique following methods described in Appenzeller et al. (2007) and Appenzeller and Oribe (1997). Data are reported in conventional delta notation relative to SMOW and methodologies for calculating hair growth rates based upon the time series of hydrogen isotope ratio measurements are described in Supplemental Text S1.

## 3. Results

### 3.1. Hydrogen isotope analysis of hair and biologic rhythms

Hydrogen isotope ratios were measured at 3 mm intervals along the length of the hairs of both subjects to allow approximate determination of hair growth rates/year and biologic rhythms (Sharp et al., 2003). The growth rate of hair for Ferrante was 12 cm/year and for Isabella 2 cm/year (normal growth rates of human scalp hair ~16 cm/year;  $P < 0.001$  vs. controls). Metabolic rhythms were determined from power spectral analysis of the hydrogen isotope ratios along the length of the hairs and the rhythms were found to be grossly deranged. The low frequency power was significantly increased ( $P < 0.004$ ) and conversely, the high frequency power of the spectra was significantly decreased ( $P < 0.007$ ); the low frequency/high frequency ratio of the spectral peak was significantly increased ( $P < 0.04$ ) in both subjects. All variances were lower in our heavy metals exposed subjects; for high frequency power (F-test,  $P < 0.001$ ), for low frequency power (F-test  $P < 0.002$ ) and for low frequency/high frequency ratios (F-test  $P < 0.04$ ).

### 3.2. Clinical symptom scores

We utilized seven scoring points obtained from twelve data sets, including living controls exposed to heavy metal pollution in contemporary Huancavelica, Peru, for comparison (Lombardi et al., 2012). Scores were based on a binary system, either present or absent (100% or 0% respectively) or on the percentage deviation from normal such as the % shorter annual growth rate compared to average normal human scalp hair growth (16 cm/year). The clinical scoring from Isabella is consistent with more severe toxicity (lower scores) on ANS function when compared to Ferrante (Fig. 1). Additionally, this scoring highlight some symptoms present in our subjects that are not specific for metal toxicity such as edema, stomatitis or fever. Such symptoms or signs may also be prominent in diseases not related to metal intoxication.

### 3.3. Modeling biologic rhythms

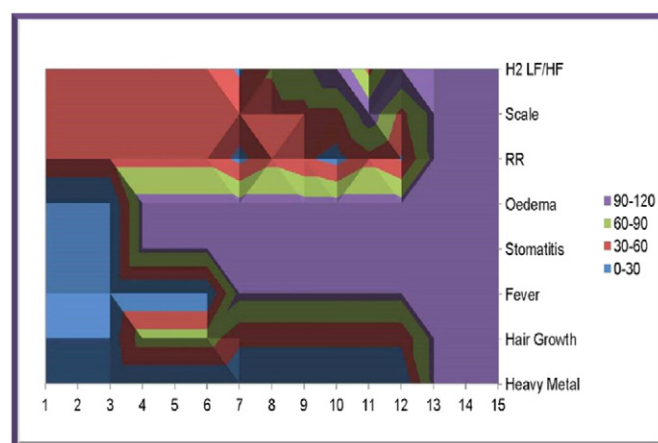
Isabella's hair showed a surprising lengthening of the quiescent period (telogen) to ~47 weeks. Ferrante's telogen was ~24 weeks (normal telogen ~13 weeks.)  $P = 0.001$ . (Fig. 2).

Modeling the lengthening quiescent periods shows that our data are about growth cycles but only when the hair is growing and not when the hair is in the quiescent period (telogen) Fig. 3.

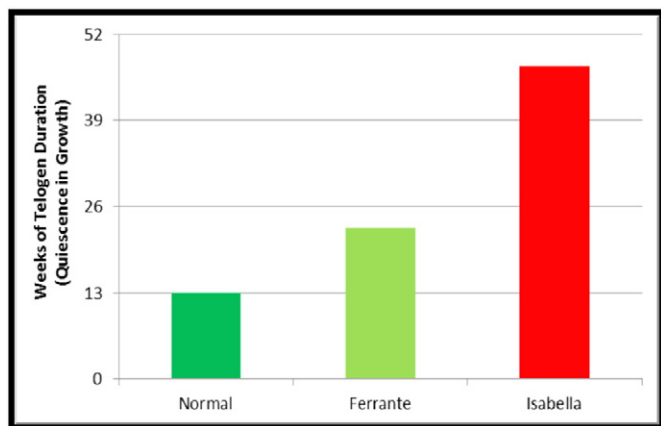
## 4. Discussion

The Anthropocene, the geological period we now live in, is characterized by ever increasing levels of neurotoxins in the biosphere resulting from human exploitation and use of natural resources. Effects of this contamination of the biosphere on contemporaneous human health, however, are not fully elucidated. During the Renaissance neurotoxins stressed human metabolism because of widespread use of mercury for medicinal purposes and lead to sweeten wine. Power plants and artisanal gold mining were not yet affecting the biosphere at that time. We hypothesized that Renaissance archeological material might provide lessons for contemporary human health.

Here we explore the effects of mercury and lead on human metabolism during the Renaissance by examining archived hair from that period. Bone and hair are considered the best archived tissues for analyses. Hair, if



**Fig. 1.** Contour plot representing the symptom or sign scores on the Y-axis and the individual data sets on the X-axis. The “critical transition” is where symptoms–signs are  $<50$  (blue). Note that symptoms such as fever and edema are non-specific; the signs — heavy metals (Pb and Hg) are present in both subjects (bottom blue–black line). Scores  $<50$  represent metabolically significant metal loads. Stomatitis is black patina on Isabella's teeth composed mainly of Hg; RR = heart rate variability spectra from our controls also exposed to heavy metals (Lombardi et al., 2012); scale = RI, repeat intervals computed hair growth rate/year; H2 = LF/HF ratios computed from hydrogen isotope ratios analyzed along the lengths of the hairs



**Fig. 2.** Weeks of telogen duration (quiescence in growth ~13 weeks/year) for normal hair growth (16 cm/year); Ferrante telogen was ~24 weeks/year (his hair grew 12 cm/year); Isabella telogen duration was ~47 weeks/year (her hair grew only ~2 cm/year).

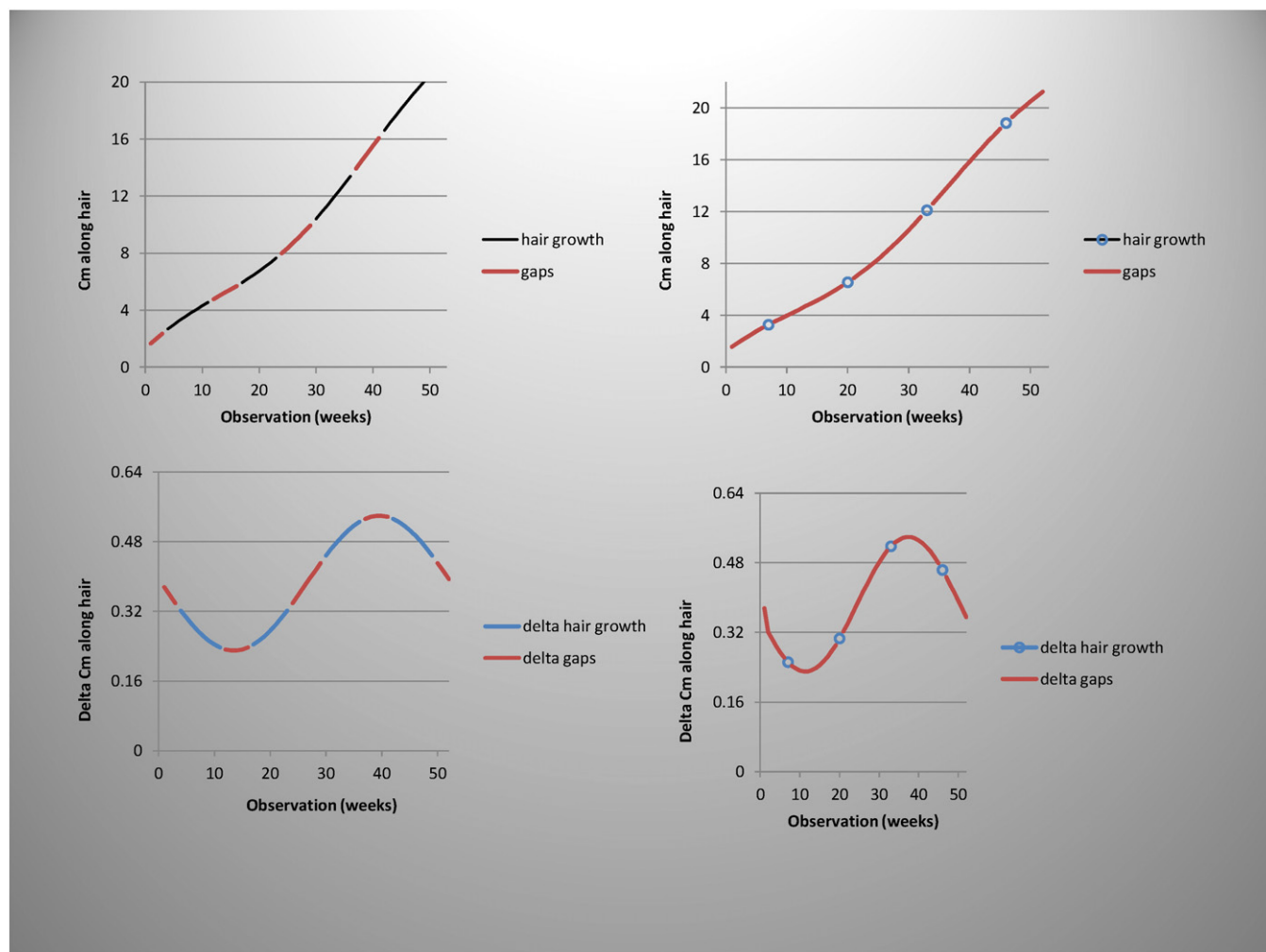
available, undergoes very little taphonomic deterioration especially when hydrogen isotope analyses are performed. Root banding, a microscopic change characterized by the appearance of a dark band near the root of

the hair has been reported (Koch et al., 2013) but this does not affect the analyses performed in this study.

The reconstruction of disease from ancient remains is fraught with difficulty and often based on unwarranted inferences. We show that modern analytical methods of archived hair of Ferrante II of Aragon, King of Naples (1469–1496) and Isabella of Aragon, Duchess of Milan (1470–1524), allowed us to infer that both nobles were exposed to toxic levels of heavy metals. This exposure resulted in abnormalities in their biologic rhythms and contributed to their early death.

Using a novel “symptom scoring” based on historical records and the results of our analyses we determined that Isabella had reached a “tipping point” in her metabolism leading to system failure induced by mercury and death. In contrast, Ferrante, exposed to toxic levels of lead, survived longer with significant symptoms of toxicity of the metal which in his case, however, had not yet reached a tipping point.

The understanding of the biology of hair, its growth cycles, biologic rhythms and metabolism is dependent on numerous interactions of a variety of systems with different dynamics which has been facilitated by mathematical modeling (Qualls and Appenzeller, 2015). Here we show that the application of modeling to Renaissance data can provide insights to life styles, environmental pollution and health of people who lived centuries ago.



**Fig. 3.** Modeling the growth cycles of Ferrante's and Isabella's hairs. Note that in Ferrante growth activity can clearly be discerned (black and blue lines) whereas in Isabella only gaps in growth (blue circles) are evident (red lines). This is consistent with her extremely short annual growth rate of only 2 cm implying impending death.



#### 4.1. Clinical histories

Both Ferrante II of Aragon (Ferrante) and Isabella died unexpectedly; Ferrante at 27 and Isabella aged 54. Ferrante's demise has been attributed to malaria; Isabella's death was associated with “dropsy”, a term no longer used. Dropsy implied an abnormal accumulation of fluid in tissues. Historically, both nobles also suffered from recurrent fevers (Lanzirotti et al., 2014; D'Errico et al., 1988).

#### 4.2. Evidence for heavy metal exposure

The evidence for mercury toxicity in Isabella and lead toxicity in Ferrante has been extensively reviewed using modern techniques for studying archived bones and other tissues found in their sarcophagi (Lanzirotti et al., 2014) both toxins affect peripheral nerves and the autonomic nervous system (ANS).

#### 4.3. Critical transitions (tipping points)

To anticipate “critical transitions” (Scheffer et al., 2012) in metabolism, that is, the point at which toxicity of heavy metals might lead to irreversible collapse of an individual's metabolism as evidenced by shortened annual hair growth and other abnormalities in ANS function, we used a clinical scoring system based on symptoms, signs and results of the numerous analyses carried out on Isabella, Ferrante and controls (for extensive details see Supplemental Text S1). We had seven available scoring points and twelve data sets for comparison. Our scores were based on a binary system, either present or absent (100% or 0% respectively) or on the percentage deviation from normal hair growth (16 cm/year). This scoring clearly distinguished between Isabella, showing more severe toxicity (lower scores), compared to Ferrante (Fig. 1). Additionally, this scoring highlighted some symptoms that had insignificant effects on metabolism scoring such as edema, stomatitis or fever which, however, may have many different etiologies, but are non-specific for heavy metal toxicity (see Supplemental Text S1). Taken together, the evidence points to the conclusion that the biologic rhythms derived from hair growth, and by extension ANS function, were abnormal in both Renaissance Italians.

Our contemporaneous controls were chosen because they live in heavily mercury polluted environments. They were clinically normal because they have partially adapted to the mercury leaking into the biosphere from the mine that has been in operation since pre-Hispanic times. Their annual hair growth rate was nevertheless markedly reduced (Lombardi et al., 2012).

When modeling such systems, a slow return to equilibrium, that is the growing phase of the hair and decreased variance of hair growth, is thought to predict the point of no return to baseline (the tipping point) (Scheffer et al., 2012). Using the longer intervals in resumption of hair growth and decreased variance as derived from hydrogen isotope ratios along the length of Isabella's hair we could deduce that she was near death (complete failure of metabolism; telogen 47 weeks; hair growth 2 cm/year) compared to Ferrante (telogen 24 weeks; hair growth 12 cm/year; Fig. 2); the tipping point was reached by Isabella but not quite by Ferrante.

Heavy metals such as lead and mercury are known to disrupt ANS function and thus in turn affect metabolism and growth (Appenzeller and Oribe, 1997). ANS disruption of biological cycles perturbs also the rates of cellular proliferation and, of course, matrix synthesis. This was the eventual result of heavy metal toxicity and the ultimate cause of Isabella's death, as tissue maintenance was no longer prioritized and new cells were not replacing old.

Evidence provided previously shows that Isabella had mercurial stomatitis due to excretion of mercury in her saliva and mercury within her hair shaft (D'Errico et al., 1988). Both would indicate metabolic absorption of mercury that could account for the modeled disturbances in biological rhythms (Fig. 4).



**Fig. 4.** Isabella's stomatitis—the black patina on the surface of her teeth (green arrows). Note this proved to be composed almost entirely of Hg.

#### 4.4. Disturbances in biological rhythms

Biologic clocks control biologic rhythms; they are pervasive throughout biology. They are present in all tissues and function to couple metabolism and growth to the rotation of the earth around the sun and to other periodic signals. Biologic rhythms are oscillators driven by changes in gene expression (Carpenter et al., 2011; Bass, 2012). The cyclical changes in gene expression in the central nervous system are transmitted to peripheral tissues by the autonomic nervous system (ANS) (Appenzeller et al., 2007). These cyclic changes couple physiologic changes to metabolism, to behavior and to other cycling such as reproduction and tissue regeneration. Heavy metals such as lead and mercury disrupt ANS function and thus in turn affect metabolism (Appenzeller and Oribe, 1997).

Absorption of environmental toxins is ubiquitous and increasing, especially due to the use of fossil fuels which add both mercury and lead to the biosphere. But as we show here in pre-industrial societies such as Renaissance Italy, the affluent were particularly vulnerable because of affordability of dietary and pharmaceutical sources of heavy metals and other toxins.

Rhythmic changes in gene expressions are linked to metabolic changes which occur at varying time intervals ranging from 24 h (circadian) to weekly (circaseptan) and monthly and even longer intervals. Such ubiquitous rhythmic time changes in biology are termed biologic rhythms.

The clinical symptom scoring presented here is consistent with grossly abnormal biologic rhythms, hair growth/year, and by extension ANS function, in both individuals.

To further define the “critical transition” we also modeled the intervals between the spurts of hair growth and the dormant stages in which the hair does not grow (telogen) based on the hydrogen isotopic composition measured at intervals along the length of the hair for each individual. The power spectra derived from the hydrogen isotope ratios of both nobles allowed us to determine growth rates in cm/year (see

Supplemental Text S1). We found that the intervals between growth resumption and quiescence were 47 weeks for Isabella and 24 weeks for Ferrante indicating a longer interval for Isabella's metabolism to return to an active phase, in keeping with the shortened annual hair growth (2 cm/year compared to Ferrante's 12 cm/year). This indicates that the stable state of the system took longer to recover after perturbation and initiate growth once more in Isabella, potentially a sign of impending irreversible failure of her metabolism and death.

## 5. Conclusions

Previous work (Lanzirotti et al., 2014) and data presented here demonstrate that remains from Ferrante and Isabella contained high amounts of heavy metals (mercury and lead). Our earlier observations are also consistent with metabolic absorption of mercury in Isabella and lead in Ferrante. Both toxins are injurious to the ANS and affect its ability to control metabolic activity.

The power spectra derived from the hydrogen isotope ratios in the hairs of both nobles allowed us to determine hair growth rates in cm/year (see Supplemental Text S1) and to model potential impacts of the neurotoxins on the ANS function.

What can be learned from paleo-autonomic neurology of fifteen's century Italian nobility? We suggest that the increasing mercury and lead pollution expected in the Anthropocene, the human-made world we now live in, may soon have parallels to the inescapable heavy metal pollution of Renaissance Europe. The effects of this toxic load on present day behavior, physiologic rhythms and other biologic cycles are not as yet predictable. However, transgenerational tolerance to mercury has been found in some heavily polluted locations in South America (Lombardi et al., 2012) and this might mitigate, in part, the expected ravages on the biology of eventual survivors of the Anthropocene.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jasrep.2016.01.013>.

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