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Intra-population variation of hair and fingernail stable hydrogen, oxygen, carbon and nitrogen isotopes in London, Ontario, Canada residents during the COVID-19 pandemic

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Abstract

Lockdowns and travel restrictions during the COVID-19 pandemic forced a significant fraction of London, Ontario, Canada residents to remain in one location for long enough to reach isotopic equilibrium with their primary drinking water source(s). This situation created ideal natural conditions for measuring the isotopic fractionation between the stable hydrogen and oxygen isotopes of drinking water and hair or nail tissues, and for determining the magnitude of intra-population variation in tissue $\delta^{2}H$ and $\delta^{18}O$. Hair and nail of participants who reported exclusively drinking London municipal tap water spanned much larger $\delta^{2}H$ and $\delta^{18}O$ ranges than their drinking water. Forensic $\delta^{2}H_{drinking\ water}$ and $\delta^{18}O_{drinking\ water}$ reconstructions presuming a constant tissue-drinking water isotopic fractionation therefore carry high uncertainty. London and southern Ontario values of $\delta^{13}C_{hair}$ and $\delta^{15}N_{hair}$ for omnivores vary by < 1 ‰ between COVID-19 and pre-COVID-19 times, suggesting that the overall dietary habits of Londoners were not affected by the pandemic.

Keywords

Stable isotopes, hair, nails, drinking water, diet, COVID-19, pandemic, London, Ontario, Canada, southern Ontario, forensic

Summary for Lay Audience

Stable isotopes are atoms of the same element that differ in their number of neutrons. Stable isotopes of hydrogen and oxygen are of particular interest to archeological and forensic investigations because they reflect the isotopic compositions of drinking water, which in turn typically reflect the isotopic compositions of local fresh water. Stable isotopes in fresh water vary predictably with geography. Hence, it is hoped that stable isotope compositions of drinking water incorporated into hair and nails can be used to reconstruct the region of origin of unknown hair and nail tissues.

Travel restrictions and lockdowns during the COVID-19 pandemic forced residents of London, Ontario, Canada – who consume municipal tap water of near constant isotopic composition – to remain in their local area long enough for the hydrogen and oxygen isotopes within their hair and nails to reflect local tap water. This created a unique opportunity to study the relationship between the stable hydrogen and oxygen isotope compositions of drinking water and hair and nail tissues grown during this period. This study found, however, that the isotopic range London municipal tap water was much smaller than the isotopic range of hair and nail of London residents consuming this water. This finding thus limits the utility of hair and nail isotopic compositions as a tracer of geographic origin in archeological and forensic investigations.

The stable carbon and nitrogen isotope compositions of hair and nails reflect one's dietary habits. The small range of carbon and nitrogen isotope compositions measured for hair and nails in London residents indicate consumption of diets with generally similar isotopic compositions. Moreover, the stable carbon and nitrogen isotope compositions of hair from London and southern Ontario omnivores collected prior to the start of the COVID-19 pandemic did not differ significantly from those collected in the present study. These similarities suggest the dietary composition of London residents has not changed significantly since the start of the pandemic.

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Chapter 1

1.0 Introduction

Increased human mobility and the emergence of globalized food production and distribution systems have presented challenges to forensic and archeological workers attempting to establish the provenance of unknown persons or reconstruct migration history and diet. The significant contribution of multiple isotopically distinct drinking waters and nonlocal food items has created a complex web of stable isotope inputs that in turn influence the stable isotope composition of bodily tissues (e.g., Chesson et al., 2010a, 2010b, 2010c, 2018; Ehleringer et al., 2008; Huelsemann et al., 2009; O'Brien & Wooller, 2007; O'Connell & Hedges, 1999). The complexity of these relationships has necessitated assumptions to be made regarding the dietary composition and residency of anonymously sampled participants or reliance on diet and beverage information in self-reported surveys to be built into any study design (e.g., Ehleringer et al., 2008; Fraser & Meier-Augenstein, 2007; Hedrick et al., 2016; Mancuso & Ehleringer, 2019; Schoeller, 1990; Schoeller et al., 1995; Thompson et al., 2010).

The COVID-19 pandemic has created a unique opportunity to improve our understanding of the relationships between the stable isotope compositions of drinking water and dietary inputs, and tissues such as hair and nails. Previous studies reported stable hydrogen and oxygen isotopes in human urine reflected local drinking water within 30 days of traveling to a new location, indicating a 90 % turnover of body water within this time (Cerling et al., 2007; Katzenburg & Krouse, 1989; O'Brien & Wooller, 2007). Shifts of hair stable isotope compositions lag shifts in body water by 5-7 days, as the hair strand must grow from the follicle to the surface of the skin before it can be sampled (O'Brien & Wooller, 2007). Fingernails of non-local travelers showed stable isotopes compositions that were indistinguishable from local residents within 90 days of arrival (Mancuso & Ehleringer, 2019).

Travel restrictions and lockdown orders imposed during the COVID-19 pandemic have forced significant portions of the population to remain in one location for long enough that the stable isotope composition of their hair and nails is expected to be in isotopic equilibrium with local drinking water and dietary supplies. This has created more ideal natural conditions under which the hydrogen and oxygen stable isotope variability in hair and nails of London, Ontario, Canada residents can be measured. As such, obtaining insight into the stable hydrogen and oxygen isotope compositions of hair and nails formed by individuals consuming a drinking water source with a known isotopic composition, and the degree of intra-population isotopic variability in hair and nails of London, Ontario, Canada residents is a primary objective of the present study. Measuring stable carbon and nitrogen isotopes in hair and nail is useful to determine if there have been substantial changes in the dietary habits of London, Ontario, Canada residents since the start of the COVID-19 pandemic by comparing values obtained in this study with published pre-COVID-19 values for hair. Dietary information provided by stable carbon and nitrogen isotopes can offer insight into the robustness and adaptability of food production and distribution networks in response to supply chain disruptions and the importance of local and non-locally produced foods in Canadian diets.

1.1 Background

For decades, stable isotopes in tissues have been used as a proxy for the dietary and migratory habits of animals and humans, both modern (Chesson et al., 2010b, 2018; Mancuso & Ehleringer, 2019; Meier-Augenstein & Fraser, 2008; Kelly, 2000; Nardoto et al., 2006, 2011, 2020; Rubenstein & Hobson, 2004) and ancient (Dupras & Schwarcz, 2001; Fauvelle & Somerville, 2021; Mabee, 2019; Plint et al., 2019; Schoeninger & Moore, 1992; Sharp et al., 2003; White et al., 2004). This has made stable isotopes a powerful tool for archeologists and forensic investigators. This is particularly true in situations where large-scale natural disasters with many casualties, or mutilation and decomposition of remains render

more traditional methods of forensic identification, such as fingerprints, dental records, or DNA impractical or impossible (de Boer et al., 2020). The use of stable isotopes to reconstruct diet and migration history is possible thanks to an understanding of the controls on the isotopic composition of drinking water and foods, and the relationship between the isotopic composition ingested food and drink, and that of tissues (Caut et al., 2009; Chesson et al., 2018; Daux et al., 2008; Ehleringer et al., 2008; Huelsemann et al., 2009; Lamb et al., 2014; Lehn et al., 2015; Longinelli, 1984; Luz & Kolodny, 1985; Macko et al., 1986; O'Connell & Hedges, 1999).

The Industrial Revolution saw the rise of a middle class, who – with newfound discretionary income and leisure time in which to spend it – powered technological innovation in the field of transportation. The development of steam engines created an obvious opportunity for mechanised propulsion systems, enabling increased human mobility, as well as economic growth through trade, and tourism. The globe promptly found its farthest reaches tied together like never before by transportation systems accessible to more people than ever before, such as railway networks and global shipping lanes, and by the early twentieth century, air travel (Gee & Fayos-Solá, 1997; Glaesser et al., 2017).

With improved access to transportation, however, has come increased complexity and challenges in reconstructing the migration history and diets of modern humans using stable isotopes. The ease and rapidity with which humans may now travel between multiple regions with isotopically distinct drinking water signatures and obtain beverages such as juices or sodas with non-local origins can confound attempts to reconstruct migration history (Bol et al., 2007; Chesson et al., 2010a, 2010c; Fraser & Kalin, 2006; Lamb et al., 2014). Additionally, the wide selection of foods with international origins available to consumers homogenizes stable isotope compositions within food items and results in a reduction of isotopic variation between localities, limiting the ability to determine the provenance of individuals based on dietary composition (Bol et al., 2007; Chesson et al., 2008, 2010b; Lee et al., 2021; Nardoto et al., 2006; Rodrigues et al., 2016). The following sections detail the stable isotopes

measured in this study, the information they provide on the dietary and travel history of study participants, and the expected changes in the stable isotope ratios of local resident's tissues due to COVID-19 related dietary changes.

1.2 Stable Isotopes

Isotopes are atoms of a given element, with all isotopes of an element containing the same number of protons and electrons, but differing in the number of neutrons, allowing them to be separated into "heavy" and "light" isotopes (e.g., protium vs deuterium). Stable isotopes are those which do not undergo measurable radioactive decay into different elements. All isotopes of a given element share the same chemical properties; however, their slight differences in mass results in some differing physical properties. Heavier isotopes react more slowly than lighter ones as they form slightly stronger bonds. Differing reaction rates tend to result in heavy or light isotopes becoming concentrated in a product or a reactant during chemical reactions, a process known as fractionation (Sharp, 2017).

Isotopes are denoted using the shorthand notation ^{Z}X, where X represents the element of interest and Z indicates the mass number of a particular isotope (e.g., ^{2}H). The ratio of heavy to light isotopes within a material is commonly denoted using delta (δ) notation, where the value of δ is given by the following equation:

$$\delta = (R_{sample}/R_{standard} - 1) \quad 1.1$$

where R represents the ratio of heavy to light isotopes of an element of interest (e.g., $^{2}H/^{1}H$), and ratios are expressed in parts per thousand, or per mil (‰) (Coplen, 2011). Sample ratios are compared against an internationally recognized standard of known isotopic composition to determine the level of isotopic enrichment or depletion in a sample relative to that standard. Samples depleted of the heavy isotope

relative to the standard material have negative δ values while samples enriched in the heavy isotope have positive δ values.

The magnitude of isotopic discrimination between two materials or phases, such as between the liquid (e.g., A) and vapor phase (e.g., B) of water, is controlled by the fractionation factor, '$\alpha_{A\text{-}B}$'. Taking the logarithm of the fractionation factor ($\ln\alpha_{A\text{-}B}$) allows for linearization of isotopic separations between phases A and B, as equilibrium fractionation curves are temperature dependent and often non-linear (Miller, 2002; Sharp, 2017). Multiplying $\ln\alpha_{A\text{-}B}$ by 1000 allows the separation between A and B to be denoted in ‰. A reasonable approximation for $1000\ln\alpha_{A\text{-}B}$ can be achieved by subtracting the values of δ_A and δ_B, which is denoted in equation 1.2.

$$1000\ln\alpha_{A\text{-}B} \approx \delta_A - \delta_B \qquad 1.2$$

Approximating $1000\ln\alpha_{A\text{-}B}$ by subtracting δ_B from δ_A in the right side of equation 1.2 is denoted using 'big delta' (written as $\Delta^Z X_{A\text{-}B}$) in equation 1.3 (Miller, 2002; Sharp, 2017).

$$\Delta^z X_{A\text{-}B} = \delta_A - \delta_B \qquad 1.3$$

Most of the light elements such as hydrogen, oxygen, carbon, and nitrogen consist of multiple stable isotopes. All isotopes of an element may be used to form 'isotopologues,' or molecules with identical chemical formulas and atomic bonding arrangements but differing molecular masses (e.g., $^1H_2{}^{16}O$ vs $^1H_2{}^{18}O$) (Sharp, 2017).

The ratio of heavy to light isotopes of each element that are incorporated into human tissues are controlled by several factors. The most significant of these factors are the isotopic composition of an individuals' primary drinking water source(s) (e.g., Ehleringer et al., 2008; Fraser & Meier-Augenstein, 2007; Longinelli, 1984; Luz et al., 1984; Sharp et al., 2003) and dietary composition (e.g., Bataille et al.,

2020; Bol et al., 2007; Brettell et al., 2012; Daux et al., 2008; Fraser & Kalin, 2006; McMullagh et al., 2005).

1.2.1 Stable Isotopes in Hair and Nails

Both hair and nails are comprised of a proteinaceous tissue called keratin. Keratinaceous tissues are particularly attractive for stable isotope investigations as they are continuously formed and do not undergo remodeling once synthesized (Chesson et al., 2020; McCullagh et al., 2005). A notable exception is non-carbon-bound H atoms, which undergo continuous exchange with ambient water and water vapor (Bartelink & Chesson, 2019; Bowen et al., 2005a; Sharp et al., 2003). This behavior contrasts with that of bone collagen, another commonly analyzed proteinaceous tissue with a similar chemical composition as keratin (McCullagh et al., 2005). Bone collagen undergoes continual remodeling and reflects average drinking water and dietary compositions over 10-25 years of life (Hedges et al., 2007).

In addition to proteinaceous collagen, stable isotopes in the mineralized components of bone, most commonly the carbonate and phosphate fractions within bioapatite, are analyzed for dietary composition and region of residence information (Daux et al., 2008; Longinelli, 1984). As with collagen, bioapatite within bones undergoes continuous remodeling over the lifetime of the individual and reflects average drinking water and dietary compositions over the last several years of life (Bartelink & Chesson, 2019). Bioapatite within tooth enamel behaves like hair and nail keratin and does not undergo remodeling once formed; however, unlike hair and nails, teeth are not continually formed throughout a person's lifetime and reflect dietary composition and residence locality during childhood, when teeth are formed (Bartelink & Chesson, 2019; Lamb et al., 2014; Webb et al., 2014).

Since hair and nails grow at an average rate 10 mm per month and 3 mm per month, respectively (Wilson & Gilbert, 2007; Zaias, 1990), they may be treated as serial recorders. By sampling hair and nail at regular intervals, diet and residential history can be reconstructed over a known period

of time spanning months to years (Chesson et al., 2018; Lehn et al., 2011; Williams & Katzenburg, 2012). The following sections discuss in further detail the stable isotopes of each element analyzed in this study, the dietary and drinking water source information obtained from each isotopic measurement, and the international standards against which such results are reported.

1.2.2 Hydrogen & Oxygen

Stable hydrogen and oxygen isotope ratios are reported relative to Vienna Standard Mean Ocean Water (VSMOW) in this study. It should be noted, however, that values of $\delta^{18}O$ are reported relative to Vienna Pee Dee Belemnite (VPDB) in some literature (Coplen, 1995). The $\delta^{18}O$ of both VSMOW and VPDB is defined as 0‰, but it is important to note the absolute $^{18}O/^{16}O$ ratios are not identical between both standards; VSMOW is offset from VPDB by ~+31‰ (Kim et al., 2015).

Stable hydrogen and oxygen isotopes of hair and nails have been used for several applications, including reconstructing recent travel history (Fraser & Kalin, 2006; Lamb et al., 2014), distinguishing local and non-local residents of a population center (Mancuso & Ehleringer, 2019; O'Brien & Wooller, 2007), and identifying the provenance of unknown human remains in forensic investigations (Chesson et al., 2018; Lehn et al., 2011). In addition to measurements made on hair and nails, oxygen isotopes of mineralized skeletal tissues such as bioapatite phosphate (PO_4^{3-}), and structural carbonate (CO_3^{2-}) have also been used as proxies for climatic conditions and migration (Longinelli, 1985; Luz & Kolodony, 1985; Luz et al., 1984).

Migration patterns, regional temperature, humidity and degree of seasonality have been reconstructed using oxygen isotope ratios measured for bioapatite phosphate from contemporary and archeological materials (e.g., Daux et al., 2008; Levinson et al., 1984; Longinelli, 1984; Mitchell & Millard, 2009; Webb et al., 2013). Such reconstructions are possible because the relationships among the $\delta^{18}O$ of drinking water, dietary inputs, and atmospheric O_2 are well understood (Bryant and Froelich, 1995; Chenery et al., 2012; Daux et al., 2008; Luz & Kolodny, 1985; Luz et al., 1984). Mineralized tissues such

as structural carbonate and bioapatite phosphate oxygen are formed in isotopic equilibrium with body water, the δ^2H and δ^{18}O of which are largely controlled by the isotopic composition of drinking water, metabolic water synthesized from ingested foods (Kohn, 1996), water contained within food, and atmospheric O_2 (Bryant and Froelich, 1995; Luz et al., 1984).

The relationship between the δ^2H and δ^{18}O values in human tissues and the hydrogen and oxygen isotopic composition of ingested foods and drinking water can be extended further. Bowen et al. (2007) measured the δ^2H and δ^{18}O in local tap water samples collected from municipalities across the continental US, finding a strong relationship between the isotopic composition of the local tap water and local precipitation. Similar relationships have been found between the δ^2H and δ^{18}O of local tap water supplies in Indian, Pakistani, Mongolian, and Chinese population centers, and the corresponding precipitation falling in these localities (Thompson et al., 2010). Correlations have also been noted between the δ^2H and δ^{18}O of non-water beverages, such as milk, soda, or beer, and the δ^2H and δ^{18}O of precipitation in the localities where each of the beverages were produced. That said, correlation between the δ^2H and δ^{18}O of beverage water and tap water at the purchase location is weak (Chesson et al., 2010a, 2010c). This outcome suggests that beverage water records the isotopic composition of precipitation falling in the region of production, but not necessarily that of precipitation in the locality where these beverages are consumed.

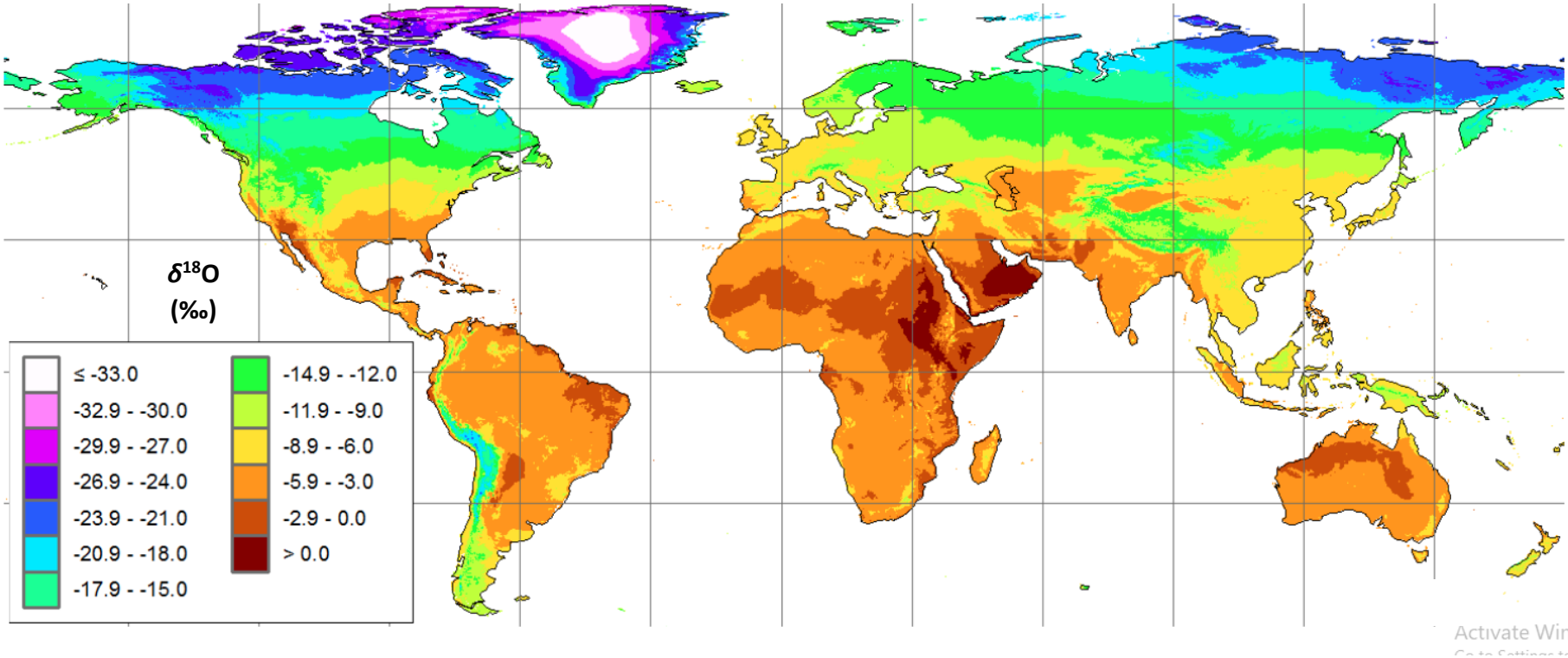
The relationship between the δ^2H and δ^{18}O of local tap water, beverage water, and precipitation are important for archeological or forensic investigations, as the isotopic composition of precipitation is in turn determined by the temperature at which precipitation condenses, the amount of rainfall, and the geographic location in which it falls (Clark & Fritz, 1997; Dansgaard, 1964). In general, moisture-laden air is moved away from its evaporation source, typically equatorial sea water, to higher latitudes and elevations. As moisture condenses and is removed from the initial cloud mass via precipitation, the heavier isotopologues of water will preferentially enter the liquid phase in accordance

with a Rayleigh distillation model. The remaining vapor will therefore be increasingly enriched in the light isotopologues of water as more vapor condenses and is rained out (Craig, 1961; Dansgaard, 1964; Garzione et al., 2000; Gat, 1996; Luz and Barkan, 2010). The δ^2H and δ^{18}O of any new condensate formed from the remaining vapor fraction will also grow progressively lower, dictated by the liquid-vapor fractionation factor at the condensation temperature, and can result in large depletions of heavy isotopes in precipitation, especially once most of the moisture has been lost from the cloud mass (Criss, 1999; Galewsky et al., 2016; Gat, 1996; Jouzel and Merlivat, 1979). The magnitude of fractionation between hydrogen and oxygen isotopes in the liquid and vapor phases of water molecules is inversely proportional to temperature (Horita & Wesolowski, 1994), and results in larger fractionations at lower temperatures.

Precipitation falling in low latitudes is subject to an 'amount effect' during massive rainouts of moisture from an air mass, such as during a monsoon (Sharp, 2017). The amount effect is the progressive reduction of precipitation δ^2H and δ^{18}O during a single rain event as the heavier isotopologues of water are initially rained out and continued precipitation is condensed from the remaining vapor that is becoming increasingly depleted in heavy isotopes. The ultimate result of all controls and relationships described in this section is a global pattern of precipitation that exhibits decreasing δ^2H and δ^{18}O with increasing latitude, elevation, and inland distance (Fig. 1.1) (Rozanski et al., 1993).

The separation of stable hydrogen and oxygen isotopes, relative to dietary and drinking water inputs, following their incorporation into human hair and nails is not well understood (Ehleringer et al., 2008; Sharp et al., 2003). That said, it is known that movement between regions with large differences in the isotopic composition of precipitation or local tap water will be sequentially recorded in the δ^2H and δ^{18}O of hair and nails (Chesson et al., 2018; Mancuso & Ehleringer et al., 2019; Sharp et al., 2003; Terzer et al., 2013).

a.


δ18O
(‰)
≤ -33.0
-32.9 - -30.0
-29.9 - -27.0
-26.9 - -24.0
-23.9 - -21.0
-20.9 - -18.0
-17.9 - -15.0
-14.9 - -12.0
-11.9 - -9.0
-8.9 - -6.0
-5.9 - -3.0
-2.9 - 0.0
> 0.0

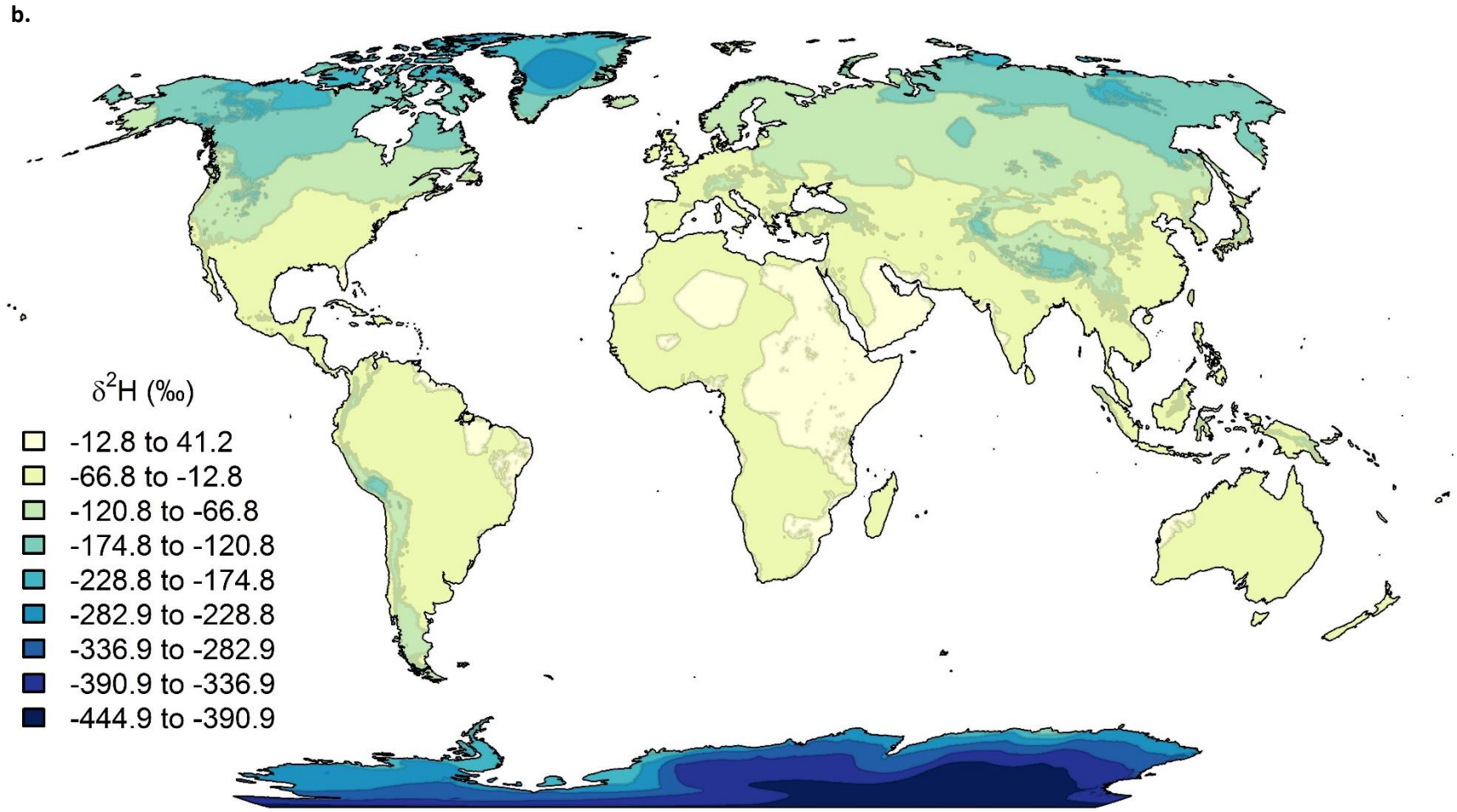


Figure 1.1. Stable isotope distributions of global mean annual precipitation: (a) $\delta^{18}O$ and (b) $\delta^{2}H$. The maps show increasing depletion of heavy isotopes with increasing latitude, distance inland, and elevation. Modified and used with permission from (a) Terzer et al. (2013) and (b) Bowen (2022), Bowen & Revenaugh (2003) and IAEA/WMO (2015).

Linear regression slopes calculated from paired values of drinking water and tissue (i.e., hair or nails) δ^2H and δ^{18}O were used to derive the fraction of hydrogen and oxygen atoms from drinking water that are incorporated into hair and nails. The fraction of H and O atoms in hair and nails derived from drinking water is strongly dependent on the proportion of locally produced foods within an individual's diet. Populations consuming larger fractions of non-local foods in their diet, such as residents of developed nations with globalized food supply chains, were found to obtain ~27-49% of the hydrogen atoms in their hair from drinking water (Ehleringer et al., 2008; Fraser & Meier-Augenstein, 2007; Sharp et al., 2003), and 32-38% of hydrogen atoms in their nails (Fraser & Meier-Augenstein, 2007; Mancuso & Ehleringer, 2019). Approximately 78% of the total hydrogen atoms in hair were derived from drinking water in pre-globalized populations, whose diets were primarily comprised of locally produced foods (Bowen et al., 2009). Paired hair and nail samples from modern developing nations derived ~42% of total hydrogen atoms in hair from drinking water (Thompson et al., 2010). The remaining fraction of hydrogen atoms in hair and nails were contributed by food, metabolic water, or by water contained within food.

A similar trend is observed with oxygen atoms in hair and nails. Residents of developed nations with globalized diets were found to obtain ~35% of the oxygen atoms in their hair (Ehleringer et al., 2008), and ~40% of oxygen atoms in nails from drinking water (Mancuso & Ehleringer, 2019). Residents of developing nations obtained ~40% of the O atoms (Thompson et al., 2010), and pre-globalized populations obtained ~70% of O atoms in hair from drinking water (Bowen et al., 2009). Food, food water and atmospheric O_2 contributed the remaining fraction of hair oxygen atoms. The emergence of a "continental supermarket" diet, characterized by homogenization of δ^2H and δ^{18}O in dietary items and corresponding tissues in developed nations, including Canada has been noted by several studies (Ehleringer et al., 2008, 2015; Manca et al., 2006; O'Brien & Wooller, 2007). The large fraction of non-

local food items that comprise “supermarket” diets likely contain significant fractions of hydrogen and oxygen atoms that do not reflect local drinking water δ^2H and $\delta^{18}O$ compositions (Bowen et al., 2009), decoupling the δ^2H and $\delta^{18}O$ of food from the isotopic composition of waters in the local hydrological regime (O’Brien & Wooller, 2007). Individuals primarily consuming locally produced foods should have hair and nails that incorporate larger fractions of H and O atoms from drinking water, as grown plants and locally raised animals should have a δ^2H and $\delta^{18}O$ that resembles the isotopic composition of local precipitation or other nearby drinking water sources. Waters contained within transpiring plant tissues, such as leaves, may be modified from the original δ^2H and $\delta^{18}O$ of local precipitation; however, the stable isotope composition of evapotranspired waters should fall along an evaporation line originating from the initial precipitation δ^2H and $\delta^{18}O$ (Bariac et al., 1989; Cernusak et al., 2016; Dongmann et al., 1974; Tuthorn et al., 2015; Yakir & Sternberg, 2000).

Despite several investigations into the relationships between δ^2H and $\delta^{18}O$ of paired drinking water and hair and/or nail samples (e.g., Ehleringer et al., 2008; Fraser & Meier-Augenstein, 2007; Mancuso & Ehleringer, 2019; O’Brien & Wooller, 2007), only one tentative mean value for $\Delta^2H_{\text{hair-drinking water}}$ of 17 ± 9 ‰ has been published (Sharp et al., 2003). To the best of my knowledge, no values for $\Delta^2H_{\text{nail-drinking water}}$ and $\Delta^{18}O_{\text{hair/nail-drinking water}}$ have been published. Such measurements are critical to studies reconstructing the isotopic composition of drinking water and other climatological conditions under which the tissues formed. Equally important is an understanding of the inherent variation of hair and nail δ^2H and $\delta^{18}O$ within a single population, as a high degree of variability within the isotopic composition of resident tissues translates into large confidence intervals for reconstructed drinking water compositions (Bump, 1991; Smithson, 2003). Large confidence intervals will bound reconstructed drinking water δ^2H and $\delta^{18}O$ with large uncertainties, seriously limiting the ability of forensic or archeological investigations to identify likely regions of origin with a high degree of certainty.

Reconstructing the isotopic composition of precipitation, and other associated climatic data using δ^2H and $\delta^{18}O$ recorded in tissue(s) requires knowledge of the isotopic separation between the stable hydrogen and oxygen isotopes in drinking water and in the tissue(s) of interest, as well as the temperature of tissue formation. The constant body temperature of mammals means the temperature term is constant in isotopic fractionations associated with metabolic reactions between various amino acids in dietary or drinking water inputs, and body water as well as amino acids in or synthesized by the body. Thus, the isotopic separation between dietary and drinking water inputs, and subsequently formed mammalian tissues, should theoretically be fixed (Kirsanow & Tuross, 2011; Matin at al., 2008).

Lockdowns and travel restrictions imposed during the COVID-19 pandemic have made the London municipal tap water supply and bottled waters, most of which are sourced from nearby (i.e., southern Ontario) groundwater sources, the most readily available drinking water sources to London residents (Bowen et al., 2005b; Gleick & Cooley, 2012). I hypothesize that by limiting the available drinking water sources, the range δ^2H and $\delta^{18}O$ of London resident's hair and nail will be smaller than previously reported measurements. Furthermore, I expect that the limited ability of London residents to consume non-local drinking water sources during lockdowns and travel restrictions should result in a smaller $\Delta^2H_{\text{hair-drinking water}}$ standard deviation than the ± 9 ‰ obtained for sampled residents of Austria and Hungary (n = 3), Poland (n = 1), and New Mexico (n = 3) (Sharp et al., 2003).

1.2.3 Carbon

All values of $\delta^{13}C$ are reported relative to the international standard VPDB, which has been assigned a $\delta^{13}C$ of 0‰ (Coplen et al., 2006). As with hydrogen and oxygen isotopes, a $\Delta^{13}C_{\text{hair-diet}}$ separation of ~ +1.0 to +3.5 ‰ exists in mammals (DeNiro & Epstein, 1978; Sponheimer et al., 2003; Tieszen et al., 1983), which must be taken into account in stable isotope dietary reconstruction. The

separation between tissues and diet, with respect to ^{13}C, is small compared to the range of measured plant $\delta^{13}C$ that comprise our diets.

Food, beverages, and atmospheric CO_2 constitute the carbon inputs to our body, although the primary control on the $\delta^{13}C$ of tissues, including hair and nails, is the mean $\delta^{13}C$ of ingested food and beverages (e.g., Bataille et al., 2020; Huelsemann et al., 2009; Nakamura et al., 1982; Nardoto et al., 2006; Yoshinaga et al., 1996). The stable carbon isotope ratios of overall dietary inputs, in turn, reflect the proportion of C_3 and C_4 photosynthetic plants that comprise an individual's diet, as well as the proportions of C_3 and C_4 plants consumed as feed by livestock animals whose meat is subsequently consumed by humans. Plants using the C_3 photosynthetic pathway, such as wheat, potatoes, rice, barley, and most fruits are more common and exhibit stronger discrimination against the heavy carbon isotope (mean $\delta^{13}C \approx -27$ ‰) than C_4 plants (mean $\delta^{13}C \approx -13$ ‰), such as corn and sugar cane (O'Brien, 2015; Smith & Epstein, 1971). Indeed, a controlled dietary study saw human $\delta^{13}C_{hair}$ shift by + 8.5 ‰ to +9.9 ‰ following a switch from a C_3 to C_4-based diet (Huelsemann et al., 2009).

Similarly, animals raised on C_4 cornmeal can be differentiated from those raised on C_3 grains, such as wheat or barley (Heaton et al., 2008; Martinelli et al., 2011). The proportions of consumed C_3 vs C_4 feed that are recorded in the $\delta^{13}C$ of animal tissues are subsequently propagated to secondary consumers, such as humans. As a result, the $\delta^{13}C$ of human hair and nails record the proportions of C_3 and C_4 plants consumed directly by a person, as well as indirectly in the form of animal proteins reared on C_3 vs C_4-based feed mixes (Caut et al., 2009; Macko et al., 1999; McCutchan et al., 2003).

Industrialization and globalization of food production and distribution systems during the 20th century has seen a remarkable increase in the variety of foods available to consumers living in large urban centers with origins spanning a wide geographic range. Expanded food selection and wider regions of origin for food items have also given rise to the 'continental supermarket' diet,

counterintuitively characterized by a homogenization of $\delta^{13}C$ in dietary inputs and smaller intra-population $\delta^{13}C$ ranges (Bataille et al., 2020; Nardoto et al., 2006). The smaller $\delta^{13}C$ range of continental supermarket diets is a result of industrial-scale production of a limited variety of food crops by a limited number of commercial farms, which is then consumed by residents of developed nations (Chesson et al., 2010b; Frison, 2016; Lin, 2011; O'Connell & Hedges, 1999)

Despite isotopic homogenization, stable carbon isotopes have proven a useful tool to help determine the geographic origins of food items, as the more water efficient C_4 plants are more common in low latitude and arid regions than C_3 plants (Martinelli et al., 2011; Still et al., 2003; Valenzuela et al., 2012). Stable carbon isotopes have also proven useful in differentiating Americans from Europeans. The North American climate is more amenable to cultivating corn than Europe (Stubbendieck et al., 2017), and government subsidies of corn encourage greater cultivation of this crop (Lin, 2011), resulting in higher $\delta^{13}C$ in hair and nails of Americans than in Europeans (Bol et al., 2007; Ritchie & Roser, 2013; Wada et al., 1991). Urban and non-urban residents of developing countries have also been differentiated though stable carbon isotopes. Nardoto et al. (2011, 2020) found higher $\delta^{13}C$ in the nails of urban Brazilians than their rural counterparts, indicating greater consumption of processed foods containing sugars and sweeteners derived from C_4 plants by urban dwellers, which is consistent with a nutritional transition from locally produced foods to a 'supermarket' diet.

Canadians, as citizens of a developed nation, exhibit a limited range of $\delta^{13}C_{hair}$ consistent with consumption of a 'supermarket' diet (Bataille et al., 2020; Lehn et al., 2015). I hypothesize that supply chain disruptions resulting from COVID-19 outbreaks, travel restrictions, lockdowns, and reduced production, particularly in large meat processing and packaging plants (Thilmany et al., 2021), will necessitate greater reliance on locally produced foods as the supply of non-local foods became more limited through limited supply and consumer panic buying and stockpiling (Hobbs, 2020). As southern Ontario and Quebec produce ~90% of all corn grown in Canada (Hamel & Dorff, 2014), increased

reliance on locally produced foods is expected to manifest as an increase in hair and nail $\delta^{13}C$ as more locally produced corn feed is substituted for non-local C3-grasses in livestock feed.

1.2.4 Nitrogen

Stable isotopes of nitrogen are reported relative to atmospheric nitrogen (AIR), which has a $\delta^{15}N$ defined as exactly 0‰ (Mariotti, 1983). Nitrogen isotopes are used in dietary reconstruction to assess the amount of plant vs animal proteins in the diet, and by extension, the trophic position of an organism within a food web. Animal tissues are generally enriched in the heavy nitrogen isotope due to preferential excretion of ^{14}N (DeNiro & Epstein, 1981; Minagawa & Wada, 1984). The process of preferential incorporation of ^{15}N into tissues creates a $\Delta^{15}N_{tissue\text{-}diet}$ discrimination of +1 ‰ to +6 ‰, with a typical enrichment of ~ +3 ‰ in most mammals (e.g., Abend & Smith, 1997; Ambrose & DeNiro, 1986; DeNiro & Epstein, 1981; O'Connell & Hedges, 1999; Sponheimer et al., 2003). Stepwise enrichment of $\delta^{15}N_{tissue}$ continues with successive increases in tropic level (Ambrose & DeNiro, 1986; Hedges & Reynard, 2007), as organisms occupying higher trophic positions consume more high-quality, animal-derived protein (Robbins et al., 2005). This progressive enrichment in ^{15}N allows distinction between primary and secondary consumers (i.e., herbivores vs carnivores). Additionally, juvenile mammals may appear to sit one tropic level above their mothers due to consumption of milk that has been enriched in ^{15}N relative to their mother's dietary inputs (Fuller et al., 2006; Jenkins et al., 2001; Polischuk et al., 2001).

Dietary protein source, particularly marine versus terrestrial protein sources, exerts a strong control on the stable nitrogen isotope composition of tissues, including hair (Huelsemann et al., 2009). The complexity of marine food webs results in a greater range of trophic positions occupied by marine organisms, relative to terrestrial food webs, that result in ^{15}N-enrichment of marine proteins relative to terrestrial proteins (Fry, 1988; Schoeninger & DeNiro, 1984). Stepwise trophic concentration of ^{15}N and

^{15}N-enrichment of marine proteins relative to terrestrial proteins permits the differentiation of populations consuming plant-based diets, terrestrial protein-based diets, and marine protein-based diets based on $\delta^{15}N_{tissue}$ (Buchardt et al., 2007; Martin, 1999; Schoeninger & DeNiro, 1984; Shoeninger & Moore, 1992; Schwarcz et al., 1985).

Considerable δ^{15}N variation has been observed within a single trophic level in both marine and terrestrial food webs (Giraldo et al., 2016; McMahon & McCarthy, 2016). In some cases the δ^{15}N variations are larger than fractionations between trophic levels (Hobbie et al., 2000; Huelsemann et al., 2009), potentially complicating efforts to identify the trophic position of an organism from which tissues are sampled. Such variation is not unexpected however, as plant δ^{15}N values are known to span a wide range due to numerous factors, including differing mechanisms for nitrogen uptake and assimilation, aridity, and soil salinity (Adams & Grierson, 2001; Evans, 2001). Nitrogen is an essential element for plant growth as much as it is for animals; however, atmospheric N_2 is directly accessible only to a minority of plants, such as legumes, that are capable of nitrogen fixation via symbiotic bacteria living in their root nodules (Ledgard & Steele, 1992). Nitrogen-fixing bacteria allow the conversion of atmospheric N_2 into bioavailable forms such as NO_3^- or NH_3 within the soil (Becana & Sprent, 1987), and these compounds may then be assimilated by plants, including those that are incapable of nitrogen fixation on their own (Frungillo et al., 2016; Lea & Miflin, 2018). As such, plant growth rates are limited, in most cases, by the amounts of bioavailable nitrogen compounds (Ågren et al., 2012).

Ammonification and nitrification reactions convert decaying organic material within the soil into isotopically light bioavailable NH_4^+, and NO_3^- as bacteria favor the lighter and less metabolically expensive ^{14}N (Deliwche & Steyn, 1970). Denitrifying reactions in the nitrogen cycle, particularly the breakdown of NH_4^+ and NO_X compounds (volatilization and denitrification respectively), favor ^{14}N in the products, causing the remaining N^-pool to become increasingly ^{15}N-enriched prior to uptake by plants (Lehmann et al., 2003). Meanwhile, artificial fertilizers applied to agricultural crops are commonly

derived from atmospheric N_2 and tend to have $\delta^{15}N$ of ~0‰ (Bateman & Kelly, 2007; Brandes & Devol, 2002; Finlay & Kendall, 2007). Anthropogenic fossil fuel combustion has resulted in substantial additions of isotopically light NO_x emissions, which may then be deposited on and later taken up by plants, further lowering their overall $\delta^{15}N$ (Savard et al., 2009). A simplified illustration of the primary sources and sinks of nitrogen in terrestrial biomes is shown in Figure 1.2. For a more in-depth review of the nitrogen cycle and the alterations caused by anthropogenic activities, see Sprent (1987) and Vitousek et al. (1997).

The COVID-19 pandemic may have driven substantial changes in the dietary habits of London, Ontario, Canada residents, which could manifest as changes in the $\delta^{15}N$ of hair and nails compared to previously published data. Dietary changes may include reduced meat consumption due to price increases, fear of viral contamination of meat products, or supply disruptions due to COVID-19 outbreaks at meat processing plants (Donaldson, 2020; Loh et al., 2021). Ontario and Quebec produced ~ 80% of all soybeans grown in Canada in 2020 (Statistics Canada, 2021), a crop capable directly assimilating isotopically light atmospheric N_2. I hypothesize that a reduction in animal protein consumption, combined with increased use of locally produced soybeans in animal feed, or direct consumption by London residents the form of soymilk, tofu, or soy flour will manifest as a decrease in $\delta^{15}N$ in London residents' hair and nails relative to pre-pandemic values (Bataille et al., 2020).

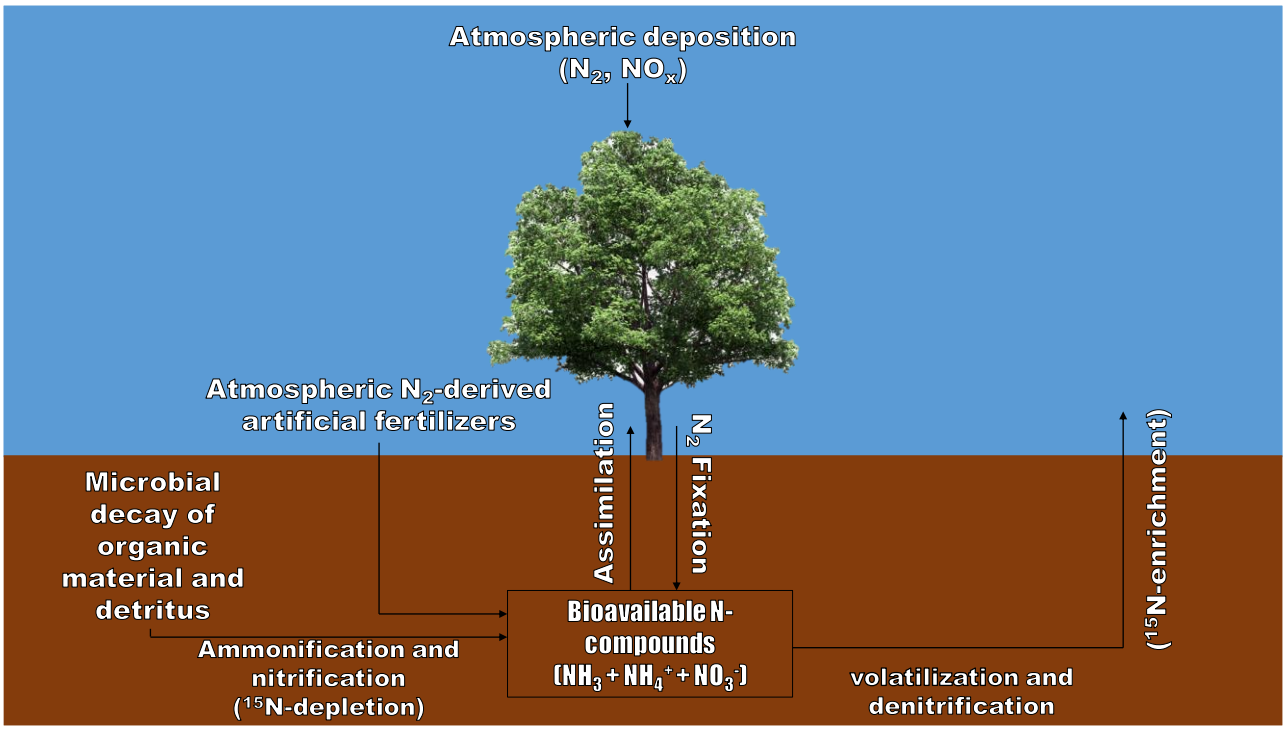


Figure 1.2. Simplified illustration outlining the primary sources and production of bioavailable nitrogen for plants in terrestrial settings.

1.3 Thesis Objectives

It is hoped that an improved understanding of the relationship between stable isotope compositions of drinking water and dietary inputs, and human hair and nails will allow for the accurate reconstruction of drinking water and dietary compositions using stable isotopes in these tissues. Stable isotope reconstruction of drinking water and diet compositions would also reduce uncertainties and personal bias associated with self-reported diet and drinking water data by providing an independent method for the ground-truthing of self-reported dietary and beverage consumption data (Hedrick et al., 2016; Schoeller, 1990; Schoeller et al., 1995).

1.4 Research Questions

This thesis explores the relationship between the isotopic composition of drinking water sources and dietary inputs, on one hand, and the isotopic composition of hair and nail tissues, on the other hand, within a geographically immobile population with the aim of answering the following questions:

i. What is the extent of δ^2H and $\delta^{18}O$ variability in the London municipal tap water supply?

ii. What is the extent of δ^2H and $\delta^{18}O$ variation in the hair and nails of London, Ontario, Canada residents who are consuming the local municipal tap water supply and how does this variation compare to previously reported isotopic variations within hair for this region?

iii. What are the values of $\Delta^2H_{hair/nail\text{-}drinking\ water}$ and $\Delta^{18}O_{hair/nail\text{-}drinking\ water}$ for London, Ontario, Canada residents, how do the values obtained in this study compare with previously reported values, and are there any trophic controls on London resident hair and nail δ^2H and $\delta^{18}O$?

iv. What effects, if any, has the COVID-19 pandemic had on the dietary composition of residents of London and the broader southern Ontario region?

1.5 Thesis Organization

This thesis contains four additional chapters beyond this Introduction. Chapter 2 details the materials and methods utilized in participant recruitment, sample collection and preparation, and isotopic analysis of sampled materials. Chapter 3 reports the results of all stable isotope analyses performed. It further documents the degree of intra-population variation across all measured stable isotopes in hair, nails, and drinking water samples and compares these results with previously reported ranges. Chapter 4 discusses the potential drivers of intra-population isotopic variation, and the potential

applications and limitations of such stable isotope analysis for forensic or archeological reconstructions of residence history, climatic conditions, and dietary habits. Chapter 5 summarizes the conclusions concerning the magnitude of intra-population isotopic variability within hair and nails in London, Ontario, Canada, and the relationships between the stable isotopic composition of drinking water and dietary inputs, and the isotopic compositions of keratinous tissues.

Chapter 2

Materials and Methods

2.1 Sampling

After obtaining ethics approval from the Western Health Science Research Ethics Board (REB) (see Appendix A), a recruitment email containing a link to an online survey asking respondents about their travel history over the most recent six months, their primary sources of drinking water, and their dietary habits (Appendix B), was circulated amongst the Earth Science department at the University *of* Western Ontario in London, Ontario, Canada. Participants were classified into the following dietary groups based on their dietary preferences:

i. Ovo-lacto vegetarians (henceforth referred to as "vegetarians", unless otherwise noted) (n = 2) – respondents who reported eating no meat, but consuming eggs and dairy products.
ii. Semi-vegetarians (n = 3) – respondents who reported eating meat once per week to once per month, in addition to eggs and dairy products.
iii. Omnivores (n = 19) – respondents who reported eating meat more than once per week, in addition to eggs and dairy products.
iv. Carnivores (n = 3) – respondents who reported a significant fraction of their daily caloric intake was sourced from meat products.

For each dietary group an average isotopic composition was calculated. For dietary groups with at least 3 individuals a standard deviation (± ‰) was calculated and is presented in the format "Mean ± SD." Respondents who had remained in the London area and primarily consumed the municipal or other isotopically consistent water source (i.e. groundwater via wells or bottled spring water) (Stichler & Moser, 1979; Krabbenhoft et al., 1990; Clark and Fritz, 1997) during the past six months are expected to

have had sufficient time for their hair and fingernails to reach isotopic equilibrium with their drinking water and dietary supplies and are considered eligible to participate in the present study.

Eligible respondents were invited to provide a sample of arm hair (henceforth referred to as "hair" unless otherwise noted). The short length of arm hair will record the most recent weeks to months of dietary and drinking water inputs that an individual has consumed and increases the likelihood that collected samples reflect equilibrium conditions with local drinking water and dietary supplies. Additionally, sampling arm hair expands the pool of eligible participants by allowing individuals with shaved heads to participate. Eligible participants were also asked to provide a sample of fingernail clippings (henceforth referred to as "nails" unless otherwise noted), and their two primary drinking water sources. If participants reported they obtained all their drinking water from only one source (e.g. their home) then only one sample of tap water was collected.

Eligible participants (*n*=27) were sampled between November 2020 and January 2021, and all samples received were assigned a 3-letter coded ID. The first letter recorded the sampling location (in this case "L" for "London"). The second letter denotes dietary group ("C" for "carnivore", "O" for "omnivore", "S" for "semi-vegetarian", and "V" for "vegetarian"). The third letter denotes the primary reported sources of drinking water ("T" for "tap" water, whether supplied by the municipal water supply or private wells, and "B" for "bottled" water). Hair samples were collected by dry shaving; or, in the case of participants who lacked sufficient arm hair to provide a usable sample, a minimum of 5mm of head hair was sampled with scissors. Head hair samples were cut as close to the scalp as possible to obtain material representing the most recent weeks of growth (O'Connell et al., 2001; Fraser & Meier-Augenstein, 2007; O'Brien & Wooller, 2007; Mant et al., 2016). Nail clippings from each finger were also obtained from participants. Samples were stored in 4 mL glass vials until cleaned and prepared for isotopic analysis.

Participants were given two 20 mL plastic sample bottles and instructed how to properly fill and seal the vials with a drinking water sample. Participants were requested to return samples to the university campus after collection. Samples were refrigerated until ready for isotopic analysis. A third water sample was collected from one participant living in the Waterloo Region of Ontario during July 2021 and compared against the first sample of tap water collected during the initial sampling period in November 2020. Unlike London, the Waterloo Region obtains ~75% of its drinking water from aquifers, which exhibit substantial spatial geochemical heterogeneity and water ages (Stotler et al., 2011), and the remaining ~25% from the Grand River (Region of Waterloo, n.d.). As fingernails grow at a rate of ~3 mm/month (Wilson & Gilbert, 2007), approximately 3-4 months would be needed before newly formed nail material reaches the tip of the finger for sampling. The second water sample from the Waterloo region should provide insight into any isotopic variations that occurred in the local tap water between the time the sampled nails were formed, and the time of tissue and initial water sampling from this participant.

2.2 Sample Preparation

Hair and nail samples were rinsed twice at room temperature for one hour in a 2:1 methanol/chloroform solution to remove any detergents, oils, or other lipids, before being allowed to air-dry at room temperature overnight in a fume hood. Samples were then rinsed twice for 20 minutes in an ultrasonic bath with deionized water, then freeze-dried (O'Connell & Hedges, 1999; O'Connell et al., 2001) and stored at room temperature while awaiting isotopic analysis. For stable carbon and nitrogen isotope analysis, ~0.25mg of dry sample was weighed into tin capsules; a similar quantity was weighed into silver capsules for oxygen and hydrogen isotope analysis. When quantities permitted, samples were prepared in triplicate.

Sample loss during preparation left insufficient hair for isotopic analyses for two participants. A further 6 samples lacked sufficient material to perform all isotopic analyses on all desired elements (carbon and nitrogen, oxygen, and hydrogen), in which cases sample material was prioritized for analysis of oxygen isotopic composition, followed by hydrogen and then carbon and nitrogen analyses. Sufficient nail material was collected from all participants for triplicate analyses, though 1 subsample was excluded from the hydrogen and the oxygen isotope dataset, 4 subsamples from the nitrogen isotope dataset, and 3 subsamples from the carbon isotope dataset due to a loss of some material from the sample capsule in the autosampler prior to analysis. Sample loss likely occurred following puncturing or tearing of the sample capsule by sharp edges of nail samples prior to analysis, most likely when the capsules were sealed for isotopic analysis, or while capsules were loaded into the autosampler. The remaining sample material (if any) within the capsule was then insufficient to obtain reliable stable isotope measurements for these samples. Appendix C details the results of all stable isotope analyses performed on drinking water, hair, and nail samples. The number of samples analyzed for each element of interest, and for each tissue, is summarized in Table 2.1.

Table 2.1. Summary count of all isotopic analyses performed for all tissues in this study.

Tissue							
Hair				**Nails**			
$\delta^{13}C$	**$\delta^{15}N$**	**$\delta^{2}H$**	**$\delta^{18}O$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{2}H$**	**$\delta^{18}O$**
49	49	55	61	78	77	80	80

2.3 Stable Isotope Analysis

All isotopic analyses were conducted at the Laboratory for Stable Isotope Science (LSIS) at the University *of* Western Ontario, London, Ontario, Canada. For all samples analyzed in duplicate or triplicate a mean isotopic composition was calculated for each analyzed element. Standard deviations (± ‰) were calculated for all for all samples prepared in triplicate and for standard reference materials used in this study to assess measurement precision.

2.3.1 Water Sample Isotopic Analysis

All water samples were analyzed using a 2006 Los Gatos Off-Axis Integrated Cavity Output Spectrometry analyzer. All water samples were inspected for signs of damage to the sample container, container seal, and any potential leakage or evaporation of water sample prior to analysis. Hydrogen and oxygen isotope ratios measured in all water samples are reported relative to VSMOW, calibrated using the standards LSD (accepted δ^2H = –161.8 ‰, accepted δ^{18}O = –22.57 ‰), MID (accepted δ^2H = –108.1 ‰, accepted δ^{18}O = –13.08 ‰), and HEAVEN (accepted δ^2H = +88.7 ‰, accepted δ^{18}O = –0.27 ‰). The analytical precision obtained for these standards over 4 analytical sessions for δ^2H was ± 0.46 (n = 19), ± 0.60 (n = 19), and ± 0.11 (n = 19), respectively. The analytical precision obtained for these standards over 4 analytical sessions for δ^{18}O was ± 0.04 ‰ (n = 19), ± 0.07 ‰ (n = 19), and ± 0.03 ‰ (n = 19), respectively. The accuracy of the calibration curve provided by the calibration standards was assessed by analyzing the in-house standard EDT (measured δ^2H = –55.10 ± 0.68 ‰, n = 19, measured δ^{18}O = –7.30 ± 0.15 ‰, n = 19) as an unknown. These results compare favorably with the accepted δ^2H and δ^{18}O of –56.0 ‰ and –7.27 ‰, respectively (Table 2.2).

2.3.2 Hydrogen Isotope Analysis of Tissues

Standards, hair, and nail samples for hydrogen isotope analysis were stored at room temperature for at least 5 days prior to analysis to allow the exchangeable fraction of H atoms in keratinous tissues to reach isotopic equilibrium with ambient atmospheric moisture in the laboratory (Sharp et al., 2003; Bowen et al., 2005a). To prevent further exchange with ambient moisture over the several days needed to complete all analytical runs, after the 5-day equilibration period, samples and standards were stored together in a bench top desiccator until ready for analysis. Samples and standards were immediately loaded into a Costech Zero-Blank autosampler after removal from the desiccator and flushed overnight under continuous helium flow. Samples were then combusted at 1120 °C using a Thermo Scientific™ High Temperature Conversion Elemental Analyzer (TC/EA). The resultant H_2 gas was swept through a gas chromatograph (GC) separation column held at 90 °C for separation using continuous helium flow as the carrier gas before entering a Delta V^{PLUS} Isotope-Ratio Mass Spectrometer (IRMS) for analysis.

All hydrogen isotope ratios are reported relative to VSMOW, calibrated using the international keratin reference standards Kudu Horn Standard (KHS) (accepted $\delta^2H = -35.3$ ‰) and Caribou Hoof Standard (CBS) (accepted $\delta^2H = -157.0$ ‰) (Coplen, 2020a, 2020b). The analytical precision obtained for these standards over 5 analytical sessions was ±2.40 ‰ ($n = 20$) and ± 1.59 ‰ ($n = 20$), respectively. The accuracy of the calibration curve provided by the KHS and CBS standards was assessed by analyzing international reference standards USGS 42 (Tibetan human hair) (measured $\delta^2H = -75.6 \pm 1.47$ ‰, $n = 14$) and USGS 43 (Indian human hair) (measured $\delta^2H = -46.8 \pm 1.42$ ‰, $n = 13$) as unknowns. These results compare favorably with the accepted respective values of –72.9‰ and –44.4 ‰ for USGS 42 and 43, respectively (Coplen, 2019a, 2019b).

2.3.3 Oxygen Isotope Analysis

Standards, hair, and nail samples were combusted at 1320 °C using a Thermo Scientific™ TC/EA. The resultant CO gas was swept through a GC separation column held at 90 °C, using continuous helium flow as the carrier gas, before entering a Delta V^{PLUS} IRMS, operating in dual-inlet mode, for isotopic analysis. All oxygen isotope ratios are reported relative to VSMOW and calibrated using the standards KHS and CBS (accepted $\delta^{18}O$ = +21.21‰ and +2.39‰, respectively) (Coplen, 2020a, 2020b). The analytical precision obtained for these standards over 5 analytical sessions was ± 0.30 ‰ (*n* = 20) and ± 0.24 ‰ (*n* = 20), respectively. The accuracy of the calibration standards was assessed by measuring the reference standards USGS 42 (measured $\delta^{18}O$ = +8.59 ± 0.21‰, *n* = 15) and USGS 43 (measured $\delta^{18}O$ = +14.14 ± 0.36‰, *n* = 14) as unknowns, which compare well with accepted values of +8.56 and +14.11‰, respectively (Coplen, 2019a, 2019b).

2.3.4 Stable Carbon and Nitrogen Isotope Analysis

Standards, hair, and nail samples were combusted at 1020 °C using a Costech 4010 Elemental Analyzer (EA). Combustion gasses were swept through a GC separation column held at 90 °C using continuous flow of helium carrier gas, before entering a DELTA V IRMS operating in continuous flow mode via a Conflo IV. Measured stable carbon and nitrogen isotope ratios are reported relative to the international reference standards VPDB and AIR, respectively. The international standards USGS 40 (L-glutamic acid, accepted $\delta^{13}C$ = –26.39‰, accepted $\delta^{15}N$ = –4.52 ‰) and USGS 41a (^{13}C and ^{15}N-enriched L-glutamic acid, accepted $\delta^{13}C$ = +36.55‰, accepted $\delta^{15}N$ = +47.55 ‰) were used for calibration to VPDB and AIR (Qi et al., 2003). The analytical precision obtained for USGS 40 (*n* = 18) and USGS-41a (*n* = 17) $\delta^{13}C$ over 4 analytical sessions was ± 0.02‰, and ± 0.23‰, respectively. The analytical precision obtained for these calibration standards for $\delta^{15}N$ was ± 0.04‰ and ± 0.22‰, respectively.

The accuracy of the calibration curve was assessed using an in-house keratin standard (measured $\delta^{13}C$ = –24.04 ± 0.05‰, and $\delta^{15}N$ = +6.40 ± 0.15‰, *n* = 31), and the international keratin reference standards USGS 42 (measured $\delta^{13}C$ = –21.12 ± 0.08‰, and $\delta^{15}N$ = +8.02 ± 0.09‰, *n* = 12), and USGS 43 (measured $\delta^{13}C$ = –21.30 ± 0.08‰, $\delta^{15}N$ = +8.43 ± 0.11‰, *n* = 12) as unknowns. The measured values compare favorably with the accepted $\delta^{13}C$ of –24.04‰, –21.09‰, and –21.28‰ for the keratin, USGS 42, and USGS 43 standards, respectively. The measured $\delta^{15}N$ of these standards compare favorably with their accepted $\delta^{15}N$ of +6.36‰, +8.05‰, and +8.44‰ for the keratin, USGS 42, and USGS 43 standards, respectively. A summary of the accuracy and precision obtained for the standard reference materials used in this study is presented in Table 2.2.

2.3.5 Carbon and Nitrogen Content & C/N Ratios of Keratinous Tissues

Carbon content was calculated for samples which contained sufficient sample material to obtain reliable $\delta^{13}C$ measurements, as indicated by a signal amplitude of at least 1000mV on the mass 44 collection cup in the mass spectrometer flight tube. Samples with a calculated carbon content exceeding 100% were omitted as such high carbon contents indicate sample contamination, or the possible addition of sample material due to two samples or sample material lost from another capsule dropping simultaneously from the autosampler into the EA. Carbon contents of remaining hair and nail samples varied between 7.9 and 86.9 %. Nitrogen content was calculated for samples that contained sufficient material to obtain reliable $\delta^{15}N$ measurements, as indicated by an amplitude of at least 1200mV on the mass 28 collection cup at the end of the mass spectrometer flight tube. Nitrogen content in hair and nails ranged between 5.86 and 47.28 %. Carbon/nitrogen (C/N) ratios ranged between 1.08 and 4.00. Some of these results are outside the accepted range of 2.9 to 3.8 and indicate potential contamination or incomplete combustion during analysis (O'Connell & Hedges, 1999). The $\delta^{13}C$ and $\delta^{15}N$ of subsamples with C/N ratios outside the accepted range were compared, when possible, against $\delta^{13}C$ and $\delta^{15}N$ measurements of subsamples from the same individual to assess if significant differences existed

between the $\delta^{13}C$ and $\delta^{15}N$ of suspect samples, on one hand, and samples within accepted C/N ranges, on the other hand. If no significant differences existed, the results were retained in the dataset. Samples outside the accepted C/N range that lacked other subsamples from the same individual for comparison were removed as a precaution. In total, 5 samples with radically different $\delta^{13}C$ and $\delta^{15}N$ from other subsamples from the same individual were removed from the nitrogen dataset, and 3 samples from the carbon dataset.

2.4 Data Analysis

All statistical tests were performed at the 5% significance level using the data analysis toolpak in Excel 2016, or in R 4.0.3.

2.4.1 Hydrogen and Oxygen Isotope Data Analysis

The average isotopic separations between hair and drinking water ($\Delta_{hair\text{-}water}$), and nail and drinking water ($\Delta_{nail\text{-}water}$) were calculated by subtracting the average drinking water $\delta^{2}H$ and $\delta^{18}O$ from the corresponding average hair and average nail isotopic compositions for participants who reported their drinking water was exclusively supplied by the London municipal water supply (n = 23). An average ± SD of all calculated $\Delta_{hair\text{-}water}$ and $\Delta_{nail\text{-}water}$ values was then computed (Sharp et al., 2003). This approach was taken to control for as much isotopic variability as possible in the isotopic composition of drinking water consumed by participants, as the $\delta^{2}H$ and $\delta^{18}O$ of bottled beverages has been found to vary based on purchase location (Chesson et al., 2010a, 2010c). Additionally, changes in the proportions of bottled water brands or bottled water source locations can result in temporally variable body water isotopic composition, which can in turn be passed to the isotopic composition of tissues incorporating hydrogen and oxygen atoms from body water (Ehleringer et al., 2008; Gretebeck et al., 1997).

Measured hair and nail $\delta^{2}H$ and $\delta^{18}O$ in this study were compared against published models of the relationships between hydrogen or oxygen isotope compositions of hair and fingernails, on one

Table 2.2. Accuracy and precision data of stable isotope reference materials used in the present study.

Standard	Mean δ^2H (‰, VSMOW)	Accepted δ^2H (‰, VSMOW)	*n*	SD (± ‰)	Mean δ^{18}O (‰, VSMOW)	Accepted δ^{18}O (‰, VSMOW)	*n*	SD (± ‰)	Mean δ^{13}C (‰, VPDB)	Accepted δ^{13}C (‰, VPDB)	SD (± ‰)	Mean δ^{15}N (‰, AIR)	Accepted δ^{15}N (‰, AIR)	SD (± ‰)	*n*
CBS	–157.0	–157.0	20	1.59	2.39	2.39	20	0.24	–	–	–	–	–	–	–
EDT	–55.1	–56.0	19	0.68	–7.30	–7.27	19	0.15	–	–	–	–	–	–	–
HEAVEN	+88.7	+88.7	19	0.11	–0.27	–0.27	19	0.03	–	–	–	–	–	–	–
Keratin	–	–	–	–	–	–	–	–	–24.04	–24.04	0.05	6.4	6.36	0.15	31
KHS	–35.3	–35.3	20	2.40	21.21	21.21	20	0.3	–	–	–	–	–	–	–
LSD	–161.8	–161.8	19	0.46	–22.57	–22.57	19	0.04	–	–	–	–	–	–	–
MID	–108.1	–108.2	19	0.60	–13.08	–13.08	19	0.07	–	–	–	–	–	–	–
USGS 40	–	–	–	–	–	–	–	–	–26.39	–26.39	0.02	–4.52	–4.52	0.04	18
USGS 41a	–	–	–	–	–	–	–	–	36.55	36.55	0.23	47.55	47.55	0.22	17
USGS 42	–75.6	–72.9	14	1.47	8.59	8.56	15	0.21	–21.12	–21.09	0.08	8.02	8.05	0.09	12
USGS 43	–46.8	–44.4	13	1.42	14.14	14.11	14	0.36	–21.30	–21.28	0.08	8.43	8.44	0.11	12

hand, and drinking water, on the other hand. Measured tissue δ^2H and δ^{18}O were plotted against measured drinking water δ^2H and δ^{18}O. These data were then compared with tissue δ^2H and δ^{18}O predicted from drinking water isotopic compositions, using models for populations consuming a globally sourced 'supermarket diet' (Fraser & Meier-Augenstein, 2007; Ehleringer et al., 2008). Measured tissue and drinking water δ^2H and δ^{18}O were further compared with predicted tissue δ^2H and δ^{18}O using a model for populations consuming a transitional diet, where locally produced foods are steadily replaced by a growing proportion of processed, non-local foods (Thompson et al., 2010). Measured tissue and drinking water δ^2H and δ^{18}O were compared against predicted tissue δ^2H and δ^{18}O using a final model for pre-globalized populations consuming a diet almost entirely composed of locally produced food (Bowen et al., 2009).

2.4.2 Stable Carbon and Nitrogen Isotope Data Analysis

Stable carbon and nitrogen isotope data for hair and nail samples of London omnivores obtained in this study were compared with pre-pandemic δ^{13}C and δ^{15}N data for London omnivores between 50 and 59 years of age (n = 3), and southern Ontario omnivores, with representatives of all age groups between 18 to 29 and 60 to 69 years of age (n = 24); the pre-pandemic data were reported by Bataille et al. (2020).

As with the analysis of δ^2H and δ^{18}O in paired hair and water samples, unpaired t-tests were used to compare groups meeting the assumptions of normal data distribution. When Shapiro-Wilk test results indicated data comprising one or both comparison groups were not normally distributed, a non-parametric Mann-Whitney U test was used (Valenzuela et al., 2012; Correia et al., 2019).

Chapter 3

3.0 Results

3.1 Drinking Water δ^2H and $\delta^{18}O$ Results

This section lists the stable isotope compositions of collected drinking water samples. In situations where participants provided multiple samples from the London municipal tap water supply, the arithmetic mean for $\delta^2H_{drinking\ water}$ and $\delta^{18}O_{drinking\ water}$ was taken. A weighted average of $\delta^2H_{\ drinking\ water}$ and $\delta^{18}O_{drinking\ water}$ was taken in situations where participants provided one sample from the London municipal tap water supply and a second sample of bottled water or well water to more accurately reflect the overall composition of ingested drinking water by those participants. The calculated mean $\delta^2H_{drinking\ water}$ and $\delta^{18}O_{drinking\ water}$ for individuals, henceforth referred to as 'drinking water,' are listed in Table 3.1, stable isotope measurements of drinking water samples are listed in Appendix C.

Values of $\delta^2H_{drinking\ water}$ span a range of 20.6 ‰. The lowest value of –73.5‰ was measured in samples collected from a participant living outside the London municipal water supply boundary and who reported bottled and well water as their primary drinking water sources. The highest $\delta^2H_{drinking\ water}$ value of –52.9 ‰ was measured for a sample of London municipal tap water. The range of $\delta^2H_{drinking\ water}$ for London municipal tap water supply samples, at 3.4 ‰, is significantly smaller than the range of all drinking water samples, with a minimum value of –56.3 ‰, and a maximum value of –52.9 ‰. The average of all London municipal water supply $\delta^2H_{drinking\ water}$ measurements performed in this study is –53.9 ± 0.8 ‰ (n = 23).

Values of $\delta^{18}O_{drinking\ water}$ span a range of 4.0 ‰, with a minimum value of –11.4 ‰, measured in in the same water samples as the minimum $\delta^2H_{drinking\ water}$, and a maximum value of –7.4 ‰, again measured in a sample of London municipal tap water. The range of $\delta^{18}O_{drinking\ water}$ for London municipal

tap water supply samples is 0.5 ‰, with a minimum value of –7.9 ‰, a maximum value of –7.4 ‰, and the average of all London municipal tap water supply $\delta^{18}O_{drinking\ water}$ measurements performed in this study is –7.5 ± 0.1 ‰ (n = 23).

The higher $\delta^{18}O_{drinking\ water}$ and $\delta^{2}H_{drinking\ water}$ values of the municipal water supply, compared to groundwater-derived bottled or well waters, are consistent with preferential groundwater recharge by isotopically lighter winter precipitation (French & Binley, 2004; Maulé et al., 1994) and evapoconcentration effects in surface water bodies, such as the Great Lakes, which supply London's drinking water (Brooks et al., 2014; Ruan et al., 2020). The small variance of ± 3.9 ‰ and ± 0.1 ‰ associated with $\delta^{2}H_{drinking\ water}$ and $\delta^{18}O_{drinking\ water}$, respectively, of London tap water is also consistent with water obtained from a source reservoir that experiences minimal temporal isotopic variation due to a long residence time, in this case, 22 years for Lake Huron (Brooks et al., 2014; Gat, 1995, 2010; Quinn, 1992).

A sample of tap water collected in November 2020 from the Waterloo region, which obtains ~75% of its drinking water from groundwater sources and the remaining portion from the Grand River (Region of Waterloo, n.d.), has a $\delta^{2}H_{drinking\ water}$ of –70.6 ‰ and a $\delta^{18}O_{drinking\ water}$ of –10.9 ‰. A second water sample, collected in July 2021 from the same participant and from the same location within the region as the first sample, had a $\delta^{2}H_{drinking\ water}$ of –69.5 ‰ and $\delta^{18}O_{drinking\ water}$ value of –10.6 ‰. Higher $\delta^{2}H$ and $\delta^{18}O$ were measured for the second sample from this individual, which was collected during summer, when warmer weather and higher evaporative potential, and therefore evapoconcentration effects on the Grand River are stronger than in November, when the first sample was collected. The 1.1 ‰ and 0.3 ‰ separation between the respective $\delta^{2}H_{drinking\ water}$ and $\delta^{18}O_{drinking\ water}$ of the two samples suggests the contribution of isotopically heavier precipitation and higher evaporative potential to the Grand River during summer has a negligible effect on the stable isotope composition of the Waterloo Region municipal water supply (Ruan et al., 2020; West et al., 2014).

3.2 Hair Isotope Results

When sample quantities permitted stable isotope measurements for multiple subsamples from the same individual, the arithmetic average was calculated. A standard deviation (SD) was calculated for samples analysed in triplicate. These average values (± SD; where applicable) are presented Table 3.1. Results for all stable isotope measurements performed for all hair subsamples are listed in Appendix C.

3.2.1 δ^2H_{hair} Results

Values of δ^2H_{hair} span a range of 24.2 ‰, with a minimum value of –86.8 ‰, and a maximum value of –62.6 ‰. The full δ^2H_{hair} range was observed in participants who reported London municipal tap water as their sole drinking water source (Table 3.1). Measured δ^2H_{hair} were plotted against measured $\delta^2H_{drinking\ water}$. These data are compared against predicted δ^2H_{hair} compositions using published models for populations consuming a 'supermarket' diet (Ehleringer et al., 2008; Fraser & Meier-Augenstein, 2007), transitional diet (Thompson et al., 2010), and pre-globalized diet (Bowen et al., 2009) in Figure 3.1. Linear regression equations used by each predictive model are listed in Table 3.2.

An isotopic separation between hair and drinking ($\Delta^2H_{hair\text{-}water}$) was calculated for participants who reported the London municipal tap water supply as their sole drinking water source. The minimum $\Delta^2H_{hair\text{-}water}$ was –9.5 ‰, and the maximum was –29.6 ‰ (Table 3.1) with a mean $\Delta^2H_{hair\text{-}water}$ of –17.1 ± 6.2 ‰ (n = 18). This result compares favorably with an average separation of –17 ± 9 ‰, reported by Sharp et al. (2003); however, the standard deviation obtained in this study is smaller than the previously reported value, as predicted in section 1.2.2.

When δ^2H_{hair} was analyzed by dietary group (Fig. 3.2), the lowest observed values (Table 3.3) were found for vegetarians with a mean δ^2H_{hair} of –86.8 ‰ (n = 2), followed by carnivores with a mean δ^2H_{hair} of –80.3 ± 5.1 ‰ (n = 3). Semi-vegetarians had the second highest mean δ^2H_{hair}, at –71.8 ± 6.6 ‰

Table 3.1. Mean participant $\delta^2H_{drinking\ water}$, $\delta^{18}O_{drinking\ water}$, $\Delta^2H_{hair\text{-}water}$, and $\Delta^{18}O_{hair\text{-}water}$ obtained in this study. Sample names encode sample collection location in the first letter (in this case 'L' for 'London'). The second letter denotes dietary group ('C' for 'carnivore', 'O' for 'omnivore', 'S' for 'semi-vegetarian', and 'V' for 'vegetarian'). The third letter denotes the primary reported sources of drinking water ('T' for 'tap' water, whether supplied by the municipal water supply or private wells, and 'B' for 'bottled' water). Data for participants who reported consuming drinking water from sources other than London municipal tap water are shown in bold font and excluded from the mean $\Delta^2H_{hair\text{-}water}$ and $\Delta^{18}O_{hair\text{-}water}$ calculation, given the potentially variable isotopic compositions of bottled and ground waters among source localities (Bowen et al., 2005b; Chesson et al., 2010a, 2010c).

Sample Name	***Mean*** $\delta^2H_{drinking\ water}$ **(‰ VSMOW)**	**Mean** δ^2H_{hair} **(‰ VSMOW)**	**Hair SD (‰)**	$\Delta^2H_{Hair\text{-}water}$ **(‰)**	**Mean** $\delta^{18}O_{drinking\ water}$ **(‰ VSMOW)**	**Mean** $\delta^{18}O_{hair}$ **(‰ VSMOW)**	**Hair SD (‰)**	$\Delta^{18}O_{hair\text{-}water}$ **(‰)**
LCT 1	–53.7	–83.3	15.3	–29.6	–7.4	10.8	0.7	18.2
LCT 2	–53.6	–74.3	8.6	–20.8	–7.5	11.1	0.3	18.6
LCT 3	–53.9	–83.1	7.1	–29.1	–7.4	9.9	0.1	17.3
LOB 1	**–73.5**	**–82.4**	**3.0**	**–8.8**	**–11.4**	**9.4**	**0.6**	**18.8**
LOT 1	–53.7	–	–	–	–7.5	–	–	–

LOT 2	–53.5	–	–	–	–7.4	11.4	–	18.8
LOT 3	–54.1	–69.5	4.0	–15.4	–7.5	10.4	0.4	17.9
LOT 4	**–62.2**	**–69.0**	**–**	**–6.8**	**–9.1**	**11.1**	**–**	**18.8**
LOT 5	–54.0	–63.5	–	–9.5	–7.7	12.0	0.1	19.7
LOT 6	–53.7	–72.4	6.4	–18.7	–7.4	11.1	0.1	18.6
LOT 7	**–71.9**	**–73.9**	**3.5**	**–1.9**	**–11.0**	**8.2**	**0.2**	**18.8**
LOT 8	–54.3	–64.6	–	–10.3	–7.5	11.7	–	19.2
LOT 9	–53.8	–65.9	0.9	–12.2	–7.6	11.1	0.3	18.7
LOT 10	–53.3	–75.0	3.8	–21.7	–7.4	11.6	1.2	19.0
LOT 11	–53.6	–69.9	1.9	–16.4	–7.6	11.4	0.2	19.0
LOT 12	–54.3	–66.4	2.4	–12.1	–7.5	10.2	0.5	17.7
LOT 13	–53.6	–68.3	–	–14.8	–7.7	9.7	0.7	17.4
LOT 14	–52.9	–62.6	–	–9.7	–7.4	10.9	–	18.3
LOT 15	–55.0	–	–	–	–7.7	–	–	–
LOT 16	–55.5	–65.6	0.9	–10.1	–7.7	11.8	0.4	19.5
LOT 17	–52.9	–76.6	11.3	–23.7	–7.5	10.3	0.4	17.8
LOT 18	–56.3	–	–	–	–7.9	11.4	–	19.3

LST 1	–53.3	–73.5	3.8	–20.2	–7.4	11.6	0.4	19.0
LST 2	–53.7	–71.6	4.2	–17.9	–7.6	11.5	0.2	19.1
LST 3	–54.1	–70.2	1.1	–16.2	–7.6	11.2	0.9	18.8
LVT 1	**–71.0**	**–86.8**	**–**	**–15.8**	**–10.4**	**10.9**	**–**	**18.8**
LVT 2	–53.8	–	–	–	–7.4	10.7	–	18.1

Table 3.2. Linear regression equations for relationships between δ^2H_{hair} and $\delta^2H_{drinking\ water}$, and $\delta^{18}O_{hair}$ and $\delta^{18}O_{drinking\ water}$ for 'supermarket,' transitional, and pre-globalized diets.

Dietary Composition	Tissue	δ^2H Equations	$\delta^{18}O$ Equations	Reference
Pre-globalized	Hair	$\delta^2H_{Hair}=0.78\delta^2H_{drinking\ water}-49.45$	$\delta^{18}O_{Hair} = 0.7\delta^{18}O_{drinking\ water} + 19.24$	Bowen et al. (2009)
Transitional	Hair	$\delta^2H_{Hair}=0.4\delta^2H_{drinking\ water}-75$	$\delta^{18}O_{Hair} = 0.4\delta^{18}O_{drinking\ water} + 16.4$	Thompson et al. (2010)
Supermarket	Hair	$\delta^2H_{Hair}=0.271\delta^2H_{drinking\ water}-79$	$\delta^{18}O_{Hair} = 0.353\delta^{18}O_{drinking\ water} + 15.2$	Ehleringer et al. (2008)
Supermarket	Hair	$\delta^2H_{hair}=0.49\delta^2H_{drinking\ water}-35.28$	–	Fraser & Meier-Augenstein (2007)
Supermarket	Nail	$\delta^2H_{nail}=0.37\delta^2H_{drinking\ water}-48.69$	$\delta^{18}O_{nail} = 0.4\delta^{18}O_{drinking\ water} – 15.1$	Fraser & Meier-Augenstein (2007)

(n = 3), and omnivores the highest, with a mean δ^2H_{hair} of –69.7 ± 5.5 ‰ (n = 18). The limited variation of δ^2H in London municipal tap water constrains responsibility for variations in δ^2H_{hair} across dietary groups to differences in dietary input δ^2H and metabolic rates between sampled individuals (Ehleringer et al., 2008; Gretebeck et al., 1997; Sharp et al., 2003). In other words, isotopically, "you are what you eat" (DeNiro & Epstein, 1976).

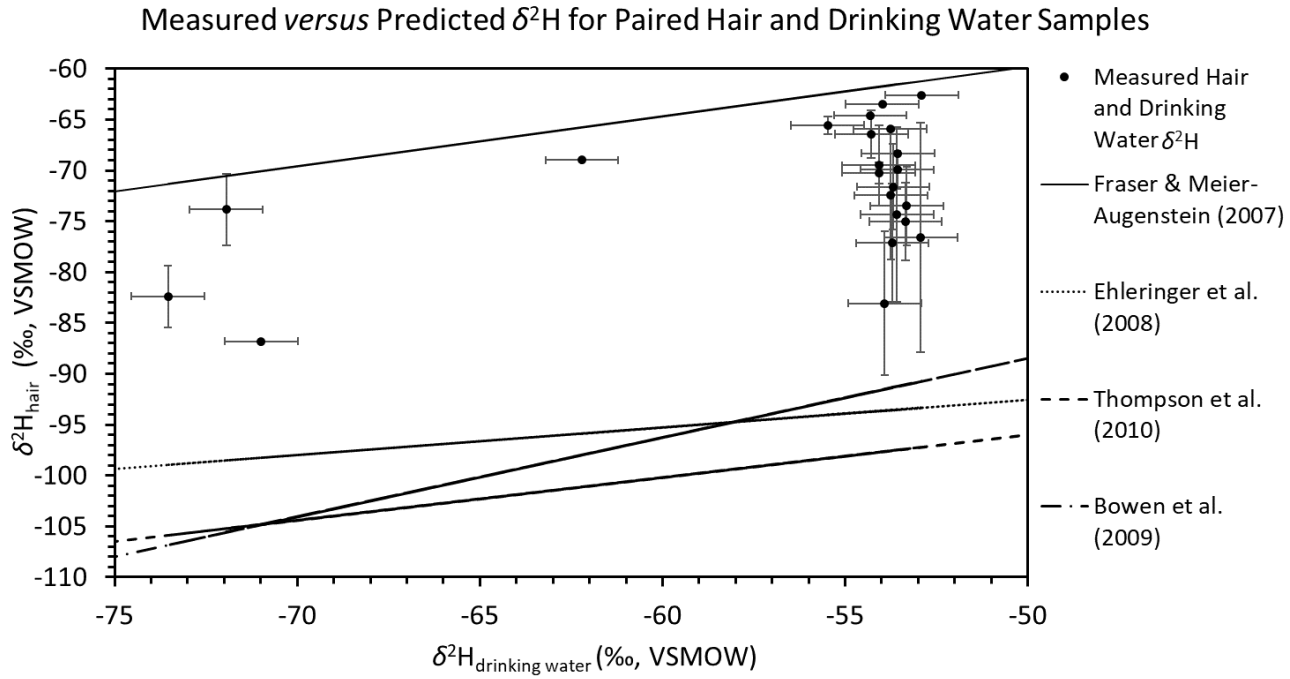


Figure 3.1. Measured δ^2H_{hair} and $\delta^2H_{drinking\ water}$ compared against predicted δ^2H_{hair} based on $\delta^2H_{drinking\ water}$ for populations consuming 'supermarket', transitional, and pre-globalized diets. Vertical error bars denote ± 1 SD for samples analyzed in triplicate, horizontal error bars denote analytical precision of ± 1 ‰ for water samples.

Results for all statistical tests performed on mean dietary group δ^2H_{hair} are listed in Table 3.4. Mann-Whitney U tests reveal statistically significant differences between δ^2H_{hair} values of omnivores and

carnivores, and between semi-vegetarians and carnivores (p = 0.002 and 0.025, respectively). No statistically significant differences were found between semi-vegetarian and omnivore δ^2H_{hair}. When semi-vegetarian δ^2H_{hair} values are included with omnivores, the mean δ^2H_{hair} of both dietary groups

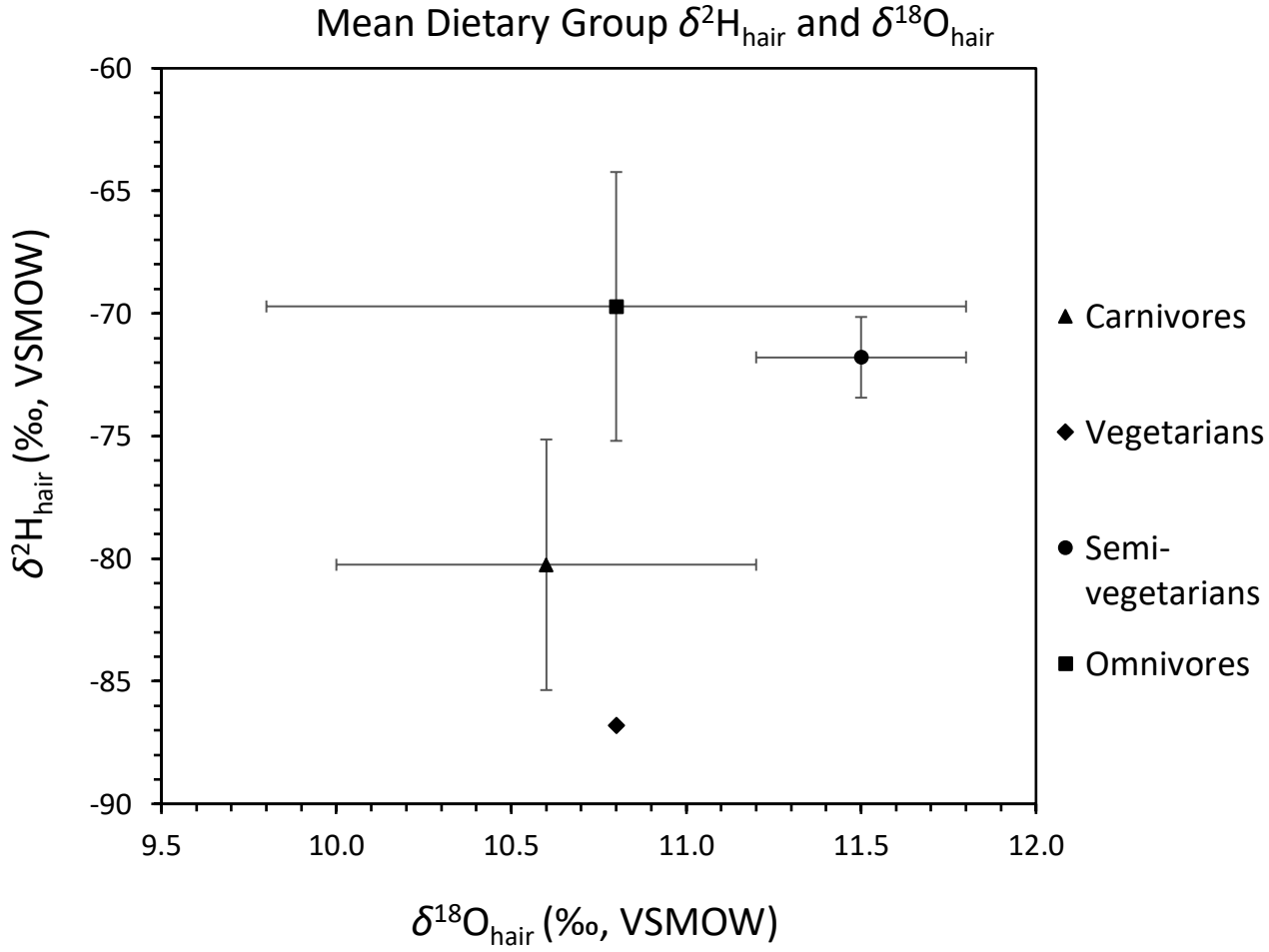


Figure 3.2. Mean dietary group δ^2H_{hair} and $\delta^{18}O_{hair}$, ± SD (error bars where possible). Note, insufficient sample material existed to perform triplicate analyses and calculate vegetarian δ^2H_{hair} and $\delta^{18}O_{hair}$ SD. Hence, this dietary group lacks error bars.

decreases by only 0.4 ‰ to –70.1 ± 5.1 ‰. When participants who reported drinking water from sources other than London municipal water were removed from the evaluation, the mean δ^2H_{hair} of the

combined London omnivores and semi-vegetarians consuming only municipal tap water increased slightly to –69.1 ± 4.3 ‰.

The occurrence of the lowest δ^2H_{hair} in vegetarians is consistent with trophic enrichment in ^2H in tissues (Birchall et al., 2005); however, the mean carnivore and semi-vegetarian δ^2H_{hair} values are not consistent with the expected trend of trophic enrichment. Mean participant $\delta^2H_{drinking\ water}$, δ^2H_{hair}, and calculated $\Delta^2H_{hair\text{-}water}$ values are summarized in Table 3.1.

Table 3.3. Mean dietary group δ^2H_{hair} and $\delta^{18}O_{hair}$. [a]Includes isotopic data for semi-vegetarians in the omnivore dietary group calculations. [b] Excludes participants who consumed drinking water from sources other than the London municipal water supply; includes semi-vegetarians. [c]Excludes participants who consumed drinking water from sources other than London municipal water. [d]Includes semi-vegetarians and carnivores in omnivore dietary group.

Diet Type	Mean δ^2H_{Hair}	SD (‰)	*n*	Mean $\delta^{18}O_{Hair}$	SD (‰)	*n*
Vegetarian	–86.8	–	1	10.8	–	2
	–69.7	5.5	15	10.8	1.0	17
Omnivore	–70.1	5.1	18[a]	11.0	0.7	14[c]
	–69.1	4.3	15[b]	10.9	0.9	23[d]
Semi-vegetarian	–71.8	1.6	3	11.5	0.3	3
Carnivore	–80.2	5.1	3	10.6	0.6	3

Table 3.4. Statistical comparisons of $\delta^{18}O_{hair}$ and $\delta^{2}H_{hair}$ across dietary groups containing at least 3 individuals. Statistically significant results (p-value ≤ 0.05) are shown in bold font. "TT" test type refers to an unpaired t-test, and "M-W" refers to a Mann-Whitney U-test.

	$\delta^{2}H$		$\delta^{18}O$	
Comparison Groups	p-value	Test Type	p-value	Test Type
omnivores versus carnivores	**0.002**	M-W	0.597	TT
omnivores versus semi-vegetarians	0.303	M-W	0.207	TT
carnivores versus semi-vegetarians	**0.025**	M-W	0.217	TT

3.2.2 $\delta^{18}O_{hair}$ Results

Values of $\delta^{18}O_{hair}$ (Table 3.1) span a range of 3.7 ‰, with a minimum value of +8.2 ± 0.2 ‰ and a maximum of +12.0 ± 0.1 ‰. The sample with the lowest $\delta^{18}O_{hair}$ was obtained from a participant in the Waterloo Region who drank a combination of municipal tap ($\delta^{18}O$ = –10.7 ‰) and bottled water (mean $\delta^{18}O$ = –13.5 ‰). The sample with the highest $\delta^{18}O_{hair}$ was obtained from a participant who exclusively drank London municipal tap water. Measured $\delta^{18}O_{hair}$ was plotted against measured $\delta^{18}O_{drinking\ water}$. These data were compared against predicted $\delta^{18}O_{hair}$ in Figure 3.3 using models for populations consuming 'supermarket' (Ehleringer et al., 2008), transitional (Thompson et al., 2010), and pre-globalized (Bowen et al., 2009) diets (Table 3.2).

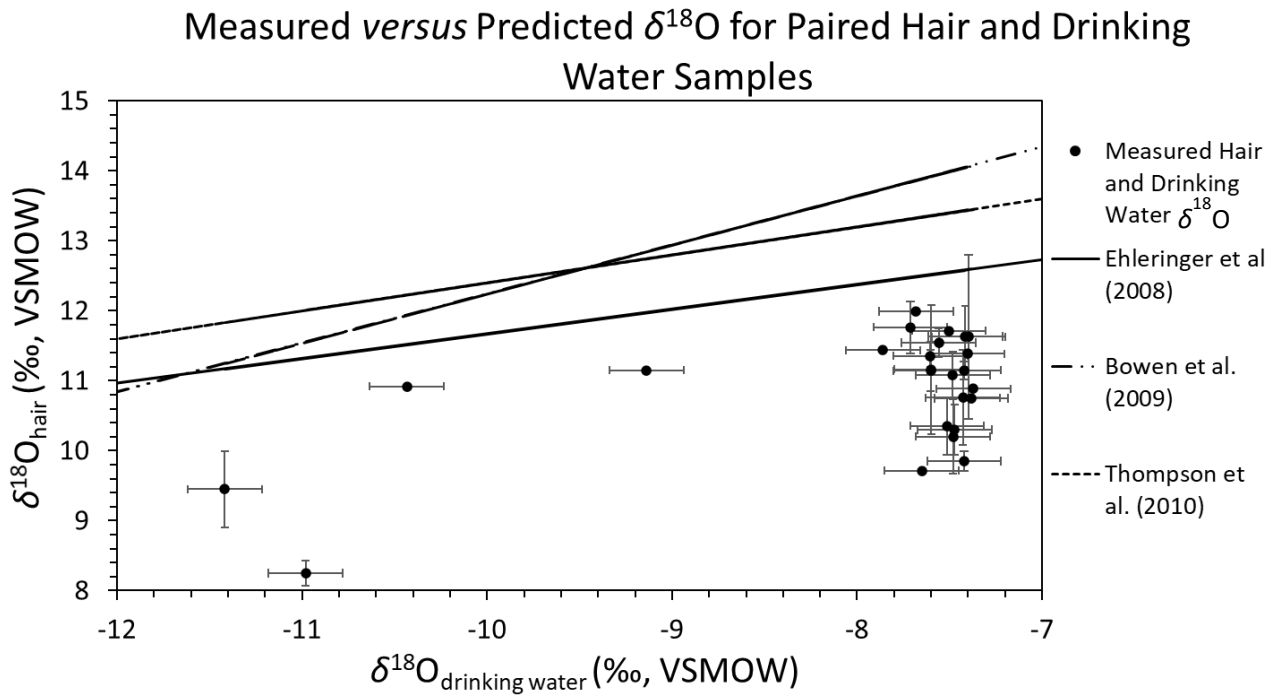


Figure 3.3. Measured $\delta^{18}O_{hair}$ and $\delta^{18}O_{drinking\ water}$ versus predicted $\delta^{18}O_{hair}$ using published models of $\delta^{18}O_{hair}$ and $\delta^{18}O_{drinking\ water}$ relationships. Vertical error bars denote ± SD for samples with sufficient material to perform analyses in triplicate. Horizontal error bars denote analytical error of ± 0.2 ‰.

An oxygen isotope separation between hair and drinking water ($\Delta^{18}O_{hair\text{-}water}$) was calculated for participants who reported the London municipal tap water supply as their sole source of drinking water. The range of $\Delta^{18}O_{hair\text{-}water}$ in the present study was 2.4 ‰, with a minimum $\Delta^{18}O_{hair\text{-}water}$ of +17.3 ‰ and a maximum of +19.7 ‰ (Table 3.1). The mean $\Delta^{18}O_{hair\text{-}water}$ for all participants consuming London municipal tap water was +18.6 ± 0.7 ‰ (n = 21). While numerous studies have used oxygen isotopes in modern human hair and nails as a migration tracer (e.g., Fraser & Kalin, 2006; Meier-Augenstein & Fraser, 2008; O'Brien & Wooller, 2007), I am unaware of studies that have investigated the separation of oxygen isotope compositions in paired tissue and drinking water samples, as has been done in the present investigation. When analyzed by dietary group (Fig 3.2), the lowest $\delta^{18}O_{hair}$ was associated with

carnivores (mean $\delta^{18}O_{hair}$ = +10.6 ± 0.6 ‰ *n* = 3), followed by vegetarians (mean $\delta^{18}O_{hair}$ = +10.8 ‰, *n* = 2) and omnivores (mean $\delta^{18}O_{hair}$ = +10.8 ± 1.0 ‰, *n* = 19), and semi-vegetarians (mean $\delta^{18}O_{hair}$ = +11.5 ± 0.3 ‰, *n* = 3). When participants who reported drinking water from sources other than London municipal water were removed from the calculation, the mean $\delta^{18}O_{hair}$ of London omnivores increased slightly to +11.0 ± 0.7 ‰ (*n* = 14) (Table 3.3).

Unpaired t-tests did not reveal any statistically significant differences between omnivores and semi-vegetarians, omnivores and carnivores, or carnivores and semi-vegetarians (Table 3.4). A summary of mean participant $\delta^{18}O_{hair}$, $\delta^{18}O_{drinking}$ and $\Delta^{18}O_{hair\text{-}water}$ values is presented in Table 3.1. As for hydrogen isotopes, the limited variation in London municipal water supply $\delta^{18}O$ suggests most variation in $\delta^{18}O_{hair}$ across dietary groups and individual participants can be attributed to differences in the $\delta^{18}O$ of foods consumed by each dietary group. To a more limited extent, inter-individual variations in metabolism, such as turnover rates of amino acids and various body water pools, are also likely contributing factors (Ehleringer et al., 2008; Gretebeck et al., 1997). When the omnivore, carnivore and semi-vegetarian dietary groups were analyzed together, the mean $\delta^{18}O_{hair}$ increased slightly to +10.9 ± 0.9 ‰.

3.2.3 $\delta^{13}C_{hair}$ Results

Mean participant $\delta^{13}C_{hair}$ ± SD are listed in Table 3.5. Values of $\delta^{13}C_{hair}$ span a range of 3.8 ‰, with a minimum of –20.6 ‰ and a maximum of –16.8 ± 0.1 ‰. The highest $\delta^{13}C_{hair}$ was obtained for a participant living near London, who reported consuming an omnivorous diet. The lowest $\delta^{13}C_{hair}$ was obtained for a participant who reported consuming a semi-vegetarian diet. When analyzed by dietary group (Fig. 3.4), the lowest $\delta^{13}C_{hair}$ was observed in semi-vegetarians, followed closely by vegetarians, omnivores, and lastly, carnivores with mean $\delta^{13}C_{hair}$ of –19.9 ± 0.9 ‰ (*n* = 3), –19.3 ‰ (*n* = 2), –18.6 ± 0.9 ‰ (*n* = 11), and –17.9 ± 0.3 ‰ (*n* = 3), respectively. This general enrichment in ^{13}C for hair from

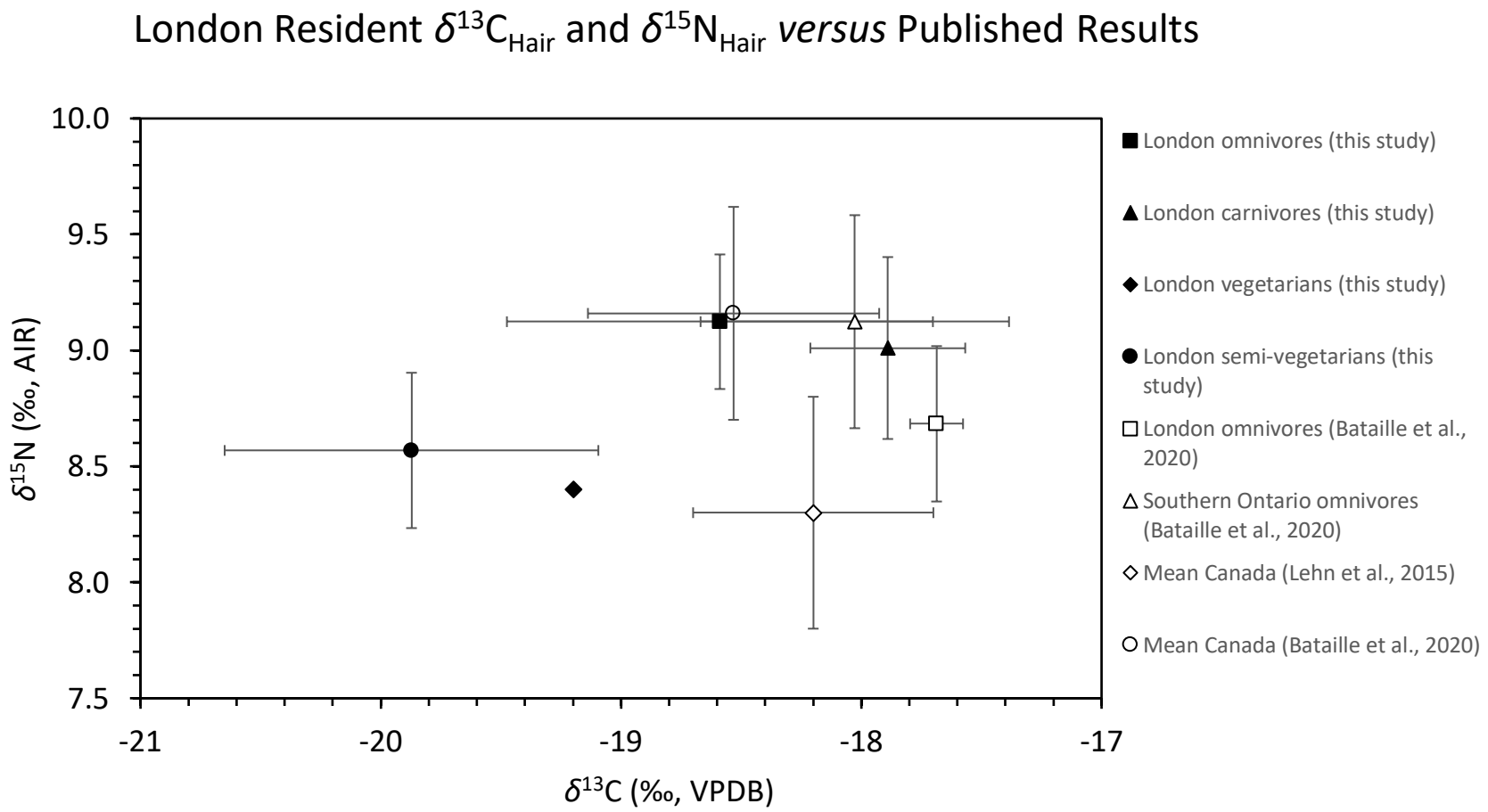


Figure 3.4. Mean dietary group $\delta^{13}C_{hair}$ versus $\delta^{15}N_{hair}$ in this study (solid markers) and previously reported mean values for omnivores living in London and the surrounding southern Ontario region. National mean stable carbon and nitrogen isotope compositions for Canada are also shown (open markers) (Bataille et al., 2020; Lehn et al., 2015).

vegetarians to carnivores is consistent with stepwise ^{13}C-enrichment of tissues between trophic levels (Fuller et al., 2006; Schoeller et al., 1986; Yoshinaga et al., 1996;).

A published $\delta^{13}C_{hair}$ of –17.7 ± 0.1 ‰ (n = 3) for London omnivores sampled prior to 2020, and before the onset of the COVID-19 pandemic (Bataille et al., 2020), is within error of the mean $\delta^{13}C_{hair}$ for London omnivores obtained in this study. A mean published $\delta^{13}C_{hair}$ of –18.0 ± 0.6 ‰ (n = 24) for southern Ontario omnivores (Bataille et al., 2020) is slightly higher than the $\delta^{13}C_{hair}$ of London omnivores obtained in the present study and slightly lower than the published London omnivore carbon isotope composition, but is still within error of both London omnivore results. Mean published southern Ontario (Bataille et al., 2020) and measured (this study) London omnivore $\delta^{13}C_{hair}$ are within error of a mean Canadian national value of –18.5 ± 0.6 ‰ (n = 581) obtained by Bataille et al. (2020) and also within error of a second, similar, national mean of –18.2 ± 0.5 ‰ (n = 15) obtained by Lehn et al. (2015). Additionally, the previously reported London omnivores (Bataille et al., 2020) and carnivores measured in the present study have mean $\delta^{13}C_{hair}$ within error of the pre-COVID-19 national mean.

The mean $\delta^{13}C_{hair}$ of vegetarians and semi-vegetarians obtained in the present study are somewhat lower than the mean Canadian values (Fig 3.4) reported by Bataille et al. (2020) and Lehn et al. (2015). Statistical tests (Table 3.7) comparing published (Bataille et al., 2020) $\delta^{13}C_{hair}$ for London and southern Ontario omnivores with $\delta^{13}C_{hair}$ obtained for London omnivores in the present study found no significant differences between the comparison groups.

3.2.4 $\delta^{15}N_{hair}$ Results

Mean participant $\delta^{15}N_{hair}$ obtained in the present study are listed in Table 3.5. Values of $\delta^{15}N_{hair}$ span a range of 1.4 ‰. A minimum $\delta^{15}N_{hair}$ of +8.2 ± 0.1 ‰ ‰ was measured for an individual who

Table 3.5. Mean hair and nail $\delta^{13}C$ and $\delta^{15}N$, and $\Delta^{13}C_{hair\text{-}nail}$ and $\Delta^{15}N_{hair\text{-}nail}$ for participants in this study. Sample names encode sample collection location in the first letter (in this case 'L' for 'London'). The second letter denotes dietary group ('C' for 'carnivore', 'O' for 'omnivore', 'S' for 'semi-vegetarian', and 'V' for 'vegetarian'). The third letter denotes the primary reported sources of drinking water ('T' for 'tap' water, whether supplied by the municipal water supply or private wells, and 'B' for 'bottled' water).

	Hair						Nail			
Sample Name	**Mean $\delta^{13}C$ (‰, VPDB)**	**SD (‰)**	**Mean $\delta^{15}N$ (‰, AIR)**	**SD (‰)**	**$\Delta^{13}C_{hair\text{-}nail}$ (‰)**	**$\Delta^{15}N_{hair\text{-}nail}$ (‰)**	**Mean $\delta^{13}C$ (‰, VPDB)**	**SD (‰)**	**Mean $\delta^{15}N$ (‰ AIR)**	**SD (‰)**
LCT 1	–18.1	0.2	+8.6	0.1	0.7	0.3	–18.9	0.1	+9.0	0.1
LCT 2	–17.7	0.0	+9.1	0.1	0.4	0.4	–18.1	0.1	+9.5	0.1
LCT 3	–17.6	0.4	+9.5	0.2	0.0	0.1	–17.6	0.3	+9.6	0.1
LOB 1	–16.8	0.1	+9.4	0.1	0.7	0.4	–17.5	0.2	+9.9	0.2
LOT 1	–	–	–	–	–	–	–19.4	0.1	+8.8	0.1
LOT 2	–	–	–	–	–	–	–18.7	0.1	+9.5	0.1
LOT 3	–18.1	0.7	+9.6	0.3	0.2	0.2	–18.3	0.0	+9.8	0.1
LOT 4	–	–	–	–	–	–	–18.3	0.1	+10.1	0.1
LOT 5	–19.4	–	–	–	0.5	0.6	–19.8	0.0	+9.5	0.1
LOT 6	–19.8	–	+9.1	–	0.2	0.4	–20.0	0.2	+9.5	0.1
LOT 7	–17.3	–	+9.5	–	0.4	0.7	–17.6	0.1	+10.2	0.1
LOT 8	–	–	–	–	–	–	–17.8	0.2	+9.2	0.1
LOT 9	–18.7	–	+8.6	–	0.1	0.6	–18.8	0.2	+9.2	–
LOT 10	–18.2	0.1	+9.3	0.1	0.6	0.3	–18.8	0.5	+9.6	–
LOT 11	–19.1	0.0	+8.9	0.0	0.6	0.7	–19.7	–	+9.5	–
LOT 12	–19.0	0.1	+9.2	0.3	0.3	9.6	–19.3	0.1	+9.6	0.1
LOT 13	–19.6	0.1	+8.9	0.1	0.2	0.5	–19.8	0.1	+9.4	–
LOT 14	–	–	–	–	–	–	–18.9	0.0	+9.8	0.1

LOT 15	–	–	–	–	–	–	–19.4	0.1	+8.8	0.0
LOT 16	–	–	–	–	–	–	–18.1	0.2	+8.9	0.5
LOT 17	–18.7	0.1	+9.0	0.1	0.1	0.6	–18.8	0.1	+9.6	0.1
LOT 18	–	–	–	–	–	–	–18.5	0.0	+9.1	0.0
LST 1	–20.6	–	+8.2	–	0.9	0.5	–21.4	0.1	+8.7	0.1
LST 2	–18.9	–	+8.9	–	2.6	0.4	–21.4	0.0	+9.4	0.1
LST 3	–20.0	–	+8.8	–	0.4	0.5	–20.4	0.1	+9.3	0.1
LVT 1	–19.1	–	+8.2	–	–0.1	1.0	–19.0	0.1	+9.2	0.1
LVT 2	–19.3	–	+8.6	–	0.6	0.0	18.6	0.2	+8.6	0.1

reported consuming a semi-vegetation diet. A maximum $\delta^{15}N_{hair}$ of +9.6 ± 0.3 ‰ was measured for an individual who reported consuming an omnivorous diet. When analyzed by dietary group (Fig. 3.4), vegetarians possessed the lowest mean $\delta^{15}N_{hair}$ (+8.4 ‰; *n* = 2), followed by semi-vegetarians (+8.6 ± 0.4 ‰; *n* = 3), carnivores (+9.0 ± 0.4 ‰; *n* = 3), and finally omnivores (+9.1 ± 0.3 ‰; *n* = 11).

Mean omnivore and carnivore $\delta^{15}N_{hair}$ are similar enough that a mean $\delta^{15}N_{hair}$ calculated from the combined values of carnivores and omnivores is +9.1 ± 0.4 ‰, a value identical to the mean $\delta^{15}N_{hair}$ for omnivores alone. As such, carnivores are included with omnivores in subsequent calculations in which London omnivore $\delta^{15}N_{hair}$ is mentioned. Similarly, when vegetarians and semi-vegetarians are lumped together, the mean $\delta^{15}N_{hair}$ changes only slightly to +8.5 ± 0.3 ‰. The general trend of increasing $\delta^{15}N_{hair}$ from vegetarians to carnivores is consistent with increasing ^{15}N-enrichment of consumed tissues that occupied higher trophic levels (Buchardt et al., 2007; Hedges & Reynard, 2007; Huelsemann et al., 2009; O'Connell & Hedges, 1999). An exception to this trend is observed in omnivore and carnivore $\delta^{15}N_{hair}$. The importance of marine versus terrestrial protein sources on $\delta^{15}N_{tissue}$ compositions, and the natural $\delta^{15}N_{tissue}$ variability within a trophic level are detailed in Section 1.2.4. Table 3.6 summarizes the proportion of terrestrial versus marine proteins that comprise participant's diets per screening questionnaire responses.

Pre-COVID-19 $\delta^{15}N_{hair}$ values for southern Ontario omnivores measured by Bataille et al. (2020), with a mean $\delta^{15}N_{hair}$ value of +9.1 ± 0.5 ‰ (*n* = 24), are identical to the mean value of London omnivores obtained in this study ($\delta^{15}N_{hair}$ = +9.1 ± 0.3, *n* = 14). A published pre-COVID-19 mean $\delta^{15}N_{hair}$ value for London omnivores of +8.7 ± 0.3 ‰ (*n* = 3) is slightly lower than the mean value of London omnivores in this study, and published southern Ontario omnivores (Bataille et al., 2020). Published (Bataille et al., 2020) and measured (this study) mean $\delta^{15}N_{hair}$ for all dietary groups are higher than a pre-COVID-19 Canadian national mean $\delta^{15}N_{hair}$ (+8.3 ± 0.5 ‰, *n* = 15) reported by Lehn et al. (2015), A second national mean $\delta^{15}N_{hair}$ of +9.2 ± 0.5 ‰, calculated from Bataille et al. (2020), is slightly higher than mean

published London omnivores and is within error for London omnivores and carnivores (this study), as well as published southern Ontario omnivores (Bataille et al., 2020).

Table 3.6. Fraction of terrestrial versus marine proteins in participant diets. A score of 1 corresponds to 100% terrestrial protein consumption and a score of 0.1 corresponds to 100% marine protein consumption. Sample names encode sample collection location in the first letter (in this case 'L' for 'London'). The second letter denotes dietary group ('C' for 'carnivore', 'O' for 'omnivore', 'S' for 'semi-vegetarian', and 'V' for 'vegetarian'). The third letter denotes the primary reported sources of drinking water ('T' for 'tap' water, whether supplied by the municipal water supply or private wells, and 'B' for 'bottled' water).

Participant ID	Proportion of Terrestrial vs. Marine Protein in Diet
LCT 1	0.9
LCT 2	0.8
LCT 3	0.8
LOB 1	0.8
LOT 1	0.5
LOT 2	0.7
LOT 3	0.9
LOT 4	0.8
LOT 5	0.3
LOT 6	–
LOT 7	0.7
LOT 8	0.8
LOT 9	0.7
LOT 10	0.9
LOT 11	0.6
LOT 12	0.7
LOT 13	0.3
LOT 14	0.5
LOT 15	0.9
LOT 16	0.7
LOT 17	0.9
LOT 18	0.8

LST 1	0.4
LST 2	0.9
LST 3	0.8
LVT 1	1
LVT 2	0.1

Mann-Whitney U tests were performed between $\delta^{15}N_{hair}$ measured for London omnivores (this study) and pre-COVID data for London omnivores published by Bataille et al. (2020), and between published data for pre-pandemic London and southern Ontario omnivores (Bataille et al., 2020). Unpaired t-tests were performed between $\delta^{15}N_{hair}$ data for London omnivores in this study and published pre-pandemic $\delta^{15}N_{hair}$ data from southern Ontario omnivores (Bataille et al., 2020). No statistically clear differences were found between any of the comparison groups (Table 3.7). Differences between the $\delta^{15}N_{hair}$ of London omnivores in this study, and pre-pandemic London omnivore values, as well as differences between pre-pandemic London and southern Ontario omnivores reported by Bataille et al. (2020), are border-line significant (p-value = 0.067 and 0.082, respectively).

Table 3.7. Statistical comparisons between measured (this study) and previously published omnivore hair $\delta^{13}C$ and $\delta^{15}N$. 'TT' and 'M-W' tests refer to unpaired t-tests and Mann-Whitney U tests, respectively.

	$\delta^{13}C_{Hair}$	$\delta^{15}N_{Hair}$
Statistical Comparison: London (this study) versus London (Bataille et al., 2020) Omnivores		
p-value	0.126	0.067
Test	M-W	M-W
Statistical Comparison: London (this study) versus southern Ontario (Bataille et al., 2020) Omnivores		
p-value	0.137	0.579
Test	TT	TT
Statistical Comparison: London (Bataille, et al., 2020) versus southern Ontario (Bataille et al., 2020) Omnivores		

p-value	0.123	0.082
Test	M-W	M-W

3.3 Nail Isotope Results

The arithmetic average was calculated for multiple nail subsamples from the same individual and these values are presented in this section. For samples analyzed in triplicate, a standard deviation was calculated and are presented in the form *mean ± SD*. Results for all stable isotope analyses are listed in Appendix C.

3.3.1 δ^2H_{nail} Results

Values of δ^2H_{nail} span a range of 18.4 ‰, with a minimum of –86.6 ± 3.1 ‰, measured for a participant living in the London area who reported primarily consuming bottled (δ^2H = –78.5 ‰) and well water (δ^2H = –68.6 ‰), and consuming an omnivorous diet. A maximum of –68.2 ± 2.6 ‰ was measured in a participant who reported exclusively consuming London municipal tap water and an omnivorous diet (Table 3.8). Measured δ^2H_{nail} were plotted against measured $\delta^2H_{drinking\ water}$. These data compared against predicted δ^2H_{nail} using models for participants consuming a 'supermarket' diet in Figure 3.5 (Fraser & Meier-Augenstein, 2007; Mancuso & Ehleringer, 2019). See Table 3.2 for equations used in the δ^2H_{nail} prediction models.

When analyzed by dietary group (Fig. 3.6), the lowest δ^2H_{nail} values were observed for vegetarians (–81.4 ‰; *n* = 2), followed by semi-vegetarians (–76.9 ± 3.3 ‰; *n* = 3), omnivores (–75.9 ± 5.0 ‰; *n* = 19), and lastly carnivores (–74.2 ± 2.8 ‰; *n* = 3). When participants consuming water from sources other than London municipal tap water were removed from the dataset, omnivore δ^2H_{nail} increased slightly to –75.0 ± 4.6 ‰ (*n* = 16) (Table 3.9). Results for all statistical tests performed on mean dietary group δ^2H_{nail} are listed in Table 3.10. Unpaired t-tests did not reveal any statistically significant differences between the δ^2H_{nail} values of any dietary groups, while Mann-Whitney U tests

revealed statistically significant differences between vegetarians and omnivores, and between vegetarians and carnivores (p-value = 0.006).

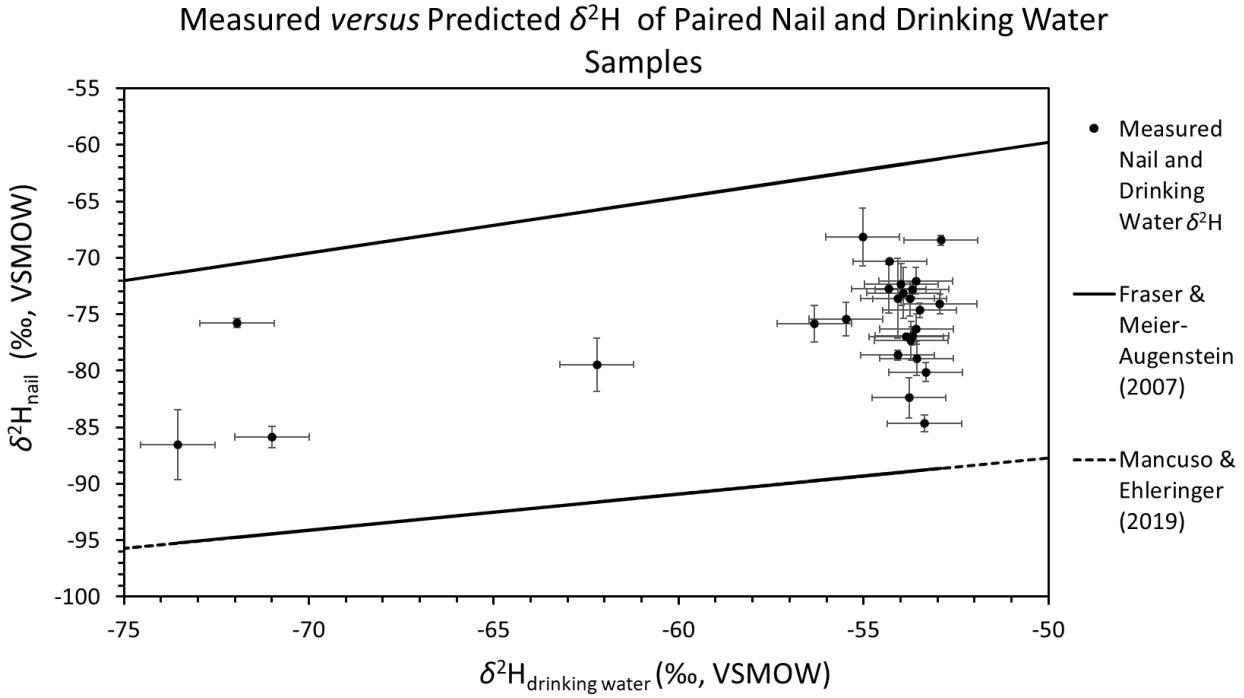


Figure 3.5. Measured δ^2H_{nail} and $\delta^2H_{drinking\ water}$ compared against predicted δ^2H_{nail} based on $\delta^2H_{drinking\ water}$ for populations consuming a 'supermarket' diet. Vertical error bars denote ± 1 SD for samples analyzed in triplicate, and horizontal error bars denote an analytical precision of ± 1 ‰ for water samples.

The observed trend of increasing δ^2H_{nail} from vegetarians to carnivores is consistent with observed patterns of ^{2}H-enrichment in tissues occupying higher trophic levels (Birchall et al., 2005; Buchardt et al., 2007). However, the similar mean δ^2H_{nail} among omnivores, semi-vegetarians, and carnivores suggests that these dietary groups consumed diets with similar hydrogen isotope compositions. When semi-vegetarian and carnivore δ^2H_{nail} values are combined with those of the

omnivores, the mean δ^2H_{nail} of the combined group changes only slightly from the original omnivore mean δ^2H_{nail} value, to –75.8 ± 4.6 ‰ (*n* = 25).

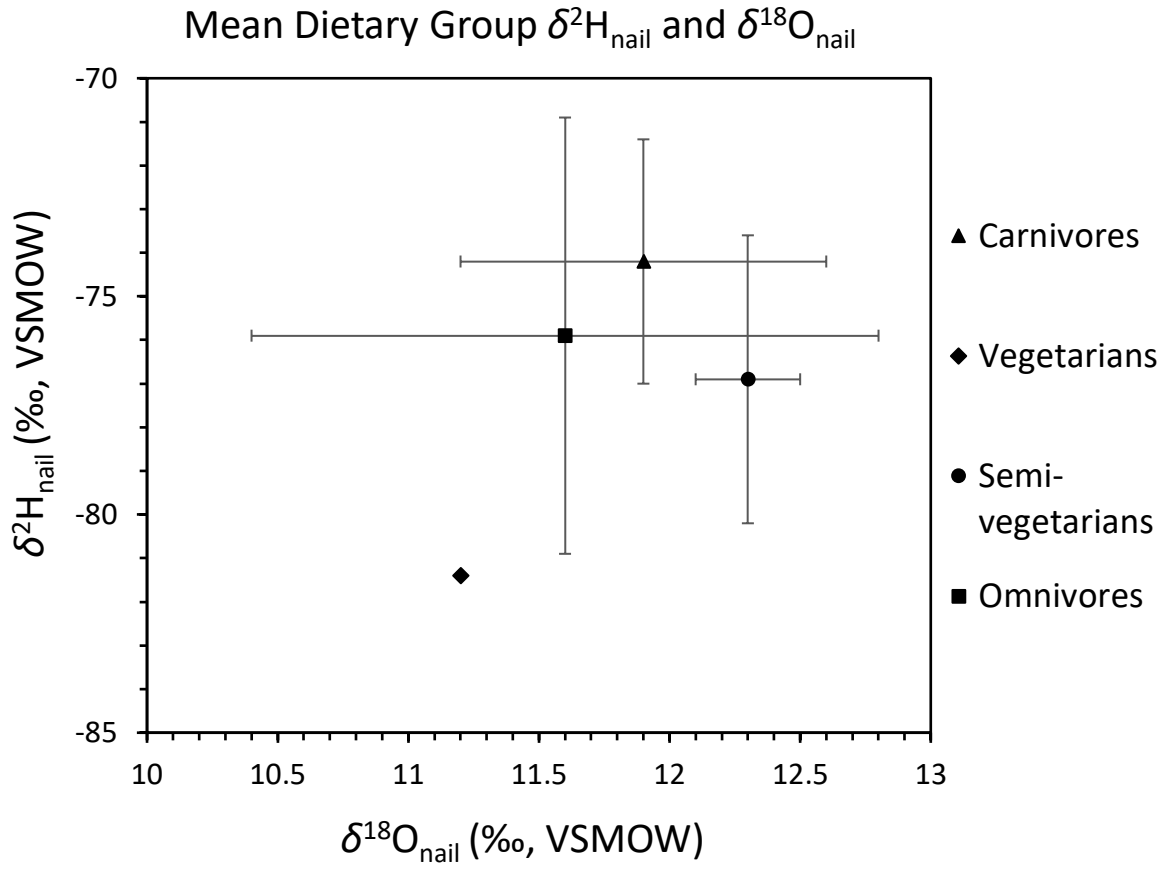


Figure 3.6. Mean dietary group δ^2H_{nail} and $\delta^{18}O_{nail}$ ± SD (error bars).

A $\Delta^2H_{nail\text{-}water}$ was calculated for all participants exclusively drinking London municipal tap water. Values of $\Delta^2H_{nail\text{-}water}$ ranged between –13.1 ‰ and –31.3 ‰, with a mean of –21.3 ± 4.3 ‰. Values of $\Delta^2H_{hair\text{-}nail}$ (*n* = 17) were highly variable and ranged between a minimum of –10.0 ‰, for a participant who reported exclusively drinking London municipal tap water and consuming a carnivorous diet, and a maximum of +16.5 ‰, for a participant who reported exclusively drinking London municipal tap water and consuming an omnivorous diet. The cause of the highly variable $\Delta^2H_{hair\text{-}nail}$ obtained in the present

Table 3.8. Mean drinking water and nail δ^2H and $\delta^{18}O$, $\Delta^2H_{nail\text{-}water}$, $\Delta^{18}O_{nail\text{-}water}$, $\Delta^2H_{hair\text{-}nail}$, and $\Delta^{18}O_{hair\text{-}nail}$ for all participants in this study. Data for participants who reported consuming drinking water from sources other than London municipal tap water are shown in bold font and excluded from the mean $\Delta^2H_{hair\text{-}water}$ and $\Delta^{18}O_{hair\text{-}water}$ calculations.

Sample Name	*Mean* $\delta^2H_{drinking\ water}$ (‰ VSMOW)	Mean δ^2H_{nail} (‰ VSMOW)	Fingernail SD (‰)	$\Delta^2H_{nail\text{-}water}$ (‰)	$\Delta^2H_{hair\text{-}nail}$ (‰)	Mean $\delta^{18}O_{drinking\ water}$ (‰ VSMOW)	Mean $\delta^{18}O_{nail}$ (‰ VSMOW)	Fingernail SD (‰)	$\Delta^{18}O_{nail\text{-}water}$ (‰)	$\Delta^{18}O_{hair\text{-}nail}$ (‰)
LCT 1	–53.7	–77.3	1.7	–23.6	–6.0	–7.4	+12.4	0.1	+19.9	–1.7
LCT2	–53.6	–72.1	1.2	–18.5	–2.3	–7.5	+12.1	0.2	+18.0	–1.0
LCT3	–53.9	–73.1	2.3	–19.2	–10.0	–7.4	+11.1	0.3	+18.6	–1.3
LOB 1	**–73.5**	**–86.6**	**3.1**	**–**	**–**	**–11.4**	**+9.7**	**0.5**	**–**	**–**
LOT 1	–53.7	–72.8	0.7	–19.1	–	–7.5	+12.6	0.1	+20.0	–
LOT 2	–53.5	–74.6	0.6	–21.2	–	–7.4	+10.6	0.1	+19.6	0.7
LOT 3	–54.1	–78.6	0.4	–24.5	+9.1	–7.5	+10.1	0.2	+17.6	0.3
LOT 4	**–62.2**	**–79.5**	**2.4**	**–**	**–**	**–9.1**	**+11.2**	**0.1**	**–**	**–**
LOT 5	–54.0	–72.3	1.9	–18.4	+8.8	–7.7	+11.9	0.1	+19.5	0.1
LOT 6	–53.7	–73.6	1.5	–19.9	+1.2	–7.4	+12.3	0.1	+19.7	–1.1
LOT 7	**–71.9**	**–75.8**	**0.4**	**–**	**–**	**–11.0**	**+8.7**	**0.6**	**–**	**–**
LOT 8	–54.3	–72.8	2.2	–18.4	+8.2	–7.5	+13.1	0.2	+20.6	–1.4
LOT 9	–53.8	–82.4	1.8	–28.6	+16.5	–7.6	+11.6	0.2	+19.2	–0.4
LOT 10	–53.3	–84.7	0.7	–31.3	+9.6	–7.4	+12.2	0.1	+19.6	–0.6
LOT 11	–53.6	–76.3	1.3	–22.7	+6.4	–7.6	+13.0	0.0	+20.6	–1.7
LOT 12	–54.3	–70.3	0.1	–16.1	+3.9	–7.5	+11.3	0.2	+18.8	–1.1
LOT 13	–53.6	–78.9	1.5	–25.3	+10.6	–7.7	+12.4	0.1	+20.1	–2.7
LOT 14	–52.9	–68.5	0.4	–15.6	+5.9	–7.4	+10.9	0.3	+18.3	–0.4
LOT 15	–55.0	–68.2	2.6	–13.1	–	–7.7	+12.0	0.1	+19.7	–

LOT 16	–55.5	–75.4	1.5	–19.9	–	–7.7	+12.3	0.1	+20.1	–0.6
LOT 17	–52.9	–74.1	0.9	–21.1	–2.5	–7.5	+10.8	0.1	+18.3	–0.5
LOT 18	–56.3	–75.8	1.6	–19.5	–	–7.9	+12.7	0.3	+20.6	–1.6
LST 1	–53.3	–80.1	0.8	–26.8	+6.6	–7.4	+12.4	0.3	+19.9	–0.8
LST 2	–53.7	–76.9	0.8	–23.2	+5.3	–7.6	+12.0	0.1	+19.6	–0.5
LST 3	–54.1	–73.6	3.5	–19.5	+3.4	–7.6	+12.4	0.1	+20.0	–1.3
LVT 1	**–71.0**	**–85.9**	**1.0**	**–**	**–**	**–10.4**	**+11.2**	**0.4**	**–**	**–**
LVT 2	–53.8	–77.0	0.2	–23.1	–	–7.4	+11.2	0.1	+18.6	–0.5

Table 3.9. Mean dietary group δ^2H_{hair} and $\delta^{18}O_{hair}$. [a]Excludes participants who reported consuming drinking water from sources other than London municipal water. [b]Includes carnivores and semi-vegetarians. [c]Includes carnivore δ^2H_{nail}.

Diet Type	Mean δ^2H_{nail} (‰, VSMOW)	SD (‰)	*n*	Mean $\delta^{18}O_{nail}$ (‰, VSMOW)	SD (‰)	*n*
Vegetarian	–81.4	–	2	+11.2	–	2
Semi-vegetarian	–76.9	3.3	3	+12.3	0.2	3
	–75.9	5.0	19	+11.6	1.2	19
Omnivores	–75.0	4.6	16[a]	+11.9	0.9	16[a]
	–75.8	4.6	25[b]	+11.9	0.9	22[c]
Carnivores	–74.2	2.8	3	+11.9	0.7	3

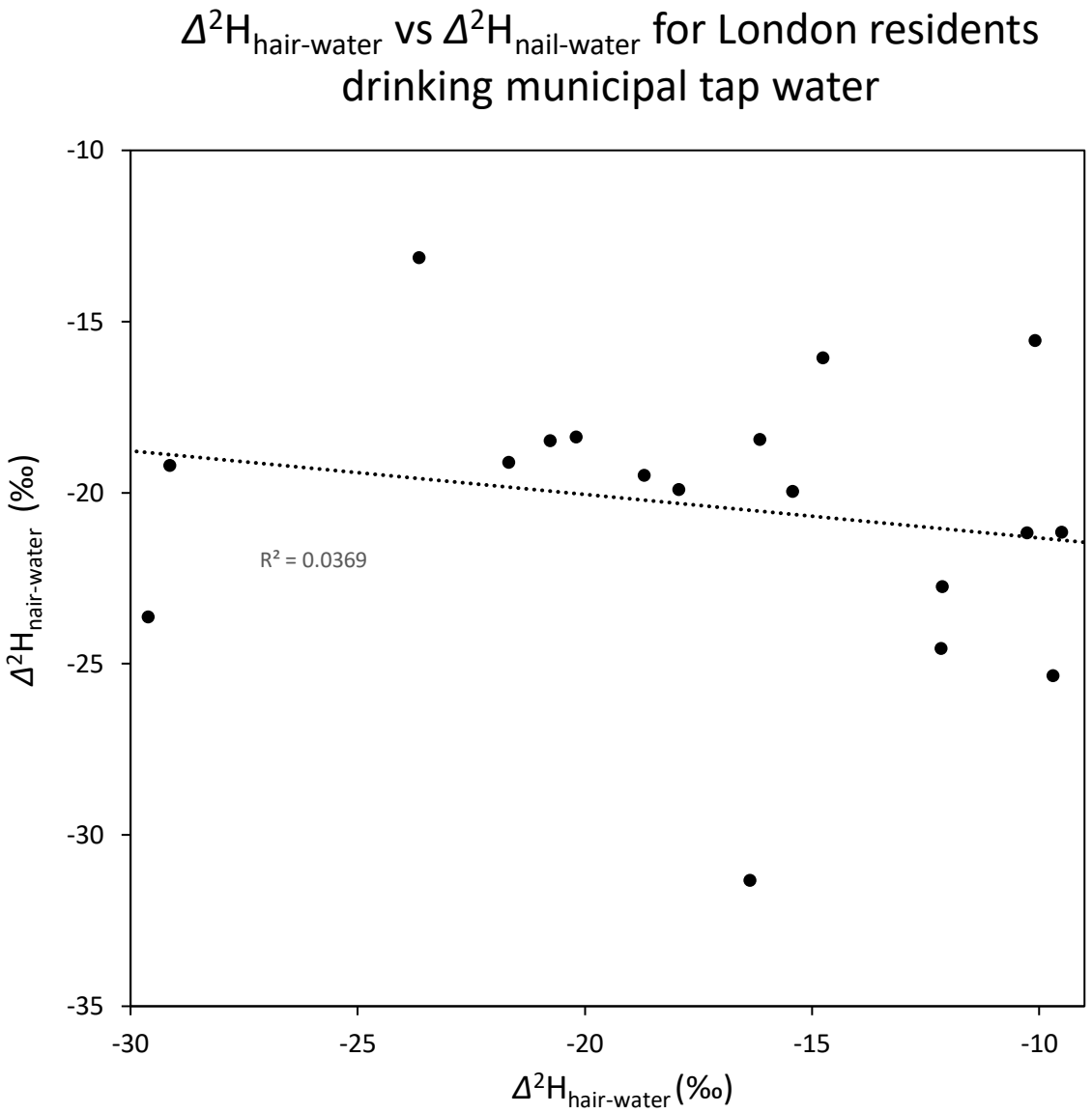


Figure 3.7. Paired $\Delta^2H_{hair\text{-}water}$ and $\Delta^2H_{nail\text{-}water}$ for participants who reported the London municipal tap water supply as their sole source of drinking water.

study is unclear. The mean $\Delta^2H_{hair\text{-}nail}$ of +4.6 ± 6.3 ‰ obtained in the present study is lower than the mean $\Delta^2H_{hair\text{-}nail}$ of +7 to + 14 ‰ calculated from data published by Fraser & Meier-Augenstein (2007) and Lehn et al. (2011).

3.3.2 $\delta^{18}O_{nail}$ Results

Values of $\delta^{18}O_{nail}$ span a range of 4.5 ‰, with a minimum of +8.6 ± 0.6 ‰ measured in a participant from the Waterloo Region who reported drinking a mixture of tap ($\delta^{18}O$ = –10.6 ‰) and bottled water ($\delta^{18}O$ = –13.5 ‰) and consuming an omnivorous diet. A maximum of +13.1 ± 0.2 ‰ was measured in a participant who reported exclusively drinking London municipal tap water and consuming an omnivorous diet (Table 3.7). Measured $\delta^{18}O_{nail}$ are plotted against $\delta^{18}O_{drinking\ water}$ and compared against predicted $\delta^{18}O_{nail}$ using models for populations consuming a 'supermarket' diet in Figure 3.8. See Table 3.2 for predicted $\delta^{18}O_{nail}$ model equations.

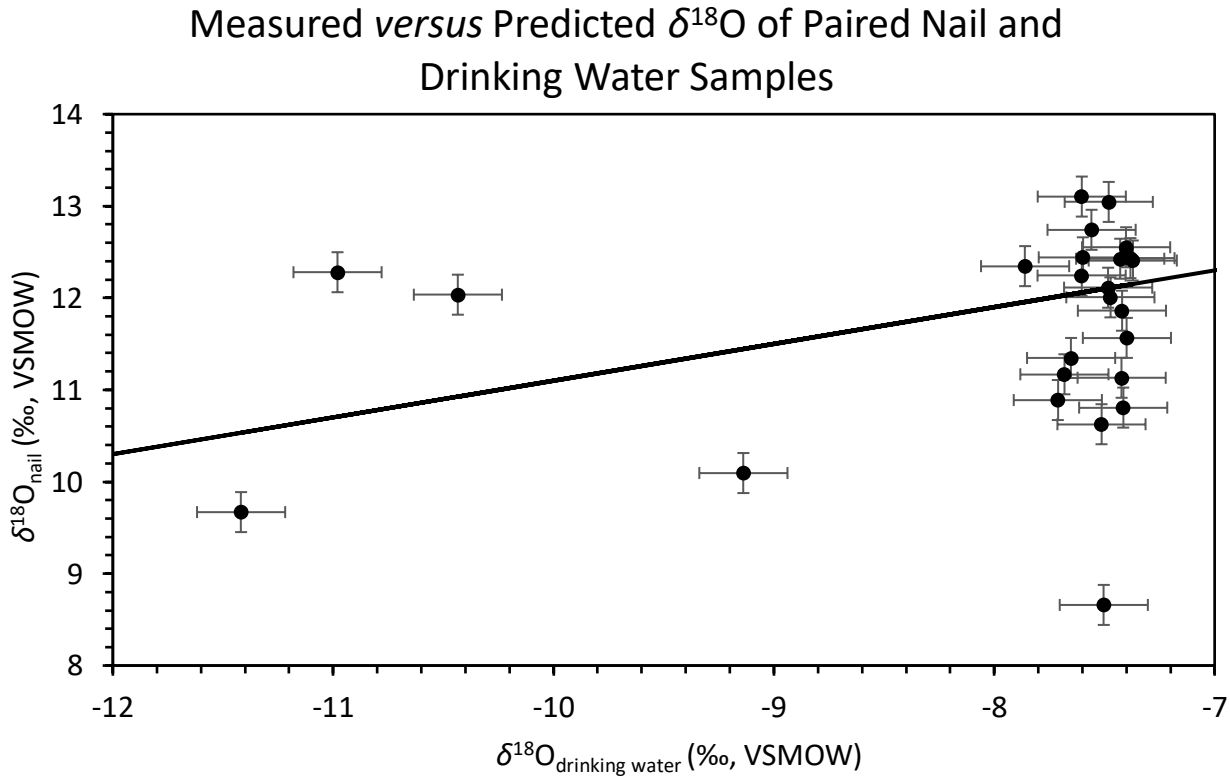


Figure 3.8. Measured $\delta^{18}O_{drinking\ water}$ and $\delta^{18}O_{nail}$ versus predicted $\delta^{18}O_{nail}$ from $\delta^{18}O_{drinking\ water}$ for London residents from this study. Filled circles represent measured $\delta^{18}O_{nail}$ and $\delta^{18}O_{drinking\ water}$, the solid line denotes predicted $\delta^{18}O_{nail}$ using a model for populations consuming a 'supermarket' diet (Mancuso &

Ehleringer, 2019). Vertical error bars denote ± SD on samples with sufficient material to perform analyses in triplicate. Horizontal error bars denote analytical precision of ± 0.2 ‰.

When analyzed by dietary group (Fig. 3.6) the lowest $\delta^{18}O_{nail}$ was observed in vegetarians, with a mean $\delta^{18}O_{nail}$ of +11.2 ‰ (*n* = 2), followed by +11.6 ± 1.2 ‰ (*n* = 19) for omnivores, +11.9 ± 0.7 (*n* = 3) for carnivores, and finally +12.3 ± 0.2 ‰ (*n* = 3) for semi-vegetarians. When participants who reported consuming water from sources other than London municipal tap water are removed from the calculation (Table 3.9), the mean omnivore $\delta^{18}O_{nail}$ increases slightly to +11.9 ± 0.9 ‰ (*n* = 16), identical to that of carnivores. When carnivores are included with omnivores, the mean $\delta^{18}O_{nail}$ are unchanged. Unpaired t-tests revealed statistically significant differences between the $\delta^{18}O_{nail}$ of the carnivore and vegetarian, and between the vegetarian and omnivore dietary groups (Table 3.10).

Table 3.10. Statistical comparisons of $\delta^{2}H_{nail}$ and $\delta^{18}O_{nail}$ across dietary groups. "TT" test type refers to an unpaired t-test, and "M-W" to a Mann-Whitney U test. Statistical tests with p-values below the significance threshold are shown in bold font.

	$\delta^{2}H$		$\delta^{18}O$	
Comparison Groups	**p-value**	**Test Type**	**p-value**	**Test Type**
omnivore vs carnivore	0.157	TT	0.575	M-W
omnivore vs semi-vegetarian	0.476	TT	0.064	M-W
omnivore vs vegetarian	**0.017**	M-W	0.238	M-W
carnivore vs semi-vegetarian	0.087	TT	0.070	TT
carnivore vs vegetarian	**0.006**	M-W	**0.012**	TT

semi-vegetarian vs vegetarian	0.263	M-W	**<0.001**	TT

Values of $\Delta^{18}O_{nail-water}$ span a range of 4.0 ‰, with a minimum of +17.6 ‰ measured for a participant who reported exclusively drinking London municipal tap water and consuming an omnivorous diet. A maximum $\Delta^{18}O_{nail-water}$ of +21.6 ‰ was measured for a participant who reported drinking a mixture of bottled water ($\delta^{18}O$ = –13.4 ‰) and London municipal tap water ($\delta^{18}O$ = –7.5 ‰) and consuming a vegetarian diet. A mean $\Delta^{18}O_{nail-water}$ of +19.4 ± 0.9 ‰ (n = 23) calculated for all participants in this study exclusively consuming London municipal tap water is somewhat lower than the mean $\Delta^{18}O_{nail-water}$ of +22.8‰, calculated using mean Salt Lake City resident $\delta^{18}O_{nail}$ and calculated precipitation $\delta^{18}O$ presented in Mancuso and Ehleringer (2019).

Values of $\Delta^{18}O_{hair-nail}$ ranged from a minimum of –2.7 ‰ and a maximum of +0.7 ‰. Both the maximum and minimum $\Delta^{18}O_{hair-nail}$ were measured in participants who reported exclusively drinking London municipal tap water and consuming an omnivorous diet. The mean $\Delta^{18}O_{hair-nail}$ of –0.8 ± 0.7 ‰ is smaller than the value of ~ –2 ‰ calculated from results reported by Fraser and Kalin (2006). When $\Delta^{18}O_{hair-water}$ calculated for individuals who reported the London municipal water supply as their sole source of drinking water were plotted against corresponding $\Delta^{18}O_{nail-water}$ (Fig. 3.9), a weak correlation (R^2 = 0.29) was observed.

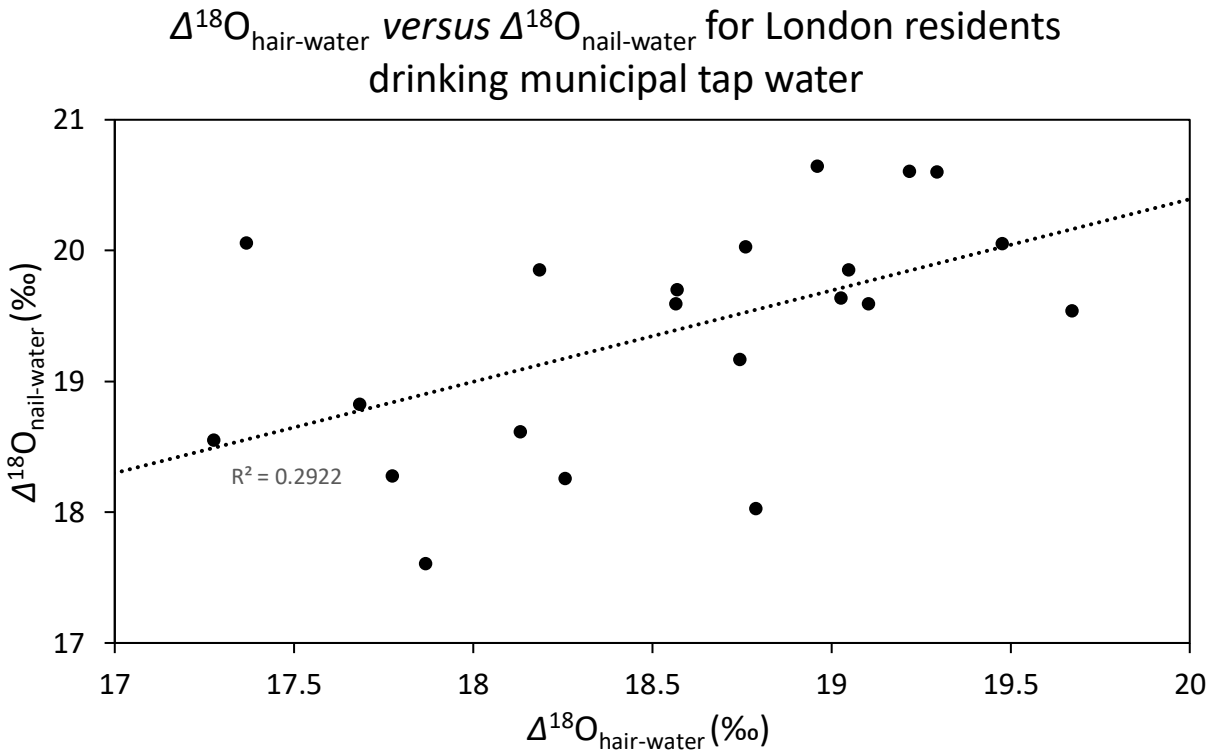


Figure 3.9. Paired $\Delta^{18}O_{hair\text{-}water}$ and $\Delta^{18}O_{nail\text{-}water}$ for participants who reported the London municipal tap water supply as their sole source of drinking water.

3.3.3 $\delta^{13}C_{nail}$ Results

Values of $\delta^{13}C_{nail}$ in the present study span a range of 4.0 ‰, with a minimum $\delta^{13}C_{nail}$ of –21.4 ± 0.2 ‰ for a participant consuming a semi-vegetarian diet, and a maximum $\delta^{13}C_{nail}$ of –17.5 ± 0.2 ‰ for a participant consuming an omnivorous diet (Table 3.5). When analyzed by dietary group (Fig. 3.10), semi-vegetarians exhibited the lowest mean $\delta^{13}C_{nail}$ (–20.5 ± 0.8 ‰; n = 3), followed by a mean of –19.4 ‰ (n = 2) for vegetarians, –18.8 ± 0.8 ‰ (n = 19) for omnivores, and –18.2 ± 0.6 ‰ (n = 3) for carnivores. When carnivores are included with the omnivore dietary group, the mean $\delta^{13}C_{nail}$ changes only slightly to –18.7 ± 0.8 ‰. The general trend of increasing $\delta^{13}C_{nail}$ with increasing consumption of animal proteins is consistent with increasing ^{13}C-enrichment in tissues occupying higher trophic levels (Buchardt et al., 2007; Fuller et al., 2006; Schoeller et al., 1986; Yoshinaga et al., 1996). Values of $\Delta^{13}C_{hair\text{-}nail}$ ranged

between –0.1 and +2.6 ‰, with nails in this study being depleted of ^{13}C by an average 0.5 ± 0.6 ‰ compared to paired hair samples. This value of $\Delta^{13}C_{hair\text{-}nail}$ compares favorably with the previously published mean $\Delta^{13}C_{hair\text{-}nail}$ of +0.2 to +0.3 ‰ measured for modern humans from Germany (Lehn et al., 2011) and from the United Kingdom (O'Connell et al., 2001).

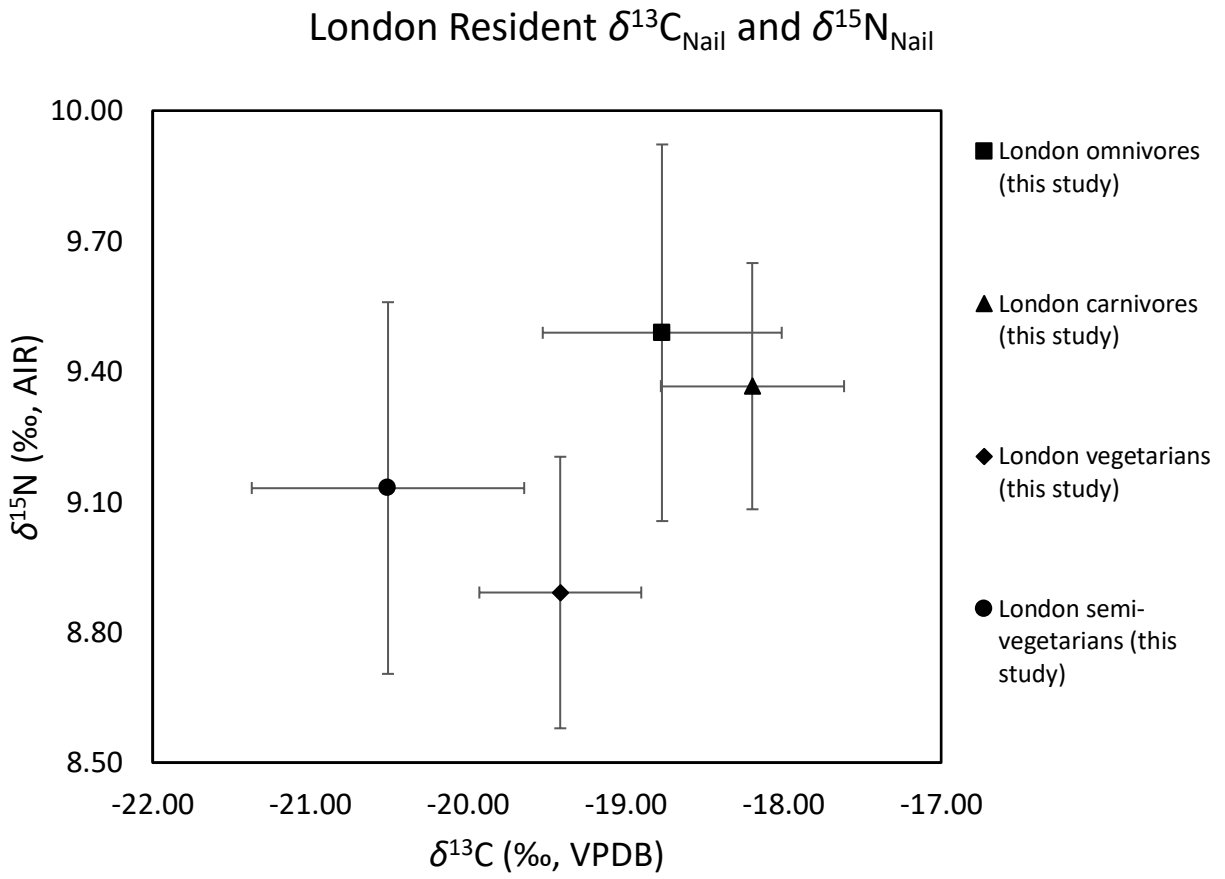


Figure 3.10. Mean dietary group $\delta^{13}C_{nail}$ versus $\delta^{15}N_{nail}$ ± SD.

3.3.4 $\delta^{15}N_{nail}$ Results

Values of $\delta^{15}N_{nail}$ values range between a minimum of +8.6 ± 0.1 ‰ and a maximum of +10.2 ± 0.1 ‰ (Table 3.5). When considered by dietary group (Fig. 3.10), the lowest mean $\delta^{15}N_{nail}$ was +8.9 ‰ (*n* = 2) for vegetarians, followed by a mean of +9.1 ± 0.3 ‰ (*n* = 3) for semi-vegetarians, +9.4 ± 0.3 ‰ (*n* =

3) for carnivores, and +9.5 ± 0.4 ‰ (n = 19) for omnivores. When carnivores are included with omnivores, the mean $\delta^{15}N_{nail}$ (n = 22) is unchanged. When data for semi-vegetarians and vegetarians are combined, the mean $\delta^{15}N_{nail}$ changes slightly to +9.0 ± 0.3 ‰ (n = 5). The trend of increasing $\delta^{15}N_{nail}$ from vegetarians to omnivores is consistent with stepwise ^{15}N-enrichment of tissues occupying successively higher trophic levels and tissues of individuals consuming greater proportions of animal-derived protein (Buchardt et al., 2007; Minagawa & Wada, 1982; Peterson & Fry, 1987). Values of $\Delta^{15}N_{hair\text{-}nail}$ range between –9.6 and 0.0 ‰, with a mean depletion of –1.0 ± 2.1 ‰ in nails compared to paired hair samples. Previously reported values of $\Delta^{15}N_{hair\text{-}nail}$ (–0.7 to –0.5 ‰; Lehn et al., 2011; O'Connell et al., 2001) are similar.

Chapter 4

4.0 Discussion

4.1 Variation of Stable Hydrogen and Oxygen Isotope Compositions in Hair, Nails, and Drinking Water

Upon initial inspection, the range of δ^2H_{hair} and of $\delta^2H_{drinking\ water}$ samples (24.2 ‰ and 20.6 ‰, respectively) are similar. The approximately equivalent range in mean δ^2H_{hair} and $\delta^2H_{drinking\ water}$ is also consistent with previous investigations into the δ^2H of paired hair and local drinking water sources across geographic isotope gradients in precipitation (Bowen et al., 2003, 2007, 2009; Ehleringer et al., 2008; Thompson et al., 2010; Wunder, 2010); this result suggests that the range of δ^2H_{hair} is primarily controlled by the range of $\delta^2H_{drinking\ water}$. This apparent correlation, however, is complicated when the $\delta^2H_{drinking\ water}$ sources each participant reported consuming and the mean δ^2H of paired hair and nail samples are examined more closely.

Surprisingly, the maximum and minimum δ^2H_{hair} were observed in participants who reported exclusively consuming London municipal tap water as their primary drinking water source, which has a δ^2H range of only 3.4 ‰. Thus, the entire 24.2 ‰ range of mean δ^2H_{hair} in this study is bookended by participants who consumed a drinking water source exhibiting much smaller variation in δ^2H. A similar observation is made with paired values of $\delta^2H_{drinking\ water}$ and δ^2H_{nail}. The lowest δ^2H_{nail} (–86.6 ± 3.1 ‰) was observed in a participant who reported consuming drinking water from bottled or well water sources that had lower $\delta^2H_{drinking\ water}$ than London municipal tap water (Table 3.8). The lowest δ^2H_{nail} measured for a participant who reported exclusively drinking London municipal tap water was –84.7 ± 0.7 ‰, a value only slightly higher than the minimum δ^2H_{nail} observed in this study. The highest δ^2H_{nail} (–68.2 ± 2.6

‰) was measured for a participant who reported exclusively drinking London municipal tap water. Thus, the range of δ^2H_{nail} of participants exclusively consuming the London municipal tap water supply is 16.5 ‰ (Table 4.1).

As with $\delta^2H_{drinking\ water}$ and paired hair and nail samples, the $\delta^{18}O$ of all measured samples spanned a comparable range, at 4.0 ‰ for drinking water, 3.7 ‰ for hair, and 4.5 ‰ for nails (Table 4.1). This observation is also consistent with previous investigations across oxygen isotope gradients of precipitation and drinking water sources (Bowen et al., 2007, 2009; Ehleringer et al., 2008; Thompson et al., 2010; Wunder, 2010). The range of mean $\delta^{18}O_{hair}$ for participants who reported London municipal tap water as their sole source of drinking water was 2.3 ‰, somewhat less than the total range of $\delta^{18}O_{hair}$ for all study participants, but still substantially larger than the $\delta^{18}O_{drinking\ water}$ range of 0.5 ‰ measured for London municipal tap water samples (Table 4.1). The lowest and highest $\delta^{18}O_{hair}$ for participants drinking only London municipal tap water was +9.7 ± 0.7 ‰ and +12.0 ‰, respectively (Table 3.1).

Table 4.1. Ranges of δ^2H and $\delta^{18}O$ in drinking water, hair, and nail samples for all participants in the present study, and for participants who reported exclusively consuming the London municipal tap water supply as their source of drinking water.

	Isotopic Range of Drinking Water, Hair, and Nail Samples (‰, VSMOW)					
Participant Subset	**$\delta^2H_{drinking\ water}$**	**δ^2H_{hair}**	**δ^2H_{nail}**	**$\delta^{18}O_{drinking\ water}$**	**$\delta^{18}O_{hair}$**	**$\delta^{18}O_{nail}$**
All Participants	20.6	24.2	18.4	4.0	3.7	4.5

London Tap Water Only	3.4	24.2	16.5	0.5	2.3	3.0

The range of $\delta^{18}O_{nail}$ for participants who reported exclusively drinking London municipal tap water (3.0 ‰) is somewhat smaller than the total range of $\delta^{18}O_{nail}$. The lowest $\delta^{18}O_{nail}$ measured among participants exclusively consuming London municipal tap water was +10.1 ± 0.2 ‰, a value somewhat higher than the minimum recorded value for all participants in the present study (+8.6 ± 0.6 ‰). Consumption of drinking water having lower $\delta^{18}O$ than London municipal tap water does not guarantee lower $\delta^{18}O$ in hair or nails than participants exclusively consuming London municipal tap water. The two smallest $\delta^{18}O_{hair}$ were measured for participants primarily drinking bottled and/or well water with lower $\delta^{18}O$ than London municipal tap water. Two participants who reported consuming bottled or other non-municipal supply water sources possessed higher $\delta^{18}O_{hair}$ than the lowest $\delta^{18}O_{hair}$ for participants exclusively consuming the London municipal tap water (Table 3.1). Similarly, the same two participants had $\delta^{18}O_{nail}$ higher than the lowest measured $\delta^{18}O_{nail}$ among participants exclusively consuming London municipal tap water (Table 3.8).

4.1.1 Overestimation of Predicted versus Measured Hydrogen and Oxygen Isotope Compositions in Hair and Nails of London Residents

Published models are unable to reliably predict the $\delta^{2}H$ and $\delta^{18}O$ of hair and nails from London residents based on the isotopic composition of local drinking water (Table 4.2). The Fraser & Meier-Augenstein (2007) model, constructed using data for modern Belfast residents, consistently overestimates the hair and nail $\delta^{2}H$ of London residents, for a given isotopic composition of drinking water, by a surprising 22 ‰ and 23 ‰, respectively (Figs. 3.1 and 3.5). All models overestimate London resident $\delta^{18}O_{hair}$ by up to 4.2 ‰ (Fig. 3.3). Overestimation of London resident $\delta^{2}H_{hair}$ is unique to the

Fraser & Meier-Augenstein (2007) model (Table 4.2). Models constructed from hair samples of modern Americans (Ehleringer et al., 2008), early twentieth century pre-industrialized natives from around the globe (Bowen et al., 2009), and industrially developing Asiatic populations (Thompson et al., 2010) underestimate the δ^2H_{hair} of London residents by 22 to 35 ‰ (Fig. 3.1).

Table 4.2. Differences between measured and predicted hair and nail δ^2H and $\delta^{18}O$ of London, Ontario, Canada residents.

	Hair		Nail	
Predictive Model	$\Delta^2H_{predicted-measured}$ **(‰)**	$\Delta^{18}O_{predicted-measured}$ **(‰)**	$\Delta^2H_{predicted-measured}$ **(‰)**	$\Delta^{18}O_{predicted-measured}$ **(‰)**
Bowen et al. (2009)	–28	4.2	–	–
Ehleringer et al. (2008)	–31	3.1	–	–
Fraser & Meier-Augenstein (2007)	22	–	23	–
Thompson et al. (2010)	–35	3.8	–	–
Mancuso & Ehleringer (2019)	–	–	–21	3.4

The propensity of available models to underestimate $\delta^{18}O_{hair}$, and hair and nail δ^2H using the Fraser & Meier-Augenstein (2007) model, of London residents may be an artefact of differences between the isotopic composition of London municipal tap water and local precipitation in regions

where food items consumed by residents are produced. The available predictive models presume the isotopic composition of local drinking water supplies are similar, if not identical to local precipitation, with some models using the mean annual $\delta^{2}H$ and $\delta^{18}O$ of local precipitation as a proxy for local drinking water (see Table 3.2 for details on published models). The $\delta^{2}H$ and $\delta^{18}O$ of food, however, reflect the isotopic composition of precipitation or irrigation water in the growing region (Chesson et al., 2008, 2010b); and, as noted previously, the London municipal water supply is enriched in ^{2}H and ^{18}O compared to average annual precipitation in southern Ontario (Brooks et al., 2014; Ruan et al., 2020). While London municipal tap water may be consumed directly or in beverages such as coffee or tea, or in foods such as soups, stews, 22 to 73% of hydrogen, and 30 to 65% of oxygen in hair and nails are sourced from food or food water (Bowen et al; 2009; Ehleringer et al., 2008; Sharp et al., 2003).

The stable hydrogen and oxygen isotope composition of plant tissues are known to be highly variable, with $\delta^{2}H$ and $\delta^{18}O$ for transpiring tissues that are far removed from local precipitation or soil water. For example, enrichment in ^{2}H and ^{18}O of leaf water due to vapor pressure differences between the heavy and light isotopologues of water during transpiration is a well-known phenomenon (e.g., Bariac et al., 1989; Cernusak et al., 2016; Dongmann et al., 1974; Yakir & Sternberg, 2000). Dongmann et al. (1974) modeled a maximum ^{18}O enrichment of 25 ‰, measuring enrichments of ~21 ‰ under natural conditions. By comparison, the $\delta^{2}H$ and $\delta^{18}O$ of water in non-transpiring plant tissues has been found to closely resemble the isotopic composition of soil water (Ehleringer & Dawson, 1992; White, 1989), indicating minimal fractionation within these tissues. Despite substantial $\delta^{2}H$ and $\delta^{18}O$ fractionation within plant tissue, the stable isotope composition of precipitation, precipitation-derived groundwater, or other sources of irrigation water represent an isotopic baseline, with respect to ^{2}H and ^{18}O, from which subsequent plant tissue $\delta^{2}H$ and $\delta^{18}O$ are modified from. The $\delta^{2}H$ and $\delta^{18}O$ of local precipitation, groundwater, or other irrigation waters will have a significant influence on the $\delta^{2}H$ and $\delta^{18}O$ of plant tissues and waters therein (Beyer et al., 2018; Penna et al., 2020).

Agricultural operations in southern Ontario primarily rely on precipitation to water crops, with most supplemental irrigation systems utilizing groundwater extracted from on-farm wells, or from surface water bodies fed by groundwater, such as dug ponds (DeLoë et al., 2001; Statistics Canada, 2013). In the latter case, there is potential for evaporative enrichment of ^{18}O and ^{2}H; however, the limited residence time of shallow dug ponds fed by groundwater limits this potential effect compared to long residence time water bodies, such as the 22-year residence time for Lake Huron (Gat, 1996; Kalvāns et al., 2020; Quinn, 1992; Zuidema, 2011). Therefore, shallow groundwater-fed dug ponds should contain water with $\delta^{2}H$ and $\delta^{18}O$ not far removed, it at all, from their source water, which in turn reflects the mean annual isotopic composition of precipitation (Sachse et al., 2006). The $\delta^{2}H$ and $\delta^{18}O$ of food, metabolic water, and water contained within non-transpiring tissues of food products, such as carrots, beets, celery, etc., should be expected to be lower than predicted water compositions contained within the same plant tissues of a hypothetical crop grown under identical conditions but irrigated using water from London's municipal tap water supply.

The $\delta^{2}H$ and $\delta^{18}O$ of water within transpiring plant tissues will be elevated relative to irrigation waters, whether the irrigation water is local precipitation, precipitation-derived groundwater, or London municipal tap water (Deák et al., 1987; Gat, 1996). Predictive models, however, calculate the $\delta^{2}H$ and $\delta^{18}O$ of waters within transpiring plant tissues based on the compositions of drinking water used in the model equations (Bowen et al., 2009; Ehleringer et al., 2008; Fraser & Meier-Augenstein, 2007; Mancuso & Ehleringer, 2019), in this case, municipal tap water. Thus, the actual $\delta^{2}H$ and $\delta^{18}O$ of water in transpiring plant tissues consumed by London residents and irrigated using precipitation or precipitation-derived ground water should be lower than what predictive models calculate for a hypothetically identical crop irrigated using London tap water, assuming all other variables such as transpiration rates, the magnitude of associated leaf water fractionation, growing season length, etc. are held constant. Incorporating ^{2}H and ^{18}O-depleted water, relative to compositions based on the $\delta^{2}H$ and

$\delta^{18}O$ of the municipal tap water, within foods into gut and body water pools will result in lower than expected mean $\delta^{2}H$ and $\delta^{18}O$ in subsequently formed tissues than if London tap water, and waters evolved from London tap water through evapotranspiration processes, were the sole source of hydrogen and oxygen inputs (Fig. 4.1).

The respective $\delta^{2}H$ and $\delta^{18}O$ of mean annual precipitation falling at London, Ontario, Canada between 2004 and 2014 are –64 ‰ and –9.6 ‰ (F. Longstaffe, personal communication, November 28, 2021), which are 10.1 ‰ and 2.41 ‰ lower, respectively, than the mean $\delta^{2}H$ and $\delta^{18}O$ of London municipal tap water measured in the present study. Measured precipitation $\delta^{2}H$ and $\delta^{18}O$ compare favorably with mean modeled values of –67 ‰ and –9.9 ‰, respectively, for precipitation falling at London, Ontario, Canada (Bowen, 2021; IAEA/WMO, 2015; Bowen & Revenaugh, 2003). The largest model overestimates of $\delta^{2}H_{hair}$, $\delta^{2}H_{nail}$, and $\delta^{18}O_{hair}$ for London residents, however, are greater than the differences between the $\delta^{2}H$ and $\delta^{18}O$ of mean annual London precipitation and mean London tap water. These two water compositions, however, do not represent the complete range of irrigation water $\delta^{2}H$ and $\delta^{18}O$ available to London residents in food (Alvarez, 2019; Open Government, 2015; Statistics Canada, 2013). The difference between London municipal tap water $\delta^{2}H$ and $\delta^{18}O$ and precipitation falling in other agricultural centers producing foods consumed by London residents, such as the Prairies, is substantially larger than that between London municipal tap water and local precipitation (Bowen, 2021; Bowen & Revenaugh, 2003; IAEA/WMO, 2015; Terzer et al., 2013).

Fluid intake is another important control on the isotopic composition of body water (O'Grady et al., 2010; Valenzuela et al., 2021), which mediates the isotopic composition of hair and nails (Bowen et al., 2009; Longinelli, 1984; Luz & Kolodny, 1985; O'Grady et al., 2012; Podlesak et al., 2012). Large body water flux through high fluid intake will drive body water $\delta^{2}H$ and $\delta^{18}O$ towards the isotopic composition of drinking water as this input will proportionally dominate the other possible hydrogen and oxygen

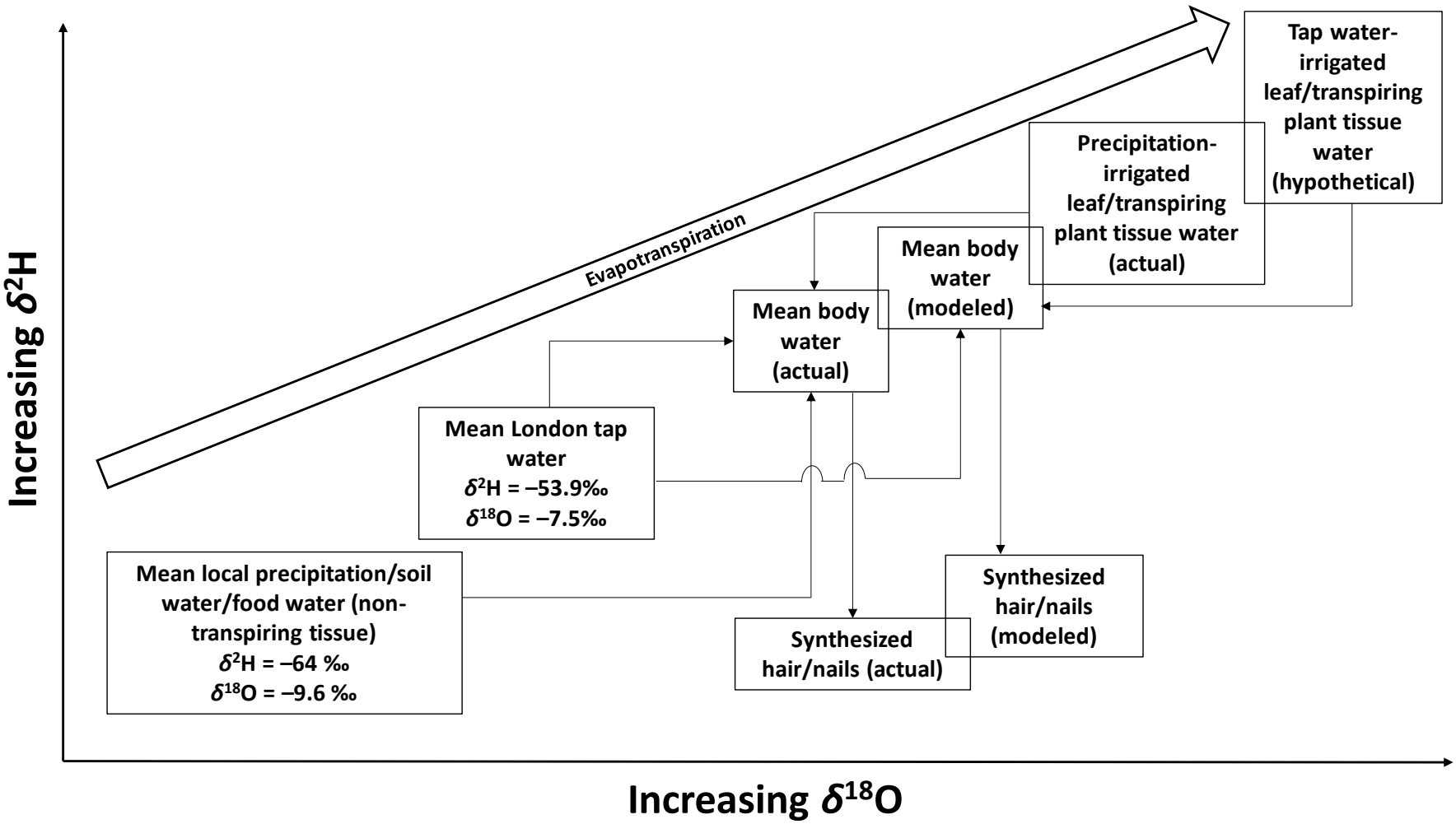


Figure 4.1. Conceptual visualization of discrepancies between actual and modeled mean hair and nail δ^2H and δ^{18}O stemming from likely isotopic differences between local London tap water and local precipitation used to grow crops consumed by London residents.

inputs to the body (Valenzuela et al., 2021). Predictive models do not take variable fluid intake across individuals into account (Bowen et al., 2009; Ehleringer et al., 2008; Fraser & Meier-Augenstein, 2007; Mancuso & Ehleringer, 2019; Thompson et al., 2010). In cases where high fluid flux drives body water δ^2H and δ^{18}O close or identical to drinking water, predictive models can overestimate the isotopic composition of body water, and by extension overestimate the δ^2H and δ^{18}O of subsequently synthesized hair and nail tissue.

4.1.2 Underestimation of Predicted versus Measured Hydrogen and Oxygen Isotope Compositions for Hair and Nails of London Residents

Underestimation of δ^2H_{hair} by the Bowen et al. (2009), Ehleringer et al. (2008), and Thompson et al. (2010) models (Fig. 3.1), and δ^2H_{nail} by the Mancuso & Ehleringer (2019) model (Fig. 3.5) cannot be explained by consumption of food water depleted of ^{2}H and ^{18}O relative to London municipal tap water. Consistent underestimation of hair and nail δ^2H and δ^{18}O could most readily be attributed to an increase in mean body and gut water δ^2H and δ^{18}O due to consumption of water within foods or beverages enriched in ^{2}H and ^{18}O relative to the local drinking water supply (Fig. 4.2). Potential contributing sources of such water to the body and gut water pools of London residents include:

i. Preferential use of ^{2}H- and ^{18}O-enriched summer precipitation and evapoconcentrated soil water by food crops (Ehleringer et al., 1991);
ii. Consumption of non-local foods and beverages from regions characterized by precipitation and/or drinking water with higher δ^2H and δ^{18}O than the local drinking water supply (Calderone & Guillou, 2008; Chesson et al., 2010b); and
iii. Significant consumption evaporatively enriched waters that have been standing in pipes for long periods, waters that have been boiled, distilled, or otherwise fermented during the preparation of hot beverages such as coffee, tea, and cocoa, alcoholic beverages such as beer and spirits, or

evaporatively reduced foods such as soups or stews (Brettel et al., 2012; Daux et al., 2008; Royer et al., 2017).

The $\delta^{2}H$ and $\delta^{18}O$ in precipitation vary seasonally, with summer precipitation typically being enriched in ^{18}O and ^{2}H compared to mean annual $\delta^{2}H$ and $\delta^{18}O$ (Dansgaard, 1964; Yurtsever & Gat, 1980). Such evaporative enrichment in the heavy isotopes of soil water utilized by plants compounds this effect (Sprenger et al., 2017). Plant water uptake from soil is dependent on climate regime and the morphology of the plant's root system. Dawson & Ehleringer (1991) and Ehleringer & Dawson (1992) measured the stable isotope composition xylem water of trees growing in the riparian zone of arid regions, finding near total reliance on deeper, more reliable groundwater sources by these plants as opposed to stream water, which may experience significant temporal variability in discharge and course. Despite the advantages of consistent access to groundwater, not all plant species rely on it to meet the entirety of their water needs.

Dawson & Pate (1996) measured the isotopic composition of xylem water in plants with both shallow lateral roots, capable of utilizing shallow soil moisture from precipitation, and deeper tap roots, capable of utilizing groundwater at various points during the Australian wet and dry seasons. Xylem water closely resembled the isotopic composition of precipitation during the wet season before shifting during the dry season to isotopic compositions that more closely resembled groundwater. Similarly, xylem water in southern Utah desert plants exhibited an isotopic composition corresponding to that of spring precipitation and snowmelt; as the growing season continued, however, the isotopic composition of xylem water began to vary across species (Ehleringer et al., 1991). Annual plants were completely dependent on summer precipitation, and herbaceous perennials also heavily relied on summer precipitation to meet water needs (Ehleringer et al., 1991).

Many staple crops such as wheat, corn, beans, oats, rice, etc., are annual crops (USDA, 2014), and their $\delta^{2}H$ and $\delta^{18}O$ can be expected to reflect a weighted average of spring and summer precipitation. This seasonal bias should result in greater enrichment in ^{2}H and ^{18}O than would be expected if crops exclusively utilized ground water to meet their water needs (Fig. 4.2). Enrichment of ^{18}O and to a lesser extent ^{2}H, in summer precipitation and soil water is further amplified by evapotranspiration and metabolic processes within the plant (Barbour, 2007; Cernusak et al., 2016; Rossmann, 2001). Higher $\delta^{2}H$ and $\delta^{18}O$, relative to precipitation and soil water, within plant tissues is propagated to body water through synthesis of metabolic waters during digestion of foods. Elevated body water $\delta^{2}H$ and $\delta^{18}O$ from metabolic and food water contributions is in turn propagated to nails and hair (Bowen et al., 2009; O'Grady et al., 2012; Podlesak et al., 2012; Valenzuela et al., 2021). Such differences between municipal tap water, annual averages for precipitation, and summer precipitation $\delta^{2}H$ and $\delta^{18}O$ are not taken into account by predictive models.

Consumption of foods or beverages imported from regions characterized by higher precipitation $\delta^{2}H$ and $\delta^{18}O$, or regions with higher evaporative potential, resulting in greater evapoconcentration of soil waters taken up by plants (Sprenger et al., 2017), relative to the local drinking water supply, could also increase the mean isotopic composition of body water. Lamb et al. (2014) found elevated bioapatite $\delta^{18}O$ in tissues of King Richard III of England that formed during his kingship, compared to tissues formed in his childhood, most likely due to consumption of imported wines and other non-local luxury food items. Similarly, dilution of wine and fruit juices by local tap water has been detected through a lowering of $\delta^{2}H$ and $\delta^{18}O$ from those measured in undiluted wines and juices produced in the same region (Calderone & Guillou, 2008). Stable hydrogen and oxygen isotope measurements of menu items available from fast food restaurant chains showed that many of the ingredients were not locally produced (Chesson et al., 2010b), creating the potential for customers to consume isotopically heavy water, relative to local tap water, contained in foods with non-local origins. Colley et al. (2022) found

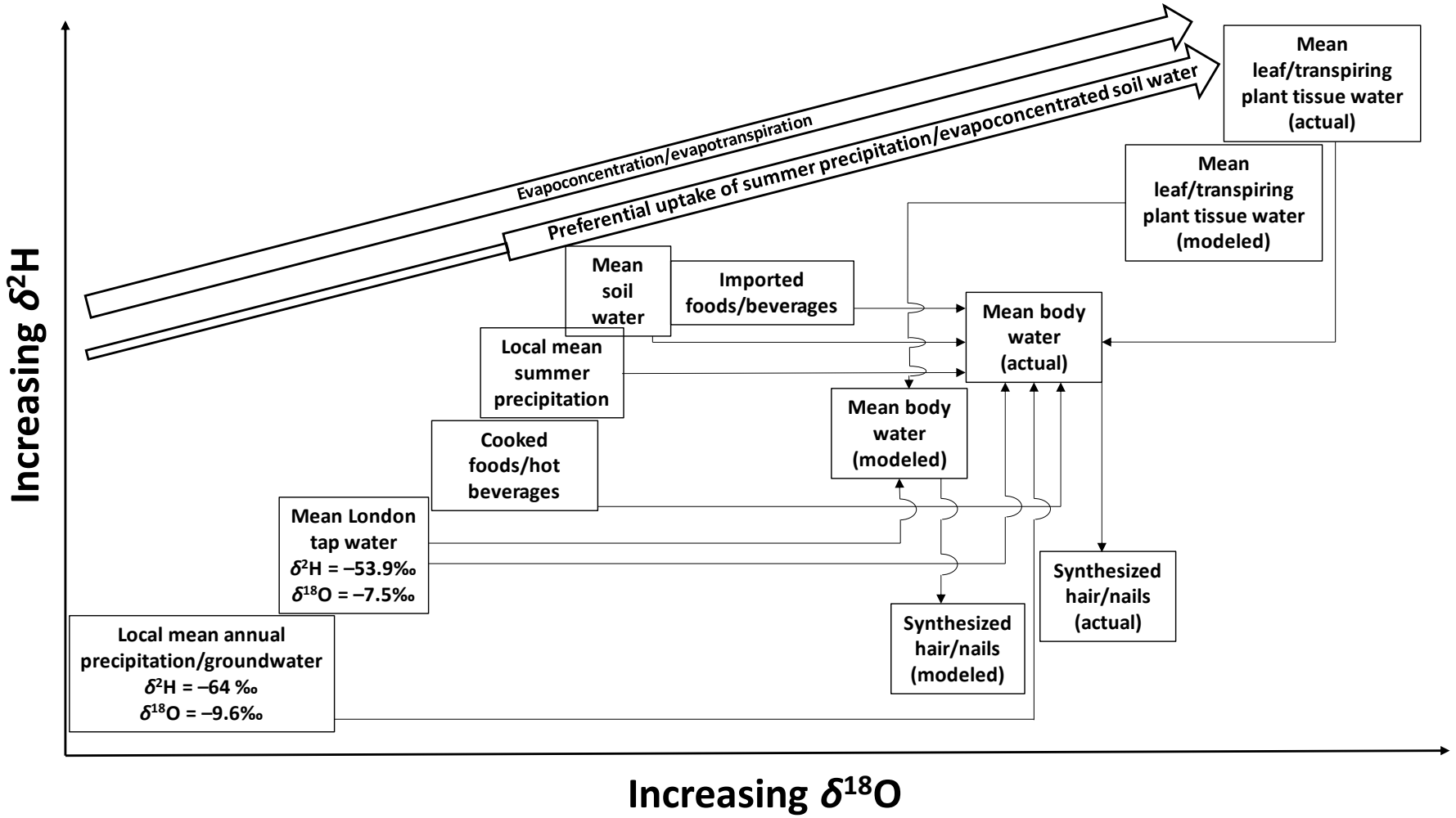


Figure 4.2. Conceptual visualization of discrepancies between actual and modeled mean hair and nail δ^2H and δ^{18}O caused by consumption of water contained in foods and beverages. Preferential uptake of more ^{18}O- and ^{2}H-rich summer precipitation and evapoconcentrated surface soil waters relative to ground water by plants is denoted by the thicker arrows.

evidence that London residents who reside in urban and suburban areas of the city exhibit lower knowledge of healthy food nutrition, selection and food-preparation skills compared to rural southwestern Ontario residents. It is therefore probable participants in the current study have hair and nail $\delta^{2}H$ and $\delta^{18}O$ that reflect consumption of non-local food items, particularly in light of reports of worldwide increases of snacking and alcohol consumption since the start of the COVID-19 pandemic (Bakaloudi et al., 2021; Chenarides et al., 2021). Increased non-local food consumption via snacking and alcohol consumption, however, may be at least partially offset by decreased fast food consumption (Bakaloudi et al., 2021; Chenarides et al., 2021).

Effects on the $\delta^{18}O$ of prepared foods and beverages due to water loss during cooking, boiling or brewing have also been shown to increase bioapatite $\delta^{18}O$ by up to 2.3 ‰ (Brettel et al., 2012). A proportionally similar enrichment in ^{2}H in prepared food and beverages, which is then recorded in hair and nails, is also to be expected. Additional alterations from the isotopic composition of raw foods by cooking are discussed in section 4.5. The abundance of annual crops that rely on summer precipitation and evapoconcentrated surface soil water, the near ubiquitous consumption of coffee, tea, and cooked foods, and the large selection of imported foods and beverages available to London, Ontario, Canada residents could explain the overestimation of hair and nail $\delta^{2}H$ and $\delta^{18}O$ by some predictive models.

A definitive explanation for the overestimation of hair and nail $\delta^{2}H$ and $\delta^{18}O$ by some predictive models, and underestimation by others cannot be determined from data collected in this study or data available in the literature. Such an explanation would first require detailed information on the source regions of foods and beverages consumed by all participants in this study. Also required would be details of the cooking and food preparation methods employed and the proportion of total body H and O inputs from each dietary component.

Values of $\delta^{18}O_{nail}$ predicted using a model constructed from modern humans from around the world by Mancuso & Ehleringer (2019) show the closest agreement with values measured for London, Ontario, Canada residents in the present study. However, this model still overestimates the $\delta^{18}O_{nail}$ of fourteen London residents and underestimates the values of a further eight, with only three $\delta^{18}O_{nail}$ measurements matching predicted values (Fig. 3.8). The distribution of measured $\delta^{18}O_{nail}$ on either side of the predicted regression line indicates the deviation from predicted values is less systematic than the over or underestimation of hair and nail $\delta^{2}H$ and $\delta^{18}O$ values by other models.

4.2 Variation of Stable Hydrogen and Oxygen Isotopes in Hair and Nails Compared to Other Tissues

There is a large amount of intra-population variation in the $\delta^{2}H$ and $\delta^{18}O$ of hair and nail tissue, even for individuals who are expected to be in isotopic equilibrium with local drinking water sources (e.g., Figs. 3.1, 3.3, 3.5, and 3.8). Hair samples of local residents from Belfast, Northern Ireland (Fraser & Kalin, 2006) and Fairbanks, AK, USA (O'Brien & Wooller, 2007) have $\delta^{2}H$ ranges within 10 ‰ of the $\delta^{2}H_{hair}$ range obtained in the present study. Residents of Belfast (Fraser & Kalin, 2006), southwest England (Bol et al., 2007), Fairbanks, and East Greenbush, New York, USA (O'Brien & Wooller, 2007) have $\delta^{18}O_{hair}$ ranges within 1 ‰ of the $\delta^{18}O$ range measured in this study. Nail samples of Salt Lake City, UT, USA (Mancuso & Ehleringer, 2019), and southwest England (Bol et al., 2007) residents have $\delta^{2}H$ and $\delta^{18}O$ ranges within 10 ‰ and 1 ‰, respectively, of those measured in the present study. However, local residents from East Greenbush, NY, USA (O'Brien & Wooller, 2007), and the Greater Toronto Area (GTA) of Ontario, Canada (Mant et al., 2016) show smaller $\delta^{2}H_{hair}$ and $\delta^{18}O_{hair}$ ranges than those measured in the present study compared to the present study. Residents of Belfast have a larger $\delta^{18}O_{nail}$ range than measured in the present study (Table 4.3), however, this may be due to frequent international travel by students and PhD lectures who constitute the sampled individuals from Belfast dataset.

Table 4.3. Intra-population δ^2H and δ^{18}O variation of hair and nails at various localities and the size of the sampled population.

			Hair		
Population Center	**δ^2H Range (‰)**	***n***	**δ^{18}O Range (‰)**	***n***	**Reference**
Belfast, Northern Ireland	17.2	5	2.7	8	Fraser & Kalin, 2006
East Greenbush, NY, USA	5.4	5	3.1	5	O'Brien & Wooller, 2007
Fairbanks, AK, USA	18.9	20	2.8	5	O'Brien & Wooller, 2007
GTA, ON, Canada	11.8	8	1.5	8	Mant et al., 2016
SW England	—	—	3.8	33	Bol et al., 2007
London, ON, Canada	24.2	22	3.7	25	This Study
			Nail		
Belfast, Northern Ireland	—	–	6.6	20	Fraser & Kalin, 2006
Salt Lake City, UT, USA	20	26	5.3	26	Mancuso & Ehleringer, 2019
SW England	—	–	3.8	33	Bol et al., 2007
London, ON, Canada	18.4	27	4.5	27	This Study

4.2.1 Hair and Nail Hydrogen and Oxygen Isotope Variation Driven by Variable Food and Beverage δ^2H and $\delta^{18}O$

As sampled populations in this study, and those described in the literature are predominantly composed of omnivores, and tissue samples were prepared and analyzed using comparable techniques (Bowen et al., 2005a; O'Connell & Hedges, 1999), it seems unlikely that differences in dietary preferences or sample preparation are to blame for the larger than expected variation of hair and nail δ^2H and $\delta^{18}O$ among London, Ontario, Canada residents. Additionally, δ^2H_{hair} ranges in East Greenbush were 18.8 ‰ smaller than the range observed in this study. The δ^2H_{hair} range for Fairbanks, however, was within 10 ‰ of the range in the current study despite the lack of statistically significant differences between the isotopic compositions of foods available for purchase at supermarkets in each locality (O'Brien & Wooller, 2007). Furthermore, the $\delta^{18}O_{hair}$ range obtained for both East Greenbush and Fairbanks is within 1 ‰ of the range obtained in the present study.

Heterogeneity in the isotopic composition of food products between sampling regions is largely mitigated in most published models by collecting tissue samples from residents of industrialized nations who are consuming a "continental supermarket" diet comprised of globally sourced foods that have stable isotope compositions reflecting industrial-scale production of a limited variety of crops in a select number of key agricultural regions (Chesson et al., 2010b; Frison, 2016; Lin, 2011; Manca et al., 2006). Residents of such countries, Canada included, can be expected to consume an isotopically homogenous continental 'supermarket' diet, limiting isotopic variation within foods between sampled population centers (Ehleringer et al., 2008, 2015; O'Brien & Wooller, 2007). Hair samples from populations consuming greater proportions of locally produced food, however, have been found to possess more variation in δ^2H_{hair} and $\delta^{18}O_{hair}$ than populations consuming more globally sourced foods (a 'supermarket' diet; Bowen et al., 2009). As over 70 % of food consumed by Canadians is domestically produced (Alvarez et al., 2019), greater variation of hair and nail δ^2H and $\delta^{18}O$ could be expected among

Canadians, and by extension, London, Ontario residents, compared to residents of the United States or EU, where larger populations or smaller transportation distances makes national, or international distribution of foods more economical.

Much of the observed intra-population δ^2H and $\delta^{18}O$ variation in hair and nails can also be attributed to isotopic differences across various H and O pools within the body that are utilized during keratin synthesis within the hair follicle or nail bed, and to variations in the δ^2H and $\delta^{18}O$ of inputs to these pools. Hydrogen and oxygen inputs to the human body are obtained from drinking water, water contained within food, and H and O incorporated into food in the form of, amino acids, lipids, and other organic dietary molecules. Unlike hydrogen, oxygen in the human body may also be sourced from atmospheric O_2 (Longinelli, 1984; Luz et al., 1984). Given keratin's proteinaceous composition (Plowman, 2007), with several of the amino acids within said proteins sourced from dietary inputs (Ehleringer et al. 2008; Newsome et al., 2020), it is unsurprising H and O atoms within ingested food have the most influence over hair and nail δ^2H and $\delta^{18}O$, with ~26-30%, and ~35% of H and O atoms in hair and nails of industrialized nation residents derived from drinking water, and the remainder sourced from food (Ehleringer et al., 2008; Sharp et al., 2003). Potential causes for variation in δ^2H and $\delta^{18}O$ of food items have already been discussed in section 4.1.1. In addition to isotopic variation of bodily hydrogen and oxygen inputs, numerous opportunities exist for *in-vivo* isotopic exchange and fractionation of H and O atoms prior to keratin synthesis, creating additional opportunities for isotopic variation.

4.2.2 Hair and Nail Hydrogen and Oxygen Isotope Variation Driven by Inter-Individual Metabolic Variations

Hydrogen atoms in proteins and amino acids may be subdivided into exchangeable and non-exchangeable categories, with all non-carbon-bonded H atoms subject to isotopic exchange (Morowitz & Chapman, 1955). Up to the time of keratin synthesis, it can be assumed that all exchangeable H atoms

have undergone complete exchange with follicle water, which can be considered a combination of blood water and water synthesized from drinking water and metabolized foods (Gretebeck et al., 1997; Kreuzer-Martin et al., 2005). Non-exchangeable H atoms in amino acids can be further classified as 'essential' (i.e., amino acids the body is not capable of synthesizing) or 'non-essential' (i.e., amino acids the body is capable of synthesizing). The compositions of the former should only reflect the δ^2H of ingested foods, as the skeletons of these amino acids are incorporated directly from food; hydrogen atoms from non-essential amino acids, by comparison, may have a δ^2H reflecting both dietary amino acids and the δ^2H of hydrogen pools within the body from which the atoms in these amino acids are also sourced (Ehleringer et al. 2008; Newsome et al., 2020).

Oxygen atoms in proteins undergo complete exchange with body and gut water during hydration, a digestive stage where peptides and amino acids are cleaved in the stomach and small intestine following hydration and adsorption through the gut wall (Schneider & Hall, 2005; Stewart et al., 2001). Subsequent oxygen isotope exchange may occur during the addition of the carbonyl-O to amino acids, but further oxygen isotope exchange is minimal while exposed to waters with a neutral pH (Angel & Orlando, 2006; Murphy & Clay, 1979). During subsequent synthesis of keratinous proteins, O atoms are either bound in the carbonyl group, or to hydrogen as a water, and the δ^{18}O of keratinous proteins should largely reflect the δ^{18}O of gut water at the time of digestion (Ehleringer et al., 2008).

A larger window for isotopic exchange of hydrogen in amino acids prior to their incorporation into hair or nail tissues, as compared to oxygen, is likely responsible for the lack of correlation ($R^2 = 0.04$) between $\Delta^2H_{hair\text{-}water}$ and $\Delta^2H_{nail\text{-}water}$ (Fig. 3.7) compared to a weak correlation ($R^2 = 0.29$) between $\Delta^{18}O_{hair\text{-}water}$ and $\Delta^{18}O_{nail\text{-}water}$ (Fig. 3.9). Body water δ^2H within a single individual continually varies by up to several per mil due to the isotopic variability of ingested foods and beverages, and differing amounts of fluid intake between individuals (Valenzuela et al., 2021). There is continuous exchange of H atoms in amino acids with body water that varies in its δ^2H up to the time amino acids are fixed into keratinous

tissue (Gretebeck et al., 1997; Kreuzer-Martin et al., 2005). This exchange will overwrite correlations between the δ^2H of amino acids and body water at the time of digestion. The faster formation rate of hair (~ 300 μm per day) versus nails (~100 μm per day) (Wilson & Gilbert, 2007; Zaias, 1990) can also overwrite correlations between the magnitude of $\Delta^2H_{hair\text{-}water}$ and $\Delta^2H_{nail\text{-}water}$; nails will integrate a longer, more variable time span of body water δ^2H compositions than hair. The large difference between hair and nail growth rates may also explain the extreme variation in $\Delta^2H_{hair\text{-}nail}$ observed in Table 3.8. Conversely, limited isotopic exchange of oxygen following adsorption of amino acids through the gut wall will better preserve correlations between the δ^{18}O of amino acids in dietary inputs and body water, and the magnitude of $\Delta^{18}O_{hair\text{-}water}$ and $\Delta^{18}O_{nail\text{-}water}$ at the time of digestion.

The supply of blood to hair follicles and nail beds may also create variability in the δ^2H and δ^{18}O of hair and nails. Blood supplying materials to form keratinous tissues, and nutrients for the follicle cells are primarily delivered through capillaries, in which laminar flow is believed to dominate (Barbee & Cokelet, 1971; Stonebridge & Brophy, 1991). Blood, being a suspension of red and white blood cells platelets, and plasma, requires turbulent flow to homogenously mix all components (Stepanchuk, 2017). There are very little data available in the literature describing the δ^2H and δ^{18}O of blood at discrete locations within a single individual, particularly within capillaries, most likely due to the difficulty in acquiring samples from such small blood vessels (Westerterp, 2017). Nevertheless, intracapillary variation of blood δ^2H and δ^{18}O may exist, and the contribution of variable isotopic composition of blood supply to hair follicle and nail bed cells towards intra-population variation of hair and nail H and O isotopes cannot be precluded.

Since hair and nail keratin, like tooth enamel, do not undergo remodelling after formation, their δ^2H, except for exchangeable fraction of H atoms, and δ^{18}O can be considered invariant after formation (Chesson et al., 2020; Meier-Augenstein & Fraser, 2008). Therefore, the isotopic composition of hair and nails will more closely reflect the instantaneous δ^2H and δ^{18}O of gut, body, and blood water pools, and

the composition of ingested amino acids. Additionally, human hair and nails grow at a rate of ~ 300 μm and 100 μm per day, respectively (Wilson & Gilbert, 2007; Zaias, 1990), much faster than tooth enamel, which forms at a rate of ~ 2.5-6.5 μm per day (Dean, 1998; Mahoney, 2008). Thus, hair and nails are hypothesized to have greater isotopic variability than tissues that undergo remodeling, or form at slower rates. The δ^{2}H and δ^{18}O of slower forming or remodelled tissues will reflect the mean values of dietary and drinking water inputs integrated over the duration of their formation or remodelling. Integrating isotopic signals over longer periods reduces isotopic variation caused by weekly-to-monthly dietary shifts that may be seen more prominently in hair and nails, as well as other faster growing tissues (Fraser & Meier-Augenstein, 2007).

Analysis of oxygen isotopes in bioapatite phosphate within bone and tooth enamel of multiple individuals residing in populations centers around the world seems to support this hypothesis. A maximum intra-population $\delta^{18}O_{PO4}$ range of 2.3 ‰ (Luz et al., 1984) was reported among residents of Ahmedabad, India, which is comparable to the smaller $\delta^{18}O_{hair}$ ranges reported for Belfast (Fraser & Kalin, 2006) and Fairbanks (O'Brien & Wooller, 2007**)** residents (Table 4.3), and to a blood water intra-population δ^{18}O range of 2.1 ‰ (n = 13), reported by Longinelli (1984). The intra-population range of δ^{18}O in enamel and bone bioapatite phosphate from several other localities (Daux et al., 2008; Levinson et al., 1987; Longinelli, 1984; Luz et al., 1984), however, remains smaller than the reported $\delta^{18}O_{hair}$ ranges of localities summarized in Table 4.3. The range of $\delta^{18}O_{PO4}$ from other studies is also smaller than any of the $\delta^{18}O_{nail}$ ranges (Table 4.3) found in the present study or previously published literature.

It is difficult to compare inherent variation of stable hydrogen isotope in human hair and nails to stable hydrogen isotope variation in other tissues. The lack of hydrogen atoms in bioapatite phosphate and structural carbonate preclude comparison with hair and nail δ^{2}H. However, H and O isotopes in bone collagen have been investigated and used in reconstruction of human dietary and migration patterns, and paleoclimatic conditions (Cormie et al., 1994; France et al., 2008; Reynard & Hedges,

2008). Recent work, however, has demonstrated the potential for isotopic exchange between H and O isotopes within bone collagen and in reagent waters during sample preparation; the magnitude of isotopic exchange depends on the on the reagents, and their isotopic compositions, used during the dissolution phase of collagen extraction (von Holstein et al., 2018). Given the influence of methodology on the final isotopic measurements, and the current limited availability of collagen O and H isotope data for modern individuals, it is likely not appropriate or even possible to assess the degree of intra-population δ^2H or $\delta^{18}O$ variability in bone collagen.

Despite the numerous non-methodological explanations for the smaller intra-population δ^2H_{hair} and $\delta^{18}O_{hair}$ ranges of East Greenbush, NY, USA, and GTA, Ontario, Canada residents, sample size appears to have a significant influence on the magnitude of H and O isotopic variation within a population. The small δ^2H_{hair} and $\delta^{18}O_{hair}$ ranges of East Greenbush and the GTA were obtained from a sample size of <10 individuals (Table 4.3). Small sample sizes appear to have poor reliability in capturing the true range of intra- population isotopic variability. To mitigate this uncertainty, sample sizes should ideally be no smaller than ~30 individuals (Perneger et al., 2015) when assessing the level of intra-population isotopic variation.

Due to the numerous steps and chemical reactions involved in protein digestion and *in-vivo* synthesis, and tissue formation—combined with variable δ^2H and $\delta^{18}O$ in dietary food items and inter-individual variations in metabolic and H and O pool turnover rates – it is unsurprising the isotopic separation between diet and drinking water inputs and body tissues is unique for every individual (Speakman, 1998). Isotopic 'noise' due to metabolic and dietary input variations are unavoidable in tissues, which can mask some of the isotopic signal from local drinking water (Bowen et al., 2009; Reynard et al., 2016). While numerous models describing the relationship between stable H and O isotopes in drinking water and hair or nail tissue have been published, including models accounting for consumption of variable proportions of locally grown food items, very few of the paired drinking water

and hair, and drinking water and nail δ^2H and δ^{18}O obtained in the present study fall on any of the model regression lines (e.g., Figs. 3.1, 3.3, 3.5, and 3.8).

4.3 Limitations on Use of Hydrogen and Oxygen Isotopes to Identify Region of Origin

The reconstruction of recent travel history and potential regions of origin of unidentified cadavers using stable isotope analysis of hair and nails has been a small but significant area of study in forensics (Bartelink et al., 2018; Chesson et al., 2018; Lehn et al., 2015; Meier-Augenstein, 2008). Numerous studies have measured the H and O stable isotope compositions of hair, nails and other tissues, over regional to global scales to create an isotopic database from which contour maps delineating regions characterized by similar δ^2H and δ^{18}O – commonly referred to as 'isoscapes' – can be developed (Bowen et al., 2007; Chesson et al., 2018; Ehleringer et al., 2010). The δ^2H and δ^{18}O of tissues that delineate an isoscape are commonly compared with corresponding δ^2H and δ^{18}O of local tap water or precipitation for the same region. The hope is that a global dataset of stable hydrogen and oxygen isotope ratios in hair, nail or other tissues can be utilized in conjunction with $\delta^2H_{drinking\ water}$ and $\delta^{18}O_{drinking\ water}$ at a given location to infer potential points of origin of unidentified persons using this information (Bartelink et al., 2018; Kemp & Meier-Augenstein, 2009; Rauch et al., 2007; Schwarcz & Walker, 2006).

There are several limitations to this approach. First, there are broad swaths of regions globally that exhibit similar precipitation δ^2H and δ^{18}O (Reynard et al., 2016; Rozanski et al., 1993). This obstacle, however, can be overcome, in some cases, by combining stable hydrogen and oxygen isotope data with those of carbon, nitrogen, and sulfur – or radiogenic isotope ratios (e.g., $^{87}Sr/^{86}Sr$) – to help discriminate among regions with similar precipitation δ^2H and δ^{18}O, but isotopically distinct dietary habits (Bol & Pflieger, 2002; Bol et al., 2007) or bedrock geology that affects the Sr-isotope composition of locally consumed food (Price et al., 2020).

A second source of uncertainty is the potential for geographic or seasonal variation of the isotopic composition of local tap water at local scales (Kennedy et al., 2011). There are two primary drivers of such variation. First, the water demands of larger urban population centers often necessitates drawing on multiple isotopically distinct water sources that may be a great distance away (Good et al., 2014; Jameel et al., 2016; Tian et al., 2020). The second is the use of surface water reservoirs that are subject to seasonal variation due to evapoconcentration to supply drinking water (Chen & Tian, 2021; Reynard et al., 2016; Ruan et al., 2020). In the present study, the 22-year residence time of Lake Huron water (Quinn, 1992), from which London, Ontario, Canada obtains its drinking water, results in minimal seasonal variation of London municipal tap water δ^2H and δ^{18}O (Table 3.1).

Third, the range of hair and nail δ^2H and δ^{18}O across London, Ontario, Canada residents consuming, and in isotopic equilibrium with, a common drinking water source, is much larger than the range of precipitation for the region in which the population lives. Hair and nails formed at a single locality are expected to have a δ^2H and δ^{18}O range that could encompass those of precipitation falling over a much more geographically extensive area than the locality of tissue growth. For example, a mean annual δ^2H of –81.5 ‰ and –64 ‰, and a mean annual δ^{18}O of –11.7 ‰ and –9.6 ‰ was measured for precipitation falling at Turkey Lake, near Sault Ste. Marie, Ontario, Canada, and London, Ontario, Canada, respectively (F. Longstaffe, personal communication, November 28, 2021). The difference between the δ^2H and δ^{18}O of precipitation falling at London, and Turkey Lake is 17.5 ‰ and 2.1 ‰, respectively, a difference about the same as the δ^2H_{nail} and $\delta^{18}O_{hair}$ range of London residents who reported exclusively drinking municipal tap water (Table 4.1). The difference between the δ^2H and δ^{18}O of precipitation falling at London and Turkey Lake is smaller still than the range of δ^2H_{hair} and $\delta^{18}O_{nail}$ in London residents drinking municipal tap water (Table 4.1). Large intra-population hair and nail H and O isotope variability casts significant doubt on the ability to provenance tissues of unknown origin using hair and nail δ^2H and δ^{18}O to reconstruct drinking water isotopic composition. Such calculated values for

drinking water δ^2H and δ^{18}O would be bounded by large confidence intervals, corresponding to broad geographic areas, and thus severely limiting the utility of this forensic technique.

Indeed, such attempts to identify the provenance unidentified human remains have been limited to semi-hemispheric or continental-scale resolution, even when all or a combination of δ^{13}C, δ^{15}N, and δ^{34}S measurements were made to further restrict potential regions of origin (Bartelink & Chesson, 2019; Chesson et al., 2018; Meier-Augenstein, 2018). Variable δ^2H and δ^{18}O of dietary inputs will drive variation in the δ^2H and δ^{18}O of body and gut water, which undergo isotopic exchange with ingested proteins, from which hydrogen and oxygen atoms in hair and nail keratin are derived (Ehleringer et al., 2008; O'Brien & Wooller, 2007). Variation in metabolic rates will also exist across individuals in all population centers; however, some drivers of isotopic variability in tissues will be more constrained in some populations compared to others. Populations consuming drinking water sources that undergo little to no temporal or spatial isotopic variation, as well as populations consuming an isotopically homogenous 'continental supermarket' diet should exhibit lower intra-population hair and nail δ^2H and δ^{18}O variability (Bowen et al., 2009). Thus, attempts to differentiate tissues from distinct regions of origin using stable H and O isotopes in hair or nail tissue will have less uncertainly when performed on populations consuming temporally or spatially invariant waters, than amongst populations with greater isotopic variability in resident tissues' (Reynard et al., 2016).

4.4 Potential Applications of Stable Hydrogen and Oxygen Isotopes in Tissues and Drinking Water

While numerous confounding variables make attempts to identify the specific region of origin of hair and nails using stable isotopes of hydrogen and oxygen isotopes alone highly uncertain, there are still useful applications of such data. As previously noted, such results for human hair and nails have been used to identify recent travel between regions with distinct drinking water δ^2H and δ^{18}O (e.g.,

Fraser & Kalin, 2006; Meier-Augenstein & Fraser, 2008; O'Brien & Wooller, 2007), and to distinguish local and non-local residents of a single population center (Mancuso & Ehleringer, 2019; Podlesak et al., 2012). The ability to classify an unknown individual as a local or non-local resident using hair or nail δ^2H and δ^{18}O is of use in situations where remains may be too badly degraded or fragmented to use conventional identification methods such as fingerprints, dental records, or DNA. In the case of the latter identification method, stable isotope analysis offers the added benefit of being far less costly and time consuming (Carreon-Martinez & Heath, 2010) and does not require comparison with extant DNA profiles of an unknown individual or their relatives (Budowle et al., 2005; Chesson et al., 2018).

Identifying an individual as a local or non-local resident of a population center can limit the number of missing persons reports that need be reviewed during a forensic investigation to learn a victim's identity. If a tissue sample from an unknown individual is found to possess similar δ^2H and δ^{18}O to the range known for the local population, review of missing persons reports can be limited to those filed by police departments within an 'isoscape' characterized by similar hair and nail δ^2H and δ^{18}O. While an isoscape may encompass a geographically large area, it may still be small enough to preclude the need for a national or international search of missing persons reports.

4.5 Oxygen and Hydrogen Isotope Variation Among Dietary Groups

The small mean δ^{18}O range of 0.9 ‰ and 1.1 ‰ among the dietary group hair and nail samples in this study (Figs. 3.2 and 3.6), respectively, indicates that the oxygen isotope composition of dietary inputs consumed by each dietary group are similar. The mean dietary group hair and nail δ^2H exhibits a more complex relationship across all groups. Early work by Estep & Dabrowski (1980) found correlations between the δ^2H of dietary inputs and tissues in mice, suggesting a relationship between tissue δ^2H and trophic position. More recent studies found bone collagen δ^2H to be higher in carnivorous species relative to omnivorous or herbivorous species (Birchall et al., 2005; Topalov et al., 2013); these studies, however,

do not account for the exchangeable fraction of H atoms within collagen previously described in section 4.2.2. Additional studies have found correlations between tissue δ^2H and $\delta^{15}N$ (Birchall et al., 2005; Soto et al., 2011; Topalov et al., 2013), further suggesting trophic δ^2H enrichment, although it is unclear if this apparent enrichment is due to metabolic effects, as with trophic enrichment of $\delta^{15}N$ (DeNiro & Epstein, 1981; Minagawa & Wada, 1984), or due to compounding environmental water contributions with increasing trophic position (Solomon et al., 2009; Soto et al., 2013).

A trend of increasing mean δ^2H_{nail} with increasing trophic position was observed in this study (Fig. 3.6), with vegetarians exhibiting the lowest mean δ^2H_{nail}, followed by semi-vegetarians, omnivores, and the highest mean δ^2H_{nail} observed for carnivores. The relationship between mean δ^2H_{hair} and trophic position is less consistent across dietary groups in this study. Vegetarians exhibit the lowest mean δ^2H_{hair}, followed by carnivores and then semi-vegetarians, with the highest mean δ^2H_{hair} exhibited by omnivores (Fig. 3.2), suggesting that trophic enrichment of δ^2H is inconsistent across tissues from the same individuals. The inconsistent relationship between tissue δ^2H and trophic position have been observed in other studies (Hobson et al., 1999; Vander Zanden et al., 2016). In contrast to δ^2H, trophic position is poorly correlated with tissue $\delta^{18}O$, limiting the applications of oxygen isotopes within food web studies (Nielson & Bowen, 2010; Schilder et al., 2015; Wang et al., 2009).

Modification of the stable isotopic compositions of raw foods during cooking (Daux et al., 2008; Royer et al., 2017; Tuross et al; 2017) may be a more important control on hair and nail δ^2H than the trophic position occupied by and individual. Alteration to the protein, carbohydrate, lipid, and water content of food (Daux et al., 2008; Weber et al., 2008), as well as cooking method (Bognár, 1998; Oluwaniyi et al., 2010; Royer et al., 2017) have been shown to change the $\delta^{18}O$ of cooked foods from their uncooked values. Royer et al. (2017) reported that $\delta^{18}O$ are lower in cooked terrestrial meats, compared to the raw food, while marine meats are ^{18}O-enriched relative to the uncooked equivalents, regardless of whether the meat was boiled, roasted, or fried. Preferential consumption of one protein source over

another has been shown to cause small $\delta^{18}O$ shifts (~0.6 ‰) in bioapatite phosphate (Royer et al., 2017). Water lost to evaporation during cooking and chemical changes to carbohydrate, lipid, and protein contents all play a large role in altering the oxygen isotope composition of cooked foods. Similar changes can be expected between the $\delta^{2}H$ of cooked food and its uncooked equivalent.

The range of $\delta^{2}H_{hair}$ across all dietary groups is 17.1 ‰, and the total range of mean dietary group $\delta^{2}H_{nail}$ is 7.2 ‰. The smaller range of $\delta^{2}H_{nail}$, compared to hair, may reflect the slower formation rate of the former, which allows more time for integration of hydrogen from all isotopic pools within the body (Fraser & Kalin, 2006).

4.6 Variation of Stable Carbon Isotopes in Hair and Nails

Stable isotopes of carbon were measured in hair and nail tissues to assess intra-population $\delta^{13}C$ variability within an urban population center. Collecting hair and nail $\delta^{13}C$ data is useful to determine if there have been substantial changes in the dietary habits of London residents since the start of the COVID-19 pandemic by comparing the values obtained in this study against published $\delta^{13}C_{hair}$ data. The calculated range of $\delta^{13}C_{hair}$ (3.8 ‰) in London omnivores sampled in this study is much larger than the range previously reported by Bataille et al. (2020) (0.2 ‰). The range of $\delta^{13}C_{hair}$ measured by Bataille et al. (2020) in pre-pandemic southern Ontario omnivores (2.6 ‰) is also smaller than the range obtained in the present study. The small sample size (n = 3) of previously published $\delta^{13}C_{hair}$ for London, Ontario, Canada residents, compared to the present study (n = 27), may be too small to capture the true extent of $\delta^{13}C_{hair}$ variation within the population.

The previously reported ranges of London and southern Ontario resident $\delta^{13}C_{hair}$ may also reflect differences in the food knowledge and dietary choices between individuals living in urban versus rural areas (Colley et al., 2022). The smaller $\delta^{13}C_{hair}$ ranges previously reported in the available literature could be due to an overrepresentation of omnivorous diets containing large proportions of corn-fed

animal products (Bol & Pflieger, 2002; O'Connell & Hedges, 1999). Corn is the largest cereal crop grown globally (Hamel & Dorff, 2014), and is both one of the most common ingredients in animal feed and agricultural products that utilizes the C_4 photosynthetic pathway (Heaton et al., 2008; Martinelli et al., 2011). Dietary groups consuming lower proportions of corn-fed animal products in their diet, if any are consumed at all, should have lower tissue $\delta^{13}C$ (Huelsemann et al., 2009). Previous studies, however, have reported the relationship between $\delta^{13}C_{hair}$ and $\delta^{13}C_{nail}$ in vegans, vegetarians, and omnivores to be inconsistent, with lower $\delta^{13}C_{hair}$ values observed in vegans and vegetarians compared to omnivores in some but not all studies (Bol & Phlieger, 2002; Nardoto et al., 2006; O'Connell & Hedges, 1999).

The respective standard deviation (± 1.0 ‰ and ± 0.9 ‰) of London resident $\delta^{13}C_{hair}$ and $\delta^{13}C_{nail}$ obtained in the present study is larger than the ± 0.5 ‰ standard deviation obtained for $\delta^{13}C_{hair}$ of Canadians at the national scale (Lehn et al., 2015). It is not known, however, what dietary habits (i.e., omnivore, vegetarian, etc.) were adhered to by the individuals who contributed the hair samples from which the national average was determined.

4.6.1 Variation of Dietary Group Mean Hair and Nail $\delta^{13}C$

The similarity between the mean $\delta^{13}C_{hair}$ of London omnivores in the present study (mean $\delta^{13}C_{hair}$ = –18.6 ± 0.9‰, n = 19) and mean previously reported values for London and southern Ontario omnivores (mean $\delta^{13}C_{hair}$ = –17.7 ± 0.1 ‰, n = 5; –18.0 ± 0.6 ‰, n = 24, respectively) would indicate that COVID-19 has had a minimal impact on the dietary habits of London and southern Ontario residents. The respective 0.9 ‰ and 0.6 ‰ decrease of London omnivore $\delta^{13}C_{hair}$ from pre-pandemic results for London southern Ontario omnivores reported by Bataille et al. (2020) would, if anything, suggest a modest reduction of corn-fed meat products in London omnivore diets (Huelsemann et al., 2009). This was an initially unexpected result, as over 60 % of the total farmland seeded for corn production in Canada is found in Ontario, and another 30 % located in the adjacent province of Quebec (Hamel & Dorff, 2014).

Thus, it might have been expected that any disruptions to the food supply chain in the London, or Southern Ontario area due to COVID-19 may necessitate a greater reliance on locally produced foods. Increased use of locally sourced corn to raise livestock would result in higher $\delta^{13}C$ in animal derived proteins, compared to animals fed a C_3 based diet of barley, rice, or oats. Instead, the slight decrease of London omnivore $\delta^{13}C_{hair}$ may reflect displacement of fast-food that contains corn-fed meats (Chesson et al., 2010b), by home-prepared meals (Bakaloudi et al., 2021)

The similarities between the mean $\delta^{13}C_{hair}$ and $\delta^{13}C_{nail}$ of London omnivores and carnivores (mean $\delta^{13}C_{hair}$ = –17.9 ± 0.3 ‰; mean $\delta^{13}C_{nail}$ = –18.2 ± 0.6 ‰, n = 3) in the present study would also indicate that both dietary groups consumed a similar diet, with respect to ^{13}C (Bol et al., 2007; DeNiro & Epstein, 1978; Huelsemann et al., 2009). The categorization of individuals into omnivore and carnivore dietary groups, despite the isotopic data that suggest each dietary group consumed similar proportions of C_3 and C_4 plants, is likely due to inadequate definition of each dietary group in the screening survey participants completed prior to sampling. Omnivores were defined to consume meat at least once per week, in addition to eggs, dairy and fish. Carnivores were defined as obtaining a significant portion of their daily caloric intake from meat or other animal products. Under these definitions, respondents may have identified themselves as carnivores, yet they may have still directly consumed substantial plant material, such as vegetables and legumes, breads, and fruit. Additionally, omnivores may consume similar amounts animal proteins as carnivores in a non-meat form. Dairy products or eggs can exhibit a similar $\delta^{13}C$ as meat products, particularly if dairy, egg and meat products are obtained from animals raised on feed with a similar composition of C_3 vs C_4 plants (Jenkins et al., 2001; Miller et al., 2008; Rogers, 2009).

Weakly statistically significant differences were found between the $\delta^{13}C_{hair}$ of London omnivores in this versus previously reported studies (Table 3.7), and between previously reported $\delta^{13}C_{hair}$ of London versus southern Ontario omnivores (Bataille et al., 2020). When the mean $\delta^{13}C_{hair}$ values of these dietary

groups are compared, however, all groups are separated by less than 1 ‰, except for the semi-vegetarians and vegetarians (Table 4.4). This small difference is a strong indication that individuals belonging to the dietary groups consumed diets having similar carbon isotope compositions. This result is not surprising. Most food consumed by London and surrounding southern Ontario residents is domestically produced, resulting in food production and distribution system that are less reliant on the predictability of supply chains (Zhu et al., 2020).

Table 4.4. Comparison of mean dietary group hair $\delta^{13}C$ and $\delta^{15}N$ for residents of London from this study with those previously reported for London and southern Ontario residents (Bataille et al., 2020), and a Canadian national mean (Lehn et al., 2015).

Dietary Group	$\delta^{13}C_{hair}$ *(‰, VPDB)*	$\delta^{15}N_{hair}$ *(‰, AIR)*	***n***
London (this study)			
Carnivore	–17.9 ± 0.3	+9.0 ±0.4	3
Omnivore	–18.6 ± 0.9	+9.1 ± 0.3	19
Semi-vegetarian	–19.9 ± 0.9	+8.6 ± 0.4	3
Vegetarian	–19.2	+8.4	1
London (Bataille et al., 2020)			
Omnivore	–17.7 ± 0.1	+8.8 ± 0.3	5
Semi-vegetarian	–17.7	8.3	1
Southern Ontario (Bataille et al., 2020)			
Omnivore	–18. ± 0.6	9.1 ± 0.5	24
Canadian National Mean (Bataille et al., 2020)			
Omnivore	–18.5 ± 0.6	9.2 ± 0.5	581

Canadian National Mean (Lehn et al., 2015)			
Omnivore	–18.2 ± 0.5	+8.3 ± 0.5	15

4.7 Variation in Stable Nitrogen Isotopes in Hair and Nails

The range of $\delta^{15}N_{hair}$ and $\delta^{15}N_{nail}$ measured in this study, at 1.4 ‰ and 1.6 ‰ (Table 3.5), respectively, is larger than the previously reported range of $\delta^{15}N_{hair}$ for London omnivores (0.6 ‰) but similar to the range of published southern Ontario omnivores (1.7 ‰) (Bataille et al., 2020). The range of $\delta^{15}N_{nail}$ values is comparable to that of hair. A smaller sample size, rather than overrepresentation of omnivorous diets, is the likely cause of the smaller range of $\delta^{15}N_{hair}$ values previously reported for London omnivores. The standard deviation of the mean $\delta^{15}N_{hair}$ of all individuals in the present study (± 0.4 ‰) is similar to those obtained using published data for omnivores from London, southern Ontario, and Canadians nationwide (± 0.3 ‰, ± 0.5 ‰, and ± 0.5 ‰, respectively) (Bataille et al., 2020; Lehn et al., 2015). This similarity suggests that the width of the $\delta^{15}N$ data distribution curve is approximately equal for all groupings. As for carbon isotopes, the separation between mean dietary group hair nitrogen isotope compositions is small, with mean $\delta^{15}N_{hair}$ for all dietary groups in this study and previously published data (Bataille et al., 2020) spanning a range of < 1 ‰ (Table 4.4). The small differences between $\delta^{15}N_{hair}$ values in this study and previously reported data for London and southern Ontario residents with similar dietary habits reinforces the assertation that COVID-19 has had a minimal impact on the dietary habits of individuals living in this region.

The mean $\delta^{15}N_{hair}$ for London omnivores in this study, and published values for southern Ontario omnivores, unlike their corresponding values of $\delta^{13}C_{hair}$, are the highest of any dietary group (Table 4.4). The slightly higher mean $\delta^{15}N_{hair}$ for London omnivores (+9.1 ± 0.3 ‰, n = 19) in the present study compared to the previously reported mean value of +8.8 ± 0.3 ‰ (n = 5) would suggest a modest increase in animal protein consumption by London residents (Huelsemann et al., 2009; Petzke et al.,

2005). This observation, however, is contradictory to the reduction of corn-raised animal product consumption by London omnivores predicted based on the mean dietary group $\delta^{13}C$ (Table 4.4). Such a contradiction may be due to a slightly increased consumption of marine meats (Schoeninger & DeNiro, 1984; Shoeninger & Moore, 1992), combined with a reduction of corn-fed fast-food meats in the diets of London residents during the COVID-19 lockdown period (Bakaloudi et al., 2021; Chesson et al., 2010b). The mean $\delta^{15}N_{hair}$ and standard deviations for southern Ontario omnivores (+9.1 ± 0.3, n = 24) reported by Bataille et al. (2020) are identical to the value obtained for London omnivores in the present study, and to the national mean of +9.2 ± 0.5 ‰ (n = 581) calculated from data reported by Bataille et al. (2020). These similarities suggest London and surrounding southern Ontario residents consume an isotopically similar diet and that the overall isotopic composition of London resident's diets have not changed substantially, with respect to ^{15}N, since the start of the pandemic. The apparent increase of London resident $\delta^{15}N_{hair}$ from pre-COVID-19 value reported by Bataille et al. (2020) to the value obtained in the present study is likely due to the small sample size of pre-COVID-19 London residents.

The highest $\delta^{15}N$ value for hair or nails was not observed in the carnivore dietary group from London, which is inconsistent with typically observed increasing concentration of ^{15}N with increasing trophic level (Buchardt et al., 2007; DeNiro & Epstein, 1981; O'Connell & Hedges, 1999). Instead, carnivores have hair and nail $\delta^{15}N$ similar to omnivores in this study. As for carbon isotopes, this result likely arises from inadequate criteria for defining each dietary group in the survey that participants completed prior to sampling. For the definitions used the in survey, omnivores who consumed meat or fish more than once per week, although perhaps not daily, could consume a similar level of animal proteins as carnivores. Section 1.2.4 describes the importance of marine versus terrestrial protein proportions in an individual's diet on the overall $\delta^{15}N$ of tissues. Diets based on marine protein sources, such as fish and shellfish, result in higher $\delta^{15}N$ of tissues than tissues formed by individuals consuming diets based on terrestrial proteins, such as chicken, pork, or beef (Martin, 1999; Schoeninger & DeNiro,

1984; Shoeninger & Moore, 1992; Schwarcz et al., 1985). The highest hair and nail δ^{15}N of omnivores – as opposed to carnivores, as expected – may be due to greater proportions of marine meats, that are typically ^{15}N-enriched relative to terrestrial meats, in the diets of omnivores than in the diets of carnivores.

Support for this explanation can be found in Table 3.6, which summarizes the dietary protein sources on a scale of 1 to 10 for participants in the present study. A score of 1 denotes 100 % marine protein consumption and a score of 10 denotes 100 % terrestrial protein consumption. Of the participants who identified as carnivores, the lowest numbered response to the screening questionnaire's protein source question was 8. Conversely, 11 participants who identified as omnivores reported obtaining at least 30 % of their protein from marine sources, with 4 of the 11 respondents reporting they obtain at least 50 % of their consumed protein from marine sources. This would allow ^{15}N-enriched marine meats to raise the mean hair and nail δ^{15}N of omnivores to a similar, or higher level than carnivores.

Chapter 5

5.0 Conclusions

Lockdowns and travel restrictions imposed during the COVID-19 pandemic have forced large fractions of the population to remain in one location long enough for hair and nails to reach isotopic equilibrium with drinking water and dietary supplies (Cerling et al., 2007; Katzenburg & Krouse, 1989; Mancuso & Ehleringer, 2019; O'Brien & Wooller, 2007). These mobility restrictions have created a unique opportunity for this thesis to explore the relationship between the δ^2H and δ^{18}O of the primary drinking water supplies of residents of London, Ontario, Canada – typically the London municipal tap water supply – and the corresponding isotopic compositions of resident's hair and nails. This study has also used the stable isotope compositions of carbon and nitrogen to investigate the effects of the COVID-19 pandemic on the dietary habits of London and surrounding southern Ontario area residents. This chapter summarizes the findings of chapters 3 and 4 and expands on them by suggesting pathways for future research on this subject.

5.1 Hair, Nail, and Drinking Water δ^2H and δ^{18}O

Values of hair, nail and drinking water δ^2H and δ^{18}O for all participants in the present study are summarized in Tables 3.1 and 3.8. All $\delta^2H_{drinking\ water}$ measurements spanned a range of 20.6 ‰. Values of δ^2H_{hair} and δ^2H_{nail} spanned a range of 24.2 ‰ and 18.4 ‰, respectively, similar to the range of all drinking water samples. Drinking water δ^{18}O spanned a range of 4.0 ‰, $\delta^{18}O_{hair}$ and $\delta^{18}O_{nail}$ spanned similar ranges of 3.7 ‰ and 4.5 ‰, respectively. Table 4.3 summarizes the ranges of intra-population δ^2H and δ^{18}O variation of hair and nails measured in this study and those reported at other localities. The range of intrapopulation δ^2H hair and nail variation in the present study are comparable, in most cases, to ranges reported for hair samples collected from other localities prior to the start of the COVID-19 pandemic (Bol et al., 2007; Fraser & Kalin, 2006; Mancuso & Ehleringer, 2019; O'Brien & Wooller,

2007). The range of δ^2H_{hair} obtained in the present study was larger than those reported for residents of East Greenbush, NY, USA (O'Brien & Wooller, 2007), and the greater Toronto area (GTA), Ontario, Canada (Mant et al., 2016). The latter also reported a smaller $\delta^{18}O_{hair}$ range than was obtained in the present study. The smaller ranges from these two comparator regions have been attributed to the small sample sizes ($n < 10$) collected.

5.1.1 δ^2H and $\delta^{18}O$ Variation in London Municipal Tap Water versus Hair and Nails

The O and H isotope ranges of hair, nail and drinking water samples are comparable at first glance. A different story emerges, however, when the δ^2H and $\delta^{18}O$ ranges of the London municipal tap water supply are compared with those of hair and nail samples collected from participants who reported London municipal tap water supply as their sole source of drinking water (Table 4.1). The δ^2H and $\delta^{18}O$ ranges of the London municipal tap water supply, at 3.4 ‰ and 0.5 ‰, respectively, are significantly smaller than the total range of all drinking water samples collected in this study. Conversely, the range of δ^2H_{hair} for participants exclusively drinking London municipal tap water remains at 24.2 ‰ and δ^2H_{nail} spans an only slightly smaller range of 16.5 ‰. The $\delta^{18}O_{hair}$ and $\delta^{18}O_{nail}$ range from participants exclusively drinking the London municipal tap water supply is also significantly larger, at 2.3 ‰ and 3.0 ‰, respectively, than their drinking water inputs. Potential explanations for these differences can be divided into two broad categories. The first is δ^2H and $\delta^{18}O$ variation within food and beverage inputs and the second is inter-individual metabolic differences and variations in the δ^2H and of body and gut water pools.

About 70-74 % of H atoms and ~ 65 % of O atoms in hair and nails are obtained from foods (Ehleringer et al., 2008; Sharp et al., 2003). Therefore, the δ^2H and $\delta^{18}O$ of ingested foods should strongly influence hair and nail δ^2H and $\delta^{18}O$. Bowen et al. (2009) observed a larger range of δ^2H_{hair} and $\delta^{18}O_{hair}$ in pre-globalized indigenous populations, which was attributed to increased consumption of

locally produced foods throughout the year and under different climatic conditions relative to the increasingly isotopically homogenous and vertically integrated supply chains that have created a 'supermarket' diet consumed by residents of modern, globalized nations (Chesson et al., 2010b; O'Brien & Wooller, 2007). That said, Canada's relatively small population, compared to its large geographic size, makes the transportation of low-cost bulk food items over long distances less economical than in smaller, more populous regions. As a result, >70 % of all food consumed by Canadians is domestically produced (Alverez, 2019) and may contribute to larger than expected variation of hair and nail δ^2H and δ^{18}O based on the O and H isotope compositions of local drinking water.

Variations between individuals in metabolic rates and the δ^2H and δ^{18}O of body water pools are another significant driver of inter-individual tissue variation. Non-carbon bonded hydrogen atoms within amino acids are assumed to undergo complete exchange with follicle water up to the point of keratin synthesis (Gretebeck et al., 1997; Morowitz & Chapman, 1955). Exchangeable H atoms should therefore reflect the instantaneous δ^2H of all ingested drinking water, as well as water synthesized from food and water within food. Nonexchangeable H atoms in essential amino acids should reflect the bulk δ^2H of amino acids within the diet. Conversely, non-essential amino acids should exhibit a δ^2H that reflects a combination of dietary amino acids and pools within the body from which hydrogen atoms may be drawn during amino acid synthesis (Ehleringer et al., 2008).

Oxygen atoms within amino acids also undergo complete exchange during hydration and peptide cleaving within the stomach and small intestine following adsorption through the wall (Schneider & Hall, 2005; Stewart et al., 2001). Subsequent O isotope exchange may occur during the addition of the carbonyl O to amino acids; however, unlike hydrogen, further O-isotope exchange is minimal while exposed to waters with a neutral pH (Angel & Orlando, 2006; Murphy & Clay, 1979). The δ^{18}O of hair and nails should therefore reflect the composition of gut water at the time of digestion.

5.1.2 Performance of Model Relationships between Hair and Nail δ^2H and $\delta^{18}O$ and Drinking Water

Previously reported models do not accurately predict London resident hair and nail hydrogen and oxygen isotope compositions based on measured values of $\delta^2H_{drinking\ water}$ and $\delta^{18}O_{drinking\ water}$. This failure is most likely due to significant incorporation of water having both higher and lower H and O isotope compositions than the readily identified drinking water. The possibilities include:

i. Differences between the δ^2H and $\delta^{18}O$ of London municipal tap water and precipitation or groundwater used to irrigate agricultural crops (Brooks et al., 2014; Ruan et al., 2020);

ii. Preferential use of ^2H and ^{18}O-enriched summer precipitation and evapoconcentrated soil water by plants during the growing season (Dansgaard, 1964; Ehleringer et al., 1991; Sprenger et al., 2017; Yurtsever & Gat, 1980);

iii. Consumption of non-local food and beverage items grown in regions with higher precipitation or irrigation water δ^2H and $\delta^{18}O$ (Calderone & Guillou, 2008; Chesson et al., 2010b; Lamb et al., 2014); and

iv. Significant consumption of evapoconcentrated waters, such as those that have been standing within pipes for long periods, waters that have been boiled to prepare hot beverages, or cooked foods that have undergone evaporative reduction, such as soups and stews foods, and whose carbohydrate, lipid and protein contents have been altered relative to pre-cooked values or during preparation (Bognár, 1998; Brettel et al., 2012; Daux et al., 2008; Royer et al., 2017; Tuross et al., 2017).

5.1.3 Limitations on the Application of Hydrogen and Oxygen Isotope Compositions in Hair and Nails

This study has demonstrated that considerable intra-population variation in tissue δ^2H and $\delta^{18}O$ exists among individuals who reported consuming a common drinking water source that exhibits minimal temporal and spatial isotopic variation and have remained in one area long enough to reach isotopic equilibrium with their locality. This study has also shown that substantial intra-population variation exists for $\Delta^2H_{tissue\text{-}water}$ and $\Delta^{18}O_{tissue\text{-}water}$. As a result, hair and nails formed at a specific locality can exhibit a range of δ^2H and $\delta^{18}O$ that could mistakenly be taken to reflect a substantially larger geographic area than is truly the case for the sampled individuals.

The magnitude of intra-population hair and nail δ^2H and $\delta^{18}O$ variability casts doubt on the ability to infer their source through reconstruction of putative drinking water isotopic compositions. Drinking water δ^2H and $\delta^{18}O$ calculated from hair and nail hydrogen and oxygen isotope compositions are bounded by large confidence intervals, corresponding to numerous potential points of origin, thus severely limiting the utility of this forensic technique.

5.2 Effects of COVID-19 on the Dietary Habits of London and Southern Ontario Residents Reflected in Hair and Nail $\delta^{13}C$

This study measured mean hair and nail $\delta^{13}C$ for London omnivores and found less than 1 ‰ of difference from previously published values (Bataille et al., 2020) for London and southern Ontario omnivores. The small change in London omnivore $\delta^{13}C$ from pre-pandemic measurements, despite being statistically significant (p = 0.038; see Table 3.7 for additional statistical comparisons) suggests that the COVID-19 lockdown and associated travel and supply chain interruptions had a minimal impact on the dietary habits of London and southern Ontario residents. No statistically significant differences were observed between published values (Bataille et al., 2020) of southern Ontario and London omnivores in

the present study (p = 0.137), supporting the assertation COVID-19 has not significantly altered the dietary habits of local residents. The slightly lower hair and nail $\delta^{13}C$ of London residents in this study, compared to previously reported values for the region and may reflect a slight decrease in the consumption of animal proteins raised on corn-based feed. Several potential reasons for this dietary change are possible, including reduced fast-food intake, meat product price increases, fear of viral contamination of meat products, supply disruptions due to COVID-19 outbreaks at meat processing plants, or ethical reasons (Bakaloudi et al., 2021; Chesson et al., 2010b; Donaldson, 2020; Loh et al., 2021).

Similar hair and nail $\delta^{13}C$ between carnivores and omnivores probably arose from inadequate criteria for defining each dietary group in the screening survey participants completed prior to sampling. Those definitions still allowed self-identified 'carnivores' to directly consume substantial amounts of non-meat items, such as, breads, and fruit, and omnivores to consume similar amounts animal proteins as carnivores in a non-meat form, e.g., eggs and dairy products.

5.3 Effects of COVID-19 on the Dietary Habits of London and Southern Ontario Residents Reflected in Hair and Nail $\delta^{15}N$

No statistically significant differences were found between the slightly higher mean $\delta^{15}N_{hair}$ measured for London omnivores in this study and previously published data for London omnivores (Bataille et al., 2020), suggesting only a modest increase in animal protein consumption by London residents during the COVID lockdown period. This modest increase is nevertheless contradictory to the slight decrease in corn-fed animal product predicted by $\delta^{13}C_{hair}$ and may be due to a combination of reduced corn-fed fast-food meat intake (Bakaloudi et al., 2021; Chenarides et al., 2021; Chesson et al., 2010b) and increased consumption of marine meats (Schoeninger & DeNiro, 1984; Shoeninger & Moore, 1992) during the pandemic. Previously published southern Ontario omnivores (Bataille et al., 2020) have

a mean $\delta^{15}N_{hair}$ identical to the value obtained for London omnivores in the present study, suggesting COVID-19 has had a minimal effect on the $\delta^{15}N$ composition of London and southern Ontario resident's diets. The assertation is supported by the <1 ‰ separation between the mean $\delta^{13}C_{hair}$ of previously published (Bataille et al., 2020) London and southern Ontario omnivore data, and the value obtained in the present study. The highest hair and nail $\delta^{15}N$ for omnivores and not carnivores may once again reflect inadequacies in the dietary group definition criteria within the screening questionnaire. Using those definitions, omnivores who consume ^{15}N-enriched meat or fish more than once per week, although perhaps not daily, could consume a similar level of animal proteins as carnivores. The small range in $\delta^{15}N$ (1.0 ‰ and 1.2 ‰ for hair and nails, respectively) among dietary groups may also simply reflect inherent intra-population variability among residents consuming similar diets.

Given the overall range of hair and nail $\delta^{15}N$, the small range of hair and nail $\delta^{15}N$ across all dietary groups, and the only minimal differences from previously published data for London and southern Ontario, it appears that COVID-19, and associated lockdowns and supply chain interruptions, have had a minimal impact on the dietary habits of individuals living in this region.

5.4 Future Work

This study has proposed that the poor predictive performance of hair and nail H- and O-isotope compositions based on $\delta^{2}H_{drinking\ water}$ and $\delta^{18}O_{drinking\ water}$ using published models reflects their failure to incorporate the contribution of other water to body water. In future investigations, steps should be taken to better understand the stable hydrogen and oxygen isotope composition of drinking water and dietary inputs. This might be accomplished by analyzing the $\delta^{2}H$ and $\delta^{18}O$ composition of foods (raw and cooked) consumed by participants over a ~30-day period. In addition to $\delta^{2}H_{drinking\ water}$ and $\delta^{18}O_{drinking\ water}$, the $\delta^{2}H$ and $\delta^{18}O$ of non-water beverages such as wine, beer, coffee or tea should be measured to gain a representative $\delta^{2}H$ and $\delta^{18}O$ baseline across all H and O inputs to the body.

To better understand proportions of plant and animal proteins that comprise an individual's diet and the stable carbon and nitrogen isotope baseline across dietary inputs, all foods consumed by study participants should be weighed and logged, as is done in several food-journal diet programs (e.g., Painter et al., 2017). Ideally, populations consistently consuming the same foods, such as students eating at school dining halls or cafeterias or armed forces personnel, who regularly eat mass communal meals prepared in bulk, could be invited to join such studies. The homogenous diets of the aforementioned population groups should help reduce $\delta^{2}H$ and $\delta^{18}O$ variability across dietary inputs in addition to reducing hair and nail $\delta^{2}H$ and $\delta^{18}O$ variability across sampled individuals. Furthermore, targeting such population groups should help alleviate issues where participants self-identify as a member of a specific dietary group (e.g., omnivores) and yet exhibit stable isotope compositions in their hair and nails that would be expected in a dietary group possibly occupying a higher trophic level.

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Appendices

Appendix A: Western University Health Science Research Ethics Board (HSREB) approval letter, and Continuing Ethics Review (CER) approval letter for methods to be used, and sample materials to be collected in the present study.

Western Research

Date: 4 November 2020

To: Dr Fred Longstaffe

Project ID: 116556

Study Title: A Verification Study of Stable Isotope Fractionations Within Human Hair and Fingernails During a Global Pandemic

Application Type: HSREB Initial Application

Review Type: Delegated

Full Board Reporting Date: 17/Nov/2020

Date Approval Issued: 04/Nov/2020 09:16

REB Approval Expiry Date: 04/Nov/2021

Dear Dr Fred Longstaffe

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above mentioned study as described in the WREM application form, as of the HSREB Initial Approval Date noted above. This research study is to be conducted by the investigator noted above. All other required institutional approvals must also be obtained prior to the conduct of the study.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
Keratin Stable Isotopes Study Protocol - Oct 25	Protocol	25/Oct/2020	7
Keratin Study Questionnaire Oct 9	Online Survey	09/Oct/2020	4
Email Recruitment Script Oct 25	Email Script	25/Oct/2020	3
Letter of Information Precise Keratin Dataset - Oct 30	Written Consent/Assent	30/Oct/2020	8

Documents Acknowledged:

Document Name	Document Type	Document Date	Document Version
Keratin Stable Isotopes Project - Budget	Study budget	09/Oct/2020	2

No deviations from, or changes to, the protocol or WREM application should be initiated without prior written approval of an appropriate amendment from Western HSREB, except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriC ouncil Policy Statement: Ethical
Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C,
Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Ms. Nicola Geoghegan-Morphet, Ethics Officer on behalf of Dr. Philip Jones, HSREB Vice-Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Western Research

Date: 14 October 2021

To: Dr Fred Longstaffe

Project ID: 116556

Study Title: A Verification Study of Stable Isotope Fractionations Within Human Hair and Fingernails During a Global Pandemic

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

REB Meeting Date: 02/November/2021

Date Approval Issued: 14/Oct/2021

REB Approval Expiry Date: 04/Nov/2022

Dear Dr Fred Longstaffe,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re- approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the Tri- Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

The Office of Human Research Ethics

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix B: Copy of the screening survey participants were asked to complete prior to admission to the study and sample collection.

Keratin Project Questionnaire PART I

Start of Block: Background/Consent

Q1 **HAVE YOU SPENT LESS THAN 1 MONTH AWAY FROM YOUR LOCAL AREA SINCE AUGUST 2020 DUE TO COVID-19? ARE YOU AT LEAST 18 YEARS OF AGE AND FLUENT IN ENGLISH? IF SO, THE LABORATORY FOR STABLE ISOTOPE SCIENCE IS SEEKING VOLUNTEERS TO PROVIDE SAMPLES OF ARM HAIR AND FINGERNAIL CLIPPINGS TO BETTER UNDERSTAND HOW STABLE ISOTOPES FROM FOOD AND DRINKING WATER ARE INCORPORATED INTO FAST-GROWING TISSUES. PLEASE SEE THE LINK TO THE LETTER OF INFORMATION INCLUDED IN THE RECRUITMENT E-MAIL FOR MORE INFORMATION. IF YOU ARE INTERESTED IN PARTICIPATING, PLEASE COMPLETE THIS SCREENING QUESTIONNAIRE AND SUBMIT IT THROUGH WESTERN'S SECURE SURVEY PLATFORM, QUALTRICS. ONCE ADMITTED TO THE STUDY YOU WILL BE CONTACTED BY EMAIL AND PROVIDED INFORMATION ABOUT THE CONSENT PROCESS AND SAMPLING.**

This survey will collect contact information, such as you name and address, that could be used to identify you. Your data security is held in the highest regard. Personal information that may be used to link your responses to your identity is confined to PART I of this survey and will be kept separate from your responses in PART II. While this study follows the recommendations of Western University's Health Science Research Ethics Board and this study incorporates measures into its design to ensure your personal information remains secure, there is always the risk of a data breach. for more information about how your information will be protected, please see the letter of information included with the recruitment e-mail you received of contact:

Dr. Fred J Longstaffe BGS 1023.

- ○ Yes, I would like to participate
- ○ No, I would not like to participate

End of Block: Background/Consent

Start of Block: PART I: Contact Information

*

Q2 Name

_____

*

Q3 E-mail

_____

*

Q4 Address

_____

Q5 If you live within London city limits, do you live North or South of Commissioners Road?

- North
- South

Q6 If you live North or South of Commissioners Road in London, have you spent significant amounts of time (e.g., working or going to school) on the opposite side of Commissioners Road where you live (i.e. living South of Commissioners but working North or vice versa) during since August 2020?

- Yes
- No

End of Block: PART I: Contact Information

Start of Block: PART II info

Q7 Thank you for completing PART I of the questionnaire. Please click the *next* button below if you would like to continue to PART II of the questionnaire. PART II will collect information about your diet, drinking water sources and recent travel history. All responses in PART II will only be associated with a coded identifier to protect your privacy, and personal information you provided in your PART I responses will be stored separately and securely at all times from your responses in PART II

Keratin Project Questionnaire PART II

Start of Block: PART II: Study Data

Q2 Are you at least 18 years of age?

- Yes
- No

Q3 Are you fluent in English?

- Yes
- No

Q4 Have you spent over 1 month outside of the London, Ontario area since August 2020?

- Yes
- No

Q5 Please rate your diet between vegan and carnivore where: **Vegan** = eat no meat, eggs, dairy or fish **Semi-vegan** = eat no meat but eat occasional (at least weekly-monthly) eggs and/or dairy and/or fish **Vegetarian** = eat no meat but eat eggs and/or dairy and/or fish **Semi-vegetarian** = eat occasional meat (at least weekly-monthly) and/or eggs and/or dairy and/or fish **Omnivore** = eat meat and/or eggs

and/or dairy and/or fish once per week or more**Carnivore =** eat primarily meat/fish/other forms of sea food

- ○ Vegan
- ○ Semi-vegan
- ○ Vegetarian
- ○ Semi-vegetarian
- ○ Omnivore
- ○ Carnivore

Q6 If you have selected vegetrarian to carnivore above, please rate your diet on scale from 1 to 10 where: **1** = eat only marine-based protein - fish, shellfish **10** = eat only land-based protein - meat, eggs, dairy

	Marine						Land			
	1	2	3	4	5	6	7	8	9	10
Type of Protein										

Q7 Do you primarily drink tap water or bottled water? Please note that coffee, tea, milk and other beverages that are not typically made using bottled water or are purchased prepackaged are considered tap water for the purposes of this study.

- ○ Tap water
- ○ Bottled water

Q8 Is your tap water supplied by the city's municipal water supply or from a well?

- ○ Municipal water supply
- ○ Well water
- ○ I don't know

Q9 If you drink more bottled water than tap water, which brand do your drink most often and what is its source?

_____

_____

_____

_____

_____

Q10 Do you wish to be notified of your results?

- ○ Yes
- ○ No

End of Block: PART II: Study Data

Appendix C: stable isotope results for all drinking water samples, and hair and nail subsamples analyzed in the present study. Methodological duplicate drinking water samples are denoted by 'D.' Bottled water samples are denoted by 'B.' Non-municipal tap water samples obtained from private wells are denoted by 'W.' [a]Denotes samples of head hair [b]denotes samples of eyebrow hair.

Participant ID	$\delta^2H_{Drinking\ Water}$ (‰ VSMOW)	$\delta^{18}O_{Drinking\ Water}$ (‰ VSMOW)	δ^2H_{hair} (‰ VSMOW)	$\delta^{18}O_{hair}$ (‰ VSMOW)	δ^2H_{nail} (‰ VSMOW)	$\delta^{18}O_{nail}$ (‰ VSMOW)	$\delta^{13}C_{hair}$ (‰ VPDB)	$\delta^{15}N_{hair}$ (‰ AIR)	$\delta^{13}C_{nail}$ (‰ VPDB)	$\delta^{15}N_{nail}$ (‰ AIR)	Notes
LCT 1	–53.4	–7.4	–80.4[b]	+11.5[b]	–76.0	+12.5	–18.1[a]	+8.8[a]	–18.9	+8.9	Head and eyebrow hair used due to insufficient arm hair.
	–54.0	–7.5	–83.7[b]	+9.9[a]	–76.8	+12.5	–18.0[a]	+8.7[a]	–18.9	+9.1	
			–72.2[a]	+10.8[a]	–79.3	+12.3	–18.0[a]	+8.6[a]	–18.8	+9.0	
			–72.2[a]	+10.8[a]			–18.4[b]	+8.7[b]			
							–18.3[b]	+8.6[b]			
							–18.0[b]	+8.5[b]			
LCT2	–53.6	–7.5	–69.7	+11.4	–72.7	+12.1	–17.8	+9.2	–18.2	+9.5	
			–69.0	+10.7	–72.7	+12.3	–17.7	+9.1	–18.1	+9.5	
			–84.3	+11.1	–70.7	+11.9	–17.7	+9.1	–18.0	+9.6	
LCT 3	–53.7	–7.4	–75.8[a] (C)	+9.7[a] (C)	–75.7	+10.8	–18.1[a] (C)	+9.4[a] (C)	–18.0	+9.6	Head hair sampled due to insufficient arm hair. Sections of hair that had been colored are denoted with 'C.'
	–54.1 (D)	–7.5 (D)	–76.0[a]	+9.7[a]	–71.8	+11.3	–17.6[a]	+9.3[a]	–17.4	+9.6	
			–83.0[a]	+10.0[a]	–71.8	+11.2	–17.5[a]	+9.5[a]	–17.4	+9.5	
			–90.2[a]	+9.9[a]			–17.3[a]	+9.7[a]			

LOB 1	–68.6 (W)	–10.8 (W)	–83.7	+9.7	–83.1	+9.2	–16.8	+9.3	–17.6	+10.0	Reported consuming ~1:1 bottled water:well water.
	–78.5 (B)	–12.0(B)	–84.5	+9.8	–87.5	+9.6	–16.8	+9.5	–17.4	+9.7	
			–78.9	+8.8	–89.1	+10.2			–17.4	+9.8	
LOT 1	–54.0	–7.5	—	—	–73.3	+12.6	—	—	–19.4	+8.9	
		–7.5			–72.3	+12.5			–19.4	+8.7	
	–53.4					+12.5			–19.3	+8.7	
LOT 2	–53.2	–7.3	—	+11.4	–73.9	+10.7	—	—	–18.8	+9.5	
		–7.5			–75.0	+10.7			–18.6	+9.4	
	–53.8				–75.0	+10.5			–18.6	+9.6	
LOT 3	–54.1	–7.5	–68.0	+10.0	–78.3	+10.0	–18.2	+9.5	–18.4	+9.7	
	–54.1 (D)	–7.5	–66.5	+10.3	–78.5	+10.0	–18.1	+9.9	–18.2	+10.0	
			–74.0	+10.8	–79.1	+10.3	–18.0	+9.4	–18.2	+9.9	
LOT 4	–72.8 (W)	–11.5 (W)	–69.0	+11.1	–81.6	+11.1	—		–18.4	+10.2	Reported consuming ~4:3 ratio of London municipal tap water:well water.
	–54.2	–7.3			–79.8	+11.2			–18.3	+10.0	
					–76.9	+11.3			–18.3	+10.0	
LOT 5	–54.0	–7.7	–63.5	+12.1	–72.2	+11.9	–19.4	+9.0	–19.9	+9.5	
				+11.9	–70.5	+11.9	–19.3	+8.8	–19.8	+9.3	
					–74.2	+11.7			–19.8	+9.6	
LOT 6	–53.7	–7.5	–66.0	+11.1	–75.1	+12.2	–19.8	+9.2	–20.0	+9.5	
	–53.8 (D)	–7.4 (D)	–72.7	+11.1	–72.1	+12.3	–19.8	+9.0	–19.9	+9.6	
			–78.7	+11.3	–73.7	+12.4	–19.7	+9.1	–19.9	+9.5	

LOT 7	–70.6 (N)	–10.8 (N)	–70.8	+8.1	–76.0	+8.0	–17.3	+9.9	–17.8	+10.2	Reported consuming 9:1 ratio of Waterloo municipal tap water:bottled water. 'N' denotes tap water sampled in November, 2020, 'J' denotes tap water sampled in June, 2021.
	–69.4 (J)	–10.6 (J)	–77.6	+8.3	–76.0	+8.9	–17.2	+9.0	–17.5	+10.3	
	–89.0 (B)	–13.7 (B)	–73.2	+8.4	–75.3	+9.0			–17.5	+10.1	
	–89.4 (BD)	–13.4 (BD)									
LOT 8	–55.2	–7.7	–64.6	+11.7	–73.6	+13.3	—	—	–17.8	+9.2	
	–53.4	–7.3			–70.3	+12.9			–17.8	+9.2	
					–74.4	+13.1			–17.6	+9.3	
LOT 9	–53.8	–7.6	–65.3	+11.0	–81.9	+11.6	–18.7	+8.6	–18.9	+9.5	
				+10.9	–84.4	+11.7			–18.6	+9.0	
			–66.6	+11.5	–81.0	+11.4					
LOT 10	–53.4	–7.4	–75.7	+10.4	–85.4	+12.3	–18.3	+9.4	–19.3	+9.8	
	–53.3	–7.4	–70.9	+11.6	–84.6	+12.3	–18.2	+9.3	–18.6	+9.4	
			–78.5	+12.8	–84.0	+12.1	–18.1	+9.4	–18.4		
LOT 11	–53.5	–7.6	–72.1	+11.1	–75.1	+13.0	–19.2	+8.9	–19.7	+9.7	
	–53.6	–7.6	–69.2	+11.4	–76.0	+13.1	–19.1	+8.8	–19.7	+9.4	
			–68.6	+11.5	–77.7	+13.1	–19.1	+8.8			
LOT 12	–53.5	–7.5	–64.1	+10.6	–70.3	+11.5	–19.0	+9.2	–19.4	+9.6	

	–55.0 (D)	–7.5 (D)	–68.8	+9.6	–70.3	+11.4	–18.9	+9.2	–19.3	+9.6
			–66.4	+10.4	–70.4	+11.1			–19.2	+9.7
LOT 13	–53.6	–7.7	–68.3	+9.2	–77.4	+12.5	–19.6	+8.9	–19.8	+9.4
				+10.2	–78.9	+12.5			–19.7	+9.4
					–80.4	+12.2				
LOT 14	–53.4	–7.5	–62.6	+10.5	–68.5	+10.9	—	—	–18.9	+9.8
	–52.4	–7.2			–68.0	+11.2			–18.9	+9.9
					–68.9	+10.6			–18.9	+9.8
LOT 15	–55.2	–7.8	—	—	–71.1	+12.0	—	—	–19.4	+8.3
	–54.8	–7.7			–66.5	+12.1			–19.3	+9.3
					–66.9	+11.9			–19.3	+8.7
LOT 16	–55.7	–7.7	–66.6	+11.5	–74.0	+12.3	—	—	–18.2	+9.0
	–55.3 (D)	–7.7 (D)	–65.0	+12.2	–75.3	+12.4			–18.2	+8.9
			–65.1	+11.6	–76.9	+12.4			–17.9	+8.9
LOT 17	–52.9	–7.5	–72.1	+10.5	–74.2	+10.9	–18.8	+9.1	–18.9	+9.6
			–68.3	+9.9	–74.9	+10.8	–18.7	+9.0	–18.8	+9.7
			–89.4	+10.5	–73.1	+10.7	–18.7	+9.0	–18.8	+9.6
LOT 18	–56.3	–7.9	—	+11.1	–75.5	+12.3	—	—	–18.6	+9.1
					–77.5	+13.0			–18.5	+9.2
					–74.3	+12.9			–18.5	+9.1
LST 1	–53.3	–7.4	–73.3	+11.8	–81.0	+12.1	–20.7	+8.3	–21.6	+8.8
			–69.8	+12.0	–80.0	+12.7	–20.6	+8.2	–21.3	+8.6
			–77.4	+11.1	–79.4	+12.4	–20.6	+8.2	–21.3	+8.7
							–20.4	+8.0		+9.4
LST 2	–53.7	–7.5	–75.6	+11.3	–77.2	+11.9	–19.3	+9.3	–19.7	
	–53.7	–7.6	–72.1	+11.6	–76.0	+12.1	–19.2	+8.7	–19.7	+9.4
			–67.2	+11.7	–77.6		–18.1	+8.8	–19.6	+9.2
LST 3	–54.1	–7.6	–70.8	+10.2	–70.2	+12.3	–20.0	+8.9	–20.5	+9.4
			–70.8	+11.2	–73.4	+12.5	–20.0	+8.8	–20.4	+9.4
			–69.0	+12.1	–77.2	+12.5			–20.4	+9.2

LVT 1	–53.5	–7.5	–86.8	+10.9	–86.5	+11.4	–19.1	+8.2	–19.1	+9.1	Ratio of bottled to tap water unknown. Arithmetic average used to calculate mean drinking water δ^2H and δ^{18}O.
	–88.4 (B)	–13.4 (B)			–86.3	+10.7			–18.8	+9.3	
					–84.8	+11.4			–19.0	+9.1	
LVT 2	–53.8	–7.4	—	+10.7	–76.9	+11.1	–19.3	+8.6	–20.1	+8.7	
					–76.9	+11.3			–19.8	+8.6	
					–77.2	+11.3			–19.8	+8.5	

Curriculum Vitae

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Honours and Awards:	National Science and Engineering Research Council of Canada Undergraduate Student Research Award 2019
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