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O tempora, O molars!: Investigating second molar stable isotope ratios of historical Dutch individuals from early medieval to early modern sites

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O tempora, O molars!: Investigating second molar stable isotope ratios of historical Dutch individuals from early medieval to early modern sites

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Preface

"One step at a time [...]. That was the only way to look at it. If you clenched your teeth hard enough, and took enough strides, you could get anywhere."

- Joe Abercrombie

Stable isotope archaeology is the closest thing to a constant I have in my academic history up to this point. Since the first lecture on it, I knew that it would be something I would work on given an opportunity, and now that this opportunity arose, and eventually with three years of hard work it coalesced into this study. I would like to thank my partner, friends, and family for their support over these years, with particular thanks to Olga for checking all paragraphs regarding Chemistry and Physics and to Tom for his help with editing and proofreading the document. I would not be able to do it without your help.

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1. Introduction

There are many ways to look at food in an archaeological context. Where it is investigated through written sources providing information about its export and sale or the agricultural trends in the investigated area, one might see it as a commodity. On the opposite end of the spectrum, urban cesspit analysis offers insights into food consumption through the lens of what was discarded. There is a tendency in archaeology to look at food exclusively in terms of consumed materials. An alternative approach would be to recognize food as something essential to the development of an individual's body. Therefore, food and its components should also be considered as the building blocks of the human body and will be investigated as such in this thesis.

The adage of 'you are what you eat' is true in a variety of areas in a person's life. Access to sufficiently nutritious food is an important factor in determining whether the human body develops at an optimal pace or not. A diet lacking essential nutrients in the early stage of growth may lead to a number of nutritional deficiencies leading to developmental disorders such as rickets (caused by vitamin D deficiency) and as a result stunting the growth of an affected child. Since variation in diet is strongly tied to sex, gender, location, and socioeconomic status (Lake et al., 2006), the resulting outcome of consuming a potentially insufficient diet can be highly informative when it comes to the lived experience of the investigated individual. Furthermore, it may shed light on social trends in the treatment of adolescents in the society they were a part of.

It is, however, crucial to remember that access to food is a multifaceted concern. Whether it is due to socioeconomic status, contemporary agricultural trends, or other more subtle factors, it has a profound effect on the body. Regardless of its result, the differences stemming from this have been found to be tangible and detectable and with the use of an appropriate methodology it can be investigated and quantified to examine them.

A common approach for reconstructing past diets is the use of stable isotope analysis. Stable isotope analysis has been employed both in contemporary contexts as well as in historical and prehistorical ones. Studies of Dutch and other Western European archaeological contexts have been a source of important developments in the reconstruction of past human lives in their respective regions and time periods (Colleter et al., 2019; Müldner & Richards, 2005a, 2007; Schats et al., 2022). Several studies have used this approach to analyze the historic skeletal assemblages across the country of the Netherlands (Kootker et al., 2016, 2019; Schats et al., 2021) The data obtained through this method has provided unique

insights into the past lives of individuals inhabiting these areas. However, when considering the existing bulk of literature pertaining to archaeological diet reconstruction studies by means of stable isotope analysis, one may observe two particular trends. First of all, the majority of stable isotope studies investigating childhood diets focusing specifically on an early aspect of development, this being postnatal weaning practices (Bourbou et al., 2013; Jay et al., 2008; Waters-Rist et al., 2022). Secondly, the majority of diet reconstruction studies as a whole focus on adult diets. While the focus on weaning practices is instrumental in investigating the earliest stages of childhood diets as well as the treatment of the most vulnerable individuals in a given society, it leaves the later stages of childhood and early adolescence uninvestigated. Further motivation for focusing on early infancy rather than on later age groups is the limited availability of skeletal remains of subadult individuals as well as limitations of the method which stem from the nature of the investigated material. Nevertheless, a potential workaround allowing for a thorough investigation of those underexplored age groups can be the utilization of dental material (Sandberg et al., 2014). Dental material, thanks to the specifics of its development provides an alternative and promising line of inquiry, which builds on its implementation in previous studies (Burt & Amin, 2014).

This study will focus on investigating the childhood diet of individuals found in five archaeological sites across the Netherlands, spanning early medieval to early modern periods through the use of obtained isotopic data. The selected sites are Alkmaar, Arnhem, Eindhoven, Klaaskinderkerke and Zwolle, and were chosen to span as much of the country's territory as was possible (Figure 1). This study will focus on the stable isotopes of carbon and nitrogen, as these elements are often considered to be among the primary building blocks of the human body and their stable isotopes are commonly used for the reconstruction of important aspects of a person 's diet. Data obtained in this way could prove to be useful in related discussions of childhood health and nutrition, and trends therein (Reitsema, 2013), within the contexts presented.

Figure 1 Map of the locations of sites from which samples selected for the study originate across the Netherlands.

1. 1. Research Aims

This study aims to obtain and analyze isotopic data derived from second molars of historical individuals that correlates to their childhood diet. Therefore, the primary question of this work is concerned with childhood diet, as reconstructed using dental material derived from individuals across the Netherlands. Using dentine collagen (carbon and nitrogen) and dental enamel (carbon) stable isotope values this study will answer the following questions:

- What can carbon and nitrogen stable isotope values of permanent human teeth from multiple archaeological sites in the Netherlands tell us about trends in childhood diet in those locations, spanning early medieval to early modern periods?

Directly related to this question is a number of supporting questions intended to exhaust the potential of the data obtained for the purpose of this study:

- What are the differences between the biological sexes?
- What are the differences between sites?
- What are the differences between time periods?
- Where direct comparison is available, how does the childhood diet compare to adult diet?

Secondarily, this study will investigate statistical relationships between the carbon isotope values of both dentine and enamel from those analyzed, as well as between carbon and nitrogen isotope values.

1. 2. Summary of proceeding chapters

The Background chapter (Ch. 2) will explain the mechanics behind the method of stable isotope analysis (SIA) as well as provide context for both the material and its sources. First, the chapter will go into sufficient detail given the nature of this study, about the underlying processes that allow for stable isotope analysis. This will include the physical, chemical, and biological aspects of stable isotope analysis, alongside the impact they have on the dental material which was utilized. Previous use of SIA in published archaeological literature, as it pertains to the scope of this study, will be presented, in order to provide a clear precedent and basis for the methodology employed in this study. Followed by that, the broad history of the locations and time periods from which the material is derived will be discussed to provide context to the samples and further discussion.

The Materials and Methods chapter (Ch. 3) will provide the necessary information on the considerations behind the sampling, as well as the describing the process of sampling of the dental material. Following that, the chapter will describe the specifics of the preparation and analysis of the extracted material. The Materials part of the chapter will specifically explain the details of the sampling and variables which will provide additional lens through which to analyze the results. The Methods part of the chapter meanwhile will go into detail on the specific methods and steps performed for the analysis to be possible. This will include the chemical protocols used to extract both of the tissues used, as well as the following analytical procedures from which the results of the study were obtained.

The Results chapter (Ch. 4) will present the obtained results in the form of tables, graphs, and short descriptions of the statistical characteristics of each grouping. These groupings are based on the aforementioned variables, such as age, location, and sex.

The Discussion chapter (Ch. 5) will expand on the obtained results in order to answer the research questions found in this chapter. This will include a discussion of the patterns and specific values reported in the results chapter. The discussion will additionally place the study and its results in the wider context of the published literature, in order to provide a broader outlook on the results and their significance.

The paper will end with the Conclusions chapter (Ch. 6), in which the study's goals, means and conclusions will be briefly summarized and will provide a few final remarks regarding the topic and potential follow-up work, which could expand upon its results.

2. Background

In this chapter the scientific foundations for the study will be outlined, including the methodology used and the validity of the research questions posed. Furthermore, the historical context of the material investigated will be provided.

2. 1. Introduction to principles of stable isotope analysis

2. 1. 1 Stable isotopes

The word 'isotopes', is formed from the Greek roots means roughly 'equal places. It refers to atoms whose nuclei contain the same number of protons but vary in the number of neutrons (Figure 1). This difference does not affect the element the nucleus belongs to, and as an extension, its chemical properties (Hoefs, 2018), hence the descriptor, 'equal places', as despite the difference in their neutron count, they regardless occupy the same place in the periodic table of elements. While the chemical properties remain unchanged with the varying number of neutrons, the physical properties differ between the isotopes of the same element.

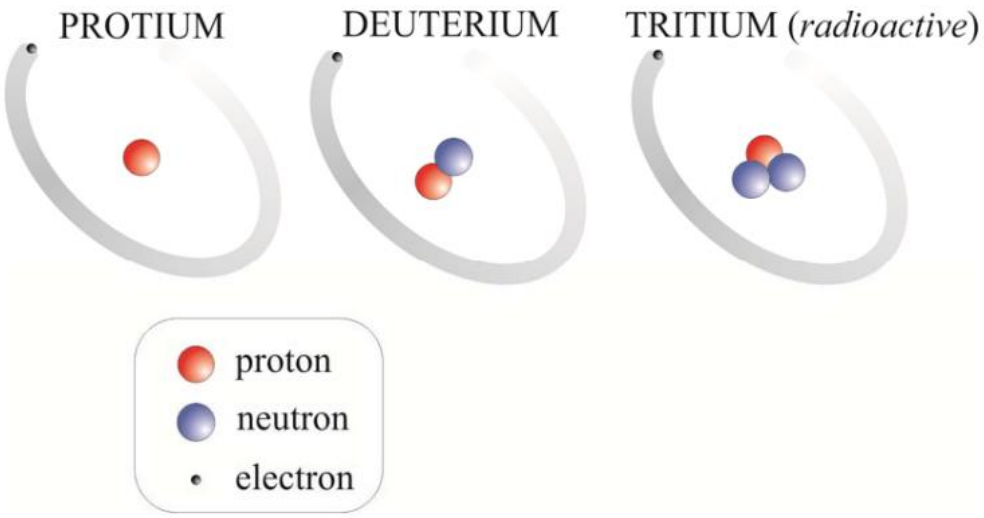


Figure 2 Depiction of hydrogen isotopes; left to right- ^{1}H, ^{2}H, ^{3}H. The number of neutrons is the main difference between the nuclei of the same element (Sharp, 2017)

One of the primary ways to categorize isotopes differentiates three kinds of isotopes, stable, unstable (also referred to as radioisotopes) and radiogenic isotopes. Unstable isotopes are defined as such through the presence of the process of radioactive decay of the nucleus,

while stable isotopes are defined as such through its absence. Radioactive decay is a process of spontaneous emission of radiation arising due to interaction of subatomic particles in the nucleus of an atom (Hoefs, 2018). The emission which occurs during it leads to a change in the nucleus of the atom. This process does not occur in stable isotopes.

Different isotopes are documented using the nuclear notation, which can be found in Figure 3. This notation is important for isotope work, as it identifies the isotope being referred to. In the notation 'X' refers to the chemical element being described. This is determined by the number of protons contained in the nucleus of the element, which in turn is noted in subscript 'Z'. Finally, the means by which we will differentiate the isotopes of the same element, found in the super-script 'A', which is the mass number. It contains the mass of the nucleus (noted in units) derived from the collective mass of the protons and neutrons. Due to the weight of protons and neutrons being approximated to 1 unit each, the subtraction of the atomic number (Z) from the mass number (A) leaves us with the number of neutrons contained in the nucleus of the referred to isotope.

$$^{A}_{Z}X$$

X= Symbol of the element

A= Mass number

Z= Atomic number

Figure 3 Isotopic notation

Due to a difference in the number of neutrons contained in the nucleus, the different isotopes vary in mass, with a difference of 1 unit per neutron. Inclusion of the heavier isotopes affects the mass of the molecules they are a part of, and that results in molecules containing the higher-mass isotope having displayed a trend to react more slowly in mass-sensitive kinetic reactions. This results in fractionation and partitioning in equilibrium states, where the presence of heavier isotopes can be distinguished from their lighter counterparts. A good understanding of these processes allows for tracking of these isotopes through what are called the 'pathways' of the elements, throughout their chemical transformations (Lee-Thorp, 2008).

The difference in the mass of the nucleus, results in the kinetic process of isotopic fractionation, where these slight differences in weight (one unit per neutron), result in differences in reactivity of those atoms, primarily in mass dependent fractionation processes. This results in differences in the strength of bonds the given atom creates, with lighter isotopes being more reactive than their heavier counterparts. This is due to the bonds created by this atom being easier to break and reconstitute, while the opposite is true of heavier isotopes, which react slower due to stronger bonds, which in turn require more energy to break and reconstitute in a new molecule. The general trend in isotopic fractionation is the decreased rate of incorporation of heavier isotopes. These differences result in changes to the ratios of light isotopes to heavy isotopes in processes like photosynthesis. These differences carry over into the products of synthesizing more complex compounds which include those atoms (Hoefs, 2018).

Sixty-one out of ninety-two natural elements exist in more than one stable isotopic form. In those 61, it can be observed that stable isotopes, having different atomic weights, do not co-exist in an equal ratio to one another. The trend among the ratios of multiple isotopic forms, is that the lighter of them will generally be the more abundant isotope, often substantially. With stable isotopes of carbon for example, carbon-12 (^{12}C) and carbon-13 (^{13}C), together make up almost 100% of earth's carbon. They coexist together with carbon-12 comprising 98.89% of carbon atoms, and carbon-13 of only 1.11% of carbon atoms. These very constant numbers are used as internationally agreed upon standards, which other values are reported in relation to. In terms of the elements whose ratios will be reported in this study, carbon and nitrogen, those standards are Vienna Peedee Belemnite (VPDB) for carbon (δ= 0.0112) (Zhang et al., 1990) and air nitrogen (AIR) (δ= 0.00367) (Junk & Svec, 1958)

Reporting the ratios between any two isotopes is done through the use of the delta notation (δ), which serves as a fraction of the investigated isotope relative to the standard employed for it (Equation 1).

$$\delta_{sample} = \left(\frac{R_{sample}}{R_{Standard}} - 1\right) x\ 1000\ (‰)$$

Equation 1 Calculation of the delta (δ) notation.

Measuring of the isotope abundance relative to the standard is done through the use of a mass spectrometer (MS). Mass spectrometry is an analytical method which separates charged atoms and molecules on the basis of their masses and their motions in a magnetic

and/or electrical field (Hoefs, 2018) and in the case of Isotope Ratio Mass Spectrometers (IRMS) precisely measures the isotopic abundance, even at very low enrichment (Meier-Augenstein, 2017).

2. 1. 2. Introduction to stable isotope analysis in dietary studies

The method implemented in this work is stable isotope analysis. It has become a rather prominent method in modern archaeological studies, having gained prominence in the last 20 years (Pestle et al., 2014). Stable isotope-based diet reconstruction is based on the simple principle; as material is incorporated into the structures of living organisms, they pass along at least in some portion, a certain ratio of stable isotopes contained in the original material. These are referred to as the isotopic pathways. This means that when consuming plant material, the stable isotopes contained in the metabolized compounds, and later incorporated into the organism, the isotopes of a given element transfer into the resulting metabolite. This process, when happening in a known ratio of incorporating the material, can be used to reconstruct the sources of the diet of an individual, based on the sum total of the isotopes of a given element contained in the original tissue.

2. 1. 2. 1. Carbon isotopes in dietary studies

Carbon has three known isotopes, two of which are stable, carbons-12, -13 and -14 (^{12}C, ^{13}C, ^{14}C respectively). Primary sources of dietary carbon are carbohydrates created by plants. During the process of photosynthesis carbon from atmospheric carbon dioxide (among terrestrial plants), is bound into multiple carbon chains, in order to produce glucose. Due to the trends in kinetic processes which were covered above, heavier isotopes react less prominently when compared to their lighter counterparts. This becomes important when considering the different photosynthetic pathways found among plants. Most common of those are the C3 and C4 pathways.

Isotopic ratios of carbon can be used to determine the origin of carbon consumed, most commonly determining whether it came from C3 or C4 plants. In dietary studies, this helps establish the source of dietary carbon consumed by the individual and in much smaller portions, consumed by the animals the individual consumed. The differences in C3 and C4 plant-based diets stem from the type of carbon-based molecules being synthesized during photosynthesis by the plant. Due to the differences in reactivity of heavier isotope containing molecules, the difference in the number of intermediate steps prior to the formation of glucose has a noticeable effect on the ratios of carbon isotopes.

The C3 pathway is most commonly found among vegetation growing in temperate climates. Their pathway involves the production of a three-carbon compound as the first step of photosynthesis. Most commonly consumed C3 plants include cereals, barley, oats, rice, wheat, soybeans, and potatoes. While this category of staple crops, dominated by cereals, are the most common C3 plants, it is important to note the inclusion of fruits, vegetables, legumes, and nuts in this group. The mean determined $\delta^{13}C$ value for C3 plants clusters between −27‰ and −28‰ (O'Leary, 1988). The C4 pathway meanwhile commonly includes plants growing in tropical and subtropical climates. Where the C3 pathway involves the production of a three-carbon compound, the first step in the C4 pathway is the production of a four-carbon compound. Commonly consumed C4 plants involve maize, millet, sorghum, and sugar cane. The mean $\delta^{13}C$ value for the C4 plants is found between −12‰ and −13‰ (O'Leary, 1988). With many varied plants which made their way to becoming cultural staples, classification of C3 and C4 plants allows us to establish with a degree of certainty supported by the literature and archaeological findings, which plants were consumed to establish the amount of carbon-13 in the analyzed tissue.

2. 1. 2. 2. Nitrogen isotopes in dietary studies

Nitrogen is a necessary element for the production of proteins and amino acids, vital building blocks of a living organism. With two stable isotopes, nitrogen-14, and nitrogen-15 (^{14}N and ^{15}N respectively). Nitrogen-15 builds up across trophic levels (i.e., 'position in the food chain'), with organisms consuming material derived from higher trophic levels containing higher levels of the heavier nitrogen isotope, due to the buildup and its lower rate of reactivity.

Nitrogen isotopic signatures in dietary studies are primarily affected by the source of dietary protein. Due to the physical processes involved in binding nitrogen to amino acids and their derivative substances, the amount of the heavier isotope (^{15}N) increases as the position in the food chain does. This means that noticeable ratios of heavier to lighter nitrogen isotopes will be lowest at the base of the food chain, specifically with the producers displaying much lower ratios when compared to consumers. The vast majority of the producers are plants, which obtain their nitrogen from the soil and decomposed material found in it. The exception to this is legumes, which obtain nitrogen directly from the air.

$\delta^{15}N$ values for the majority of plants are around +5 ‰ while the legumes, due to sourcing of atmospheric nitrogen, are closer to 0‰, ranging from 0 to +4 ‰ (DeNiro & Hastorf, 1985). Meat has a higher $\delta^{15}N$, when compared to diet, resulting in further increases in the

ratio along higher positions up the food chain, also known as higher trophic levels. This change varies anywhere between 3 and 5 ‰ (Bocherens & Drucker, 2003). When the sources of variation of $\delta^{15}N$ are known, they can be used to investigate the consumption of plant and meat derived protein as well as potentially an insufficient protein intake which can result in elevated $\delta^{15}N$ (Fuller et al., 2005).

Everything mentioned above is true primarily of terrestrial sources of nitrogen, however this does change when considering marine sources. Sources of nitrogen in marine food chains are primarily nitrates dissolved in water. These food chains are enriched in $\delta^{15}N$ compared to terrestrial food chains at their corresponding trophic levels (Schoeninger et al., 1983; Schoeninger & DeNiro, 1984), finding the $\delta^{15}N$ values of marine fish to be between +11 and +16‰, and marine mammals around +11 and +23‰ (Schoeninger & DeNiro, 1984).

2. 1. 2. 3. Human diet reconstruction

When investigating human diet, the correlation between stable isotope values in the diet and that in tissues becomes central. When the tissue in question is analyzed, the isotope ratio is treated as a mean result of the consumption of food during its development. However, the ratios of the isotopes found in the individuals' diet are not equal to those incorporated into their tissues. $\delta^{13}C$ values in human collagen for instance, have been found to be enriched by ~5‰ compared to the diet of the individual, while $\delta^{15}N$ of the same tissue will be 3-5‰ greater than that found in the diet (Ambrose, 1993). Understanding these correlations is the core of diet reconstruction. If the ratios at which a tissue accumulates the heavier isotopes into its structure is known, it forms the basis for the reconstruction of the pathway.

One of the primary issues with the analysis of organic material is the possibility of its degradation. To determine whether the degradation of the extracted collagen will not affect the analysis, the ratio of carbon to nitrogen atoms in the sample is compared to the reported atomic ratio in intact collagen, that being between 2.9 and 3.6 (Deniro, 1985a; Guiry & Szpak, 2020). Values falling beyond this range are considered to be too degraded to provide reliable information.

While dental anatomy is well documented and understood, and often used in archaeological contexts for a number of different analyses, the specific relationships of carbon isotope ratios in the teeth themselves are not yet thoroughly investigated. Previous studies investigated the relationship between isotopes derived from a selection of animal

material, including human material. Investigations into the relationship between $\delta^{13}C$ in collagen and $\delta^{13}C$ in carbonate have discovered them to be positively correlated with each other (Codron et al., 2018) with similar results between $\delta^{13}C$ in collagen and apatite (Schats et al., 2022). This line of inquiry into the relationships between stable isotope ratios in human tissues, expands into carbon isotopes from other sources. A study by Kusaka et al. (2015) found a relationship between carbon isotope ratios between carbon derived carbon in tooth enamel and bone collagen. Similarly, Loftus & Sealy (2012) found a correlation between enamel and collagen, and a weaker correlation between enamel and bone apatite. Previous investigations have not extended yet to either North-Western European, or especially Dutch samples or the relationship between dental collagen and enamel samples.

2. 1. 2. 4. Stable isotope-based diet reconstruction in archaeology

Since the connection between stable isotope ratios and dietary patterns was established, so were its archaeological uses to reconstruct trends in diet, as well as other aspects of an individual's past life. The primary motive behind this was to investigate the patterns of consumption within investigated communities. The method proved particularly useful in cases where previous information required verification, such as known written records, refuse, known agricultural practices, etc.

Other studies used methods for distinguishing between populations on the basis of different diets, such as classifying individuals with sufficiently different dietary records, as originating from a different location (Bartelink et al., 2014). This, and studies like it, exemplify just how much new insight can be gained from the analysis of carbon and nitrogen isotopes in human tissues.

The primary source of stable isotope signatures used for these studies have been sternal rib ends, preferably from the 4^{th} rib, the reason for this is down to the rates at which the 4^{th} rib undergoes turnover, as well as the continuous ossification of the sternal end, as the cartilaginous material ossifies throughout life.

2. 2. Dental material

The implementation of stable isotope analysis for dietary reconstruction purposes involves the choice of the analyzed tissue. With vast differences between the ages at which they develop the choice is crucial to the kind of data that will be obtained through its sampling and analysis. In archaeological studies, however, an additional area of concern is preservation, which can vastly limit the kind of material which is available. Out of the

material available in an archaeological context, dental material derived dentine and enamel has been found to have survivability often surpassing that of bone (Beaumont, 2020). This resiliency comes down to a number of compounding factors, the most important of which, for the purposes of stable isotope analysis is the chemical makeup of the tissues which comprise the teeth. The aspect of dental tissues, which distinguishes them from other tissues used for isotopic analysis, is that dental tissues usually do not remodel during life (White et al., 2012). Unlike bone collagen, where the turnover causes the obtained isotopic values to average out the dietary protein values obtained during a number of years prior to death (Hedges et al., 2007), with the turnover rates being dependent on the bone in question, dentine's turnover is virtually nonexistent, reflecting the diet during the formation of the tooth (Beaumont et al., 2013).

Teeth are composed of different tissues, the most important of which for the purpose of this study are enamel and dentine. The harder of which, enamel, is present in the majority, and in some cases the totality of the tooth exposed from the gums, while dentine comprises the tooth root and encapsulates the tooth cavity. The exposed area of enamel is referred to as the crown. Enamel itself is mainly composed of an inorganic component. This mineral component is primarily composed of hydroxyapatite and in a smaller portion, of calcium carbonate, calcium fluoride and magnesium phosphate. Its formation on the tooth is not uniform, with varied thickness found to be thickest around the cusps in molars (Schwartz, 2007). This inorganic phase of dental enamel, otherwise referred to as enamel (en), is the source of carbon and oxygen isotope ratios in stable isotope analysis for the purposes of this study.

Dentine comprises the roots of the teeth which embedded in the alveoli, connect to the maxilla or the mandible of the respective bone. The primary dentine layer, which makes up most of the root, is a continuous tissue surrounding the insides of the tooth. While the composition of enamel is primarily inorganic, dentine has a much more pronounced organic component, with a hardness second to enamel among the tissues making up teeth. The organic component of dentin is primarily made up of type 1 collagen, a fibrous structural protein which also makes up the organic phase of bone. Protein collagen (co) is a source of carbon and nitrogen for the purposes of this study.

2. 2. 1. Dental development

The formation of dental hard tissues begins during the fetal stage of development, with the formation of deciduous teeth lasting into early childhood. It is then followed by

formation and eruption of permanent teeth in middle childhood as they gradually replace the deciduous teeth. Because this development is a continuous process, the permanent dentition, which erupts later in life is formed during early childhood finishing during adolescence (Table 1). Teeth that develop latest in life are the second and third permanent molars, in regard to second molars, the hard tissue formation begins around two and a half to three years after birth, with the crown completing around seven to eight years after birth and the roots being completely formed between fourteen and sixteen years of age (Schuurs, 2012).

Tooth	Initiation of the tooth germ	Calcification of the crown		Root completed
		Start of calcification	Enamel formation complete	
Deciduous maxillary teeth				
Central incisor	7 weeks *in utero*	3–4 months *in utero*	1–4 months	1.5–2 years
Lateral incisor	7 weeks *in utero*	4.5 months *in utero*	2–5 months	1.5–2 years
Canine	7.5 weeks *in utero*	5.5 months *in utero*	9 months	2.5–3.3 years
First molar	8 weeks *in utero*	5 months *in utero*	6 months	2–2.5 years
Second molar	10 weeks *in utero*	6 months *in utero*	10–12 months	3 years
Deciduous mandibular teeth				
Central incisor	7 weeks *in utero*	4.5 months *in utero*	4 months	1.5–2 years
Lateral incisor	7 weeks *in utero*	4.5 months *in utero*	4.5 months	1.5–2 years
Canine	7.5 weeks *in utero*	5 months *in utero*	9 months	2.5–3.3 years
First molar	8 weeks *in utero*	5 months *in utero*	6 months	2–2.5 years
Second molar	10 weeks *in utero*	6 months *in utero*	10–12 months	3 years
Permanent maxillary teeth				
Central incisor	5–51/4 months *in utero*	3–4 months after birth	4–5 years	10 years
Lateral incisor	5–5.3 months *in utero*	10 months after birth	4–5 years	11 years
Canine	5.5–6 months *in utero*	4–5 months after birth	6–7 years	13–15 years
First premolar	Birth	1.5 years	5–6 years	12–13 years
Second premolar	7.5–8 months	2 years	6–7 years	12–14 years
First molar	3.5–4 months *in utero*	0 years	2–3 years	9–10 years
Second molar	8.5–9 months	2.5 years	7–8 years	14–16 years
Third molar	3.5–4 years	7–9 years	12–16 years	18–25 years
Permanent mandibular teeth				
Central incisor	5–5.3 months *in utero*	3–4 months after birth	4–5 years	9 years
Lateral incisor	5–5.3 months *in utero*	3–4 months after birth	4–5 years	10 years
Canine	5.5–6 months *in utero*	4–5 months after birth	6–7 years	12–14 years
First premolar	Birth	1–2 years	5–6 years	12–13 years
Second premolar	7.5–8 months	2–2.5 years	6–7 years	13–14 years
First molar	3.5–4 months *in utero*	0 years	2.5–3 years	9–10 years
Second molar	8.5–9 months	2–3 years	7–8 years	14–15 years
Third molar	3.5–4 years	8–10 years	12–16 years	18–25 years

Table 1 Chronology of dental development (Schuurs, 2012)

This, in conjunction with the aforementioned isotopic recording of dietary data in these tissues allows potential analysis of the diet of the individual. Since this analysis is based on the food ingested up to the aforementioned age ranges when the specific tissue would

finish forming, locking in the isotopic ratios, the reconstruction would focus on childhood diet of the analyzed individuals if permanent second molar's enamel and dentine are used.

2. 3. Use of stable isotope analysis for diet reconstruction in the Netherlands and North-Western Europe

Relative to the surrounding countries of North-Western Europe, the Netherlands does not have a large amount of stable isotope data focused on paleodiet. Those previous studies touched on the collagen derived stable isotope ratios of carbon and nitrogen, allowing the researchers to reconstruct the diets of historical individuals from historical cities like Alkmaar (Schats et al., 2022), Eindhoven (Simpson, 2019) and Oldenzaal (Kootker et al., 2019). Those applicable for direct comparison will be later used for reference.

The surrounding countries making up the North-Western Europe region and contemporary to the historical contexts analyzed here, have also been extensively investigated in published literature. The previous studies similarly looked at carbon and nitrogen stable isotopes primarily derived from bone collagen. Nearby countries of Belgium (Quintelier et al., 2014), France (Colleter et al., 2019) and United Kingdom(Müldner & Richards, 2005a) all have published dietary data reconstructing the diet of, historical individuals and will therefore serve as useful reference points for comparison of the results. Those studies form a crucial foundation for expansion of the comparative material for further discussion. Inclusion of studies on the past diets of individuals from the broader North-Western Europe serve the purpose of more accurately reconstructing the diet based on the material analyzed in this study, through comparison to pre-published data from those regions as well as their historical contexts and backgrounds.

2. 4. History of the sites

The sites of Arnhem, Alkmaar, Eindhoven, Klaaskinderkerke and Zwolle have been chosen to be sampled for both enamel and dentine. What follows is a brief overview of the history of the sites and collections which were sampled as well as their categorization into historical time periods.

2. 4. 1. Arnhem

Churches as well as the cemeteries around them, such as the Eusebiuskerkhof from which the samples were taken, served as places of burial for the general population in the Middle Ages, with the exception of those who died by execution or suicide.

The cemetery surrounding Eusebiuskerk in Arnhem was initially in use between the 14th and 17th centuries, with the first text mentioning the use of the cemetery around Eusebiuskerkhof in the year 1361. Following a declaration of its closure written by the Burgermeister and aldermen of Arnhem in 1636, due to the overcrowding of the cemetery during a time of the plague, closure of the cemetery was deemed necessary. This caused dissatisfaction among the residents of the city, resulting in further expansion of the cemetery soon after the decision was made. Finally, the cemetery ceased operation around the year 1829, due to the regulations of burials within city limits. Thanks to the documented closure and expansion following the plague, the cemetery can be divided into younger (1636-1829) and older phases (1361-1636), a division useful for dating the material.

Additionally, there has been prior work done on Eusebiuskerkhof's osseous material, resulting in obtaining stable isotope signatures for carbon and nitrogen, as derived from bone collagen (Zielman & Baetsen, 2020)

The two phases during which the cemetery operated place it in the wide range of late medieval to early modern for the purposes of discussion of dietary trends across theses time periods, to avoid misattributing the age of individual burials to the wrong time periods they will be discussed as a single origin.

2. 4. 2. Alkmaar

Excavated from the Paardenmarkt site in Alkmaar, the skeletal remains utilized here were found buried in a Franciscan monastery of Alkmaar. The monastery served as a place of burial likely between 1448 when it was founded and 1572 when the burials ceased alongside its demolition as a result of the Eighty Years War and reformation, with additional evidence in the form of two mass graves, tying the site to the siege of Alkmaar in 1573, a case made clear by the existence of gunshot wounds among the buried.

The first signs of human burials on the now converted Paardenmarkt (horse market) were bones found in 1800 and 1853 during tree planting and gas factory construction respectively. The site was first excavated initially in 2005 as part of exploratory excavations commissioned by the municipality of Alkmaar, and once human remains were located, a full excavation was deemed to be necessary, following that, was a large-scale excavation in 2010, which uncovered 189 primary inhumations, several ossuaries, and loose bones from disturbed contexts (Schats, 2016).

Alkmaar based on the recorded age will be discussed as a post medieval site, with the narrowest historical age range.

2. 4. 3. Eindhoven

The human material from Eindhoven was derived from St Catharine's church, Eindhoven's only medieval church, whose bordering graveyard's choir part was excavated with the intention of recovering potential DNA material from those interred there. This church was the only one with a graveyard in Eindhoven providing burials at least from the first written record in 1340 until 1793 when burials have stopped with the samples utilized for this study belonging to estimated historical age range of 1500-1850. As the church was built around the graveyard, there's good ground to reason that the burials predate the building. It's assumed that the graveyard was predominantly used for the townspeople of Eindhoven. While originally buried in sandy soil, the remains were well preserved, likely due to the runoff of numerous pieces of construction debris rich in lime.

Previous analysis of human remains from Eindhoven was undertaken. Stable isotope analysis on a small number of individuals was performed on skeletal remains and DNA extraction and analysis was done using dental material. In 381 out of 754 excavated individuals (50.4%) the extraction of molars for DNA material was possible. In 88% of those, sex determination was possible through the means of DNA material providing sex determination not based on skeletal characteristics.

Previous stable isotope studies were done on nitrogen and carbon isotopes from ribs for the purpose of dietary reconstruction (Simpson, 2019), the results showed that across the time periods covered in the sites history, there was no significant difference between food consumption at young and later age, with diets consisting mainly of cereals, milk, cheese, eggs, and meat including freshwater fish (Van Oosten, 2019).

This site can be categorized into a range of post medieval to early modern time periods and will be discussed as such when it comes to differences between time periods.

2. 4. 4. Klaaskinderkerke

Among the sites utilized for the study is the site of Klaaskinderkerke, a rural village (1286-1570) (Saers et al., 2017) located in the current province of Zeeland, on the island of Schouwen-Duiveland (Trimpe Burger & Huizinga, 1964). While the information available on the village is limited, Saers et al. (2017) infers the following about the potential rural, late-medieval life in Klaaskinderkerke from more general historical data on Zeeland; the village

is likely to have shifted its focus away from pastoral farming and towards labor intensive crops for the urban centers during the late-medieval period (Steensel, 2012). What is additionally of note here in regard to the samples being analyzed on the basis of their stable isotope ratios, is that from thirteenth century onwards, fishing for herring and other fish became a significant activity in the region and timeframe of the studied location (Bennema & Rijnsdorp, 2015; Steensel, 2012; Unger, 1998)

Historically the most notable event known regarding Klaaskinderkerke, occurred in 1570 when Zeeland was struck by a heavy storm which resulted in flooding the entire island of Schouwen. There is no evidence of rehabitation of the village of Klaaskinderkerke following that event. As a result of another flooding in 1953, topsoil of a cemetery site of Klaaskinderkerke was removed exposing human remains, the site was excavated completely in 1959, revealing the foundation of a church's western wall, alongside patches of debris marking its outline. The site contained 54 in situ burials and 57 isolated crania and other skeletal parts (Schats, 2016).

The site's age places it in the late medieval to post medieval time period, also making it the earliest site among those selected for the study, it will be discussed as such for the purpose of discussing the differences between time periods.

2. 4. 5. Zwolle

The location from which the human remains of Zwolle were derived is that of Broerenkerk, a smaller church when compared to the city's main Grote Kerk church. Those buried in the Broerenkerk included those of a 'middle class'. Members of groups such as craftsmen, lower officials and military of lower rank were buried in the Broerenkerk, while the Lower-class people were buried outside of it. Human remains found during the excavation of the site were estimated to date between the 17th and 19th century, with a significant number of them being confirmed to be buried between 1819-1828 (Oosten, 2019).

The estimated date of burials for the individuals found in the site, would place them in the historical category of early modern individuals, as such they will the discussed as belonging in that time period for the purpose of investigating potential differences between time periods. Among those individuals found during the excavation there, a small but significant number of whom were recognized to have had rickets during their lives (5.3%, n= 95), an important point for analysis of childhood diet, due to the nature of the pathology. As for dental pathologies, due to newly acquired access to refined beet sugar and lack of dental hygiene, dental pathologies were quite frequent.

3. Materials and Methods

This chapter will include the reasoning behind the sampling and the criteria for the choice of material used for analysis. The manner with which the required material was obtained is described in detail, including the extraction of the parent material as well as the chemical treatment required for analysis. The chapter also includes an in-depth description of the analytical methods utilized on the gathered material, including methods used to obtain isotopic data as well as the methods used to analyze the obtained data.

3. 1. Materials

The material chosen for analysis is dentine and enamel from second molars from historical Dutch individuals. In order to answer the research questions, second molars were chosen for analysis. This choice was due to the ages at which their dentine and enamel each finish development, those ages being ages of 14 to 16 years of age for dentine and 7 to 8 years of age for enamel (Schuurs, 2012). This spread allowed for reconstruction of the carbon component of past diets at two distinct points in the development of the analyzed individuals. The choice for sampling of the molars was based on relatively simple criteria. Those were: access to the remains stored in the University of Leiden's Faculty of Archaeology, good preservation of the area surrounding the second molar's alveola, lack of significant pathologies on the second molar. Samples chosen from each location/city were additionally required to originate from the same historical context. This was all done to limit errors potentially caused by diagenesis and dental pathologies as well as to ensure cohesive results in regard to their analysis in historical context.

3. 1. 1. Choice of locations

The sites of Alkmaar, Arnhem, Eindhoven, Klaaskinderkerke and Zwolle have been chosen to be sampled for this study. This choice was motivated by a number of factors, specifically:

- Availability of material from the site

All of the sites were found in the collection of the Universiteit Leiden's Faculty of Archaeology, and therefore available for study. Permission for destructive analysis of the material was granted by the required parties.

- Whether the site was previously analyzed in regard to stable isotope analysis

From the sites available, those which were not previously used for stable isotope analysis in the past were prioritized, in order to obtain new data from the collections available.

- Historical context of the site

In order to keep the focus on the historical context of early medieval to early modern Netherlands, sites which fell outside of this temporal range were therefore excluded.

- State of skeletal material in the collection

In order to obtain reliable data from dental material with minimal error caused by diagenesis, analyzed skeletal material needed to be in good condition, i.e., free of visible pathologies which would change the composition and structure of the crown or root. Visible damage to the alveoli of the second molars were also considered in this manner. Burials where such pathologies were present, were excluded from the study.

- Geographical location of the site

The sites chosen for this study were decided to spread across the geographical area of the contemporary borders of the Netherlands. The sites were prioritized when the geographic location of all of them together would spread across the country in a relatively dense and encompassing manner.

3. 1. 2. Sexual variation in selected samples

Prior to sampling, the material was chosen with the intent of obtaining a ratio of individuals assigned male to individuals assigned female which was as close to 1:1 as it was possible. While not always possible, due to the limitations of the collections used, the sites were sampled with a balance between Male and Female assigned individuals. While all of the locations contained a majority of individuals assigned Male during examination, the Female assigned individuals took priority. The final sex ratio is shown in Table 2.

Site	Male	Female	Indeterminate	Total
Alkmaar	7	8	0	15
Arnhem	10	7	1	18
Eindhoven	11	5	0	16
Klaaskinderkerke	7	5	1	13
Zwolle	12	2	0	14
Total	38	22	2	76

Table 2 Variation among assigned sex of individuals chosen for the study.

The final male to female ratio was established as close to a balanced ratio as it was possible given the specifications of the study and limitation of the source material, with Zwolle being the outlying site with only 2 applicable assigned Female individuals. The resulting ratio of 19/11 is skewed towards individuals assigned male, and as such, the results will be related with that limitation in mind.

3. 2. Skeletal Material

As this study was concerned with researching the dietary record in human dentine and enamel, the choice of material was narrowed down from the inception of the study. The use of 2nd molars and their dentine and enamel was deemed the optimal choice due to a variety of factors. The concerns about the destructibility and preservation of the utilized material were among the primary motivating factors for the choice of the material. Another factor was the availability of material among a wide choice of locations, where different choices would likely lead to limiting the total sample size of the study. Finally, due to turnover of skeletal material, the choice of a different bone as the source of collagen would alter the analysis of the isotope ratios and their correlation.

The main possible alternative, the use of costal (rib) bone material for a source of collagen, which notably is considered standard practice for reconstruction of past diets, would involve sampling multiple pieces of skeletal material for analysis, one for enamel and one for collagen, it is important to note that many of the individuals chosen for analysis were lacking either costal material or 2nd molars. Additionally, the usage of costal material would carry with it the issue of varied quality of the extracted collagen, which dental collagen does not share. Therefore, the use of 2nd molars would provide the required material i.e., collagen (as extracted from dentine) and enamel, while only utilizing a single source of said material. The same issue applies to the possible use of other teeth.

With those considerations, for optimized results, this study was planned around the use of 2nd molars. This choice would minimize the destruction of preserved material, lower the required work involved in sample preparation as well as increase the chances of extracting high quality collagen. Additionally, since the study's aims are better served by looking into a similar originating material, the choice of 2nd molars was deemed to be the best possible option available.

For clarity, in the remainder of the text, dentine will be used to refer to the dental tissue, while collagen will refer to the biological material extracted and analyzed from it.

3. 3. Extraction of material

3. 3. 1. Extraction of teeth

Extraction of the dental material from the sampled teeth was done in multiple stages. The chosen skulls were collected from storage, their respective mandibular or maxillary bones were examined for possible pathologies which could affect the enamel layer or the roots of the teeth, if none were found the second molar was extracted manually with care as not to affect the structure of either the tooth or the surrounding bone. In a case where pathologies were found, the tooth was re-examined to determine the presence of an area unaffected by the pathologies, so that sampling could continue.

3. 3. 2. Enamel

The sampling of enamel was done first. This was done due to the mechanical factor of handling a small object that is a second molar, as sampling the roots first would make handling the enamel much harder. This sequence of sampling also allowed for minimizing potential needless damage to the tooth as it was sampled, as mishandling it in this way would result in a loss of material.

The tooth enamel portion of the crown was scoured using a PROXXON GG12 glass engraving tool. Scouring was done over an area cleaned in the same manner as the sampling, done until the area being sampled was of a relatively uniform coloration. The glass engraving tool was equipped with a diamond tipped bit, which was cleaned in between uses. The bit was cleaned by submerging it in hydrochloric acid and submerged in Mili-Q water immediately after, in order to avoid contamination between samples. The crown of the tooth was scoured to sample approximately 5 mg of white dental enamel powder. Following that, the sampled material was deposited into a marked 5ml glass vial for storage. From this

material 0.3 ± 10% mg were weighed using a Sartorius Genius series analytical balance and subsampled into marked clean glass Exetainer® vials.

The subsampled enamel samples were transferred to the stable isotope laboratory in the Vrije Universiteit Amsterdam for combined O-C isotope analysis which was done using a Thermo Finnigan GasBench II device interfaced with a Thermo Finnigan Delta + mass spectrometer.

In line with O-C isotope analysis, Vienna Peedee Belemnite (VPDB) was used alongside an in-house carbonate reference material (VICS). They were calibrated against NBS19 and LSVEC certified reference materials. Instruments were checked using the IAEA-603 (calcite, CaCO3) which across all O-C analyses (n=26) returned an average reading of 2.44‰ for carbon and -2.33‰ for oxygen, with a reproducibility of 0.16 for carbon and 0.24 for oxygen.

3. 3. 3. Dentine

Collagen extraction from dentine was done using a Dremel Multitool (Model 3000) to separate the smallest tooth root found on the sample from the dental crown. The separation was done specifically using an ore-cleaned circular cutting disc. If the roots weight was deemed insufficient for collagen extraction (i.e., less than 200mg) a sufficient part of another root of the same tooth was added to the sample until the combined weight was ranging from 200 to 300 mg. In rare cases where the material did not allow for sampling within this weight range, the extraction was modified to better serve the process.

3. 3. 3. 1. Collagen extraction

The collagen extraction protocol utilized for this part of the study is modified from a method of collagen extraction from bone originally used by Müldner & Richards (2005). All of the steps of the protocol were performed in Universiteit Leiden's Faculty of Archaeology.

3. 3. 3. 1. 1. Preparation

The first step was the labeling of samples according to their storage identification number and giving each one its ID number. Each sample was weighted on a Sartorius Genius series analytic balance. In the previously mentioned cases of a sample falling outside of the range accounted for in the protocol (200-300 mg), the volume of chemicals used in step 2, 3 and 4 was modified in based on the 'sample weight: volume of chemicals used' ratio derived from the volume used in the protocol.

Samples were analyzed in bulk according to which site and context they originated from. This was done with the intention of observing the extraction process as it developed in each context separately. Observing the samples in this manner would allow for estimation of the quality of the material from each location and possibly counteracting or accounting for issues related to diagenesis of the samples.

3. 3. 3. 1. 2. Demineralization

Following their organizing and weighing, the tubes were demineralized through an acid-wash. Each sample was kept in approximately 10ml 0.6 M HCl, shaken and refrigerated at 4˚C for 48 hours. After that, the acid was decanted and centrifuged and replaced with a fresh portion of acid of the same volume and molarity and placed back into refrigerated conditions of 4˚C. This step was repeated for approximately 6-10 days or until no indication of further reactions in the form of air bubbles being released, was visible inside the tube. Each sample was then decanted, rinsed in Milli-Q and centrifuged (~2000rpm, 1 minute) two to three times to ensure separation of material and quality of the sample.

3. 3. 3. 1. 3. Purification

In order to purify the sample, 10ml of 0.125 M NaOH was added to each tube and kept closed at room temperature for approximately 20 hours. This was done in order to remove contaminants, such as humic acids from the sample. After 20 hours the samples were examined, decanted, rinsed in Milli-Q, and centrifuged between each of these steps (~2000 rpm, 1 minute). This step was repeated 2-3 times. As this step is likely to reduce collagen yield in the sample, additional consideration was given to smaller samples, or samples with low expected collagen yields.

3. 3. 3. 1. 3. Gelatinization

In order to gelatinize them the samples 9ml of 0.001M HCl was added to the vials and kept heated in an oven at 80˚C initially for 24 following which they were examined and possibly placed back in the oven for another 48 hours.

3. 3. 3. 1. 4. Ezee-filtering

Once cooled down to a temperature at which they could be handled safely, the sample tubes were stirred using a Vortex, in order to limit the amount of material gathering at the bottom of the tube and the dissolved fluid was then filtered using Ezee-filters. The filtered solution was then poured into new pre-weighted tubes.

3. 3. 3. 1. 5. Freeze-drying

Prior to the freeze-drying process, the caps for each tube were replaced with perforated parafilm, intended to provide a way for vapor to escape during the lyophilization process, following that the samples were placed in the freezer overnight and freeze-dried the following day. The material obtained this way was then weighed along with the tube and its corresponding cap to calculate the collagen yield.

3. 3. 3. 1. 6. Sample aliquot

The samples were then moved from the Leiden University chemical lab to the Vrije Universiteit Amsterdam for subsampling. Using a micro-balance, the weighed subsamples of ~0.5mg of purified collagen were placed into tin packets alongside tin packets containing standards (USGS 40, 41, 42) on the sampling tray and taken for stable isotope analysis.

3. 4. Statistical analysis

To assess the hypothesis of existing correlation between $\delta^{13}C_{co}$ and $\delta^{13}C_{se}$, linear regression and Pearson's correlation coefficient were originally considered for application to the obtained data. Both methods are used to determine association and correlation between two sets of continuous variables. However linear regression was found to be not entirely suitable for this purpose due to both variables present in the study being independent of each other, as such Pearson's correlation was chosen as the statistical method most suitable for determining correlation between the $\delta^{13}C_{se}$ and $\delta^{13}C_{co}$ values, as it doesn't distinguish between independent and dependent variables.

Microsoft Excel software was chosen to perform the calculations in. The calculation of the correlation coefficient (r) was done using Equation 1 where n stands for the number of pairs of $\delta^{13}C$ ratios obtained, and x and y standing for values of $\delta^{13}C_{co}$ and $\delta^{13}C_{se}$ respectively. The correlation coefficient was converted into a t-statistic (t) using Equation 2. Finally, the p-value was required to determine the probability of obtaining a result equal to or more extreme to what was observed, while working under the assumption of a null hypothesis. In order to calculate it, Student T test was performed on the obtained data under the assumption of a two tailed distribution.

$$\mathbf{r} = \frac{\mathbf{n}(\sum \mathbf{xy}) - (\sum \mathbf{x})(\sum \mathbf{y})}{\sqrt{[\mathbf{n}\sum \mathbf{x^2} - (\sum \mathbf{x})^2][\mathbf{n}\sum \mathbf{y^2} - (\sum \mathbf{y})^2]}}$$

Equation 2 Pearson correlation coefficient

$$t = \frac{r \times \sqrt{n-2}}{\sqrt{1-r^2}}$$

Equation 3 Conversion of the correlation coefficient to the t-statistic

To investigate other relationships between the obtained values, the values of isotope spacing were additionally employed. Isotope spacing is the absolute difference between the ratios of isotopes of carbon-13, here derived from structural enamel and collagen ($\Delta_{en\text{-}co} = \delta^{13}C_{en} - \delta^{13}C_{co}$)

4. Results

This chapter will report on the results of the extraction of material and stable isotope analysis performed for the study. The results are reported in accordance with suggestions found in the published literature (Bond & Hobson, 2012; Coplen, 2011; Roberts et al., 2018) on reporting stable isotope results and use of terminology. The results will be reported in their totality, divided into location they were derived from, as well as into groups based on assigned age groups and assigned sex groups. These categories were introduced in order to answer the research questions posed in Chapter 1 and are based on information obtained during the sampling process. Finally in order to answer the question regarding the correlation between $\delta^{13}C$ values of collagen and carbonate, the results of correlation coefficient calculations will be presented.

The inclusion of dental enamel in stable isotope analysis for this study, on top of obtaining $\delta^{13}C_{se}$ results by design in obtaining results for $\delta^{18}O_{se}$ as well. These results will not be reported on or discussed as they fall outside of the scope of this study, they will however be included in Appendix 2.1. in order to ensure full transparency in regard to the results obtained during the course of the study.

All of the teeth (n=76) selected for enamel analysis were successfully sampled, subsampled, and analyzed. This stands in mild contrast to the collagen results, where out of 76 dentine samples, 68 (89%) had collagen successfully extracted and analyzed. Sample preparation failed in the eight samples, due to instrumental issues during the freeze-drying process resulting in a loss of material beyond what was required for analysis.

4.1 Quality Control

The C/N ratios were found to have an average of 2.8 (g/g) and 3.3 (mol/mol) (Table 4). Both values falling within the accepted range for reliable collagen results as reported by DeNiro (1985) allowing for the results to be treated as reliable for the purpose of their further analysis and discussion, as no values used to calculate the average fell outside of the accepted range. Due to instrumental errors leading to obvious discrepancies in measuring of the weight of the collagen samples, the collagen yield was not possible to be calculated. Inclusion of other, aforementioned quality control measures was deemed sufficient for the data analysis to continue.

Location	N	Nitrogen (%)	Carbon (%)	C/N (g/g)	C/N (mol/mol)
Alkmaar	13	15	41	2.8	3.3
Arnhem	12	15	42	2.9	3.3
Eindhoven	16	15	42	2.9	3.3
Klaaskinderkerke	13	15	42	2.8	3.3
Zwolle	14	15	42	2.9	3.3
Total	68	15	42	2.8	3.3

Table 3 Average carbon and nitrogen quality control results for collagen samples.

4.2 Carbon and nitrogen isotope analysis results

The $\delta^{13}C_{en}$ data varied between −14.9 ‰ and −11.8‰ making for a range of 3.1‰. Both the average and median results were found to be −13.1‰ (Table 5). The $\partial^{13}C_{co}$ signatures varied between −20.9‰ and −19.0‰, with an average and median of −20.0‰. The collagen $\partial^{15}N_{co}$ values were between 10.9‰ and 15.1‰ and had an average of 13.1‰ and median of 13.3‰ (Table 5).

Material	Site of origin	N	Min (‰)	Max (‰)	Average (‰)	Median (‰)	Q1 (‰)	Q3 (‰)
$\partial^{13}C_{se}$ (‰ vs VPDB)	Alkmaar	15	−14.9	−12.7	−13.5	−13.3	−13.3	−12.9
	Arnhem	18	−13.8	−12.2	−13.1	−13.1	−13.7	−12.8
	Eindhoven	16	−13.6	−12.1	−12.9	−13.0	−13.2	−12.6
	Klaaskinderkerke	13	−14.4	−11.8	−12.9	−12.8	−13.6	−12.8
	Zwolle	14	−14.4	−12.3	−13.1	−13.1	−13.5	−12.4
	Total	76	−14.9	−11.8	−13.1	−13.1	−13.5	−12.7
$\partial^{13}C_{Co}$ (‰ vs VPDB)	Alkmaar	13	−20.5	−19.4	−19.9	−20.0	−20.2	−19.7
	Arnhem	12	−20.9	−19.6	−20.1	−20.1	−20.2	−19.9
	Eindhoven	16	−20.6	−19.3	−20.0	−20.0	−20.1	−19.9
	Klaaskinderkerke	13	−20.5	−19.0	−19.9	−19.9	−20.3	−19.7
	Zwolle	14	−20.8	−19.1	−20.2	−20.2	−20.4	−20.1
	Total	68	−20.9	−19.0	−20.0	−20.0	−20.0	−19.2
$\partial^{15}N_{Co}$ (‰ vs Air)	Alkmaar	13	11.5	14.1	12.8	12.8	12.1	13.4
	Arnhem	12	11.9	14.6	13.2	13.3	12.6	13.5
	Eindhoven	16	11.6	14.9	13.6	13.7	12.9	14.4
	Klaaskinderkerke	13	10.1	14.0	12.3	12.8	11.2	13.3
	Zwolle	14	10.9	15.1	13.7	13.9	13.0	14.8
	Total	68	10.1	15.1	13.1	13.3	12.5	13.9

Table 4 Average, median, highest, lowest first and third quartile values of stable isotope analysis of $\delta^{13}C_{se}$, $\partial^{13}C_{co}$ and $\partial^{15}N_{co}$ grouped by location.

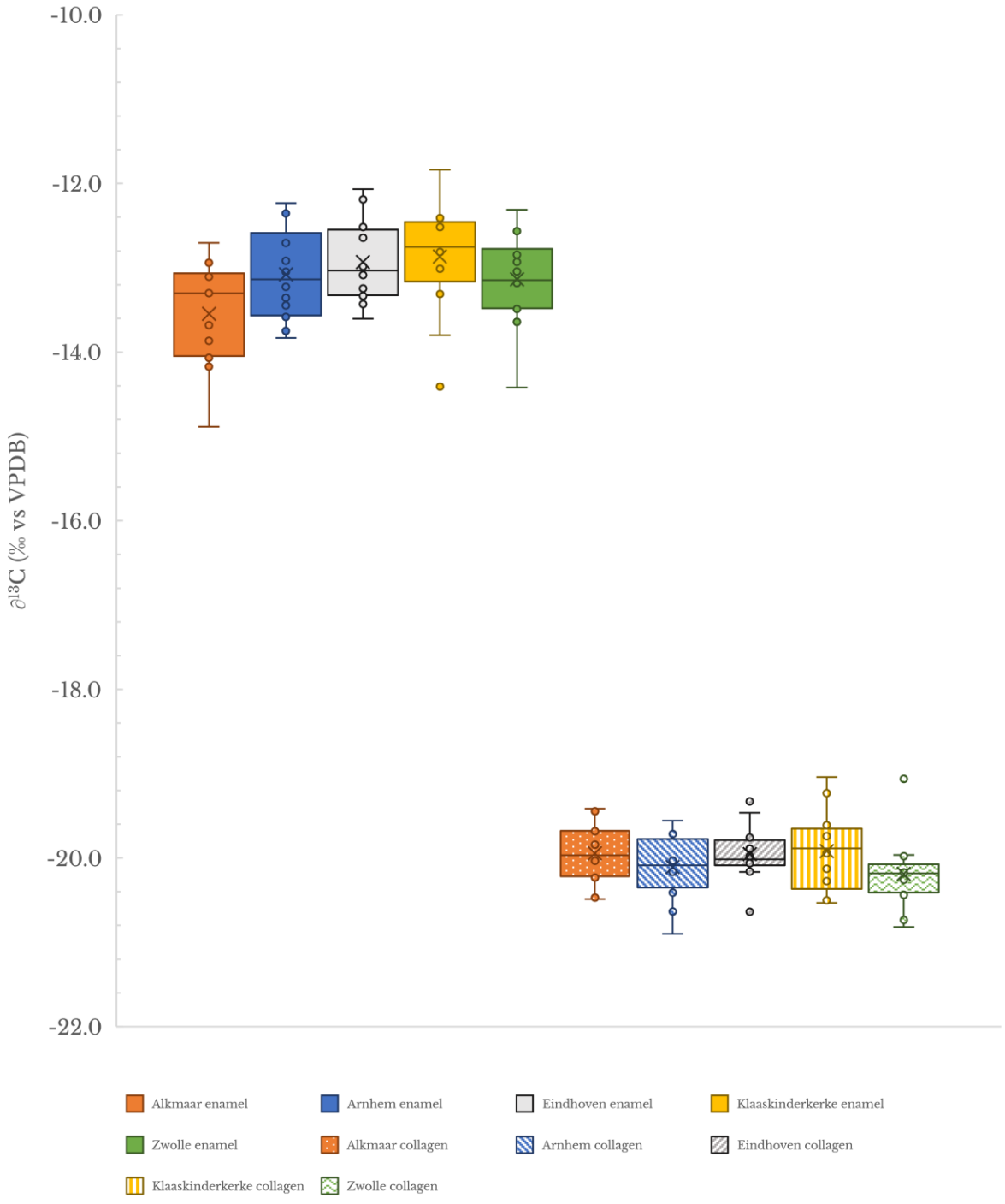


Figure 4 Obtained carbon isotope values from enamel and collagen per site.

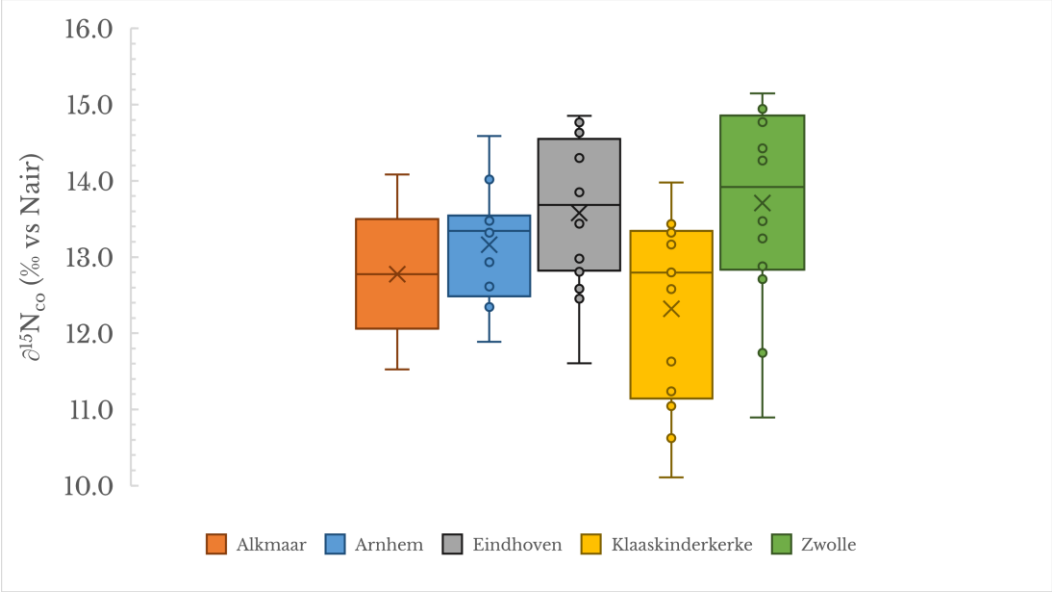


Figure 5 Obtained nitrogen isotope ratios from collagen per site.

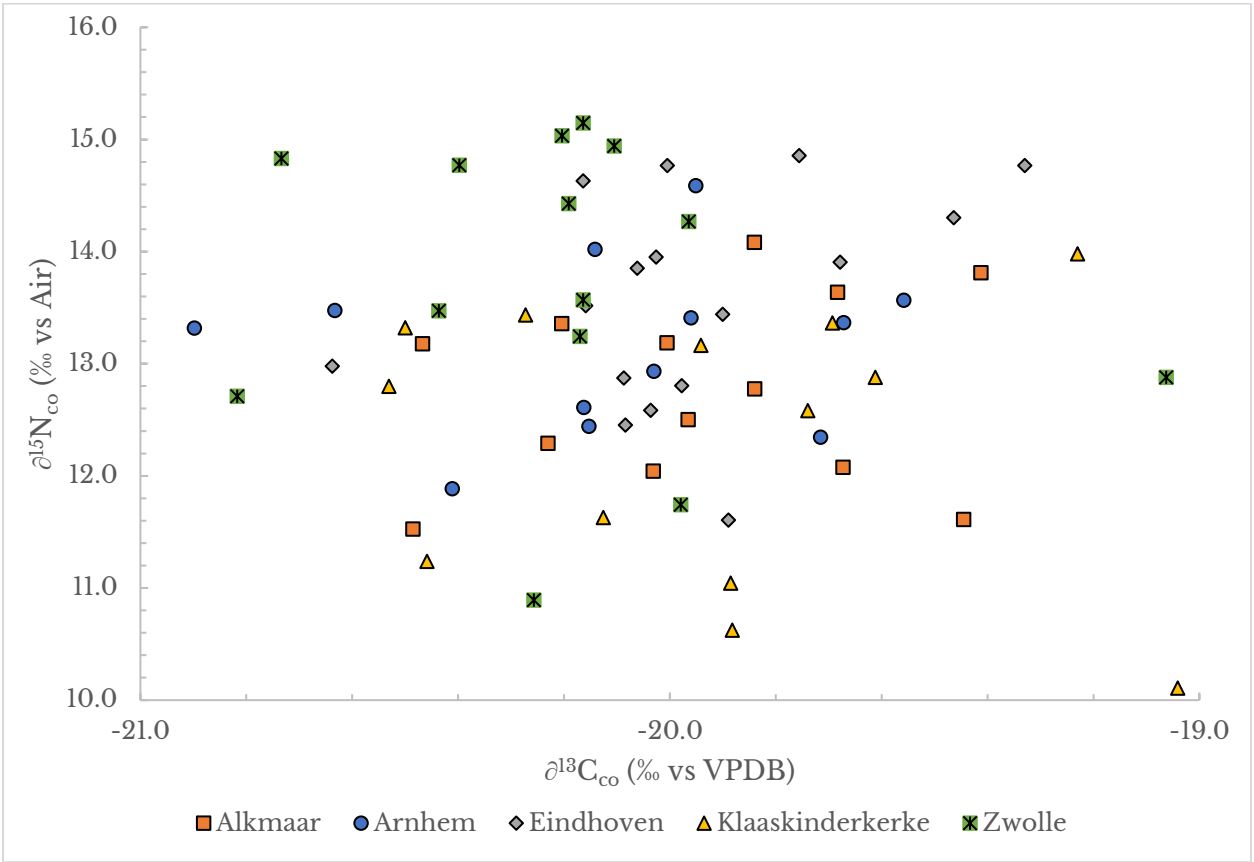


Figure 6 Carbon and nitrogen results of collagen analysis

4. 3. Population variation

Results were divided into two groups (Female/Male) based on the sex assigned to the individuals through the means of osteological analysis of skeletal remains, in order to analyze the data obtained through this lens and answer the research questions posed in chapter 1. The obtained data allowed for analysis of differences in paleodiet between individuals from those assigned sex groups. What should be repeated from chapter 3, is the discrepancy between the sample sizes of Female and Male groups with the final ratio (F/M) being 11:19. The results are first presented excluding their archaeological context and including it for presentation of intersite ranges and values.

4. .3. 1. Sex

The $\partial^{13}C_{se}$ values of the female group (Figure 7) ranged between –14.9‰ and –11.8‰, with an average and median both of –13.1‰ with an interquartile range of the female assigned group between–13.7‰ and –12.7‰. The values of the male assigned group ranged between –14.0‰ and –12.1‰ with an average and median of –13.1‰ and an interquartile range of –13.3‰ and –12.6‰. A single outlying individual with $\partial^{13}C_{se}$ value of –14.4‰ was excluded from discussion.

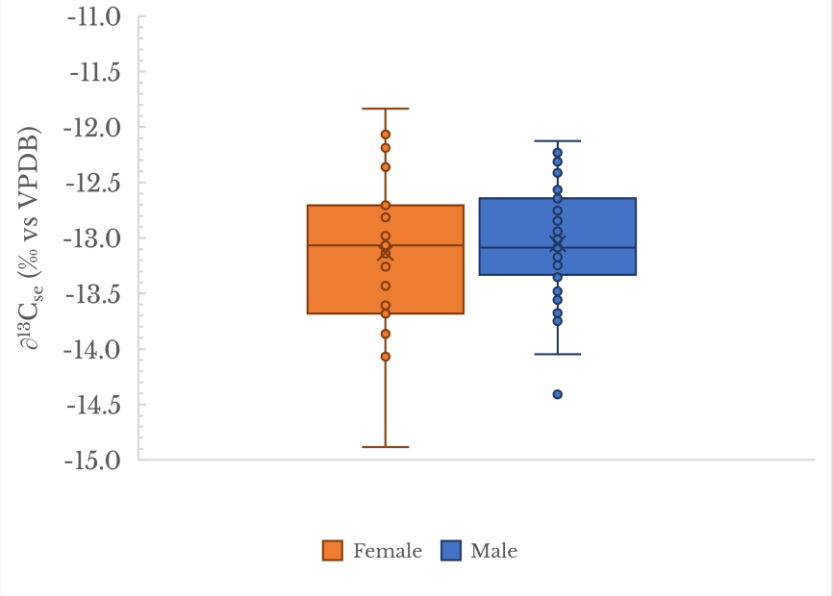


Figure 7 Carbon isotope ratios of structural enamel divided by assigned sex of the individuals.

The $\partial^{13}C_{co}$ values of the female group ranged between -20.6‰ and -19.3‰, with an average and median both of -20.1‰ and an interquartile range of –20.5‰ to –19.8‰. The Male

groups values meanwhile ranged between −20.9‰ and −19‰ with an average and median of −20‰ and an interquartile range of −20.2‰ and −19.8‰ (Figure 8).

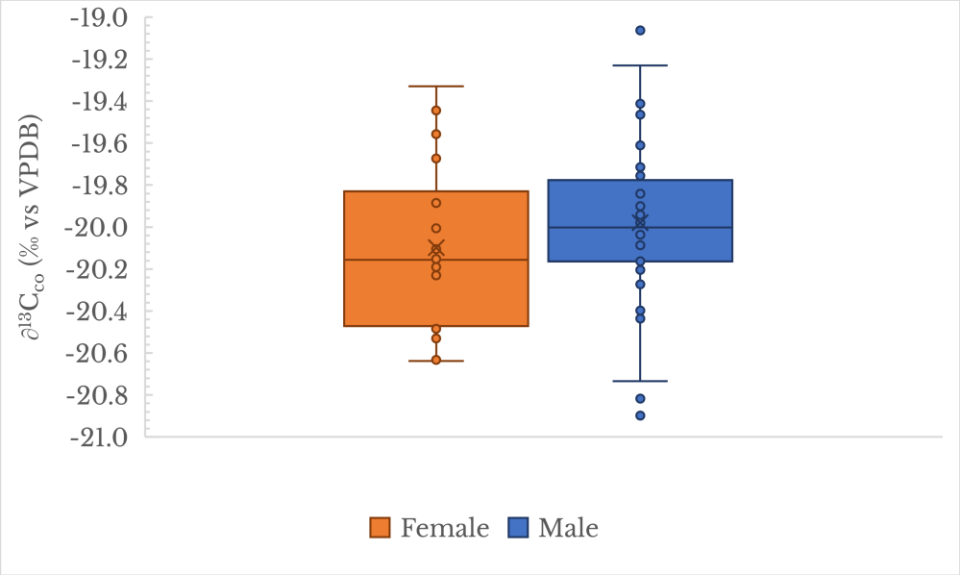


Figure 8 Carbon isotope ratios of dentine collagen divided by assigned sex of the individuals.

The $\partial^{15}N_{co}$ values of the Female group ranged between 10.6‰ and 14.9‰, an average of 13‰ and a median of 13.2‰ and an interquartile range of 12.0‰ to 13.8‰. The Male groups values meanwhile ranged between 10.1‰ and 15.2‰ with an average of 13.2‰ and a median of 13.3‰ and an interquartile range of 12.6‰ to 14.0‰ (Figure 9).

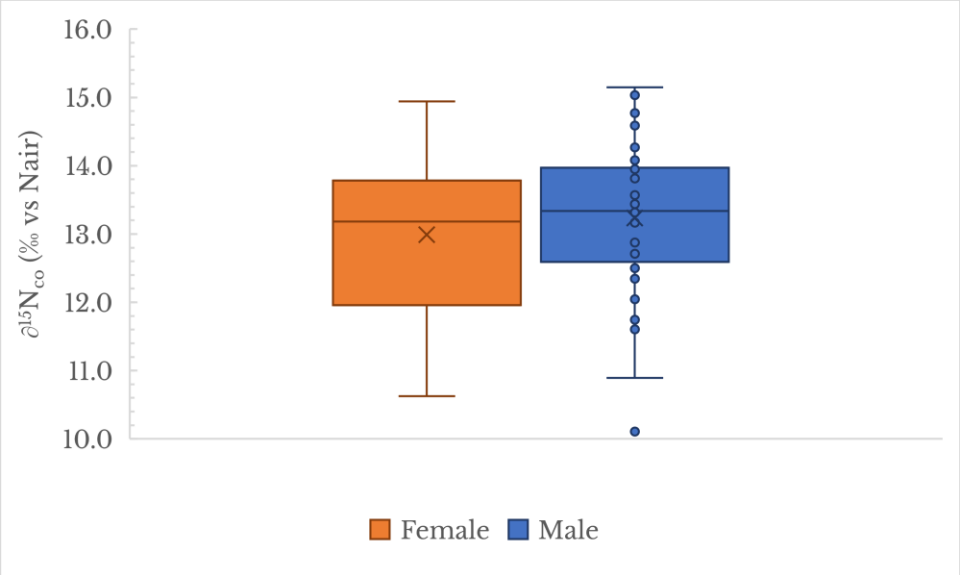


Figure 9 Nitrogen isotope ratios of dentine collagen divided by assigned sex of the individuals.

4. 3. 1. 1. Carbon Enamel

Values of $\delta^{13}C_{se}$ exhibit significant differences based on assigned sex group and location of origin. Presented data is visualized on corresponding figures, while tables containing the discussed presented data can be found in Appendix 1. for reference.

$\delta^{13}C_{se}$ values of female assigned individuals from Alkmaar were found to range between –14.0‰ and –12.4‰ with an average of –13.1‰ a median of –13.2‰ and an interquartile range of –13.5‰ to –12.9‰. Meanwhile $\delta^{13}C_{se}$ values from Alkmaar's male assigned individuals range between –13.3‰ and –12.8‰ with an average of –13.2‰ and a median of –13.1‰. Interquartile range of this group was found to be –13.3‰ and –12.9‰.

$\delta^{13}C_{se}$ values of female assigned individuals from Arnhem were found to range between –14.9‰ and –12.2‰ with an average of –13.3‰ a median of –13.4‰ and an interquartile range of –13.8‰ to –12.9‰. $\delta^{13}C_{se}$ values of the male assigned group from Arnhem were found to have a range of –13.3‰ to –12.5‰, an average of –12.9‰ a median of –12.8‰ and an interquartile range of –13.3‰ to –12.5‰.

$\delta^{13}C_{se}$ values of female assigned individuals from Eindhoven were found to range between–13.7‰ and –12.3‰ with an average of –13.1‰ a median of –13.0‰ and an interquartile range of –13.4‰ to –12.6‰. $\delta^{13}C_{se}$ values of the male assigned group from Eindhoven were found to have a range of –13.7‰ to –12.3‰, an average and median of –13.0‰ and an interquartile range of –13.2‰ to –12.5‰.

$\delta^{13}C_{se}$ values of female assigned individuals from Klaaskinderkerke were found to range between –14.2 and –12.1 with an average of and median of –13.2‰ and an interquartile range of –13.7‰ to –12.8‰. $\delta^{13}C_{se}$ values of the male assigned group from Klaaskinderkerke were found to have a range of –13.7‰ to –13.0‰, an average of –13.1‰ a median of –13.2‰ and an interquartile range of –13.3‰ to –13.0‰.

$\delta^{13}C_{se}$ values of the two female assigned individuals from Zwolle were found to range between –12.7 and –12.4‰ with an average of –12.5‰. $\delta^{13}C_{se}$ values of the male assigned group from Zwolle were found to have a range of –13.6‰ to –12.2, an average and median of –12.9 and an interquartile range of –13.5‰ to –12.3‰

Site	Female Q1 $\delta^{13}C_{se}$ (‰)	Female Q3 $\delta^{13}C_{se}$ (‰)	Male Q1 $\delta^{13}C_{se}$ (‰)	Male Q3 $\delta^{13}C_{se}$ (‰)
Alkmaar	−13.5	−12.9	−13.3	−12.9
Arnhem	−13.8	−12.9	−13.3	−12.5
Eindhoven	−13.4	−12.6	−13.2	−12.5
Klaaskinderkerke	−13.7	−12.8	−13.3	−13.0
Zwolle	−12.7	−12.3	−13.5	−12.3
Total	−13.7	−12.7	−13.3	−12.6

Table 5 First (Q1) and third (Q3) quartile $\delta^{13}C_{en}$ values of assigned sex groups based on their site of origin and in totality.

Site	Min $\delta^{13}C_{en}$ (‰)		Max $\delta^{13}C_{en}$ (‰)		Average $\delta^{13}C_{en}$ (‰)		Median $\delta^{13}C_{en}$ (‰)	
	F	**M**	**F**	**M**	**F**	**M**	**F**	**M**
Alkmaar	−14.0	−13.3	−12.4	−12.8	−13.1	−13.2	−13.1	−13.1
Arnhem	−14.9	−13.3	−12.2	−12.5	−13.3	−12.9	−12.8	−12.8
Eindhoven	−13.7	−13.7	−12.3	−12.3	−13.1	−13.0	−13.0	−13.0
Klaaskinderkerke	−14.2	−13.7	−12.1	−13.0	−13.2	−13.1	−13.2	−13.2
Zwolle	−12.7	−13.6	−12.3	−12.2	−12.5	−12.9	N/A	−12.9

Table 6 $\delta^{13}C_{en}$ (% VPDB) lowest (Min) highest (Max) average and median values of female and male individuals grouped by location of origin.

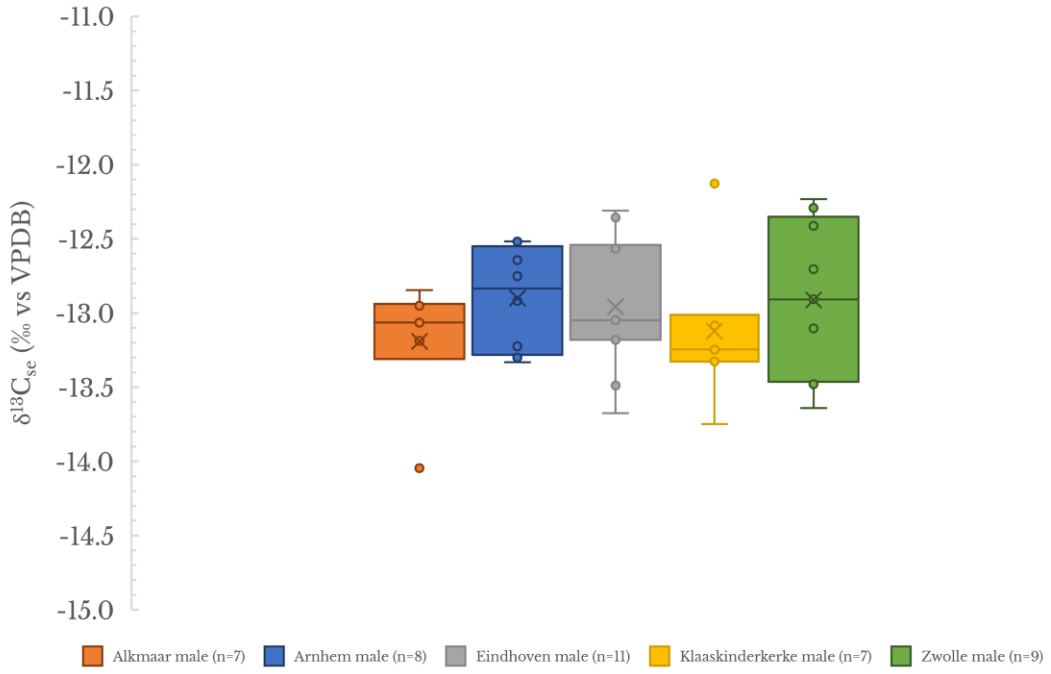
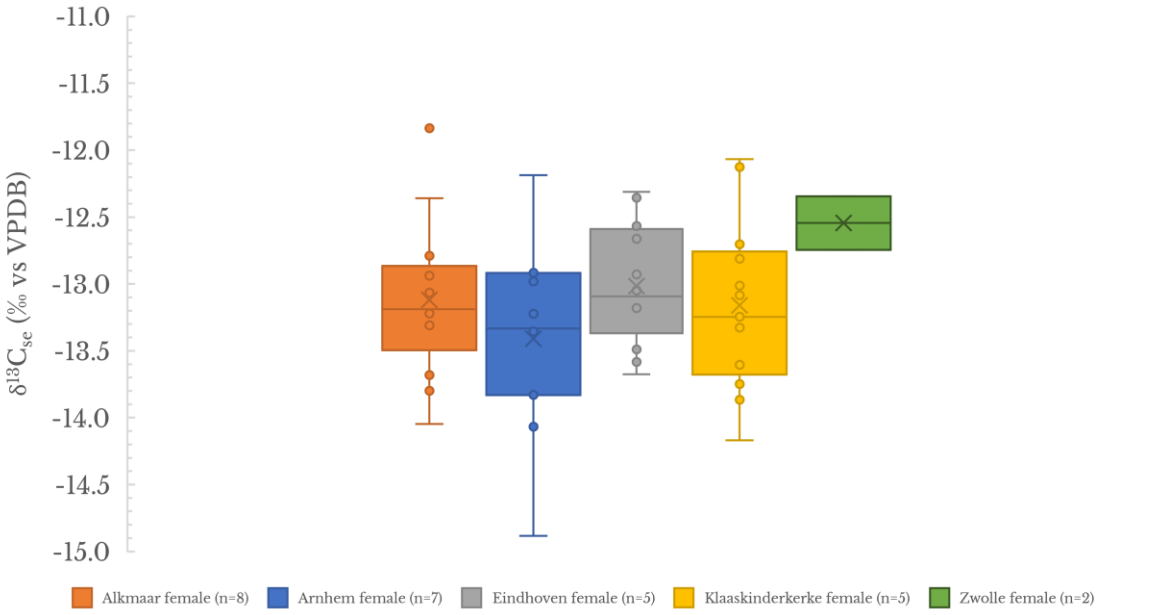


Figure 10 Carbon enamel ratios of male assigned individuals grouped by location of origin.



4. 3. 1. 2. Collagen

Due to collagen extraction not being successful in all samples, collagen samples and their values are not representative of all individuals selected for the study. Regardless, the obtained results show.

4. 3. 1. 2. 1. Carbon

Values of $\delta^{13}C_{co}$ exhibit significant differences based on assigned sex group and location of origin. These values are presented and described here.

$\delta^{13}C_{co}$ values of female assigned individuals from the site of Alkmaar were found to have a range of –20.5‰ to –19.4‰, an average and median of –20.0‰ and an interquartile range of –20.2‰ to –19.8‰. Male assigned individuals were in turn found to have $\delta^{13}C_{co}$ values ranging from –20.2‰ to –19.4‰, an average and median of –19.8 with an interquartile range of –20.0‰ to –19.7‰.

$\delta^{13}C_{co}$ values of female assigned individuals from the site of Arnhem were found to have a range of –20.9‰ to –19.6‰ with an average and median of –20.1‰ and an interquartile range of –20.5‰ to –19.7‰. Male assigned individuals were in turn found to have $\delta^{13}C_{co}$ values ranging from –20.2 to –19.7, and an average and median of –20.0 and an interquartile range of –20.1‰ to –19.9‰.

$\delta^{13}C_{co}$ values of female assigned individuals from the site of Eindhoven were found to have a range of –20.2 to –19.5‰ and an average and median of –20.0‰ and an interquartile range of –20.1‰ to –19.8‰. Male assigned individuals were in turn found to have $\delta^{13}C_{co}$ values ranging from –20.1‰ to –19.6‰ with an average of –19.9 a median of –20.0 and an interquartile range of –20.1 to –19.8.

$\delta^{13}C_{co}$ values of female assigned individuals from the site of Klaaskinderkerke were found to have a range of –20.5‰ to –19.0‰ with an average and median of –19.9‰ and an interquartile range of –20.4‰ to 19.6‰. Male assigned individuals were in turn found to have $\delta^{13}C_{co}$ values ranging from –20.3‰ to –19.0 with an average and median of –19.7‰ and an interquartile range of –20.1 to –19.2.

$\delta^{13}C_{co}$ values of the two female assigned individuals from the site of Zwolle were found to have a range and interquartile range of –20.2‰ to –20.1‰, with an average of –20.1‰. Male assigned individuals were in turn found to have $\delta^{13}C_{co}$ values ranging from –20.4‰ to –20.0‰, with an average of –20.3‰ and median of –20.2‰ and an interquartile range of –20.4‰ to –20.2‰.

Site	Female Q1 $\delta^{13}C_{co}$ [‰]	Female Q3 $\delta^{13}C_{co}$ [‰]	Male Q1 $\delta^{13}C_{co}$ [‰]	Male Q3 $\delta^{13}C_{co}$ [‰]
Alkmaar	−20.2	−19.8	−20.0	−19.7
Arnhem	−20.5	−19.7	−20.1	−19.9
Eindhoven	−20.1	−19.8	−20.1	−19.8
Klaaskinderkerke	−20.4	−19.6	−20.1	−19.2
Zwolle	−20.2	−20.1	−20.4	−20.2
Total	−20.5	−19.8	−20.2	−19.8

Table 7 First (Q1) and third (Q3) quartile values $\delta^{13}C_{co}$ of female and male individuals grouped by location of origin.

Site	Min $\delta^{13}C_{co}$ (‰)		Max $\delta^{13}C_{co}$ (‰)		Average $\delta^{13}C_{co}$ (‰)		Median $\delta^{13}C_{co}$ (‰)	
	F	M	F	M	F	M	F	M
Alkmaar	−20.5	−20.2	−19.4	−19.4	−20.0	−19.8	−20.0	−19.8
Arnhem	−20.9	−20.2	−19.6	−19.7	−20.1	−20.0	−20.1	−20.0
Eindhoven	−20.2	−20.1	−19.5	−19.6	−20.0	−19.9	−20.0	−20.0
Klaaskinderkerke	−20.5	−20.3	−19.0	−19.0	−19.9	−19.7	−19.9	−19.7
Zwolle	−20.2	−20.4	−20.1	−20.0	−20.1	−20.3	N/A	−20.2

Table 8 δ13Cco (‰ v VPDB) lowest (Min) highest (Max) average and median values of female and male assigned individuals grouped by location of origin.

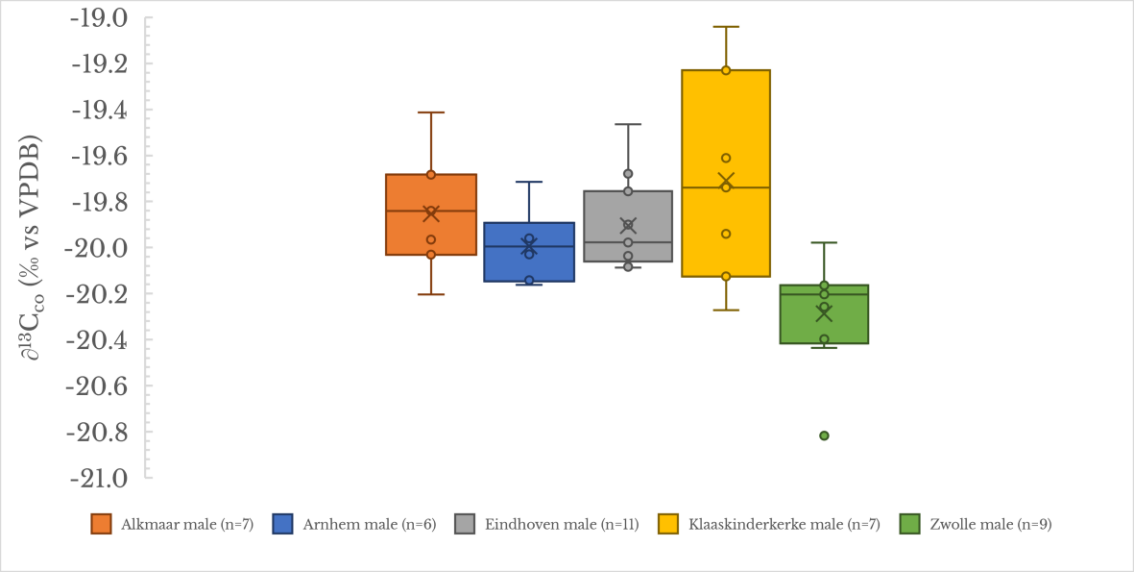


Figure 11 $\partial^{13}C_{co}$ values of male assigned analyzed individuals grouped by location of origin.

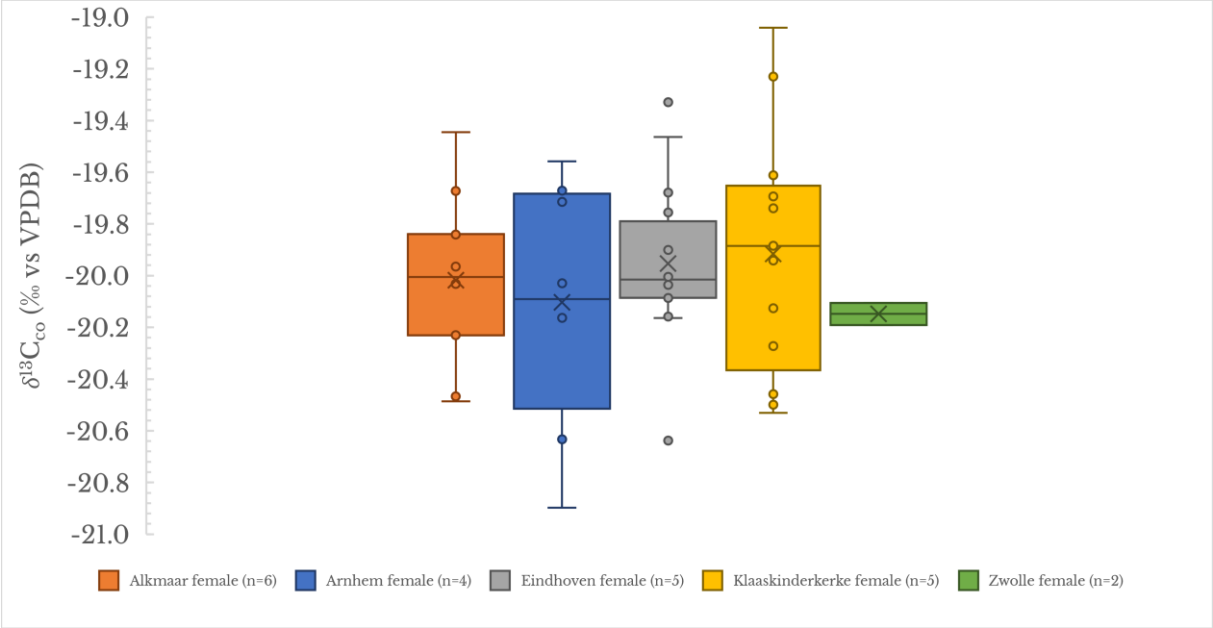


Figure 12 $\partial^{13}C_{co}$ values of female assigned analyzed individuals, grouped by location of origin.

4. 3. 1. 2. 2. Nitrogen

Values of $\delta^{13}C_{en}$ exhibit significant differences based on assigned sex group and location of origin. These values are presented and described here.

$\delta^{15}N_{co}$ values of female assigned individuals from the site of Alkmaar were found to have a range of 11.6‰ to 14.1‰, an average of 12.5‰ a median of 12.6‰ and an interquartile range of 12.5‰ to 13.4‰. Male assigned individuals were in turn found to have $\delta^{15}N_{co}$ values ranging from 12.0‰ to 14.1‰, an average of 13.2‰ a median of 13.4‰ with an interquartile range of 12.5‰ to 14.2‰.

$\delta^{15}N_{co}$ values of female assigned individuals from the site of Arnhem were found to have a range of 12.3‰ to 13.6‰ with an average of 13.0‰ a median of 13.1‰ and an interquartile range of 12.0‰ to 13.2‰. Male assigned individuals were in turn found to have $\delta^{15}N_{co}$ values ranging from 12.3‰ to 14.6‰, and an average of 13.3‰ a median of 13.2‰ and an interquartile range of 12.5‰ to 13.8‰.

$\delta^{15}N_{co}$ values of female assigned individuals from the site of Eindhoven were found to have a range of 11.6‰ to 14.8‰ and an average of 13.6‰ a median of 13.7‰ and an interquartile range of 12.8‰ to 14.5‰. Male assigned individuals were in turn found to have $\delta^{15}N_{co}$ values ranging from 11.6‰ to 14.8‰ with an average of 13.3‰ a median of 13.4‰ and an interquartile range of 12.6‰ to 14.0‰.

$\delta^{15}N_{co}$ values of female assigned individuals from the site of Klaaskinderkerke were found to have a range of 10.1‰ to 14.0‰ with an average of 12.3‰ and median of 12.8‰ and an interquartile range of 11.1‰ to 13.3‰. Male assigned individuals were in turn found to have $\delta^{15}N_{co}$ values ranging from 10.1‰ to 14.0‰ with an average of 12.5‰ and median of 12.9‰ and an interquartile range of 11.6‰ to 13.4‰.

$\delta^{15}N_{co}$ values of the two female assigned individuals from the site of Zwolle were found to have a range and interquartile range of 14.4‰ to 14.9‰, with an average of 14.7‰. Male assigned individuals were in turn found to have $\delta^{15}N_{co}$ values ranging from 10.9‰ to 15.1‰, with an average of 13.4‰ and median of 13.5‰ and an interquartile range of 12.6‰ to 14.0‰.

Site	Female Q1 $\delta^{15}N_{co}$ (‰)	Female Q3 $\delta^{15}N_{co}$ (‰)	Male Q1 $\delta^{15}N_{co}$ (‰)	Male Q3 $\delta^{15}N_{co}$ (‰)
Alkmaar	12.5	13.4	12.5	14.2
Arnhem	12.0	13.2	12.5	13.8
Eindhoven	12.8	14.5	12.6	14.0
Klaaskinderkerke	11.1	13.3	11.6	13.4
Zwolle	14.4	14.9	12.3	14.9

Table 9 First (Q1) and third (Q3) quartile $\delta^{15}N_{co}$ values of the female and male groups, grouped by location of origin.

Site	Min $\delta^{15}N_{co}$ (‰)		Max $\delta^{15}N_{co}$ (‰)		Average $\delta^{15}N_{co}$ (‰)		Median $\delta^{15}N_{co}$ (‰)	
	F	**M**	**F**	**M**	**F**	**M**	**F**	**M**
Alkmaar	11.6	12.0	14.1	14.1	12.5	13.2	12.6	13.4
Arnhem	12.3	12.3	13.6	14.6	13.0	13.3	13.1	13.2
Eindhoven	11.6	11.6	14.8	14.8	13.6	13.3	13.7	13.4
Klaaskinderkerke	10.1	10.1	14.0	14.0	12.3	12.5	12.8	12.9
Zwolle	14.4	10.9	14.9	15.1	14.7	13.4	N/A	13.5

Table 10 Lowest (Min), highest (Max) values, averages, and medians of $\delta^{15}N_{co}$ (‰ vs Nair) of female (F) and male (M) groups, grouped by location of origin.

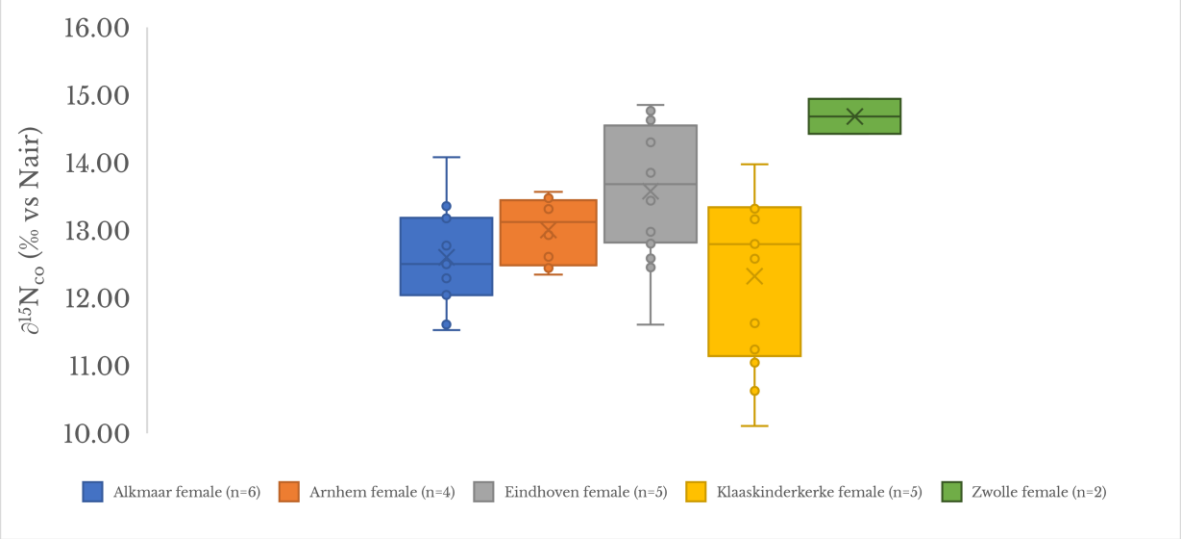


Figure 13 $\partial^{15}N_{co}$ (‰ vs Nair) values of female assigned individuals grouped by location of origin.

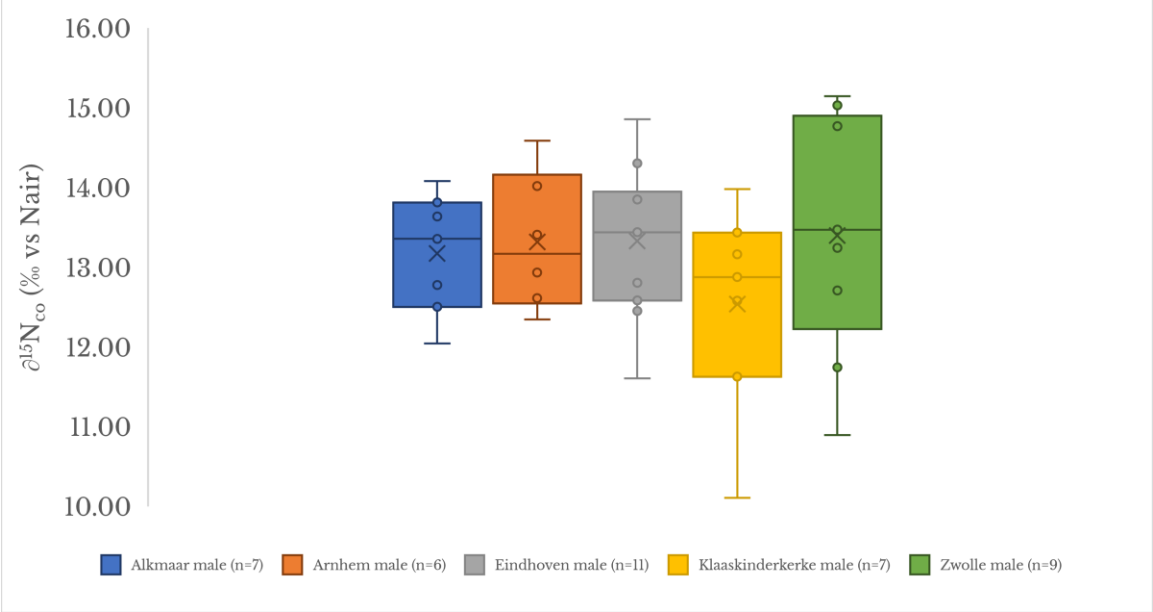


Figure 14 $\partial^{15}N_{co}$ (‰ vs Nair) values of male assigned individuals grouped by location of origin.

4. 4. Statistical analysis

The relationship between the obtained $\partial^{13}C$ (‰ vs VPDB) data originating from dentine collagen and enamel was investigated and returned with largely null results in regard to a possible correlation between the values. The Pearson Correlation Coefficient returned with a value of −0.0166 (R^2=0.001) determining a lack of correlation between the values ($P < 0.05$) (Figure 8).

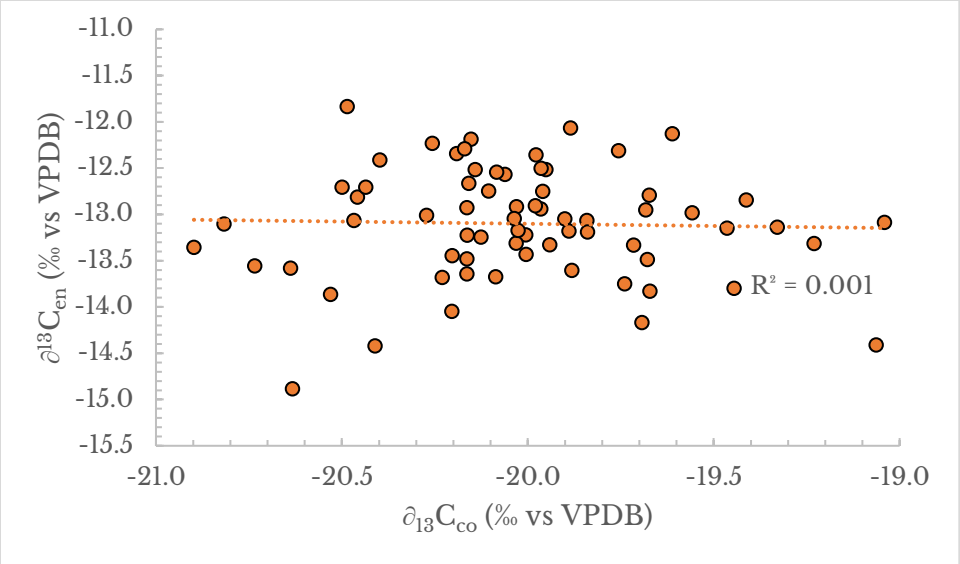


Figure 15 Correlation between carbon collagen and enamel isotope values

The spacing between carbon carbonate and carbon collagen values ($\Delta_{en\text{-}co}$) was calculated for the applicable individuals where collagen results were available, resulting in values ranging between 5.9 and 8.5, with an average and a median of 7.0. Values of $\partial^{13}C_{en}$ and $\Delta_{en\text{-}co}$ (Figure 19) were found to have a Pearson's correlation coefficient of 0.8. Results of applying this calculation toward individual sites found the $\partial^{13}C_{en}$ and $\Delta_{en\text{-}co}$ correlation to vary between them by small increments, all however displaying strong positive correlation, with sites of Alkmaar Klaaskinderkerke and Zwolle's Pearson correlation coefficients of 0.8, Arnhem with a correlation coefficient of 0.7 and Eindhoven's equal to 0.9. All of these results represent a very strong positive correlation between the two values regardless of the site of origin.

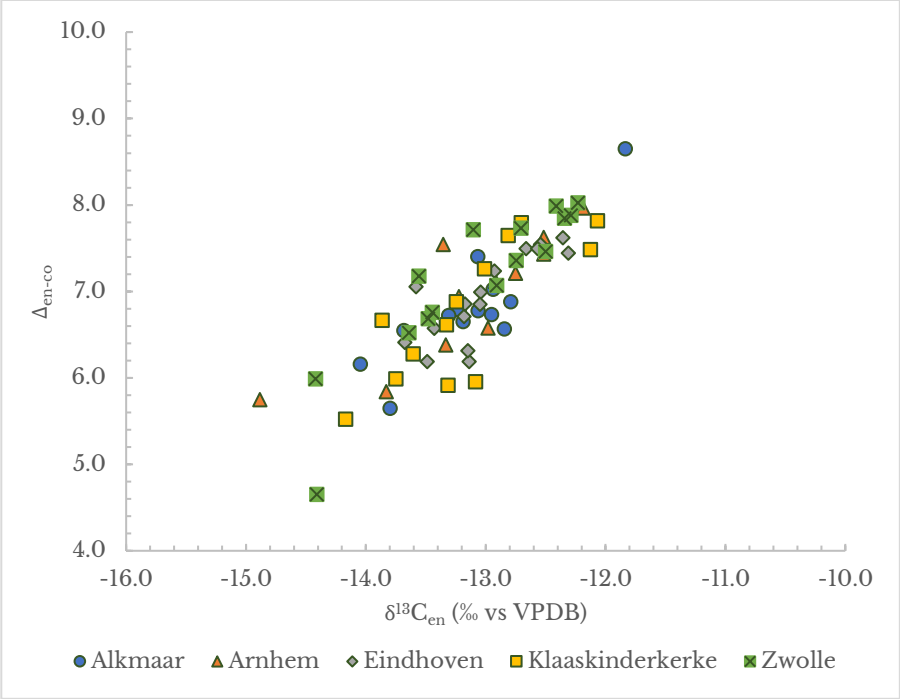


Figure 16 Spacing between carbon-13 ratios of enamel and collagen plotted against $\delta^{13}C_{co}$ (‰ vs VPDB)

Relationship between $\Delta_{en\text{-}co}$ and $\partial^{13}C_{co}$ (Figure 20) in the form of correlation between the two values shows inverse results when compared to the aforementioned $\partial^{13}C_{en}$ and $\Delta_{en\text{-}co}$. The correlations were found to be strong but negative (Figure above). Overall correlation between spacing and carbon collagen has a Pearsons correlation coefficient of −0.3, with individual variation between the sites. Alkmaar and Eindhoven have a correlation coefficient of −0.6, while Arnhem displays the lowest correlation coefficient of −0.3. Klaaskinderkerke and Zwolle show the highest negative correlation coefficients of the group, at −0.8. The strong negative correlation between the two values is varied across locations, but present regardless.

Figure 17 Spacing between carbon-13 ratios of enamel and collagen plotted against $\delta^{13}C_{co}$ (‰ vs VPDB)

Finally, the relationship between carbon spacing and nitrogen collagen was investigated in the same manner, revealing the biggest variability among the investigated ratios. Overall, the relationship between nitrogen–15 ratios and carbon spacing has a Pearson coefficient of −0.1 with extremely low negative correlation, however, between individual sites, the correlation varies more significantly. In the site of Arnhem, the relationship was found to be strongly negatively correlated (r=−0.7), while Alkmaar and Eindhoven were mildly negatively correlated (r=−0.3) while Klaaskinderkerke was inversely positively correlated (r=0.3), and Zwolle not correlated at all (r= 0)

5. Discussion

Utilization of stable isotope analysis of carbon and nitrogen derived from human tissues in an archaeological context, allows for reconstruction of past diets of the individuals analyzed, when applied to a larger context, it furthermore allows us to make claims regarding trends in consumption of certain types of food or differences in consumption between groups and time periods. Stable isotope ratios of carbon can provide information about sources of dietary protein when derived from collagen, or about sources of dietary carbon when derived from enamel, meanwhile stable isotope ratios of nitrogen provide information on the trophic level of consumed foods, allowing for differentiation between terrestrial and marine derived diets.

This chapter will present and place the results in the wider context of published material and analyze it to answer the research questions. The discussion of the data will follow previously established baselines for dental development, as mentioned in the background chapter. The development of dental tissues locks isotopic ratios in the dental material, therefore the result from the analyzed material serves as averaged values of the consumed diet from the duration of the dental development. The use of dental material derived from second molars, means that the values obtained from enamel are representative of the diet consumed between 2.5 and 8 years of age, while collagen values are representative of diet up to the age of 16 years. The data analysis and discussion will follow this developmentary timeline, dividing the discussion into individual results and trends in childhood and in adolescence, allowing for discussion of how these values and therefore diet, changed from young age to late adolescence. This will allow for a wider and more detailed view of sub-adult diet in early medieval to early modern Netherlands. Additionally, interpretation of $\Delta^{13}C_{en\text{-}co}$ and related values will supplement the dietary analysis, to arrive at more detailed conclusions regarding the composition of past diets in contexts discussed.

5.1. Historical trends

The sites investigated during this study were part of many historical periods relative to other studies focusing on medieval sites. The youngest site in the set is Klaaskinderkerke (early medieval), followed by Arnhem (late to post medieval), Alkmaar (post medieval) Eindhoven (post medieval) and Zwolle (post medieval to early modern). The largest overlap between the time periods when the sites were in use lies between in the early 15th and late 16th centuries with Arnhem, Alkmaar Eindhoven and Klaaskinderkerke. This overlap will be used to analyze the data from this time period and make more concrete conclusions

regarding trends in childhood and adolescent diet in the Netherlands. Importantly for the purposes of comparison, Klaaskinderkerke is rural, as opposed to the remaining urban sites. This distinction allows for comparison of not only temporal trends, but also to compare diets of rural and urban areas.

In regard to temporal continuity between the sites, previously published results from Blokhuizen fit themselves into the timeline recorded in this study, as it dates to early to central Middle Ages, placing itself as older than the sites examined. While the study (Schats et al., 2022) utilized collagen derived from bones, it is reflective of adult diet of the individuals rather than childhood or adolescent diet recorded here, it is nonetheless an extremely useful baseline. The trends found therein will provide an extension to the timeline and expand the discussion about changes in diet over time.

Stable isotope ratios derived from Blokhuizen (Mean $\delta^{13}C_{co}$= −20.6‰ SD= 0.4; Mean $\delta^{15}N_{co}$= 11.8‰ SD= 0.9) (Schats et al., 2022) have been previously determined to be based on consumption of largely terrestrial sources, proteins from herbivores and omnivores as well as sheep and goat products in form of meat and dairy products with minimal contribution of fish, which as stated before translates to adult diet reliant on those sources.

Stable isotope values of individuals from Klaaskinderkerke, compared to the remaining sites, were found to have noticeably enriched carbon enamel ratios, suggesting high consumption of carbon-13 enriched foods such as C4 plants or marine foods, however low nitrogen-15 ratios found in collagen samples from Klaaskinderkerke suggest that the protein sources were of lower trophic level, and less likely to have been marine in origin. Carbon collagen ratios were likewise enriched relative to other sites, suggesting carbon-13 enriched protein sources, possibly including omnivore protein with an inclusion of marine protein. Three individuals were found fall below the defined range with anywhere from marginal to significant depletion of nitrogen-15. This number of outlying individuals from this site reinforces its differences from the remaining late and post medieval sites. The ratios of the individuals in question suggest extremely low rates of omnivore and marine protein in adolescent individuals, likely consuming herbivore, and plant protein in larger quantities. The comparison of enamel and collagen data suggests that individuals from Klaaskinderkerke likely had a similar diet between the ages of eight and sixteen.

Enriched stable isotope ratios of enamel derived from Arnhem suggest that the analyzed individuals consumed carbon-13 enriched foods, such as marine foods in some proportion. Carbon collagen values meanwhile were found to be similarly enriched to other

post medieval sites, suggesting consumption of omnivore and marine foods. Nitrogen-15 ratios however seem to suggest that protein sources were of a lower trophic level, which could mean continuous consumption of omnivore derived foods at a higher rate during adolescence.

Carbon enamel stable isotope ratios from Alkmaar were found to be uniquely depleted relative to other locations, suggesting a decreased consumption of carbon-13 enriched foods relative to the other sites analyzed, this could be explained through decreased consumption of marine foods, which in turn would be explained by Alkmaar's inland location. Carbon collagen ratios on the other hand were found to be of similar value to other pre-modern sites, likely meaning that adolescent individuals consumed significant amounts of carbon-13 enriched foods. Nitrogen isotope ratios were marginally depleted, suggesting consumption of more terrestrial foods. The results suggest that childhood and adolescent diets of individuals from Alkmaar, involved a minimal inclusion of marine foods, with a heavier reliance on terrestrial sources, with a change to more carbon-13 enriched foods such as omnivore products in adolescence.

Previously mentioned research of Alkmaar in comparison found significantly more varied and on average more depleted carbon collagen values (Mean $\delta^{13}C_{co}$= −20.3‰; SD= 0.8), as well as significantly more enriched nitrogen values (Mean $\delta^{15}N_{co}$= 13.2; SD= 0.9). This was suggested to be due to increased consumption of marine foods or other higher trophic level foods, the depleted carbon collagen values, relative to now known wider context, seem to suggest that adult diet in Alkmaar more likely consisted largely of omnivores, rather than marine fish, explaining both values.

Stable isotope values of individuals from Eindhoven found carbon enamel ratios of average enrichment relative to majority of sites analyzed, suggesting similarities in diet to all sites with the exclusion of Alkmaar, with foods relatively enriched in carbon-13, such as larger quantities of omnivore foods and products and marine foods. Carbon collagen values were similarly enriched to the other post medieval sites, suggesting consumption of omnivore and marine foods, while nitrogen collagen values were found to be significantly enriched among the analyzed sites, suggesting increased consumption of marine foods relative to the other sites with the exception of Zwolle. Comparison of enamel and collagen ratios suggests continuous consumption of carbon-13 enriched foods between childhood and adolescence.

Previous research into the diet of individuals from Eindhoven, included stable isotope analysis, with obtained $\delta^{13}C_{co}$ and $\delta^{15}N_{co}$ values for, among others seven individuals also sampled for this study. Those individuals (Table 11) have had their rib collagen analyzed for carbon and nitrogen, due the turnover of rib collagen, the data obtained from those individuals is reflective of a later point in their lives, therefore direct comparison of their corresponding data from dentine collagen would be comparing their diets from adolescence to a point later in life, allowing for comparison between those two diets. Among those directly comparable, three individuals (EHV 1108, EHV 2828 and EHV 3463) were found to have their rib collagen carbon-13 and nitrogen-15 values to be depleted compared to their dentine values. This translates into a potential shift away from consumption of marine foods and towards a more terrestrial sources which occurred between young adulthood and a period prior to their death. Other individuals (EHV 2593, EHV 2817) were found to have only minimal differences between their isotopic ratios suggesting that the individuals in question had a very similar diet during young adulthood and the period prior to their death. Individual EHV 2885's isotope ratios of carbon collagen and nitrogen collagen were both depleted relative to their rib counterparts, suggesting an increase in consumption of marine protein, as well as increased consumption of carbon enriched foods. Individual 3536's isotope ratios of rib carbon-13 were depleted relative to tooth collagen, while nitrogen-15 ratios of the rib collagen were enriched relative to tooth collagen, suggesting an increase in marine food consumption and a decrease in the consumption of terrestrial foods.

Sample ID	Tooth collagen $\delta^{13}C$ (‰)	Tooth collagen $\delta^{15}N$ (‰)	Rib collagen $\delta^{13}C$ (‰)	Rib collagen $\delta^{15}N$ (‰)
EHV 1108	–20.0	14.8	–20.4	13.9
EHV 2593	–19.9	11.6	–19.7	12.0
EHV 2817	–19.8	14.9	–19.7	14.7
EHV 2828	–20.2	13.5	–20.8	12.8
EHV 2885	–20.1	12.9	–19.6	13.2
EHV 3463	–20.0	14.0	–20.2	13.1
EHV 3536	–20.0	13.9	–19.4	13.7

Table 11 Comparison of new collagen data from dentine with previously published rib collagen data (Simpson, 2019)

Stable isotope values of enamel from Zwolle with their relatively similar values to remaining sites, seem to suggest that childhood diets of individuals from this site were comparable to the remaining locations, with similar consumption of carbon-13 enriched foods. Carbon collagen ratios in comparison were depleted, suggestive of decreased

consumption of omnivore protein, corroborated by the uniquely enriched nitrogen-15 ratios, suggestive of a dietary shift from childhood to adolescence, with an increased consumption of high trophic level and carbon-13 enriched foods, such as marine foods. This comparison suggests an increased consumption of marine foods between childhood and adolescence.

The mean deviation of stable isotopes was found to be fairly small and comparable to the closest published equivalent (Schats et al., 2022) ($\delta^{13}C_{en}$= 0.5 ‰; $\delta^{13}C_{co}$= 0.3 ‰; $\delta^{15}N_{co}$= 0.9) with carbon collagen having the same mean deviation and nitrogen collagen having an even smaller one. The relatively small deviations between the sites suggest continuity of dietary habits. In summary, the results of this study present a trend in increased consumption of marine foods by adolescent individuals, from early medieval to early modern time periods with varying rates of consumption. This trend was found to not be uniform, with individuals displaying depleted values regardless of the site and time period. The depleted nitrogen-15 ratios of Klaaskinderkerke can also be explained by the site being rural, as opposed to the remaining urban sites.

In Blokhuizen the average $\delta^{15}N$ value was found to be 11.8‰, meanwhile all sites investigated in this study found significantly higher average values. Previous studies concluded that the low ratio found in Blokhuizen was reflective of a diet comprising primarily terrestrial animal protein. On average, the values found in this study, with a total average of 13.1 and individual site related values (Table 5) all were significantly enriched relative to the aforementioned depleted values of nitrogen-15. using Blokhuizen as a baseline for a closely related location exhibiting minimal contribution of fish in the diet of past individuals, we can arrive at a conclusion individuals derived from the sites examined in this study on average consumed more fish than those of Blokhuizen. While individual outliers falling outside of the 5% range exist in the dataset and will be discussed further, it is also notable that diet comparable to that of Blokhuizen, was still found in later contexts, especially that of Klaaskinderkerke.

Urban sites have been found to have on average a significantly higher $\delta^{15}N_{co}$ ratio compared to the rural site of Klaaskinderkerke. This comparison is in keeping with previous results from Alkmaar and Blokhuizen (Schats et al., 2022), former being an urban site and latter being rural. Previous work done comparing rural and urban Netherlands in regard to isotope archaeology, has shown a trend of analyzed rural populations consuming protein from lower trophic levels, while urban populations shown a more widespread consumption

of protein from higher trophic levels. This trend could be explained by more common consumption of fish in urban areas for reasons previously described in the context of medieval England. where it was hypothesized to be due to strict adherence to Christian fasting rules, which precluded consumption of meat on Fridays and Saturdays. This would significantly limit the rate of consumption of meat in those populations and increase the rates of consumption of fish, as they were a source of animal protein otherwise allowed by the rules of the fast (Müldner & Richards, 2005, 2007).

5.2. Sex

Mentioned in the preceding chapters, the results of the study when divided into groups based on assigned sex, need to be seen through the lens of the ratio between Female to Male assigned individuals being 11:19. While significant trends can be derived from this dataset, the bias towards male individuals in the sample set is significant enough to be aware of.

Carbon enamel values for males with the exclusion of a single outlier (at -14.4‰) range between -14.0‰ and -12.1‰. Meanwhile the smaller female assigned group has shown a larger variability with no clear outliers in regard to their $\delta^{13}C_{en}$. Carbon enamel values of both groups average at -13.1, which despite broader variability in the values themselves, suggest the two groups overall had comparable diet in regard to protein sources up to the age of eight years of age (Figure 6).

When cross referenced with other datasets, the same individual can be found to have outlying values for carbon collagen as well, however while carbon enamel was depleted, carbon collagen values were enriched relative to the sampled population, as well as the results in total. This change in values between enamel and collagen, due to dental development chronology mentioned previously, signifies that the outlying individual experienced a significant change in the consumed source or abundance of enriched dietary protein, such as omnivore or marine food between the ages of approximately eight and sixteen.

Carbon enamel values for the two sex-based groups originating from the site of Alkmaar, fell withing similar range of each other following the exclusion of outliers, with male and female interquartile ranges displaying a very significant overlap between each other, meaning that both male and female individuals from Alkmaar consumed a similar diet in regard to dietary carbon sources. The trend is further seen in carbon collagen values from Alkmaar, similarly, suggesting that adolescent diets did not significantly vary between

male and female assigned individuals and in comparison, to their childhood diet. With male individuals displaying a narrow and somewhat enriched range while female assigned individuals displaying a range that is slightly broader, if depleted by a small margin. This result suggests that the analyzed individuals shared an adolescent diet to a certain degree, with the female assigned group being more reliant on terrestrial protein sources compared to the male group. Nitrogen collagen values supports that, showing a significant variation between each other where male assigned individuals exhibit a significantly more enriched range of values, compared to the female group. This comparison suggests that male individuals consumed marine foods at a significantly higher rates than individuals from the female group. This multi-isotope comparison can be concluded to mean, that while childhood diet was not significantly different, during adolescence, it began to shift and differ between the two sex-based groups resulting with the male assigned individuals displaying diets aligned with higher consumption of marine foods.

Carbon enamel values for the two sex-based groups originating from the site of Arnhem, show less overlap and more variability, with male individuals displaying a narrower and overall, more enriched range of values compared to female assigned individuals from Arnhem, whose isotope ratios in turn were significantly more depleted. This result suggests that the childhood diet of male individuals was likely richer in marine or C4 derived foods, while female childhood diet was more reliant on terrestrial foods, additionally due to a very narrow range of values among male individuals it can be concluded that their diet was also too consistent to a degree among all male assigned individuals of that site, compared to the more varied childhood female diet. Differences in diet between the two sex groups continue into adolescent diet. Based on carbon collagen values of the two groups originating from Arnhem likewise vary significantly. Male assigned individuals display enriched but very narrow range of values and female assigned individuals displaying more widely varying values ranging. With the narrow range of the male assigned group, interpretation of the clustered data is that the male individuals analyzed had a limited if consistent adolescent diet. This stands in contrast to the female assigned group, with a wider if overall more depleted range, suggesting that the adolescent diet of female individuals originating from the site was different to that of male individuals and included more terrestrial protein sources. Nitrogen collagen values alike show variation among individuals exhibiting a similarly varied values, with the male assigned group displaying significantly more enriched values, suggestive of consumption of more marine foods. In summary, isotopic composition of tissues from Arnhem suggests that the individuals analyzed differed in diet throughout

childhood up to their adolescence with assigned sex being a significant factor, with male individuals favoring marine food sources over terrestrial foods more widely consumed by the female individuals.

Carbon enamel values for the two sex-based groups originating from the site of Eindhoven, show similarities between the two groups, with male assigned individuals and female individuals displaying very similar ranges of values. This overlap between the two groups suggests that in the sampled population, there was little difference between the childhood diets of male and female assigned individuals. The male group regardless displayed a somewhat narrower range of values, compared to their female counterparts with mean differences of 0.4 for the male group and 0.3 for the female group, suggesting a minimal difference between the two. Carbon collagen values similarly show very clear similarities, with ranges of values for both groups overlapping significantly. Male assigned individuals from Eindhoven and female assigned individuals both display an extremely similar range. This similarity suggests that the adolescent diet of both groups was largely consisted of the same protein sources and was not significantly affected by sex. Nitrogen collagen values for the two sex-based groups however show significant variation, with male assigned individuals exhibiting an overall more depleted range compared to that of female assigned individuals which in turn was significantly more enriched. This suggests that during childhood individuals from Eindhoven shared a similar diet in regard to sources of dietary carbon, with similar sources of dietary protein during adolescence, however adolescent diet was shown to differ significantly in regard to consumption of higher trophic level foods, with female individuals consuming more foods from said higher trophic levels compared to males.

Carbon enamel values for the two sex-based groups originating from the site of Klaaskinderkerke vary significantly. Male assigned individuals display a narrow and depleted rang of values, while female assigned individuals display a more varied range. This suggests that the childhood diet of analyzed male individuals was more stable and consistent in regard to their protein sources, while the childhood diet of female individuals was more varied, with more depleted and more enriched values alike, for a mean difference of 0.6‰, while male groups mean difference was significantly lower, at 0.3‰. This suggests that while male individuals consumed on average more $\delta^{13}C$ enriched foods, childhood diet of female individuals either included a wider variation of foods, or alternatively, varied in the proportions of consumed $\delta^{13}C$ enriched foods. Carbon collagen values of male individuals similarly show a narrow if overall more enriched range when compared to the values

exhibited by female individuals, who in turn were found to have a slightly more varied if depleted range of values. This comparison suggests male individuals overall had a diet more consistently including marine protein, when compared to the female group which favored terrestrial protein sources. Nitrogen collagen values for the two sex-based groups originating from the site of Klaaskinderkerke likewise show significant differences between each other. Male assigned individuals exhibit a narrow and enriched range of values in comparison to the female assigned individuals. This suggests that male assigned individuals consumed food from higher trophic levels more often than female individuals. In summary, childhood diet of individuals from Klaaskinderkerke had noticeably different diets, a trend which continued into adolescence, with male individuals consuming more $\delta^{13}C$ enriched foods, including marine protein.

Carbon enamel values of the male assigned group from Zwolle display a wide range, especially compared to other locations. Due to the extremely low number of females assigned individuals from this location, comparison of the two groups is limited to mentions of complete overlap between the two groups, with carbon enamel of the female assigned individuals being found within the range of values of the male assigned group. This suggests commonality in childhood diet of male and female individuals. The carbon collagen and nitrogen collagen values of the female group were also found within the range established by the male group. In all cases, the female individuals displayed enriched values comparable to the most enriched values of their male counterparts. This comparison, in light of its limitations, suggests that the two groups were shared as similar diet.

Differences between nitrogen values between the sexes were previously noted and presented between past individuals from Alkmaar and Blokhuizen with the same trend of significantly higher values among males (Schats et al., 2022). As presented here, this trend can be found throughout the analyzed populations of Arnhem and Alkmaar, while evidence from Eindhoven shows the opposite trend, with adolescent male individuals being relatively depleted compared to their female counterparts. Klaaskinderkerke and Zwolle meanwhile were found to have similarly enriched values. The persistence of this trend suggests that adolescent individuals from Arnhem and Alkmaar consumed omnivore and marine protein at a higher rate compared to that of their female counterparts, while the remaining sites did not share this trend.

In summary, the intrasite variation within each site with the exception of Zwolle shows a trend of much greater variabilitiy on female diet, when compared to their male

counterparts. Additionally, despite the larger variability among female group, regardless of their site of origin, they contain both more enriched and more depleted values when compered to the male counterparts. A previous study looking into dental collagen of archaeological origin, found male and female falues to display little to no overlap and significant differences between the two sex-based groups (Colleter et al., 2019). The findings of this study suggest that on average, male and female indviduals largely shared a similar diet in the locations and time periods investigated.

5.3 Statistical trends

The largest previous study of correlation between carbon isotopes from tissues of the same individual found positive correlation (Loftus & Sealy, 2012), leading to a conclusion that carbon isotope values of one tissue can be used to reliably approximate the values of the other. However, in regard to correlation between stable isotope ratios of carbon in dentine and enamel these results were not replicated, with a correlation coefficient displaying no correlation. From this it can be concluded that at least in regard to carbon isotopes of dentine and enamel, neither tissue can be used to approximate the values of the other. This result could be partly explained by the breadth of time between the formation and eruption of the two tissues, as enamel of the second molar ceases formation around the age of 8, while dentine of the second molar finishes formation at the age of 16. As neither tissue remodels, the isotope ratios found in them are representative of only the time of formation, therefore the two tissues are not representative of the same diet. A follow-up study should investigate if the same is true of other teeth and investigating whether a correlation can be found between tissues of different teeth, ideally between enamel of second molar compared to dentine of a first molar or dentine of the second molar and enamel of a third molar. The suggestions are based on a better alignment of eruption and formation times for either set of teeth.

Carbon spacing was proven to reflect the ratio of consumption of meat and plants in the diet of the given individual (Clementz et al., 2009; Lee-Thorp et al., 1989). When examining the obtained carbon isotope spacing values (Appendix 2. Table 15), a number of conclusions can be reached. Majority of $\Delta^{13}C_{en\text{-}co}$ values were found between 6.0‰ and 7.5‰, suggesting diets ranging from omnivorous to predominantly herbivorous. From all the analyzed sites, Zwolle displayed the most consistently high spacing values, this suggesting that across the sites chosen for the study, childhood, and adolescent diets of individuals from Zwolle were to a significant degree plant based, compared to the other sites where a larger

inclusion of animal product and protein could be inferred from the spacing. Sites of Arnhem Eindhoven and Klaaskinderkerke, based on their comparable $\Delta_{en\text{-}co}$ values can be assumed to have included similar if varied proportions of animal and plant foods. Notably few individuals displaying $\Delta_{en\text{-}co}$ values below 6.0‰, all being relative outliers to both their site and dataset analyzed, can be assumed to have consumed larger quantities of animal meat or products. Inversely, a single individual from Alkmaar displayed the highest $\Delta_{en\text{-}co}$ value found in the dataset at 8.6‰, which is highly suggestive of a predominantly plant-based diet. Alkmaar notably displayed the narrowest range of values, with exclusion of significant outliers ranging between 6.2‰ and 7.4‰.

Previous study analyzing Japanese pre-historic individuals, investigated among other things, the statistical relationship between carbon collagen derived from ribs and third molars carbon enamel, finding strong correlation between the two values (depending on the site 0.26, 0.38, 0.39 0.39 for an average of 0.35). rib collagen data from which was used in the aforementioned study found carbon and nitrogen ratios from collagen to be significantly positively correlated with each other (Kusaka et al., 2010). Data from this study has not corroborated those findings, instead finding that dentine from the second molar carbon and nitrogen are not correlated with each other.

The relationship between the obtained $\partial^{13}C$ (‰ vs VPDB) data originating from dentine collagen and structural enamel was investigated and returned with null results in regard to a possible correlation between the values. The Pearson Correlation Coefficient returned with a value of −0.0166 (R^2=0.0003) determining a lack of correlation between the values ($P < 0.05$) (Figure 8). This result displays a lack of a correlative relationship between δ13C collagen and δ13C enamel in the analyzed group. Previous study (Loftus & Sealy, 2012) found correlation between carbon collagen and carbon enamel values to be closely correlated, with an $r^2 = 0.71$ with the removal of outliers from the calculations. Results obtained in this study however do not replicate this relationship among the analyzed second molars of Dutch medieval and early modern individuals. These results suggest an inability to approximate the diet of subadult individuals based on their dental tissues. While past literature has shown and used enamel and collagen to approximate the diet of past individuals using the two tissues, these results show that at least in the context of Dutch medieval to early modern subadults. While further comparison of enamel and collagen data from dental tissues is recommended and required prior to making further conclusions, the findings so far conclude that withing this context, due to the differences between diets during the development of both tissues and likely a plethora of other factors, enamel and collagen

carbon values should not be used interchangeably for approximation of diet of subadults when investigating the diet in the same age of the individual. Sampling of the teeth from a more correlated developmental time period would likely change this result, such as by using the enamel of third molars alongside collagen of second molars. As things are, the two tissues are incompatible to be used as proxies for each other, possibly setting a limit for their use. Further data should include the use of second and third molar data alongside investigated diet and carbon diet values, for comparison and correlation.

5.4. Outliers

Outliers for the purpose of discussion of the obtained data in totality were defined as falling outside the 95th and 5th percentile of the data distribution. For analysis of intersite and intrasite trends however, outlying values were defined as those falling outside of the interquartile range (25%-75%). This stipulation was added to discuss trends in data based on a narrower and more clustered set of values to avoid conclusions on trends being based on outlying results. First and third quartile values determining the interquartile range based on the permutations of data can be found in Tables 5, 6, 8, 10, 12, 13 and 14, while complete list of values and individual identifiers for outliers can be found in Appendix 2.

Outlying $\delta^{15}N_{co}$ values (11.1‰ - 14.8‰) include three individuals from Eindhoven, who were found to be above the values by a small margin, suggesting slightly increased consumption of marine or omnivore protein compared to the remaining sites. Klaaskinderkerke meanwhile was found to have three individuals falling below the range by anywhere between 0.1‰ and 1.0‰. This trend suggests that consumption of marine and omnivore protein in Klaaskinderkerke was lower than in other sites. Zwolle had the most individuals whose nitrogen values fell outside of the standard range, with four individuals above the range and one below, all by a relatively small margin. Given the sample size of fourteen for Zwolle five individuals being counted as outliers relative to the remaining locations, suggests a breakaway from the otherwise clear dietary continuity seen in the remaining sites.

Three individuals from Alkmaar were found to have carbon enamel values falling below its interquartile range, one by a small margin and two being significantly depleted,

Five individuals from Alkmaar were found to have carbon collagen values falling outside of its interquartile range. Three of them were enriched relative to the mean established for its site, one by a small margin and two significantly enriched. Two individuals were depleted relative to the mean value for their site, both by a significant margin.

Three individuals from Alkmaar were found to have nitrogen collagen values above its interquartile range, one being enriched by a small margin, while two were significantly enriched.

Ten individuals from Arnhem were found to have carbon enamel values falling outside of its interquartile range. Five individuals displayed values enriched relative to the mean value of the site, with three being enriched by a significant margin and two being enriched by a small margin. Five individuals meanwhile displayed value depleted relative to the mean value of the site two of which were depleted by a small margin, while three were depleted by a significant margin.

Six individuals from Arnhem were found to have carbon collagen values falling outside of its interquartile range. Three of which were enriched by a small margin, relative to the mean value for the site, while the remaining three were significantly depleted.

Five individuals from Arnhem were found to have nitrogen collagen values falling outside of its interquartile range. Two values were enriched, one by a small margin and the other my a significant one, while three values were depleted relative to the mean value for the site. Two of which were depleted by a small margin and one by a significant one.

Eight individuals from Eindhoven were found to have carbon enamel values falling outside of its interquartile range, four of which displayed values enriched relative to the remaining one and four displaying depleted values. Two of the enriched values were higher than Q3 value by a small margin, while two others, were significantly enriched. All four relatively depleted individuals fell below Q1 value by a small margin.

Seven individuals from Eindhoven were found to have carbon collagen values falling outside of its interquartile range, four of which displayed values enriched by a significant margin, relative to the mean value of the site, while three individuals displayed values depleted by a small margin relative to the mean value of the site.

Eight individuals from Eindhoven were found to have nitrogen collagen values falling outside of its interquartile range, four of which displayed values enriched by significant factor, relative to the mean value of the site, while four individuals displayed values depleted by a small margin relative to the mean value of the site.

Six individuals from Klaaskinderkerke were found to have carbon enamel values falling outside of its interquartile range, three of which displayed values enriched relative to the remaining ones, and three displaying depleted values. Two of the higher values were

significantly enriched relative to the remaining individuals, and one only marginally enriched. Two of the lower values were marginally depleted, and one significantly depleted. These outliers exhibit individuals whose diet varied significantly as displayed by the more distant outliers, suggestive of differences in source of dietary protein, with the lower values being representative of higher consumption of terrestrial protein, while the higher values being representative of higher consumption of marine protein.

Six individuals from Klaaskinderkerke were found to have carbon collagen values falling outside of its interquartile range. Three of them display values marginally below the interquartile range suggesting slightly higher consumption of C3 plants or C3 plant fed animals. One individual displayed marginally higher values representative of slightly higher consumption of C4 plants, marine foods, or other carbon-13 enriched foods, while two individuals displayed significantly higher values, suggesting significant increase in consumption of C4 plants, marine foods, or other carbon-13 enriched foods.

Eight individuals displayed carbon enamel values falling outside of the interquartile range established for the site of Zwolle. Four out of which had carbon enamel values slightly higher than the upper quartile, those individuals are assumed to have higher inclusion of marine protein in their diet. On the other end, four individuals were found to have values below the lower quartile. Three individuals were depleted by a small margin, suggesting slightly increased rates of consumption of C3 plant and C3 plant fed animals, or increased consumption of C4 or marine foods relative to the remaining individuals. One outlying individual was found to have more significantly depleted values ($\delta^{13}C_{en}$= −14.4‰), suggestive of a diet largely devoid of marine protein and more highly reliant on C3 plants or C3 plant fed animals.

Eight individuals displayed carbon collagen values falling outside of the interquartile range established for the site of Zwolle. Three out of which displayed values enriched by a small margin and a single one enriched by a significant margin, suggesting that three individuals analyzed consumed marine protein, and one who consumed significantly more marine protein. Four different individuals displayed values below the interquartile range, two of which were depleted by a small margin and two depleted by a significantly larger margin. These outliers suggest dominance of terrestrial protein sources, relative to remaining individuals from the site.

Eight more individuals displayed nitrogen collagen values falling outside of the interquartile range established for the site of Zwolle. Two individuals displayed values

marginally below the range, one significantly and one extremely below the range. Four individuals were found to have values falling marginally above the quartile value. These results exhibit marginal variability around the upper and lower quartile values in the analyzed samples. Two of the more depleted individuals have values suggestive of increased consumption of lower trophic level protein, such as terrestrial animals compared to the remaining values found in the site.

6. Conclusion

In summary, when looking at the Intrasite variation of results, it can be noted that female assigned group was depleted in the sites of Alkmaar and Arnhem conversely the two male groups were more enriched in carbon-13. The site of Eindhoven showed the two groups aligning closely, suggesting that the diet in this context was similar between male and female individuals. Isotope ratios of enamel obtained from Klaaskinderkerke shows that the analyzed female assigned individuals as a group had a more variable diet compared to the consistent diet exhibited by the male assigned group. Ratios from Zwolle while limited in comparison potential due to a limited female assigned sample size, suggest that the diets of the two groups were similar to a very limited extent.

Nitrogen values of the sex-based groups of all sites analyzed display consistent differences between the male and female groups from the same site. Alkmaar and Arnhem both display male individuals to have more enriched nitrogen 15 ratios. Eindhoven meanwhile contains female group with nitrogen 15 ratios more enriched compared to their male assigned counterparts of the same site. Klaaskinderkerke is the only site of the five analyzed to have overlapping and comparable values, suggesting that the diet of those individuals was not influenced by their assigned sex. Zwolle, while not reliable for investigation of intrasite trends, shows.

Three sites were found to have diets that did not differ between the two groups, Alkmaar, Eindhoven, and Zwolle. This suggest that in those three sites, both male and female assigned individuals had comparable diets. Meanwhile the sites of Arnhem and Klaaskinderkerke were both found to have significant differences between the two groups, with the female assigned groups of both locations being depleted relative to their respective values of the male groups.

Chronological comparison of the sites, intending to investigate differences in sub-adult diets revealed an overall trend towards increased consumption of nitrogen-15 enriched foods, suggesting an increase in consumption of marine foods across time.

The relationships between the ratios were investigated through their correlation between each other and calculating spacing between values. Correlation between carbon enamel and carbon collagen, if positive would prove to be capable of approximating the value of both tissues, however the results found that in this context, the two values were not correlated to any degree. Meanwhile, carbon enamel to carbon collagen spacing values were

used to discover high-to-moderate rates of consumption of plant based foods among individuals investigated.

The trends observed in this study regarding changes in childhood and adolescent diet across time periods and locations regardless provide important insights into lived experiences of past Dutch subadults. Similar carbon enamel values suggest that individuals of all analyzed sites with the exclusion of Alkmaar, shared a similar diet in regard to consumption of C3 and C4 plants or plant-fed animals, and marine foods, with a significant depletion of carbon enamel ratios in Alkmaar being suggestive of lower consumption of those carbon isotope sources. Carbon collagen values meanwhile displayed similar values, with minor intersite variations and a significantly wide spread of values in Klaaskinderkerke, suggesting that while individuals from all sites consumed similar sources of protein in their adolescence, Klaaskinderkerke, the earliest and rural site, had the most varied diets relative to others. Nitrogen-15 ratios showed more significant variation. Eindhoven and Zwolle were found to have the most nitrogen-15 enriched diets, suggestive of consumption of higher trophic level food sources, likely meaning that individuals from those sites consumed higher ratios of marine and omnivore foods. Remaining sites of Alkmaar and Arnhem displayed fewer variable values, suggesting more consistent diets, with smaller inclusion of marine and omnivorous foods, and finally Klaaskinderkerke was found to likely consume the least amount of omnivore and marine foods out of the analyzed sites.

While varied between sites, childhood diet, and adolescent diet, when compared to the relatively limited data from adult Dutch individuals, has shown that individuals as old as eight many components with the diet of adolescent and adult individuals, potentially presenting evidence that childhood diet was not significantly different from that of adults.

Male and female individuals were also examined for between the sexes, the results of that comparison have shown a significant overlap between the values of male and female assigned individuals, suggesting that in the sites and contexts investigated, the childhood and adolescent diets do not differ between individuals of different sexes.

The limitations of the study largely lie in limited scope of comparisons to past local diets. Having produced significant amount of data, the study regardless created a meaningful base for future research of childhood and adolescent diet of past Dutch individuals. Inclusion of more local faunal stable isotope ratios would allow for more detailed results, further grounded in current research.

In follow-up studies, due to differences in age at which the dental tissues of second molars develop, the correlation should be further investigated between collagen ratios of the second molars and enamel of the third molars, however this comparison might be limited due to the inherently lower availability of third molars in archaeological collections and assemblages. Alternatively for a larger possible available selection of samples, the same follow-up should investigate the correlation between $\partial^{13}C$ values of enamel of second molars and dental root collagen of first molars. This is due to the ages at which the formation of enamel crown and roots formation finishes, namely enamel formation of second molar finishing between the ages of seven and eight, and third molar between the ages of twelve and sixteen, while roots of the first molar finish between the ages of nine and ten, and ages of fourteen and sixteen for the second molar (Schuurs, 2012).

7. Abstract

In this study, five archaeological sites across the Netherlands from early medieval to early modern time periods were sampled to reconstruct past diet of the individuals found therein. The sites chosen for the study were Alkmaar, Arnhem, Eindhoven, Klaaskinderkerke and Zwolle. The focus of the diet reconstruction was to analyze the childhood and adolescent diet using stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen isotopes ($\delta^{15}N$). Through the use of tissues which are underutilized in archaeological diet reconstruction, dental enamel (en) and dentine (co) in conjunction, derived from second molars, it was possible to obtain isotopic ratios representative of childhood diet (enamel) and adolescent diet (dentine collagen). The results obtained were then used to analyze trends between the sites, between assigned sex and statistical relationships which served to provide grounds for further improvement of the methodology.

Sampling and analysis of enamel was 100% successful, while sampling and analysis of collagen was 89% successful. The respective 76 and 68 samples were analyzed using a mass spectrometer and returned with reliable values. Results of intersite comparison aligned the, obtained results with past published literature regarding historical trends found in across the analyzed time periods, including increased consumption of marine fish in younger and more urban sites. Comparison of male and female assigned individuals has shown significant overlap between their values, suggesting that male and female individuals from the sites analyzed shared very similar diets during their childhood and adolescence. The investigation of isotope spacing of carbon values ($\Delta_{en\text{-}co}$) allowed for determining that the diets of individuals analyzed was in large portion plant based, with significant inclusion of omnivore and marine foods as determined by isotopic ratios of carbon-13 and nitrogen-15.

Analysis of statistical relationships between $\delta^{13}C_{en,}$ $\delta^{13}C_{co}$ and $\Delta_{en\text{-}co}$ has found a lack of correlation between $\delta^{13}C_{en}$ $_{\delta}{}^{13}C_{co}$, a positive correlation between $\Delta_{en\text{-}co}$ and $\delta^{13}C_{en}$ and a negative correlation between $\Delta_{en\text{-}co}$ and $\delta^{13}C_{co}$.

Follow-up studies should continue to investigate the recorded isotopic ratios in dental tissues, utilizing first and third molars as sources of further data capable of reconstructing sub-adult diet, as well as analyze local fauna to improve the accuracy of dietary reconstruction. The use of first and third molar isotopic ratios would also prove invaluable for investigating the statistical relationship between the tissues, due to the age of dental development and its relationship to diet reconstruction.

Appendix

Appendix 1. Full results

Enamel			$d^{13}C$ (‰ vs VPDB)		$d^{18}O$ (‰ s VPDB)			Collagen					Nitrogen			Carbon						
Date	Analysis	Identifier 1	Average	SD	Average2	SD 3	Amp l44	Date	Analysis	Amount	Line	Identifier 1	Area 28	$\delta^{15}N$ (‰ vs N_{air})	N (mg)	Area 44	$\delta^{13}C$ (‰ vs VPDB)	Carbon (mg)	C/N (g/g)	C/N (mol/mol)	Estimated Age Range	Estimated Sex
12/07/21	47141	IOPAA 304 1812	-13.22	0.06	-4.03	0.16	900	01/26/22	66222	0.454	32	IOPAA-1012	64.87	12.08	0.07	38.47	-19.67	0.19	2.82	3.29	36-45	Female Probable
12/01/21	46811	IOPAA 309-842	-13.06	0.08	-5.85	0.08	2119	01/26/22	66233	0.497	43	IOPAA-842	74.57	12.29	0.08	43.36	-20.23	0.21	2.78	3.24	18-25	Female
12/07/21	47145	IOPAA 311 1041	-12.94	0.07	-5.39	0.09	1950	01/26/22	66228	0.546	38	IOPAA-1041	81.96	11.61	0.08	48.03	-19.44	0.23	2.81	3.27	36-45	Female
12/07/21	47151	IOPAA 327 872	-14.17	0.07	-4.45	0.09	1213	01/26/22	66223	0.532	33	IOPAA-872	79.73	11.52	0.08	47.10	-20.49	0.23	2.83	3.30	36-45	Female Probable
12/01/21	46828	IOPAA 364 1017	-13.30	0.10	-4.59	0.08	1581														18-25	Female
12/07/21	47143	IOPAA 403 1081	-13.87	0.08	-5.35	0.07	1351	01/26/22	66224	0.466	34	IOPAA-1081	71.47	13.19	0.07	41.62	-20.01	0.20	2.78	3.24	15-18	Female
12/07/21	47148	IOPAA 403 1269	-12.70	0.05	-4.33	0.12	1455	01/26/22	66234	0.518	44	IOPAA-1269	75.59	12.04	0.08	44.21	-20.03	0.21	2.80	3.26	35-46	Male
12/07/21	47152	IOPAA 404 1032	-14.88	0.06	-5.12	0.08	1980	01/26/22	66229	0.484	39	IOPAA-1032	67.85	13.36	0.07	39.70	-20.20	0.19	2.79	3.26	18-25	Male
12/07/21	47142	IOPAA 404 1070	-13.81	0.10	-4.32	0.16	973	01/26/22	66232	0.51	42	IOPAA-1070	75.00	12.78	0.08	44.04	-19.84	0.21	2.81	3.27	26-35	Male
12/07/21	47150	IOPAA 404 962	-13.68	0.04	-4.38	0.07	1313	01/26/22	66231	0.478	41	IOPAA-962	67.23	14.08	0.07	39.07	-19.84	0.19	2.77	3.23	15-18	Male
12/07/21	47144	IOPAA 404 966	-13.26	0.07	-8.12	0.27	2017	01/26/22	66221	0.525	31	IOPAA-966	76.65	12.50	0.08	44.58	-19.97	0.22	2.78	3.25	15-18	Male
12/01/21	46826	IOPAA 507 1243	-14.05	0.05	-4.68	0.10	2517														36-45	Female Probable
12/01/21	46825	IOPAA 531 1111	-13.07	0.09	-4.93	0.08	1685	01/26/22	66220	0.48	30	IOPAA-1111	69.30	13.18	0.07	40.44	-20.47	0.20	2.78	3.25	18-25	Female
12/07/21	47149	IOPAA 584 1276	-13.10	0.08	-4.00	0.09	1400	01/26/22	66225	0.505	35	IOPAA-1276	75.11	13.64	0.08	43.89	-19.68	0.21	2.79	3.26	26-35	Male
12/01/21	46827	IOPAA 586 1267	-14.07	0.06	-3.93	0.08	1907	01/26/22	66219	0.481	29	IOPAA-1267	70.27	13.81	0.07	41.32	-19.41	0.20	2.81	3.27	46+	Male
03/15/22	51961	EKV-S3864	-13.43	0.09	-5.43	0.12	1181	09/12/22	71209	0.535	22	EKV-S3864	68.95	14.63	0.08	31.53	-20.16	0.22	2.86	3.34	n/a	Female
03/15/22	51942	EKV-S1108	-13.19	0.12	-4.67	0.09	1295	10/04/22	71577	0.497	26	EKV-S1108	71.39	14.77	0.07	108.51	-20.00	0.21	2.84	3.32	20-29	Female
03/15/22	51943	EKV-S1430	-12.95	0.09	-6.40	0.19	921	09/12/22	71210	0.466	23	EKV-S1430	60.26	12.58	0.07	27.78	-20.04	0.20	2.90	3.38	50-59	Male
03/15/22	51944	EKV-S1911	-13.33	0.28	-5.43	0.29	2631	10/04/22	71578	0.458	27	EKV-S1911	65.78	14.77	0.07	100.58	-19.33	0.19	2.86	3.34	50-59	Female
03/15/22	51945	EKV-S2038	-13.32	0.12	-5.84	0.17	1363	09/12/22	71206	0.532	19	EKV-S2038	69.15	13.91	0.08	31.51	-19.68	0.22	2.85	3.33	n/a	Male
03/15/22	51946	EKV-S2150	-13.25	0.14	-6.61	0.13	699	09/12/22	71220	0.455	33	EKV-S2150	58.29	14.30	0.07	26.77	-19.46	0.19	2.89	3.37	n/a	Male

03/15/22	51947	EKV-S2515	-13.61	0.12	-5.17	0.08	1654	08/23/22	70297	0.522	23	EKV-S2515	81.79	12.45	0.08	39.78	-20.08	0.22	2.70	3.15	n/a	Male
03/15/22	51950	EKV-S2538	-12.07	0.11	-5.67	0.17	704	08/23/22	70293	0.478	19	EKV-S2538	74.62	12.80	0.07	36.45	-19.98	0.20	2.73	3.18	40-49	Male
03/15/22	51951	EKV-S2593	-13.09	0.05	-4.53	0.08	1681	09/12/22	71204	0.502	17	EKV-S2593	64.46	11.61	0.07	29.86	-19.89	0.21	2.90	3.39	n/a	Male
03/15/22	51952	EKV-S2817	-12.13	0.06	-8.13	0.21	2040	09/12/22	71233	0.488	46	EKV-S2817	60.85	14.85	0.07	28.67	-19.76	0.20	2.95	3.45	50/59	Male
03/15/22	51953	EKV-S2828	-12.52	0.11	-4.64	0.10	1813	09/12/22	71200	0.55	13	EKV_S2828	71.48	13.52	0.08	33.00	-20.16	0.23	2.89	3.37	40-49	Female
03/15/22	51954	EKV-S2885	-12.19	0.11	-5.55	0.04	1141	09/12/22	71205	0.516	18	EKV-S2885	65.07	12.87	0.07	30.29	-20.09	0.22	2.92	3.40	40-49	Male
03/15/22	51955	EKV-S3314	-12.92	0.10	-4.95	0.09	1235	09/12/22	71202	0.453	15	EKV-S3314	58.08	13.44	0.07	26.85	-19.90	0.19	2.91	3.39	40-49	Male
03/15/22	51958	EKV-S3424	-13.33	0.08	-5.63	0.06	1546	09/12/22	71234	0.494	47	EKV-S3424	62.30	12.98	0.07	29.18	-20.64	0.21	2.94	3.42	40-49	Female
03/15/22	51959	EKV-S3463	-12.98	0.06	-5.50	0.12	1681	09/12/22	71217	0.534	30	EKV-S3463	69.35	13.95	0.08	31.65	-20.03	0.22	2.86	3.34	40-49	Male
03/15/22	51960	EKV-S3536	-12.64	0.09	-4.84	0.13	1389	09/12/22	71201	0.544	14	EKV-S3536	69.90	13.85	0.08	32.16	-20.06	0.23	2.88	3.36	60-69	Male
11/30/21	46796	KKK59-80966	-12.75	0.07	-5.57	0.18	1186	01/26/22	66213	0.498	23	KKK-966	70.99	11.24	0.07	42.34	-20.46	0.20	2.85	3.32	36-45	Female
11/30/21	46797	KKK49-52951	-12.52	0.09	-5.81	0.10	1333	01/26/22	66205	0.509	15	KKK-951	70.50	12.80	0.07	43.44	-20.53	0.21	2.94	3.42	17-30	Female
11/30/21	46800	KKK59-12930	-12.41	0.09	-4.94	0.11	1234	01/26/22	66208	0.537	18	KKK-930	79.22	13.43	0.08	46.92	-20.27	0.23	2.83	3.31	36-45	Male
12/01/21	46818	KKK59-27940	-12.50	0.06	-5.89	0.13	1067	01/26/22	66209	0.486	19	KKK-940	67.84	13.36	0.07	40.41	-19.69	0.20	2.84	3.31	16-21	N/A
11/30/21	46801	KKK59-46945	-12.75	0.05	-5.72	0.10	2455	01/26/22	66207	0.512	17	KKK-945	76.55	13.16	0.08	45.37	-19.94	0.22	2.83	3.31	26-35	Male
12/01/21	46802	KKK59-47946	-14.41	0.08	-4.71	0.08	2474	01/26/22	66211	0.503	21	KKK-946	73.10	13.98	0.07	42.97	-19.23	0.21	2.81	3.28	26-35	Male
12/01/21	46824	KKK59-50949	-13.31	0.04	-5.88	0.10	2441	01/26/22	66204	0.504	14	KKK-949	74.27	11.63	0.08	44.26	-20.13	0.21	2.85	3.32	26-35	Male
12/01/21	46812	KKK59-51950	-12.79	0.08	-5.78	0.04	2390	01/26/22	66217	0.524	27	KKK-950	79.38	10.62	0.08	46.97	-19.88	0.23	2.83	3.30	26-35	Female
12/01/21	46817	KKK59-55954	-13.80	0.07	-4.48	0.10	2421	01/26/22	66212	0.484	22	KKK-954	68.52	12.58	0.07	41.15	-19.74	0.20	2.86	3.34	36-45	Male
12/01/21	46813	KKK59-59957	-11.84	0.06	-6.35	0.07	1307	01/26/22	66206	0.504	16	KKK-957	71.49	11.04	0.07	42.12	-19.88	0.20	2.81	3.28	26-35	Female Probable
12/01/21	46819	KKK59-68959	-12.36	0.08	-5.58	0.12	1048	01/26/22	66216	0.49	26	KKK-959	68.79	13.32	0.07	40.32	-20.50	0.20	2.80	3.26	17-30	Female
12/01/21	46820	KKK59-69960	-12.81	0.06	-5.55	0.12	3421	10/04/22	71574	0.52	23	KKK-960	74.03	10.11	0.08	116.87	-19.04	0.22	2.96	3.45	26-35	Male
12/01/21	46821	KKK59-8926	-13.01	0.06	-5.28	0.13	2088	01/26/22	66218	0.538	28	KKK-926	78.80	12.88	0.08	46.80	-19.61	0.23	2.84	3.31	26-35	Male

08/23/22	59923	S759-V1500	-13.75	0.08	-6.38	0.07	2893	09/12/22	71216	0.545	29	S754-V1500	71.07	14.59	0.08	32.45	-19.95	0.23	2.86	3.34	26-35	Male Probable
08/23/22	59917	S1045-V2101	-13.22	0.06	-5.69	0.09	2427	09/12/22	71222	0.496	35	S1045-V2101	63.46	12.44	0.07	28.93	-20.15	0.21	2.86	3.34	26-35	Female Probable
08/23/22	59916	S1055-V2114	-13.35	0.07	-4.41	0.07	2579														18-25	Female Probable
08/23/22	59933	S1272-V2489	-13.83	0.10	-6.04	0.12	2228	09/12/22	71213	0.485	26	S1272-V2489	62.28	13.48	0.07	28.67	-20.63	0.20	2.89	3.37	17-30	Female Probable
08/23/22	59929	S253-V1743	-12.92	0.06	-4.95	0.12	2367														18+	Female
08/23/22	59934	S371-V481	-13.50	0.05	-5.18	0.10	2396	09/12/22	71226	0.459	39	S371-V481	59.83	12.61	0.07	27.56	-20.16	0.20	2.89	3.38	36-49	Male
08/23/22	59937	S418-V560	-12.23	0.04	-4.68	0.13	2260	09/12/22	71225	0.459	38	S418-V560	60.57	11.89	0.07	27.95	-20.41	0.20	2.90	3.38		
08/23/22	59938	S438-V687	-12.34	0.07	-5.78	0.08	2573														26-35	Female Probable
08/23/22	59915	S570-V973	-13.56	0.06	-7.02	0.10	2392	09/12/22	71228	0.515	41	S570-V973	66.23	13.32	0.07	30.37	-20.90	0.22	2.87	3.35	36-49	Male Probable
08/23/22	59930	S604-V1087	-12.91	0.05	-5.09	0.09	2009	09/12/22	71203	0.467	16	S604-V1087	60.87	13.37	0.07	28.58	-19.67	0.20	2.94	3.43	36-49	Female Probable
08/23/22	59931	S643-V1253	-12.29	0.06	-4.92	0.19	1709	09/12/22	71207	0.495	20	S643-V1253	63.31	12.34	0.07	29.02	-19.72	0.21	2.88	3.36	26-35	Male
08/23/22	59932	S669-V1318	-13.45	0.09	-6.00	0.14	2134	10/04/22	71580	0.514	29	S669-V1318	73.98	12.93	0.07	110.50	-20.03	0.21	2.79	3.26	36-49	Male
08/23/22	59924	S711-V1585	-12.70	0.11	-5.12	0.10	2838	08/23/22	70288	0.531	14	S711-V1585	81.63	13.57	0.08	40.74	-19.56	0.23	2.77	3.23	26-35	Female Probable
08/23/22	59925	S790-V1621	-12.36	0.09	-6.40	0.14	2149														26-35	Male Probable
08/23/22	59926	S791-V1623	-12.66	0.06	-4.88	0.10	2370														16-21	Male Probable
08/23/22	59922	S918-V1881	-13.68	0.08	-6.44	0.11	1941	09/12/22	71231	0.545	44	S918-V1881	70.77	13.41	0.08	32.53	-19.96	0.23	2.88	3.36	26-35	Male Probable
08/23/22	59921	S921-V1889	-13.05	0.07	-6.12	0.10	3174														36-49	Male Probable

08/23/22	59918	S929-V1901	-13.58	0.05	-6.28	0.07	2524	08/23/22	70287	0.494	13	S929-V1901	76.11	14.02	0.08	37.85	-20.14	0.21	2.77	3.24	26-35	Male
06/17/22	57346	ZW87-002	-13.17	0.11	-5.16	0.08	1025	09/12/22	71229	0.49	42	ZW87-002	63.10	15.15	0.07	29.02	-20.16	0.21	2.89	3.37	40-50	Male
06/17/22	57347	ZW87-020	-13.48	0.06	-4.17	0.08	2546	09/12/22	71219	0.462	32	ZW87-020	55.42	10.89	0.06	25.15	-20.26	0.18	2.86	3.34	36-49	Male
06/17/22	57348	ZW87-026	-12.85	0.06	-6.87	0.17	1229	09/12/22	71218	0.486	31	ZW87-026	63.27	14.77	0.07	29.21	-20.40	0.21	2.90	3.38	26-35	Male
06/17/22	57349	ZW87-034	-12.31	0.07	-8.93	0.19	1044	08/23/22	70300	0.485	26	ZW87-034	78.86	12.71	0.08	37.88	-20.82	0.21	2.67	3.12	18-25	Male Probable
06/17/22	57350	ZW87-035	-13.64	0.06	-7.30	0.06	2264	10/04/22	71573	0.501	22	ZW87-035	72.46	14.27	0.07	111.30	-19.96	0.21	2.87	3.35	26-35	Male Probable
06/17/22	57353	ZW87-042	-13.04	0.08	-6.07	0.08	1055	09/12/22	71214	0.459	27	ZW87-042	59.15	14.43	0.07	27.14	-20.19	0.19	2.88	3.37	36-49	Female
06/17/22	57354	ZW87-049	-13.14	0.11	-5.99	0.13	955	09/12/22	71215	0.549	28	ZW87-049	70.42	14.83	0.08	32.37	-20.73	0.23	2.88	3.36	36-49	Male Probable
06/17/22	57355	ZW87-050	-12.57	0.12	-7.41	0.09	1423	09/12/22	71230	0.484	43	ZW87-050	61.61	11.74	0.07	28.71	-19.98	0.20	2.92	3.41	36-49	Male
06/17/22	57356	ZW87-054	-12.93	0.06	-5.74	0.17	912	08/23/22	70292	0.487	18	ZW87-054	75.28	13.24	0.07	37.24	-20.17	0.21	2.76	3.22	36-49	Male
06/17/22	57357	ZW87-055	-13.49	0.21	-5.63	0.20	693	10/04/22	71581	0.465	30	ZW87-055	67.55	14.94	0.07	102.34	-20.11	0.19	2.83	3.31	26-35	Female
06/17/22	57358	ZW87-063	-13.15	0.10	-6.79	0.11	977	09/12/22	71227	0.508	40	ZW87-063	67.65	12.88	0.08	30.98	-19.06	0.22	2.87	3.35	26-35	Male Probable
06/17/22	57361	ZW87-070	-12.54	0.09	-7.02	0.17	979	09/12/22	71232	0.537	45	ZW87-070	68.79	13.57	0.08	31.85	-20.16	0.23	2.90	3.38	50+	Male
06/17/22	57362	ZW87-077	-13.18	0.10	-7.04	0.13	704	10/04/22	71579	0.458	28	ZW87-077	65.34	15.03	0.07	100.25	-20.20	0.19	2.87	3.35	36-49	Male
06/17/22	57363	ZW87-094	-14.42	0.04	-6.17	0.10	1339	08/23/22	70289	0.526	15	ZW87-094	81.16	13.47	0.08	40.26	-20.44	0.22	2.75	3.21	36-49	Male

Table 12 Entirety of stable isotope analysis results for both structural enamel and collagen

Appendix 2. Spacing related results and values

ID	$\Delta_{en\text{-}co}$
IOPAA 304 1812	6.5
IOPAA 309-842	7.2
IOPAA 311 1041	6.5
IOPAA 327 872	6.3
IOPAA 364 1017	
IOPAA 403 1081	6.1
IOPAA 403 1269	7.3
IOPAA 404 1032	5.3
IOPAA 404 1070	6.0
IOPAA 404 962	6.2
IOPAA 404 966	6.7
IOPAA 507 1243	
IOPAA 531 1111	7.4
IOPAA 584 1276	6.6
IOPAA 586 1267	5.3
EKV-S3864	6.7
EKV-S1108	6.8
EKV-S1430	7.1
EKV-S1911	6.0
EKV-S2038	6.4
EKV-S2150	6.2
EKV-S2515	6.5
EKV-S2538	7.9
EKV-S2593	6.8
EKV-S2817	7.6
EKV-S2828	7.6
EKV-S2885	7.9
EKV-S3314	7.0
EKV-S3424	7.3
EKV-S3463	7.0
EKV-S3536	7.4
KKK59-80 966	7.7
KKK49-52 951	8.0

KKK59-12 930	7.9
KKK59-27 940	7.2
KKK59-46 945	7.2
KKK59-47 946	4.8
KKK59-50 949	6.8
KKK59-51 950	7.1
KKK59-55 954	5.9
KKK59-59 957	8.0
KKK59-68 959	8.1
KKK59-69 960	6.2
KKK59-8 926	6.6
S759-V1500	6.2
S1045-V2101	6.9
S1055-V2114	
S1272-V2489	6.8
S253-V1743	
S371-V481	6.7
S418-V560	8.2
S438-V687	
S570-V973	7.3
S604-V1087	6.8
S643-V1253	7.4
S669-V1318	6.6
S711-V1585	6.9
S790-V1621	
S791-V1623	
S918-V1881	6.3
S921-V1889	
S929-V1901	6.6
ZW87-002	7.0
ZW87-020	6.8
ZW87-026	7.6
ZW87-034	8.5
ZW87-035	6.3
ZW87-042	7.1
ZW87-049	7.6
ZW87-050	7.4

ZW87-054	7.2
ZW87-055	6.6
ZW87-063	5.9
ZW87-070	7.6
ZW87-077	7.0
ZW87-094	6.0

Table 13 Δen-co values calculated per analyzed sample

Δ_{en-co}: N_{co} correlation	Pearson's correlation coefficient
Total	-0.1
Alkmaar	-0.3
Arnhem	-0.7
Eindhoven	-0.3
Klaaskinderkerke	0.3
Zwolle	0.0
Δ_{en-co}: C_{co} correlation	Pearson's correlation coefficient
Total	-0.6
Alkmaar	-0.6
Arnhem	-0.3
Eindhoven	-0.6
Klaaskinderkerke	-0.8
Zwolle	-0.8
Δ_{en-co}: C_{ce} correlation	Pearson's correlation coefficient
Total	0.8
Alkmaar	0.8
Arnhem	0.7
Eindhoven	0.9
Klaaskinderkerke	0.8
Zwolle	0.8

Table 14 Results of Pearson's correlation coefficient between Δen-co and analyzed stable isotope ratios obtained ($^{13}C_{en}$, $^{13}C_{co}$ and $^{15}N_{co}$) based on all values and per site

Appendix 3. Outliers

Site	ID	$\delta^{13}C_{en}$ (‰ vs VPDB)	$\delta^{13}C_{co}$ (‰ vs VPDB)	$\delta^{15}N_{co}$ (‰ vs Nair)
Alkmaar	s309 v842	–13.7	–20.2	
	s304 v1012		–19.7	
	s311 v1041	–13.8	–19.4	
	s327 v872		–20.5	
	s403 v1269	–13.3		
	s404 v1032	–14.0		
	s404 v962			14.1
	s531 v1111		–20.5	
	s584 v1276			13.6
	s686 v1267		19.4	13.8
Arnhem	S1045 v2101	–12.2		12.4
	S1055 V2114	–14.1		
	S1272 V2489	–14.9	–20.6	
	S253 V1743	–13.8		
	S530 V973		–20.9	
	S604 V1087	–13.8	–19.7	
	S643 V1253		–19.7	12.3
	S711 V1585		–19.6	13.6
	S759 V1500	–12.5		14.6
	S790 V1621	–12.6		
	S918 V1881	–12.8		
	S929 V1901	–12.5		
	S418 V560	–14.4	–20.4	11.9
Eindhoven	1108	–13.4		14.8
	1430			12.6
	1911		–19.3	14.8
	2038	–13.5	–19.7	
	2150		–19.5	
	2515	–12.5		12.5
	2538	–12.4		12.8

	2593			11.6
	2817	−12.3	−19.8	14.9
	2828		−20.2	
	2885	−13.7	−20.1	
	3428	−13.6		
	3536	−12.6		
	3864		−20.2	14.6
Klaaskinderkerke	52 951	−13.9	−20.5	
	12 930			13.4
	27 940	−14.2		13.4
	47 946		−19.2	14.0
	51 950			10.6
	55 954	−13.7		
	59 957	−12.1		11.0
	68 959	−12.7	−20.5	
	69 960		−19.0	10.1
	8 926	−12.1	−19.6	
	80 966		−20.5	
Zwolle	002	−13.5		15.1
	020	−12.2		10.9
	026	−12.4	−20.4	
	034		−20.8	12.7
	035		−20.0	
	042	−12.3		
	049	−13.6	−20.7	14.8
	050		−20.0	11.7
	054	−12.3		
	055		−20.1	14.9
	063	−14.4	−19.1	12.9
	070	−13.6		
	077			15.0
	094		−20.4	

Figure 18 Individuals and values falling outside their respective intersite interquartile ranges

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