博士論文

Interpretation of Both Food Acquisition and Social Structures
in the Southwest Asian Communities Effected from Neolithic Agriculture
Achieved by the Combination of Isotopic Analyses.

(同位体分析による食性復元から見た、
食料生産の開始と西アジア新石器社会の食物利用と社会構造の変化)

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Interpretation of Both Food Acquisition and Social Structures in the Southwest Asian Communities

Effected from Neolithic Agriculture Achieved by the Combination of Isotopic Analyses.

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Abbreviations

AMS : accelerator mass spectrometer

cal BC : calibrated Before Christ based on the radiocarbon dating

EA–IRMS : elemental analyser–isotope ratio mass spectrometer

Fa : fraction animal protein in total terrestrial protein

Fa_{AA} : fraction animal protein in total terrestrial protein based on the TP_{ter}

Fa_{col} : fraction animal protein in total terrestrial protein based on the $\delta^{15}N_{col}$

GC–C–IRMS : gas chromatography–combustion–isotope ratio mass spectrometer

Qa : quantitative estimate of aquatic resource consumption

PDB : Pee Dee Belemnite

PPN : Pre-Pottery Neolithic

PPNA : Pre-Pottery Neolithic A

PPNB : Pre-Pottery Neolithic B

PN : Pottery Neolithic

TP : trophic position

TP_{ter} : trophic position in terrestrial ecosystems

TP_{aqua} : trophic position in aquatic ecosystems

$\delta^{13}C$: the isotopic ratio of carbon ($^{13}C/^{12}C$) in the delta value unit comparing with the PDB standard

$\delta^{13}C_{col}$: $\delta^{13}C$ value of collagen

$\delta^{15}N$: the isotopic ratio of nitrogen ($^{15}N/^{14}N$) in the delta value unit comparing with AIR

$\delta^{15}N_{col}$: $\delta^{15}N$ value of collagen

$\delta^{15}N_{Glu}$: $\delta^{15}N$ value of glutamic acid

$\delta^{15}N_{Phe}$: $\delta^{15}N$ value of phenylalanine

Summary

   The aim of this study is to reconstruct the temporal changes in food acquisition and social complexity of early to late Neolithic societies in southwest Asia, by the relationship between the individual diets and the burial profiles such as sex, age and buried location, within each archaeological site. The temporal changes of neither the quantity of each food resource nor the supply to individuals in a community during Neolithic period have been revealed sufficiently yet, despite the importance in human history. Therefore, I mainly apply isotopic analysis of bone collagen and individual amino acids extracted from skeletal remains for these questions. Even though, the contributions of cereals, terrestrial animal proteins and freshwater resources to Neolithic southwest Asian human diets have been indeterminate, only by the isotopic compositions of collagen, as a commonly applied method. By contrast, the combination of two isotopic analyses by collagen and individual amino acids is able to precise estimate to the fraction of animal protein in total protein intake, free of influence from the difference of animal species. Additionally, we indicate that nitrogen isotopic compositions of amino acids are powerful tool to identify smaller contribution of freshwater resources in human diets of a community depending primarily on the terrestrial resources. Because the isotopic analyses of both collagen and individual amino acids enhance the resolution of diets ever in inland environment such as interior regions in southwest Asia, and especially make it possible to accurately estimate the consumption rate of animal protein, these analyses reveal the food sharing within the prehistoric community and the social structures based on food acquisition. In the results, the difference in the consumption rates of animal protein between male and female had already been small at early Neolithic hunter-gatherer than previously thought. On the other hand, accomplished farmer societies in the late Neolithic period seem to have had similar consumption rates of animal protein despite the variations in composition of animal species among sites.

1. General Introduction and Background

1.1. Archaeological background

The Neolithic period of the southwest Asia is the topic of the attention in human history as one of the first origin of food production and complex society, and these innovations are collectively called "Neolithisation". The region called "southwest Asia" or "Near East" corresponds to region including Turkey, Syria, Lebanon, Israel, Jordan and Iraq in historical and archaeological context (Fig. 1.1). Furthermore, southwest Asia is roughly separated to Anatolia as nearly Asian part of Turkey, Levant as Syria, Lebanon, Israel and Jordan, and Mesopotamia as a part of Iraq. Cereal cultivation as the first steps of food production essentially has been thought to have arisen mainly in Levant (Bar-Yosef and Belfer-Cohen, 1989; Bar-Yosef and Meadow, 1995), although recently it is thought the process of the origin of cereal cultivation was more complex (Zender, 2011). Thus, the archaeological studies about early phase of Neolithisation and also the previous phase as sedentary lifestyle had an interest in mainly Levant and the surrounding regions in southwest Asia (Hillman et al., 1989; Bar-Yosef and Belfer-Cohen, 1989; Bar-Yosef and Meadow, 1995; Bar-Yosef, 1998; Moore et al., 2000). Furthermore, the oldest domestication of animals is thought to have started in the northern part of Fertile Crescent such as southeast Turkey and north Syria (Helmer et al., 2005).

1.2. Chronology of Neolithic period in southwest Asia

Primarily, during Pleistocene modern humans (*Homo sapiens*) had been living on a nomadic life in cold and dry climate, also in southwest Asia (Bar-Yosef and Belfer-Cohen, 1989). This modern human's culture is generally called Upper Paleolithic based on using chipped stone tools. In the last point of the Upper Paleolithic period, the warming and wetting at terminal of the Pleistocene produced bountiful forests and abundant variety of foods for human in the Levant (Goring-Morris and Belfer-Cohen, 2008). Then, at 12,500 cal BC (calibrated Before Christ, i.e. calibrated radiocarbon age), Epipaleolithic humans broke away from the nomadic life and started sedentary hunting–gathering life called the Natufian culture depending on the ample wild foods (Bar-Yosef, 1998; Moore et al., 2000; Bar-Yosef, 2002; Goring-Morris and Belfer-Cohen, 2008).

Afterward, the following culture is called Neolithic based on using additionally polished stone tools. In

Neolithic period (9700–5000 cal BC), southwest Asian humans started a gradual progress of a food production system (Fig. 1.2). Namely, Neolithic period divide into general three periods; Pre-Pottery Neolithic A (PPNA) 9700–8500 cal BC, Pre-Pottery Neolithic B (PPNB) 8500–7250 cal BC and Pottery Neolithic (PN) 7250–5000 cal BC by archaeological artifacts and cultures (Fig. 1.2). The distinctive feature of PN separating from PPN is the advent of the pottery-ware.

Early agriculture in the southwest Asia is characterized by a combination of animal herding and cultivation of cereals and legumes (Zohary, 1996). Conservatively, it has been recognized that the domestication of plants and animals occurred in a gradual sequence of phases during the Neolithic period (Bar-Yosef, 2002). However, recent studies have revealed that the transition from hunting-gathering to food producing was complex and multi-faceted, thus challenging the interpretation of a simple and gradual development from hunting–gathering to food production (Conolly et al., 2011; Larson et al., 2014).

Actually, recent research has revealed that the domestication of plants occurred differently in different areas. For example, evidence from early farming settlements shows that between 11,500 and 9000 cal BC during PPNA, einkorn, emmer, barley, chickpea, lentil and broad bean were domesticated in various parts of the southwest Asia (Fig. 1.2), meanwhile especially wild wheats were domesticated in southeastern Turkey and northern Syria (Lev-Yadun et al, 2000; Willcox et al., 2008; Willcox, 2013). At Tell Abu Hureyra, located in northern Syria, a large variety of wild plants (more than 150 species) have been found in late Epipaleolithic layers, whereas domestic cereals and legumes have been recovered from late PPN layers (Hillman et al., 1989). However, because plant domestication generically starts in a habitat of the wild species, it is not so easy to demonstrate influence of domesticated plants in human subsistence. Also, early plant cultivation seems to have been coexistent to the gathering of wild plants. Even so, it has also been reported that the percentage of domestic plants in total plant remains slowly increased for two millenniums during the Neolithic period at six sites located in northern Syria and southeastern Turkey (Tanno and Willcox, 2006a).

On the other hand, the domestication of goats and sheep in the southwest Asia probably began in the northern part of the Zagros Mountains at southeastern Turkey by 9000 cal BC during PPNB (Zeder, 2008; Conolly et al., 2011). Conventionally, it has been thought that the domestication of cattle and pigs were preceded by goats and

sheep. However, it was recently reported that the domestication of pigs in southeastern Turkey started by 8500–8000 cal BC (Zeder, 2008). Furthermore, some research suggests that in the upper Euphrates, cattle might have been domesticated nearly simultaneously with goats, sheep, and pigs (Helmer et al. 2005; Hongo et al., 2009). Thus the northern Levant and southeastern Anatolia regions were a locus of 'Neolithisation', and changes in subsistence that occurred in the region were presumably reflected in the content and/or quantity of food consumed by Neolithic populations.

Besides, the consumption of aquatic resources is generally considered to be one of the important adaptations for anatomically modern humans (Erlandson, 2001). Previous archaeological studies demonstrated the importance of freshwater fish as food resources for hunting-gathering communities in inland region during the late Upper Paleolithic periods (e.g. Bonsall et al., 1997; Zohar, 2003; Van Neer et al., 2005; Miracle, 2007; Naito et al., 2013; Richards et al., 2015). Interest of these studies was the potential use of aquatic foods, as well as terrestrial resources, by inland community at the water side environments. Then, at inland sites in north Europe, Latvia, freshwater fish was thought to have predominated in the diet of the inhabitants during Mesolithic–Neolithic periods (Eriksson et al., 2003; 2013). Also, in inland environment of Japan, freshwater fish was considered as one of important foods for inhabitants of Jomon period, comparable to the Neolithic culture (Hongo, 1989; Yoneda et al., 2004). On the other hand, at Körtik Tepe, a Neolithic site close by upper Tigris, many fish and waterfowl remains and several fishing hooks were found from the PPNA occupation layer (Arbuckle and Özkaya, 2006; Özkaya, 2009). Additionally, a high frequency of auditory exostosis, specific trauma of diving under water, has been observed among the human skeletons (Coşkun et al. 2010). Likewise, from Hasankeyf Höyük, one of the earliest Neolithic sedentary settlements at upper Tigris, the large quantity of freshwater fish bones was found in addition to terrestrial animals (Miyake, et al., 2012). Therefore, these archaeological remains suggest that freshwater resources could play an important role coupled with terrestrial resources in the diets of an early sedentary hunter-gatherer along the river and lake. In contrast, about Neolithic early farmer the importance of freshwater resources as food became less noted as against produced food resources.

1.3. Problems in Neolithisation in southwest Asia

As above stated, during the Neolithic period, human subsistence in the southwest Asia changed from hunting–gathering to cultivation of plants and herding of animals (Bar-Yosef and Meadow, 1995). This transition, known as the 'Neolithic revolution' (Childe, 1942), was accompanied by marked changes in demography and social organization in prehistoric southwest Asian populations (Bocquet-Appel and Bar-Yosef, 2008). It is possible that these innovations in food production clearly influenced the dietary habits of humans (Armelagos and Cohen, 1984; Richards, 2002). However, PPN inhabitants were not evaluated having nutrient conditions better than Epipaleolithic Natufians, on the basis of enamel hypoplasia analysis (Hershkovitz and Gopher, 2008). Thus, the adaptive significance of food production in Neolithic southwest Asia seems to be not the simple contribution to human health.

Though, only because there were produced food resources in the site, does not mean the society depended on the produced foods such as cultivated cereals and livestock. Also, it is important to elucidate when the human diets changed during Neolithic period. Then, the contribution of freshwater resources needs to be elucidated in particular for a riverside community. However, it has been inapparent how important aquatic resources were as compared to terrestrial resources in southwest Asian Neolithic communities. Because freshwater fish bones scrapped by humans show different breaking process from mammalian bones (Zohar et al., 2008), a simple amount comparison of freshwater fish bones with mammalian bones does not indicate the composition of animal protein resources. Additionally, uses of animal and fish bodies are not always food resources, e.g. ornaments made from the bone and the fur (e.g. Cristiani and Borić, 2012). To that end, these quantitative changes of food consumption should be verified by also evidence different from conventional archaeological approach based on the composition of animal and plant remains. Also, to understand the impact of Neolithisation, especially food production, we need to reveal human diet before food production as a standard of comparison, in addition to human diets in early farmer communities.

1.4. Change of social structures

During Neolithic period, dynamics of population in southwest Asia were said to have been drastically changed

through the change of subsistence (Bocquet-Appel and Bar-Yosef, 2008; Kuijt, 2008). Nomadic hunter-gatherer usually had birth interval of one mother more widely than sedentary farmer, because they had high mobility and less weaning foods made of cereal (Tuross and Fogel, 1994; Bellwood, 2004). In addition, the food productions such as cultivation permitted more population capacity per unit of area than the hunting-gathering economy. Thus, the beginning of sedentary and agriculture operated on a growth of population to southwest Asian society.

Furthermore, the economic transition from hunting-gathering to agriculture progressed from the PPN to PN period. It is considered that this transition was accompanied by marked changes in dynamics of Neolithic populations, e.g. in PPNA the largest settlements were approximately 1 ha, whereas in middle PPNB the largest settlements averaged over 10 ha (Kuijt, 2000; Bocquet-Appel and Bar-Yosef, 2008). Additionally, recently the increase of Neolithic settlement sizes was interpreted as not only the increase of population but a change of social structure, such as a gender division and an unit size of economic activity and storage (Bocquet-Appel and Bar-Yosef, 2008; Kuijt, 2008).

To understand the social structure, hierarchical stratum and gender division of subsistence in the prehistoric community, the burial practice is predominant approach (Binford, 1971; Kuijt and Goring-Morris, 2002; Hershkovitz and Gopher, 2008; Peterson, 2010; Pearson et al., 2013). In the PPN periods, the dead humans were commonly buried under the floor of living spaces. Moreover, as a common burial practice, isolated skulls were frequently found from the Natufian through PPNB in southwest Asian sites (Kuijt, 2008, Kanjou et al., 2013). The special building called "skull buildings" or "charnel room" which many skulls were collected to, have been excavated from several PPNB settlements, such as Çayönü Tepesi and Tell Abu Hureyra (Bienert, 1991; Moore and Molleson, 2000). In previous studies, it was argued that the corporate ritual urged to share worldviews and untied social inequality within a large settlement (Kujit, 2000; 2008; Verhoeven, 2002). In addition, these burial practices of collecting skulls were noted to the relationship to ancestor worship (Kenyon, 1956; Bienert, 1991). Actually, skulls collected in the building have been suggested that they had different diets than others of whole body, and were special status in Çayönü Tepesi (Pearson et al., 2013). However, when Pottery Neolithic period comes, burial rituals were seen to alter the appearance and nuance. Altogether, burial in the residence became uncommon except infant child (Kuijt and Goring-Morris, 2002). This change of burial ritual perhaps seems to

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have been linked to the decrease in size and the dispersal of settlements at PN (Bocquet-Appel and Bar-Yosef, 2008).

Also to reveal the change of social structure during Neolithic period, some archaeological studies proposed hypotheses as link the size of household to the activity and rituals of the community, mainly in southern Levant (Flannery, 1972; Kujit, 2000; Kuijt and Goring-Morris, 2002; Kadowaki, 2012). Incidentally, the household is defined to one actual situation unit collaborating for the subsistence and sharing ownership, and different from a cognate based on the marriage and the consanguinity (Kadowaki, 2012). In a hypothesis, the change of household size was discussed by size of residence for house and food storage (Kuijt, 2008). Some opinions explain a change of the household structure during the late Neolithic period was induced by the inception and development of the food production (Byrd et al., 2000; 2005). Specifically, the collaboration of the whole males in a community, called "social hunting", was probably effective for Neolithic hunter (Driver, 1995). And the division of labor between males and females was perhaps efficient relative to the independent subsistence of each family. In contrast, developed food producing activities may not need collaborative work by many members, because the average size of settlement became small to 1–2 ha by late PN period (Kuijt, 2008). But this hypothesis about the change of household size was suggested only indirectly by residence form and size.

According to these hypotheses, the differences of the diet between male and female in early Neolithic hunter-gatherer societies performing social hunting might well have been larger than that of late Neolithic farmer, especially after start of animal herding. Similarly, in early Neolithic societies males collaborating, and also females, are thought that they consumed uniform food resources according to sexual role in a community. Then, the differences of the diets between sexes might well have been decreasing with the growth of importance of livestock in the diet during PPNB and PN. However, this change might have been not completely synchronous to the start of animal herding, and/or cereal cultivation, because continuing to use wild animals and plants too.

To test these hypotheses, a method more focusing to individual variation of food consumptions is required, because the conventional researches based on animal and plant remains basically indicate the utilization of food resources by a whole community. Incidentally, the approaches by the personal data of human skeletons were conducted to argue the social complex within a community (Hershkovitz and Gopher, 2008). For example,

markings of muscle attachment on the bones are parameter of physical activities, and then the difference of these markings between males and females suggested the division of labor with sexes (Eshed et al., 2004). The females of Levantine hunter-gatherer before Neolithic period showed muscle's markings indicating a stone-ground activity on the bone more strongly than males. This clearer difference in sex of Epipaleolithic hunter-gatherer compared to Neolithic early farmer may have indicated the decrease of the difference in sex of activity during Neolithic period.

Also, the individual diet estimated based on physical and chemical analyses is one of a few ways as with the burial practice to understand the information about the social complexity in the prehistoric community. Furthermore, the food sharing among households, not just only individual food consumptions, would yield conclusive information to reveal a social structure in early sedentary settlements.

Namely, the carbon and nitrogen isotopic compositions of human bone collagen are used to identify the different group of the custom within the community by the diets (e.g. Richards et al., 1998; Schutkowski et al., 1999; Kusaka et al., 2008), because information of the personal diet is evidence about the subsistence. For example, in early Mediaeval Germany the individuals separated by isotopic composition of collagen showed different concentration of trace element related to habitat (Schutkowski et al., 1999). The human diets estimated by isotopic composition of collagen probably were related to the difference of lifestyle in a community. In addition, within the cemetery at England of Roman period the carbon isotopic compositions of individuals were separated based on the burial type, such as lead coffin and wooden coffin (Richards et al., 1998). Individuals from lead coffins might have been high status and eaten more marine resources than individuals of wooden coffin. Moreover, Kusaka et al. (2008) showed that groups having different pattern of ritual tooth ablation had different isotopic compositions of carbon and nitrogen in the case of Jomon culture. These studies showed that within a society there were some social stratums with the different consumption of marine resources, and isotopic compositions of human bone collagen were able to identify the difference of diets.

Therefore in this study, in an attempt to analyze the human diets of Neolithic communities in the southwest Asia, I employed stable isotopic analyses of prehistoric bones from Neolithic sites to detect changes of human diets, activity and social complexity during Neolithic period.

1.5. Previous isotopic studies in this region

Stable isotopic compositions of elements constituting the organism slightly vary according to environment on the earth. Generally, isotopic composition of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are shown in the form of deviation from standard by per mill (‰, see Section 2.1 for details). Consequently, carbon and nitrogen isotopic compositions of each food resource are specific based on their position in the food chain and ecology (e.g. Fig. 1.3). And result of isotopic analysis for human responds to the proportion of the food resources in the diet.

Paleodietary reconstructions based on stable isotopes in bone collagen have been used in previous archaeological studies to provide direct information on past human activity (Schoeninger and DeNiro, 1984). For example, isotopic data revealed a rapid and dramatic change from a marine to a terrestrial diet at the Mesolithic–Neolithic transition in north and central Europe (Richards et al., 2003b; 2003c; Eriksson et al., 2008). In England, though some latest hunter-gatherers depend strongly on aquatic resources, early farmers in the same place seem to have consumed little aquatic resources.

In the southwest Asia, a number of studies have been conducted on isotopic compositions of bone remains of Neolithic populations, such as at Çatalhöyük (Richards et al., 2003a; Styring et al., 2015), Nevalı Çori (Lösch et al., 2006), Aşıklı Höyük (Pearson et al., 2010), Çayönü Tepesi (Pearson et al., 2010, 2013) and Aktopraklık (Budd et al., 2013) (Fig. 1.1).

The inhabitants of these Neolithic sites probably consumed mainly C_3 plants and terrestrial animals consuming C_3 plants according to the $\delta^{13}C$ value of collagen ($\delta^{13}C_{col}$). Typically, the $\delta^{13}C$ value of an organism is used primarily as an indicator of habitat environment and ecosystem (Fig. 1.3). In Neolithic southwest Asian sites, almost terrestrial animals, both domestic and wild, seem to have consumed little or no C_4 plant, because the $\delta^{13}C_{col}$ of these animals showed approximately −20‰ or less. Generally, C_4 plant consumers show a $\delta^{13}C_{col}$, approximately −7‰, higher than C_3 plant consumers. Similarly, as marine source consumers have high $\delta^{13}C_{col}$ value, they can be distinguished from terrestrial dependent consumer by $\delta^{13}C$ values. However, a part of sheep from Çatalhöyük have high $\delta^{13}C_{col}$ (−16‰) than the value of usual C_3 plant consumer, a part of livestock has possibilities that they fed C_4 plants (Richards et al., 2003a). Also the $\delta^{13}C$ values of humans in Neolithic southwest Asian sites showed low values approximately −20‰.

Moreover, in the case of prehistoric southwest Asia, significant differences in the $\delta^{15}N$ values of bulk bone collagen ($\delta^{15}N_{col}$) among terrestrial herbivore species such as cattle, sheep and goat from a single site have been frequently observed (e.g., Richards et al., 2003a; Lösch et al., 2006; Pearson et al., 2007; Fig. 1.4). Therefore, animal proteins consumed by humans in Neolithic southwest Asia seem to have had various $\delta^{15}N$ (Fig. 1.5).

Subsequently, humans of the southeast Anatolia PPNB sites, Çayönü Tepesi and Nevalı Çori, showed lower $\delta^{15}N_{col}$ than those of other Neolithic sites in the southwest Asia (Lösch et al., 2006; Pearson et al., 2010, 2013). In addition, the PPNB humans at Aşıklı Höyük appeared to have lower $\delta^{15}N_{col}$ values than PN humans at Çatalhöyük in central Anatolia (Richards et al., 2003a). Furthermore, late Neolithic and early Chalcolithic inhabitants at Aktopraklık, located in the northwest Anatolia, showed the highest $\delta^{15}N_{col}$ values (~9.0‰) among any humans in Neolithic Anatolia (Budd et al., 2013). These differences in the $\delta^{15}N_{col}$ values of humans at PPNB sites in Anatolia can be explained by differences in the compositions of consumed plant and animal species (Pearson et al., 2010), because cattle were minor animal remains in the southeast Anatolian sites (Hongo et al., 2009). Also, in central Anatolia, cattle represented 9% of the mammal fauna at Aşıklı Höyük (Baird, 2012), whereas at Çatalhöyük, cattle represented 20% of the mammal fauna (Russell et al., 2005).

Altogether, these studies have shown that the $\delta^{15}N_{col}$ values of human remains are generally higher at later Neolithic (PN) sites than those at early Neolithic (PPNB) sites. However, at earliest period (PPNA), few studies have reported ancient human diets by isotopic analysis. Close comparisons of the diets of Neolithic hunter-gatherers and farmers in specific regions have not, however, been conducted. Furthermore, in the Levant, the west half of the Fertile Crescent and the cradle region of wheat cultivation, the Neolithic diet reconstructed from isotopic data has been unreported. To interpret the impact of food production strategies in Neolithic southwest Asian society, dietary information of both Neolithic hunter-gatherers and Levantine inhabitants in addition to previous studies are essential.

However, many of previous dietary studies for prehistoric societies by isotopic analysis of collagen for regions other than southwest Asia mainly identify food resources collected from different trophic positions and ecosystems, such as C_3 plant ecosystem *vs.* C_4 plant ecosystem, terrestrial resources *vs.* marine resources (Vogel et al., 1977; Richards et al., 1998; Yoneda et al., 2004; Kusaka et al., 2008). By contrast, generically the human

diets of Neolithic southwest Asia consisted of mainly C_3 plant ecosystem and possibly freshwater resources showing near isotopic compositions, according to archaeological researches and previous isotopic studies. Signatures of these food resources in isotopic compositions of human collagen are not easily discriminated from each other by conventional isotopic analysis of collagen. Therefore, only by carbon and nitrogen isotopic compositions in collagen it may be insufficient both to elucidate a breakdown of the individual diets and to discuss about the difference of diets in the community, because consumable food resources around these interior Neolithic settlements show similar range of isotopic compositions.

1.6. Problem on isotopic studies using bone collagen

First of the problems is large differences in the $\delta^{15}N$ values of bulk bone collagen among different herbivore species within a site. The dietary reconstruction by stable nitrogen isotopic composition of bulk bone collagen is based on the isotopic discrimination that occurs during assimilation/dissimilation processes; on average, animal tissues are enriched in ^{15}N by ~3.4‰ relative to their prey (e.g., DeNiro and Epstein, 1981; Minagawa and Wada, 1984; McCutchan et al., 2003).

However, in the case of Neolithic southwest Asia significant differences in the $\delta^{15}N_{col}$ (>2.4‰) among different herbivore species from a single site have been frequently observed (e.g., Richards et al., 2003; Lösch et al., 2006; Pearson et al., 2007), even though the trophic position (TP) of the herbivores should be equivalent to that of a primary consumer (i.e., TP = 2.0). For example, at Geoktchik Depe, an Iron Age site in Turkmenistan, Bocherens et al. (2005) reported that $\delta^{15}N_{col}$ values in cattle were enriched by 5.0‰ relative to those in goats, a value which is greater than the ~3.4‰ difference attributed to isotopic discrimination between different trophic levels; thus, these isotopic data are not consistent with the fact that both cattle and goats are herbivores.

This contradiction has generally been explained by the fact that bulk $\delta^{15}N_{col}$ values reflect not only trophic position, but also temporal and spatial variations in the isotopic composition of feed plants in the environment (Hartman, 2011) and physiological effects (e.g., the degree of nitrogen efflux to urine and feces) on ^{15}N-enrichment in each herbivore (Sponheimer et al., 2003b). For whatever actual reason, the variation in the $\delta^{15}N_{col}$ of herbivores generates ambiguity when interpreting the isotopic data for estimation of ancient human

diets. Altogether, the estimated proportion of animal protein in an individual diet is dramatically varying percentage based on each animal in the same site. To estimate the fraction animal protein in total terrestrial protein (Fa) of human diet accurately, we used animal $\delta^{15}N_{col}$ value as value of animal protein resource (Minagawa and Wada, 1984; Post et al., 2002; Hedges and Reynard, 2007; Styring et al., 2015). I considered that the isotopic enrichment factor from diet to consumer ($\Delta^{15}N_{consumer-diet}$) is +3.4±1.1% for bulk collagen (Minagawa and Wada, 1984). This simple calculation model is based on the premise that both forage eaten by animals and cereal eaten by human had the same $\delta^{15}N_{col}$ values, and this "herbivore" does not include freshwater species.

$$Fa_{col} (\%) = \frac{(\delta^{15}N_{col}[human] - \delta^{15}N_{col}[herbivore])}{\Delta^{15}N_{consumer-diet}}$$

Altogether, the Fa_{col} of an individual who have the same $\delta^{15}N$ values is estimated varying percentage based on each animal in the same site (Fig. 1.5).

Second of the problems is contribution of freshwater resources to human diets in Neolithic southwest Asia. The contribution of freshwater resources is difficult to quantitatively identify from isotope signature of humans in Neolithic sites. Because freshwater fishes usual have higher $\delta^{15}N_{col}$ compared to terrestrial mammals, Paleolithic human that consumed much freshwater resources had high $\delta^{15}N_{col}$ values, approximately 15‰ (Baird et al., 2013; Richards, et al., 2015). Also, Neolithic north European considered as freshwater fish consumer showed $\delta^{15}N_{col}$ value, approximately 12.5‰ (Eriksson et al., 2003; 2013). However, these values of Paleolithic human estimated as freshwater fish consumer were significantly higher than those of humans at several Neolithic sites, such as Çatalhöyük producing a lot of aquatic remains (10.9‰; Richards, et al., 2003). Additionally, the carbon isotopic compositions of C_3 plants, terrestrial herbivores based on C_3 plants, and typically freshwater fishes are close in all food resources (Fuller et al., 2012), because the source of carbon for both terrestrial and freshwater plant derived from atmospheric CO_2 and carbonate from rocks and soils (Katzenberg and Weber, 1999). Thus, it is difficult to isolate the consumption of freshwater fishes from terrestrial mammals by carbon isotopic composition, unlike marine fishes.

Furthermore, sometimes the bulk $\delta^{15}N$ values of freshwater and terrestrial plants overlap, because the nitrogen in freshwater environment such as river often derives from terrestrial environment (Fig. 1.3; Finlay and Kendall,

2007). Additionally, estimated proportions of animal protein on the terrestrial resources have ambiguity when interpreting the isotopic data of collagen in Neolithic southwest Asia. In the case of Neolithic southwest Asia significant differences in the $\delta^{15}N$ values of bone collagen among different herbivore species from a single site have been frequently observed (Richards et al., 2003a; Lösch et al., 2006; Pearson et al., 2010). Because the bulk $\delta^{15}N$ values were varied over 3.4‰ among herbivores, estimated fraction of animal protein in human diet vary based on animal species as consumed meat. Especially cattle had similar $\delta^{15}N_{col}$ (9.4‰) to freshwater fish (9.3‰), and also it was higher than those of other terrestrial herbivore at Epipalaeolithic sites, Pınarbaşı and Boncuklu Höyük (Baird et al., 2013).

1.7. Avail of isotopic analysis of amino acids

Therefore, to clarify both dietary animal protein fraction in total terrestrial protein intake and quantitative evaluation of aquatic resource consumption versus terrestrial resource in Neolithic southwest Asia, I employ the nitrogen isotopic composition of individual amino acids in bone remains. The method has recently been applied in a number of ecological studies of modern animals (e.g., Ohkouchi et al., 2013; Chikaraishi et al., 2014) and in a few studies of ancient animal and human remains (e.g., Naito et al., 2010; 2013; Styring et al., 2010; 2015).

Dietary reconstruction by isotopic analysis of individual amino acids is based on a significant difference in the trophic isotopic discrimination of two common amino acids: in the transition from prey to consumer, the $\delta^{15}N$ value of glutamic acid ($\delta^{15}N_{Glu}$) increases by +8.0±1.1‰, while that of phenylalanine ($\delta^{15}N_{Phe}$) increases by only +0.4±0.4‰ (Chikaraishi et al., 2010). Because there is little change among foods and consumer, the $\delta^{15}N$ values of phenylalanine in animals mainly reflect those of the plants upon which they feed, which in turn depend on the growth environment (e.g., temperature and precipitation). In contrast, the trophic position (TP) of animals can be estimated by differences in the $\delta^{15}N$ values of glutamic acid and phenylalanine, as follows (Chikaraishi et al. 2010).

$$TP_{Glu/Phe} = [(\delta^{15}N_{Glu} - \delta^{15}N_{Phe} + \beta) / 7.6] + 1$$

This "β" shows the difference of nitrogen isotopic compositions between glutamic acid and phenylalanine in primary producers (−3.4‰ for aquatic cyanobacteria and algae, +8.4‰ for terrestrial plants; Fig. 1.6). Therefore,

this method operates different calculation to aquatic and terrestrial ecosystems by the difference of metabolism between cyanobacteria and terrestrial plant (Chikaraishi et al. 2007; 2010; Naito et al., 2013). Thus animals of aquatic ecosystem are isolated from animals of terrestrial ecosystem by the difference between $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ (e.g. aquatic first consumer, 11.0‰; terrestrial first consumer, −0.8‰). Accordingly, Naito et al. (2010; 2013) identified aquatic dependent consumer from terrestrial dependent consumer in prehistoric humans through the use of isotopic analysis of individual amino acids. In addition, this method does not require the information on the $\delta^{15}N$ values of food resources to estimate trophic position (TP) of an animal, because the TP of animals and human can be estimated by differences in the $\delta^{15}N$ values of glutamic acid ($\delta^{15}N_{Glu}$) and phenylalanine ($\delta^{15}N_{Phe}$) of individual.

However, paleodietary studies by isotopic analysis of amino acids for Neolithic southwest Asia have been yet confined to 5 individuals at Çatalhöyük, as a part of a report about validation of fraction of animal protein in total terrestrial protein based on several calculations (Styring et al., 2015).

1.8. Goals of this study

To elucidate the adaptive significance of Neolithisation for southwestern Asian human, I validate the evidence of dietary changes during the Neolithic period. To that end, the dietary reconstruction by isotopic analysis of both Neolithic hunter-gatherer and Neolithic Levantine inhabitants yet unanalyzed is necessary. Additionally, whether the dietary difference among individuals within a community increased or decreased before and after food production should be validated also.

Therefore, I report nitrogen isotopic compositions of amino acids, in addition to stable carbon and nitrogen isotopic compositions of collagen, in human and faunal remains from 4 Neolithic sites, Tell Qaramel and Tell Ain el-Kerkh in north Levant, Hasankeyf Höyük and Hakemi Use in southeast Anatolia. These are the first isotopic data on a Levantine Neolithic populations and PPNA hunter gatherers in southwest Asia to be reported. Therefore, I can distinguish terrestrial and aquatic foods and estimate dietary animal protein intake ratio by analyze of individual amino acids. And I aim at the resolution of the food acquisition and sharing in the early Neolithic societies, by the relationship between the individual diets and the burial group within the same site.

Based on these data, I obtained information about the diets of the populations in northern Levant and southeast Anatolia, which allowed us to interpret temporal changes in human diets and the food distribution within the each site throughout the entire Neolithic period, thus allowing a comparison of the isotope signatures of human remains before and after the rise of food production.

2. Methodology

2.1. Isotopic compositions of carbon and nitrogen

On the earth, atmospheric CO_2 contains two stable carbon isotopes, approximately 98.89% of carbon–12 (^{12}C) and 1.11% of carbon–13 (^{13}C) (Nier, 1950). Generally, carbon isotopic composition is represented as proportion of sample to standard limestone (belemnite) from the Pee Dee Formation in South Carolina, called PDB (Craig, 1957) and the values are usually presented by per mil ($\delta^{13}C$).

$$\delta^{13}C\ (‰) = [(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{PDB}]\ /\ [(^{13}C/^{12}C)_{PDB}] \times 1000$$

The process of changing inorganic carbon like CO_2 to organic material such as carbohydrate is called carbon fixation. Carbon fixation by photosynthesis in plants is accompanied by isotopic fractionation of carbon as proportional change of ^{12}C and ^{13}C (O'Leary, 1988; Farquhar et al., 1989). It is known that the apparent fractionation of isotopic composition with the C_3 photosynthetic pathway ($-18‰$) is larger than C_4 photosynthetic pathway ($-4‰$) (O'Leary, 1981), according to a difference of efficiency with carbon fixation between C_3 and C_4 plants. Because C_3 photosynthetic pathway has a CO_2 ejection larger than C_4 photosynthetic pathway by photorespiration, C_3 plants have large fractionation of $\delta^{13}C$ in carbon fixation. Besides, aquatic plants often have higher $\delta^{13}C$ more than terrestrial C_3 plants, because diffusion of CO_2 in water is slow than that in atmosphere (O'Leary, 1981). Thus, organisms of each ecosystem depending on the primary producers, are identified by the $\delta^{13}C$ of body tissues. Furthermore, it is known that there is small isotopic fractionation between body tissues and the diet ($+0.5–2‰$) with predation and assimilation (e.g. DeNiro and Epstein, 1978; Sponheimer et al., 2003a).

Similarly, two stable isotopes, approximately 99.64% of nitrogen–14 (^{14}N) and 0.36% of nitrogen–15 (^{15}N), account for all of isotopes in atmospheric N_2 (Dole et al., 1954). Nitrogen isotopic composition is represented as proportion of sample to atmospheric N_2 and the values are usually presented by per mil ($\delta^{15}N$).

$$\delta^{15}N\ (‰) = [(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{AIR}]\ /\ [(^{15}N/^{14}N)_{AIR}] \times 1000$$

In photosynthetic pathway, there are some mechanisms to assimilate inorganic nitrogen, such as N_2 and NOx, to organic material (Handley and Raven, 1992). Because the nitrogen isotopic composition of primary producer is hardly changed during nitrogen assimilation, the $\delta^{15}N$ of plant tissues are affected by that of the inorganic

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nitrogen source (Evans, 2001). Hence, the variation in the δ^{15}N of terrestrial plants generally ranges 0 to 10‰ depending on that of the soil (Handley and Raven, 1992). Because δ^{15}N of muscle tissues and bone collagens of predator are 1.5–5‰ higher than that of the diet, within the same environment the δ^{15}N of each organism vary depending on the trophic positions (Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; McCutchan et al., 2003; Hedges and Reynard, 2007). Therefore, the δ^{15}N of animal tissue is a useful indicator to understand prey–predator relationship for animals and humans. Furthermore, marine animals consumed by human belong to trophic positions higher than terrestrial animals because the food chains in marine ecosystem are generically more extended than those of terrestrial ecosystem. Therefore, marine resource consumer is able to be isolated from terrestrial resource consumer according to nitrogen isotopic composition of body tissues (Schoeninger and DeNiro, 1984).

Meanwhile, the ^{15}N fractionations of individual amino acids constituting proteins of animal by amino acid metabolism from diet to predator were revealed by some studies (McClelland and Montoya, 2002; McClelland et al., 2003; Chikaraishi et al., 2007; 2010). Interestingly, essential or non-essential amino acid is not necessarily congruent to the degree of the fractionation in δ^{15}N (McClelland and Montoya, 2002). Otherwise, it may be caused by a possibility which animals in the nature practically assimilate directly from almost dietary amino acid to body tissues, even if it is non-essential amino acid. Actually, some amino acids, such as alanine, valine, leucine, isoleucine and glutamic acid, show significant ^{15}N–enrichment along assimilation. These amino acids involve the removal of the amine functional group in metabolic process to alter to keto acids (Fig. 2.1; Bender, 2014). Thereby the removed amine functional group is enriched in ^{14}N, whereas remained amino acid is enriched in ^{15}N (Chikaraishi et al., 2007). In contrast, the δ^{15}N of methionine and phenylalanine show little change along predation and assimilation (Chikaraishi et al., 2007), because their dominant metabolic processes are not related to functional groups involving nitrogen (Fig. 2.1; Bender, 2014). On the other hand, specific mechanisms controlling the ^{15}N–enrichment of glycine and serine along assimilation are unclear, because these are synthesizes from several precursors through different pathways (Chikaraishi et al., 2007). Therefore, the ^{15}N-enrichments of bulk proteins reflect total of the ^{15}N-enrichment on each amino acid according to the amino acid composition.

2.2. Collagen extraction from bone

Collagen samples (i.e., gelatin consisting mainly of collagen) were extracted from bones by gelatinization, based on an improved method used in previous research (Longin, 1971; Yoneda et al., 2002). First, the bone fragments were cleaned by brushing and ultrasonic cleaning. Humic acid and fulvic acid were removed by soaking in 0.2 M NaOH at 4°C for 8 hours, and the samples were then washed with Milli-Q water. The cleaned and freeze-dried bone samples were then ground to a fine powder. Hydroxyapatite was removed from the powdered bone by reacting with 1 M HCl in cellulose tubes at 4°C for overnight. The remains were heated in Milli-Q water at 90°C for 12 hours to extract the gelatin, and the dissolved gelatin was then filtrated and freeze-dried to obtain collagen samples.

2.3. Preparation of charred kernels

Charred kernels were prepared by non-based pre-treatment as follows, based on an improved method by reference to previous research (Kanstrup et al., 2013). First, the charred kernels were cleaned by ultrasonication in Milli-Q water for 10 min at room temperature. Then, samples were washed by soaking in 0.1 M HCl for 10 min at room temperature to remove attached carbonate, and then rinsed repeatedly with Milli-Q water. The kernels were then freeze-dried and milled to a fine powder. The percent carbon (%C) and nitrogen (%N) and the stable isotopic compositions of carbon and nitrogen were measured.

2.4. Stable isotopic measurement of carbon and nitrogen by EA–IRMS

The stable isotopic compositions of the collagen and charred kernel samples were determined by an elemental analyser–isotope ratio mass spectrometer (EA–IRMS) (Flash 2000 EA coupled to a Delta V Advantage IRMS; Thermo Fisher Scientific) at the Laboratory for Radiocarbon Dating in the University of Tokyo and EA–IRMS (Flash 2000 EA coupled to a MAT 253 IRMS; Thermo Fisher Scientific) in the National Museum of Nature and Science. The analytical precision (1σ) based on replicate analyses of reference alanine was <0.2‰. The purity of the collagen samples was evaluated on the basis of the carbon (%C) and nitrogen (%N) content in the extracted collagen samples. The atomic C/N ratio was expected to be in the range of 2.9–3.6 (DeNiro, 1985), and extracted

gelatin yields were expected to be >1% (Ambrose, 1993); otherwise, data were eliminated from discussion. Because the isotopic compositions of body tissues are more positive than those of dietary materials, by 0.5‰ in the $\delta^{13}C$ value of collagen ($\delta^{13}C_{col}$) and by 3.4‰ in the $\delta^{15}N$ value of collagen ($\delta^{15}N_{col}$) (Minagawa and Wada, 1984; Ambrose, 1991), the values for foods were calculated by applying these corrections to the compositions for bone collagen.

2.5. Preparation of individual amino acids in bone collagen

Amino acids were extracted from approximately 2 mg of the collagen samples by 12 M HCl hydrolysis at 110°C for 12 h, and were then derivatized for the isotopic analysis, following Chikaraishi et al. (2010). The hydrolyzed samples were derivatized using thionyl chloride/2-propanol (1/4, v/v) at 110°C for 2 h and subsequently using pivaloyl chloride/dichloromethane (1/4, v/v) at 110°C for 2 h. After derivatization, the derivatives of the amino acids were extracted by liquid–liquid extraction with n-hexane/dichloromethane (3/2, v/v) and distilled water.

2.6. Nitrogen isotopic measurement for individual amino acids by GC–C–IRMS

The $\delta^{15}N$ values of the amino acids were determined using a gas chromatography–combustion–isotope ratio mass spectrometer (GC–C–IRMS) (Agilent 6890GC coupled to a ThermoFinnigan Delta^{plus}XP IRMS via a GC–C/TC III interface to combustion at 950ºC and reduction furnaces at 550ºC) at Japan Agency for Marine-Earth Science and Technology (JAMSTEC) (Chikaraishi et al., 2010). The derivatives of amino acids were injected using a programmable temperature vaporizing (PTV) injector (Gerstel) into an HP Ultra-2 capillary column (50 m; i.d. 0.32 mm; film thickness 0.52 μm; Agilent Technologies). The carrier gas (He) flow rate was controlled using a constant flow mode at 1.4 ml min^{-1}. Standard mixtures of 10 amino acids (alanine, glycine, valine, leucine, norleucine, aspartic acid, methionine, glutamic acid, phenylalanine, and hydroxylproline; SI Science Co., Ltd) with known $\delta^{15}N$ values were analyzed for every 5 runs. The analytical precision (1σ) on replicate analyses of reference amino acids was <0.5‰ for more than 2 nmol N of each amino acid.

The trophic position (TP) was estimated from the $\delta^{15}N$ values of glutamic acid ($\delta^{15}N_{Glu}$) and phenylalanine

($\delta^{15}N_{Phe}$), using the following equations (Chikaraishi et al., 2007; 2009; 2010; 2011):

$$TP_{aqua} = [(\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - 3.4) / 7.6] + 1 \text{ in aquatic ecosystems}$$

$$TP_{ter} = [(\delta^{15}N_{Glu} - \delta^{15}N_{Phe} + 8.4) / 7.6] + 1 \text{ in Terrestrial ecosystems}$$

The error of the estimated trophic position is 0.20 (1σ; for details, see Chikaraishi et al. 2010).

2.7. Radiocarbon dating by AMS

For the ^{14}C measurements, the preparation and graphitization methods of collagen followed those described by Yoneda et al. (2002). Briefly, 2.5 mg of collagen (containing ~1 mg of carbon) was purified by chemical treatment, and carbon dioxide was produced by combustion at 850°C in an evacuated and sealed quartz tube; the CO_2 was then cryogenically purified in a vacuum system (Minagawa et al., 1984). Then, graphite was produced by the catalytic reduction of CO_2 with iron powder (Kitagawa et al., 1993). The radiocarbon content of the graphite was measured using accelerator mass spectrometers (AMSs) at the National Institute for Environmental Studies-Tandem accelerator for Environmental Research and Radiocarbon Analysis (NIES-TERRA) (Tanaka et al., 2000), the Micro-Analysis Laboratory Tandem accelerator (MALT) at the University of Tokyo (Matsuzaki et al., 2007) and Paleo Labo Co. Ltd., Japan (Kobayashi et al. 2007). In the AMS analyses, the new oxalic acid standard (NBS RM-4990C) and ANU sucrose (IAEA-C6) were usually loaded concurrently with unknown samples. The radiocarbon dates were statistically analyzed and calibrated using OxCal (Bronk Ramsey, 1995, 2009) and IntCal13 calibration data (Reimer et al., 2013).

2.8. Statistical Analysis

The statistical software R version 3.2.3 (R Core Team, 2015) was used for all statistical analysis (Welch's *t* test, Mann–Whitney U test, Kruskal–Wallis test). Statistical significance was evaluated using the *p* values of 0.05.

3. The Temporal Changes of Isotopic Compositions of Hunter-Gatherer and Early Farmer by Conventional Collagen Analysis

3.1. Introduction

The northern Levant and southeast Anatolia regions were locus of 'Neolithisation', and changes in subsistence that occurred in the region were presumably reflected in the content and/or quantity of food consumed by Neolithic populations. However, the reports of the result of the dietary reconstructions about Neolithic southwest Asian sites were limited to Pre-Pottery Neolithic B (PPNB) and Pottery Neolithic (PN) in time and Anatolia region. Thus, in this section, in an attempt to analyze the human diets of Neolithic communities in the north Levant and southeast Anatolia in addition to previous studies, I employed conventional carbon and nitrogen isotopic analyses of bone collagens to detect dietary changes occurring during Neolithisation.

This study here reports on stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic compositions of bone collagen in human and faunal remains from two northern Levantine sites, Tell Qaramel and Tell Ain el-Kerkh, and two southeast Anatolian sites, Hasankeyf Höyük and Hakemi Use, which include remains from the Pre-Pottery Neolithic A (PPNA), PPNB and PN periods. Based on the results, the impact of food production in Neolithisation to human diets is evaluated by the temporal change of isotopic signature in collagen during Neolithic period.

3.2. Materials

3.2.1. Tell Qaramel

Tell Qaramel consists of artificial mound, called Tell in the local language, located in the Qoueiq River basin, ~25 km north of Aleppo, northwest Syria (see Fig. 1.1). This site contains evidence of occupations in the Proto-Neolithic, early PPNA and Ottoman periods. In archaeological excavations conducted in 1999, 34 adult skeletons were recovered from 23 graves in PPNA horizon H3, dated to 9820–8710 cal BC (Mazurowski, 2003; Kanjou, 2009; Mazurowski et al., 2012; Kanjou et al., 2013). Most adult skeletons from horizon H3 were separated into skulls and post-cranial parts according to burial customs, but in a few cases consisted of the entire skeleton (Kanjou, 2009; Kanjou et al., 2013). Skull removal had been a common burial practice from the

Natufian through PPNB in southwest Asia communities (Kuijt, 2008).

The PPNA people of Tell Qaramel gathered wild fruits, cereals and pulses (Willcox and Herveux, 2012), and the Tell Qaramel remains also include numerous fossils predominantly of wild cattle, as well as of wild sheep and goats. Equidae fossils are common, and pigs, fishes, turtles, molluscs, birds and snails have also been recovered from PPNA layers (Mazurowski, 2003; Grezak, 2009). At Tell Qaramel, hunting was the primary means of obtaining meat, perhaps complemented by fishing and the collection of other aquatic resources (Grezak, 2009).

Although the majority of bones were in poor condition, I tried to extract and analyze the collagen from 12 human skeletons and 6 animal bones from the PPNA layer and 10 human skeletons from the Bronze Age layer of Tell Qaramel. The animal bones included one auroch (*Bos primigenius*), two onagers (*Equus hemionus*), two pigs (*Sus scrofa*) and one caprine (sheep or goat), all of which originated from wild animals. In addition, the nitrogen isotopic compositions of 17 charred einkorn (*Triticum monococcum*) grains from Tell Qaramel were reported in a previous study (Araus et al., 2014).

3.2.2. Tell Ain el-Kerkh

Tell Ain el-Kerkh is one of three large tells at el-Kerkh, located in the south Rouj Basin, ~75 km southwest of Aleppo, northwest Syria (see Fig. 1.1). Excavations at Tell Ain el-Kerkh, started in 1997, have revealed layers containing Neolithic settlements (Iwasaki and Tsuneki, 2003). The layers date to the early PPNB in the northwest part of the area, and the late phase of the PN (latest Neolithic) in the central area. The Neolithic layers span ca. 3000 years, from 8700 to 5700 cal BC, based on ^{14}C dating of charcoal samples (Tsuneki, 2010).

The excavations at Tell Ain el-Kerkh produced a Neolithic (middle PN) cemetery containing more than 240 individual burials (Tsuneki, 2011, 2012, 2013). The cemetery is one of the earliest examples of an outdoor communal cemetery in the southwest Asia (Tsuneki, 2013). In the PN phase, all generations and sexes of individuals were buried within the same area, using similar burial practices. In this study, perinatal indicates child from ninth month of pregnancy to first month after birth, then infant indicates child from birth to first birthday (Hironaga, 2013). Though juvenile indicate young estimated from 1 year to 12 years old, I additionally

separated young-juvenile, 1–5 years old, and elder-juvenile, 6–12 years old. Sub-adult was defined as teen, then adult was separated to young-adult (20–35 years old), middle-adult (36–50 years old) and old-adult (older than 50).

Many child skeletons were found under building floors in the late PPNB layers at Tell Ain el-Kerkh, and a few adults were found in the deposits (Tsuneki et al., 2007). In addition, PN layers 2–4 produced entire skeletons of 3 adults and 5 adolescents, as well as some disturbed skeletons, layers 4–5 produced at least 40 skeletons, layers 5–6 produced 56 skeletons and layer 6 produced 29 skeletons. The cemetery shows that burial practices changed dramatically from the PPNB to the PN phase of settlement. In the PN phase, all generations and sexes of individuals were buried within the same area, using similar burial practices.

The Tell Ain el-Kerkh excavations produced remains of cattle, pigs, sheep, goats and dogs, all of which were likely domesticated, in addition to wild animals including gazelles, red deer, fallow deer, cats and rodents. From water floatation sampling, fallfish, catfish and frog bones were also discovered (Tsuneki et al., 2006). In addition, einkorn and emmer were found in PPNB layers, in addition to various species of pulses, such as chickpea and faba bean (Tanno et al., 2006a, b).

In this study, I extracted collagen for carbon and nitrogen isotopic analyses from the Tell Ain el-Kerkh samples, including from 7 human skeletons and 10 animal bones from the PPNB, 63 human skeletons and 21 animal bones from the PN. Animal bones were including cattle (*Bos taurus*), pigs (*Sus scrofa*), sheep (*Ovis aries*), goats (*Capra hircus*), gazelle (*Gazella* sp.), hare (*Lepus* sp.), wolf (*Canis lupus*), fox (*Vulpes* sp.) and freshwater fishes (Cyprinidae). Because the stable isotopic compositions of breast-feeding infants are higher than those of the mothers (Fuller et al., 2006, Tsutaya et al., 2013, Reynard and Tuross, 2015), I excluded infants under 4 years old from the study. In addition, cereal remains collected in square E271 of PN layer 6, were identified as emmer (*Triticum turgidum* ssp. *dicoccum*), and isotopic compositions were analyzed in 10 of the grains.

3.2.3. Hasankeyf Höyük

Hasankeyf Höyük is located in the upper Tigris valley in Batman, Turkey (see Fig. 1.1). This site is located on the riverside of the Tigris, about 2 km east of the medieval site of Hasankeyf. The excavations of this site, which

will be submerged by the construction of the Ilısu Dam, were carried out within the framework of the Hasankeyf rescue projects by Batman University. They conducted the first excavation in 2009, and then University of Tsukuba continues excavation since 2011 to 2014.

Hasankeyf Höyük is a roughly circular mound of 150 m in diameter and 8 m higher than the surrounding plain. Except for some storage pits from the Iron Age and the Hellenistic periods dug into the prehistoric layers, all the archaeological deposits are from the 10th millennium cal BC. Charcoal remains have been radiocarbon dated to the 10th millennium (Miyake et al., 2012).

At Hasankeyf Höyük, many human burials have been found under floors of several residences. Furthermore, because the many skeletons are kept in an anatomical position, therefore there were a primary burial. Additionally, not just adult male, some female and child had burial goods at Hasankeyf Höyük. In this study, 34 human bones from PPNA layer were treated by gelatinization. In addition, 5 human bones from Iron Age layer were treated.

A large number of animal bones were recovered from Hasankeyf Höyük, mostly from the buildings. Among the mammals, wild caprine is dominant, accounting for about 60% of the identified specimens (NISP). Also the percentage of wild sheep in the caprine at lower layers was quadruple of that of wild goats, and then increased to sextuple at layer of Group 2. In addition, wild pig and red deer are also common, and they each comprise 10% of total faunal fragments. Fox was found from this site, accounting for 5% of total faunal fragments. Gazelles, badger and tortoises are also included, but wild cattle have little found in the assemblage. In this study, analyzed animal bones were including wild cattle (*Bos primigenius*), pigs (*Sus scrofa*), sheep (*Ovis orientalis*), goats (*Capra aegagrus*), red deer (*Cervus elaphus*), badger (*Meles meles*) and tortoise.

In addition, a large quantity of fish and water bird bones recovered by 4 mm-mesh dry sieving. These specimens have been identified as Cyprinidae, *Barbus* sp. and *Capoeta* sp. These remains of aquatic animals probably suggest that freshwater resources were consumed by Hasankeyf Höyük inhabitants.

Interestingly unlike Levantine sites as origin of cereal cultivation at similar PPNA period, little evidence about consumption of cereals such as wheat was found, but almonds, pistachio, hackberry, lentil and other nuts from this site (Miyake et al., 2012). The lack of cereals was also known from neighboring PPN sites, such as Körtik

Tepe, Hallan Çemi and Demirköy Höyük (Coşkun et al. 2010; Riehl et al., 2012).

3.2.4. Hakemi Use

Hakemi Use is located in the upper Tigris valley in Bismil, Turkey (see Fig. 1.1). The mound was excavated by Hacettepe University (Tekin, 2005). The excavations at this site provided five occupation layers in the Late Neolithic period corresponding approximately to PN, dating to 6100-5950 cal BC (Erdal, 2013).

At Hakemi Use, many human burials have been found. Many of the skeletons in the burials are infants and children. Many of the graves were found from buildings and almost graves were primary burials at this site (Tekin, 2007; Erdal, 2013).

At Hakemi Use, the majority of animal bones were identified as domestic animals, and domestic sheep (*Ovis aries*) and goats (*Capra hircus*) dominated to 60% of total number of animals (Omar, 2013). Then wild and domesticated pigs (*Sus scrofa*) dominated to 13% of the total faunal remains, second after sheep and goat. Because wild animals show a low ratio relative to domesticated animals in animal remains, intensive hunting might not have been essential activity in this site (Tekin, 2007).

Cereals identified as barley and wheat, were found from the ash in the hearths (Tekin, 2005), and these were thought to have been cooked. According to the botanical investigations, these wheat and barley were considered to be cultivated plants (Tekin, 2007). In addition, because a large amount of grinding stones and pestles were found, cereal cultivation was thought to have been important activities.

In this study, 19 human bones were treated by gelatinization. But animal remains have not been collected yet.

3.3. Results

3.3.1. Faunal isotopic compositions

I attempted to extract collagen from eight animal bones from Tell Qaramel, but only four bones contained sufficient amounts of material for isotopic analysis. And no one showed C/N ratios typical of biological materials (2.9–3.6).

In addition, 31 animal bones from Tell Ain el-Kerkh were treated by gelatinization, but only 21 specimens

showed biologically acceptable C/N ratios (see Table 2.1). A gazelle and a hare sample with C/N ratios out of the biological range were excluded from the following discussion. The $\delta^{13}C$ and $\delta^{15}N$ values of faunal samples are plotted in Fig. 3.1.

At Tell Ain el-Kerkh, cattle exhibited the highest $\delta^{15}N_{col}$ values (7.7±0.6‰; mean ± 1 s.d.) of all terrestrial herbivores. Pigs exhibited a wide range of $\delta^{15}N_{col}$ values (4.3‰–8.8‰; mean, 6.5±1.8‰). In sheep, the mean $\delta^{15}N_{col}$ value was 6.1±0.6‰; in goats, the mean $\delta^{15}N_{col}$ value was 4.6±1.1‰, which is lower than those of other ruminants. Among ruminant species, significant variability was observed in $\delta^{15}N_{col}$ values, which is similar to that observed at other Neolithic sites in Anatolia, including at NevalıÇori (Lösch et al., 2006), Çayönü Tepesi (Pearson et al., 2007, 2013) and Çatalhöyük (Richards et al., 2003a). A fox showed a $\delta^{15}N_{col}$ value of 9.6‰, which is higher than those of other herbivores reflecting its habit as a carnivore. A cyprinid freshwater fish showed a $\delta^{15}N_{col}$ value of 11.0‰, which is higher than those of other terrestrial animals, suggesting that isotopic compositions within each species maintain their ecological signatures with respect to collagen values.

The $\delta^{13}C_{col}$ values of herbivores were very similar to one another, around −21‰. A fox exhibited a $\delta^{13}C_{col}$ value of −18.7‰. The low $\delta^{13}C_{col}$ values of terrestrial herbivores from both the PPNB and PN (approximately −20‰ or less) show that they consumed little or no C_4 plant material.

Besides, collagens of ten animal bones in 20 samples from Hasankeyf Höyük showed biological C/N range of collagen between 2.9 and 3.6. Among terrestrial herbivores, significant variability was observed in the $\delta^{15}N$ value of collagen, as with Tell Ain el-Kerkh. Cattle exhibited the highest $\delta^{15}N_{col}$ values among all terrestrial herbivores (10.5‰). By contrast, the mean $\delta^{15}N_{col}$ value among goats was the lowest in terrestrial animals (6.0±0.3‰). The pig, red deer, and badger have intermediate $\delta^{15}N_{col}$ between cattle and goats (Table 3.1). A fox showed a $\delta^{15}N_{col}$ value of 9.5‰, which is higher than those of other herbivores except for cattle, reflecting it as a carnivore.

Because the collagens of sheep, a tortoise and cyprinid freshwater fish samples from Hasankeyf Höyük show C/N ratios out of the biological range of collagen (2.9–3.6), their $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ were excluded from the following discussion.

The $\delta^{13}C_{col}$ values of herbivores were similar to one another, −20‰. A fox exhibited a $\delta^{13}C_{col}$ value of −19.1‰.

The low $\delta^{13}C_{col}$ values of terrestrial herbivores from Hasankeyf Höyük (−20‰ or lower) show that they consumed little or no C_4 plant material.

3.3.2. Isotopic compositions of charred kernels

The $\delta^{13}C$ and $\delta^{15}N$ values of 10 kernels from Tell Ain el-Kerkh are presented in Table 3.2 and plotted in Figure 3.1. In these kernels, the mean $\delta^{13}C$ value was −22.4±0.4‰ and the mean $\delta^{15}N$ value was 2.2±0.6‰.

3.3.3. Human isotopic compositions and radiocarbon dates at Tell Qaramel

Of 11 human bone samples from PPNA layer at Tell Qaramel, 7 contained amounts of collagen sufficient for conducting isotopic analyses. In addition, the collagen showed biologically acceptable C/N ratios, 2.9–3.6, in six of the seven samples. I measured radiocarbon dates for one human sample, which yielded ages of 8260 and 8625 cal BC; these ages are slightly younger than the charcoal dates for H3 previously obtained by Mazurowski et al. (2012). And six of 9 human individuals from Bronze Age layer at Tell Qaramel show biological C/N ratios.

The $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ values of collagen extracted from the human bone samples from PPNA layer at Tell Qaramel are shown in Figure 3.1 and Table 3.3. The mean $\delta^{13}C_{col}$ value of PPNA humans at Tell Qaramel was −19.8±0.3‰ (range between −20.4‰ and −19.4‰) and the mean $\delta^{15}N_{col}$ value was 8.7±1.2‰ (range between 6.7‰ and 10.0‰). In contrast, the mean $\delta^{13}C_{col}$ value of Bronze Age humans at Tell Qaramel was −20.5±0.1‰ (range between −20.6‰ and −20.3‰) and the mean $\delta^{15}N_{col}$ value was 8.2±0.6‰ (range between 7.5‰ and 9.2‰). Because Qaramel animal bones did not contain sufficient amounts of collagen or biologically biologically acceptable C/N ratios, I compared the Qaramel human data with those of Tell Ain el-Kerkh animals.

The Tell Qaramel human skeletons were preserved according to two types of burial practices (primary burials *vs.* skull removal from the primary burials; Kanjou, 2009; Kanjou et al., 2013). A comparison of the isotopic results shows that nitrogen values were not significantly different (Mann–Whitney U test: U = 3, p = 0.51, primary: 8.2±1.1‰ and skull: 9.1±0.7‰) but that carbon values were significantly different (U = 0, p = 0.046, primary: −19.5±0.1‰ and skull: −19.6±0.3‰) for samples representing the two burial practices.

3.3.4. Human isotopic compositions and radiocarbon dates at Tell Ain el-Kerkh

I determined the carbon and nitrogen stable isotopic compositions of six human bone samples and radiocarbon dates for seven human bone samples from the PPNB layer at Tell Ain el-Kerkh. Of the 63 human bones in the PN layer at the same site, 47 were analyzed for carbon and nitrogen isotopic compositions. And then 17 samples were measured by radiocarbon dating. None of the cremated skeletons contained sufficient collagen required for measurements.

The PPNB human bones were dated at 7500–6800 cal BC, which is in close agreement with previous charcoal dates from the late PPNB (Tsuneki, 2012). Human remains from the PN cemetery were dated to 6450–5650 cal BC. These results show that the layers 6 and 5 are of middle PN, and that the layers 4–2 are of late PN period (Tsuneki, 2012).

The $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ values of all humans are shown in Figure 3.1 and Tables 3.3. The mean $\delta^{13}C$ value of PPNB samples at Tell Ain el-Kerkh was −20.0±0.2‰, and the mean $\delta^{15}N_{col}$ value was 8.7±0.9‰. In addition, the mean $\delta^{13}C_{col}$ value of PN samples was −20.1±0.6‰, and the mean $\delta^{15}N_{col}$ value was 7.9±0.8‰.

3.3.5. Human isotopic compositions at Hasankeyf Höyük

Fifteen out of 34 human individuals from PPNA layer at Hasankeyf Höyük show biological C/N ratios, 2.9–3.6 (Table 3.3, Fig. 3.2). The $\delta^{15}N_{col}$ values of PPNA humans range from 7.4 to 9.7‰ with the mean of 8.6±0.8‰. The $\delta^{13}C_{col}$ values of PPNA humans range from −20.2 to −19.0‰ with the mean value of −19.5±0.3‰.

In contrast, five human individuals from Iron Age layer at Hasankeyf Höyük show biological C/N ratios (Table 3.3, Fig. 3.2). The $\delta^{15}N_{col}$ values of Iron Age humans range from 6.2 to 7.4‰ with the mean of 6.7±0.5‰. The $\delta^{13}C_{col}$ values of Iron Age humans range from −20.0 to −19.0‰ with the mean value of −20.0±0.5‰.

3.3.6. Human isotopic compositions at Hakemi Use

Seventeen out of 19 human individuals from Hakemi Use show biologically acceptable C/N range (Table 3.3, Fig. 3.2).

The $\delta^{15}N_{col}$ values of humans range from 5.8 to 11.2‰ with the mean of 7.4±1.3‰. The $\delta^{13}C_{col}$ values of humans range from −20.2 to −19.2‰ with the mean value of −19.7±0.3‰.

3.4. Discussion

3.4.1. Temporal changes of human diet in the northern Levant

Isotopic analyses of charred cereals from archaeological sites in Syria have shown that $\delta^{15}N$ values of cereals before cultivation were higher than those after cultivation (Araus et al. 2014). The mean $\delta^{15}N$ value of emmer grains from the PN layer at Tell Ain el-Kerkh (2.2±0.6‰) was lower than that for einkorn at Tell Qaramel (6.1‰), but similar to the value in durum (*Triticum durum*) from the Tilbeshar Bronze Age site (1.7‰) (Araus et al. 2014). Thus, the differences in the $\delta^{15}N$ values of consumed plants between sites might have affected human $\delta^{15}N$ values.

At Pınarbaşı, an Epipaleolithic site in central Anatolia, the $\delta^{15}N_{col}$ value of inhabitants was approximately 15‰, and these inhabitants are considered to have consumed abundant fresh water foods (Baird et al. 2013). For reference, fish remains had isotopic composition approximately 10‰ in nitrogen and −21‰ for carbon at early Neolithic Boncuklu Höyük located in Central Anatolia (Baird et al., 2013). Despite the findings of fish, turtle and mollusc remains at Tell Qaramel and Tell Ain el-Kerkh, Neolithic inhabitants had the $\delta^{15}N_{col}$ values lower than Pınarbaşı and freshwater resources were not an important food source of those sites.

As another possibility, the variation of human nitrogen isotopic compositions might be caused by a difference of animals consumed intensively. Faunal $\delta^{15}N_{col}$ values at Tell Ain el-Kerkh were highly variable among different herbivore species (Table 3.3 and Fig. 3.1). Therefore, the higher $\delta^{15}N$ values of some individuals could be accounted for not only by increased meat consumption but also by more intensive consumption of cattle.

In the result of temporal comparison, the $\delta^{15}N_{col}$ values of PPNB and PN individuals at Tell Ain el-Kerkh showed no significant differences (U = 72, p = 0.06), and no significant differences in $\delta^{15}N_{col}$ values were observed between individuals at Tell Qaramel and Tell Ain el-Kerkh (PPNA *vs.* PPNB: U = 16, p = 0.74; PPNA *vs.* PN: U = 75, p = 0.07; see Fig. 3.3).

Then, I compared isotopic data among sub-layers of the PN at Tell Ain el-Kerkh. The $\delta^{15}N_{col}$ values of layers 5

– 6 were significantly lower than the values observed in other layers (layers 5 – 6 $vs.$ layers 2 – 3: p = 0.004; layers 5 – 6 $vs.$ layers 4 – 5: p = 0.0007; layers 5 – 6 $vs.$ layer 6: p = 0.029; Fig. 3.4). Similarly, individuals from layers 5 – 6 show $\delta^{13}C_{col}$ values lower than those of layer 6 (p = 0.007). However, the differences in the $\delta^{13}C_{col}$ values among other layers are not significant. Altogether, the differences in the $\delta^{15}N_{col}$ values of sub-layers within the PN layer at Tell Ain el-Kerkh are larger than those among the PN, PPNB at Tell Ain el-Kerkh and PPNA at Tell Qaramel.

Despite variations in styles of subsistence, no significant differences were observed in the $\delta^{15}N_{col}$ values of human remains from the PPNA layer at Qaramel and the PPNB and PN layers at Tell Ain el-Kerkh. In contrast, though the fact that cultural characteristic were not substantively different among sub-layers of the PN at Tell Ain el-Kerkh (Akira Tsuneki, $pers.\ comm.$), the differences in the $\delta^{15}N_{col}$ values among sub-layers within the PN are larger than those among the PPNA, PPNB and PN layers. Furthermore, inhabitants of Tell Qaramel during the Bronze Age (8.2±0.6‰) have $\delta^{15}N_{col}$ values as high as inhabitants of Tell Qaramel during the PPNA. Based on the isotopic compositions of bone collagen, the variations in diet associated with subsistence during the Neolithic period were perhaps smaller than those associated with short-term trends among PN inhabitants in the northern Levant.

3.4.2. Temporal changes of human diet in the southeast Anatolia

The inhabitants at Hasankeyf Höyük have the $\delta^{15}N_{col}$ (8.6±0.8‰) lower than cattle (10.5‰). It probably indicates that cattle were rarely consumed at Hasankeyf Höyük. The species with low $\delta^{15}N_{col}$ values (Table 3.3), were important as terrestrial animal protein to inhabitants at Hasankeyf Höyük.

Despite the findings of a large quantity of fish remains at Hasankeyf Höyük, the $\delta^{15}N_{col}$ of humans are not so sufficiently high to indicate a significant contribution of freshwater resources to their diet. Similarly, at Körtik Tepe, a neighbor Neolithic site of Hasankeyf Höyük, the collagen $\delta^{15}N$ values of human did not indicate significant contribution of freshwater resources in diet, despite of the large quantity of fish bones from this site (Coşkun et al., 2010). Furthermore, at Aktopraklık in northwest Anatolia, human $\delta^{15}N_{col}$ evidence (~9.4‰) did not indicate significant consumption of freshwater resources during the late Neolithic and Chalcolithic periods,

despite that this site was near by both freshwater and marine environments (Budd et al., 2013). If freshwater resources did not contribute considerably, the nitrogen isotopic composition of humans is interpretable whether the presence or absence of consumption of freshwater resources in southwest Asia.

Next, because individuals at Hasankeyf Höyük show $\delta^{13}C_{col}$ values lower than $-18‰$, they might have consumed little or no C_4 plant. Similarly, at Hakemi use, inhabitants might have consumed little or no C_4 plant. In addition, marine resources made quite small contribution to human diets at Hasankeyf Höyük and Hakemi Use, because marine resources alike are distinguished from terrestrial food chain by $\delta^{13}C$ value.

The $\delta^{15}N_{col}$ values of humans from Hasankeyf Höyük (8.6±0.8‰) were higher than those of early farmers of PPNB at Çayönü Tepesi (6.0‰; Pearson et al. 2013) and Nevalı Çori (6.6‰; Lösch et al. 2006) in southeast Anatolia. Also, farmers at Hakemi Use have the $\delta^{15}N_{col}$ (7.4±1.3‰) lower than hunter-gatherers at Hasankeyf Höyük, though two individuals have $\delta^{15}N_{col}$ as high as Hasankeyf Höyük. Furthermore, inhabitants of Hasankeyf Höyük during the Iron Age (6.7±0.5‰) have $\delta^{15}N_{col}$ values lower than inhabitants of PPNA at Hasankeyf Höyük. In addition, the $\delta^{15}N_{col}$ of each animal from these sites were similar to each other (e.g. goat; 6.0‰ at Hasankeyf Höyük, 5.3‰ at Çayönü Tepesi, 5.9‰ at Nevalı Çori). If interpreting these results according to principle of isotopic fractionation, the carnivory of early farmer seem to have declined from hunter-gatherer in the southeast Anatolia.

3.4.3. Regional difference of temporal dietary changes of Neolithic southwest Asia

The differences in the $\delta^{15}N_{col}$ values of humans in Anatolia could be explained by differences in the compositions of consumed plants and animal species in previous studies (Richards et al., 2003a; Lösch et al., 2006; Pearson et al., 2010, 2013; Budd et al., 2013). Also, in Neolithic central Anatolia, the $\delta^{15}N_{col}$ values of PPNB humans tended to be lower than those of late PN humans (Richards et al. 2003a; Pearson et al. 2010). Actually, cattle represented 9% of the mammal fauna at PPNA of Aşıklı Höyük (Baird, 2012), whereas in PN of Çatalhöyük, cattle represented 20% of the mammal fauna (Russell et al., 2005).

To compare this result with previous researches on Neolithic southwest Asian humans and to explore the trends through time, I have plotted the nitrogen isotopic compositions of humans from ten sites (Fig. 3.5).

Interestingly, the $\delta^{15}N_{col}$ values of humans in the PPNA layer at Tell Qaramel and the PPNB layer at Tell Ain el-Kerkh both in Levant are higher than those from the PPNB sites in Anatolia (Fig. 3.5). Thus, to compare of carnivory among humans of each sites, I require the calculation of the Fa_{col}. However, the Fa_{col} of an individual who have similar $\delta^{15}N_{col}$ values is estimated varying percentage based on $\delta^{15}N_{col}$ of animal consumed as animal proteins in the calculation. According to zooarchaeological studies, the excavated animal remains differed in composition of species by sites (Yano, 2000; Russell et al., 2005; Grezak, 2009; Baird, 2012; Omar, 2013).

Indeed, the diet of the PPN Levantine inhabitants seems to be apparently more carnivorous than that of the Anatolian PPN inhabitants based on the $\delta^{15}N_{col}$, but these high $\delta^{15}N_{col}$ of Levantine inhabitants might be due primarily to the consumption of cattle having higher $\delta^{15}N_{col}$ than other herbivores. In fact, large ruminants constituted 37% of the faunal remains at Tell Qaramel, the majority of which were aurochs (Grezak, 2009). Likewise, cattle were important livestock since the beginning of herding at Tell Ain el-Kerkh; the cattle to animal remain ratios were 25% in the PPNB and 35% in the PN (Yano, 2000). Furthermore, at Tell Aray, a PN site located 12 km from Tell Ain el-Kerkh, cattle were considered the most important component of the animal fauna (Hongo, 1996). The higher $\delta^{15}N_{col}$ values observed at the northern Levant sites indicate that cattle were important providers of meat, as were sheep, goats and pigs at these sites. By contrast to PN inhabitants of Tell Ain el-Kerkh and Çatalhöyük, those of Hakemi Use have $\delta^{15}N_{col}$ as lower as PPNB inhabitants in southeast Anatolia. Thus, these isotopic compositions are consistent to archaeological aspects, because the cattle remains were rarely excavated from Hakemi Use (Omar, 2013). At Hasankeyf Höyük, also cattle probably were rarely consumed as at Hakemi Use.

Hence, so the difference of the $\delta^{15}N_{col}$ among Neolithic sites in southwest Asia is decided by not only the contribution of terrestrial animal protein, but the difference of animal species mainly consumed. Therefore, even if the $\delta^{15}N_{col}$ of humans varied according to periods, it does not necessarily indicate the change of consumption rates of terrestrial animal proteins between communities. Though temporal changes of $\delta^{15}N_{col}$ during Neolithic period differ by regions in southwest Asia, it is difficult to determine the factor of the difference on the basis of only carbon and nitrogen isotopic compositions of collagens.

3.5. Conclusion

The carbon and nitrogen isotopic compositions of collagens provide new insights into the dietary shifts associated with Neolithisation, which showed substantial variations among different regions of the southwest Asia. These regional differences were probably related to local adaptations of subsistence styles to regional environmental conditions prior to Neolithisation. However, only by carbon and nitrogen isotopic compositions it may be insufficient to elucidate about dietary resources of Neolithic humans, because consumable food resources around this inland Neolithic settlement were limited to terrestrial resources, and perhaps freshwater resources. Hence, we need to both clarify the quantity of animal protein in the diets and identify main source animal from terrestrial resources such as livestock in inland communities such as Neolithic southwest Asia on the basis of different biological index.

4. Nitrogen Isotopic Compositions in Amino Acid in Relation to Large Variability among Herbivore Collagen

4.1. Introduction

In the case of Neolithic southwest Asia significant differences in the $\delta^{15}N$ values of bulk bone collagen among different herbivore species from a single site have been frequently observed. Then, it is thought that the primary decision factors of the variations in the $\delta^{15}N_{col}$ of terrestrial mammalian herbivores are two main hypotheses; (1) the difference in the isotopic composition of fed plant according to soil nitrogen and water conditions in the habitat and (2) the difference of nitrogen metabolism among animal species. For instance, herbivores in hot and arid environments often have $\delta^{15}N_{col}$ values higher than herbivores in temperate environments, despite similar trophic position (e.g. Heaton et al, 1986).

In Hartman (2011), because the $\delta^{15}N$ of herbivores showed a positive correlation with rainfall and the $\delta^{15}N$ values of plants, the primary factor affecting $\delta^{15}N_{col}$ of terrestrial herbivores was interpreted as $\delta^{15}N$ of plants as forage, not metabolism of animals. Certainly, $\delta^{15}N$ values of plants show varied distribution of 10‰ according to aridity (Heaton, 1987; Hartman and Danin, 2010) and the concentrations of inorganic nitrogen in soil (Hobbie et al., 2000). Furthermore, in Syrian Neolithic sites, cereal grains from sites those beside Euphrates had $\delta^{15}N$ higher than that from sites far from river (Araus et al., 2014). Thus, if each herbivore species ate plants in different specific environments, the $\delta^{15}N$ of forage might be the cause of the difference of the $\delta^{15}N_{col}$ among species excavated from a same site.

By contrast to environmental factor, other studies explained the $\delta^{15}N$ of herbivores as the result of a physiological response in animals such as water stress and assimilation of protein. The $\delta^{15}N$ value of urea in herbivore's urine is lower than that of the forage of herbivores (Steele & Daniel, 1978). Therefore, excretion of urea is thought one of a cause of elevation of $\delta^{15}N_{col}$ values (Ambrose, 1991). Ambrose and DeNiro (1986) deduced that mammalian herbivores adapting to arid environments excrete more ^{15}N-poor urea in urine to conserve water and increase urine osmolality. Thus, in an arid environment these herbivores adapting to arid environment are thought to have $\delta^{15}N_{col}$ higher than other herbivores. Also, in temperate and wet environment their $\delta^{15}N_{col}$ should be similar to other sympatric herbivores. For instance, kangaroos in Australia showed a

species-specific difference of the $\delta^{15}N_{col}$ in addition to a correlation between $\delta^{15}N_{col}$ and rainfall (Gröcke et al., 1997).

Besides, Fizet et al. (1995) suggested the cause of the variation in the $\delta^{15}N_{col}$ among ancient herbivores as a difference of excretion of urea according to a content rate of protein in the forage. In controlled feeding study, herbivore individuals fed high-protein (19%) forage showed larger ^{15}N-enrichment than herbivores fed low-protein (9%) forage (Sponheimer et al., 2003b). Thus, the high $\delta^{15}N_{col}$ of bovid was attributed to a selective consumption of high-protein plants by bovid in Marillac, a Late Pleistocene site located in France (Fizet et al., 1995), because bovid need high-protein diet to survive compared to equid (Guthrie, 1982). Therefore, a protein selective feeding by each species might trigger the variation in the $\delta^{15}N_{col}$ of herbivores in the same environment.

On the other hand, when receiving the same forage, both high (19%) and low (9%) protein forages, each species had different ^{15}N-enrichments (Sponheimer et al., 2003b). Especially in ruminants, a part of urea is returned to the rumen for microbial activity to synthesize bacterial protein (Schmidt-Nielsen et al., 1957). Then the bacterial proteins are assimilated again by host animal. If the degrees of urea recycling differ by species when exposed to low protein diets, urea recycle may raise the interspecies difference of the $\delta^{15}N_{col}$ (Sealy et al., 1987).

Also, because ruminants assimilate directly not plant organs but rather bacterium assimilating plants in rumen, ruminants consuming rumen bacterium might not be veritable herbivore (Wattiaux and Reed, 1995; Hristov, 2002). In this case, ruminants such as cattle and goat are thought to have the $\delta^{15}N_{col}$ and trophic position higher than anti-ruminant, like as omnivore and carnivore. Furthermore, the $\delta^{15}N_{col}$ might be varied according to efficiency of ruminant activity among ruminants.

Probably, either the environment of habitat or the metabolisms of animal are capable of becoming a reason of the variability of $\delta^{15}N_{col}$ among sympatric animal species in principle. So I used the herbivore samples to attempt to resolve the actual cause of the differences in the $\delta^{15}N$ values of bulk collagen among the different species in the Neolithic southwest Asia. Thereby, I validate the wide distributions of nitrogen isotopic composition among herbivores by three archaeological ruminants (cattle, sheep and goats) from the Neolithic Tell Ain el-Kerkh site in Syria. The isotopic analysis of amino acids directly verifies whether these herbivores lived in isotopically

distinct environments, or were characterized by different ^{15}N-enrichment factors. Moreover, the observed results will provide an opportunity to further evaluate the method of using amino acid δ^{15}N data for reconstructing ancient diets.

4.2. Materials

Tell Ain el-Kerkh is a Neolithic settlement site located 75 km southwest of Aleppo, northwest Syria (Fig. 1.1). At the site, remains of animals such as domesticated cattle, sheep, and goats, and several wild animals have been collected from late Neolithic layers (Tsuneki et al., 2006). This study examined faunal bone collagen from three domestic herbivorous animals: cattle (Cattle, n = 3), sheep (Sheep, n = 2), and goats (Goat, n = 3); the specimens were excavated from a site which is dated to the PN period (6600–6100 cal BC) (Tsuneki, 2010).

I measure the δ^{15}N values of five amino acids (glycine, proline, glutamic acid, phenylalanine, and hydroxyproline), because these amino acids are relatively abundant in the collagen (Eastoe, 1955) and always show baseline resolution on GC–C–IRMS chromatograms.

4.3. Results

The δ^{15}N values of bulk collagen were 7.5±0.7‰ (mean ± 1σ) for cattle, 4.3±1.2‰ for goats, and 6.3±0.8‰ for sheep (Table 4.1). The Kruskal–Wallis test to compare the δ^{15}N values of each animal suggested a significant difference (p-value < 0.05) in the δ^{15}N values of bulk collagen among cattle, sheep, and goats (χ^2 = 6.25, p = 0.044). These results support previous findings. For example, it was reported from other Neolithic sites in the southwest Asia that cattle bone collagens are enriched in δ^{15}N relative to values in caprine animals (Lösch et al., 2006; Pearson et al., 2007). Also, each herbivore from PN layer showed no difference in the same species from PPNB layer at Tell Ain el-Kerkh (Table 3.1), despite around a thousand year difference. I conclude therefore that the δ^{15}N values of bulk collagen in herbivores in a closed region may exhibit a specific ordered relationship with some factor.

I determined the nitrogen isotopic composition of a series of amino acids to confirm whether the herbivores exhibit large differences in the δ^{15}N values of different amino acids (Table 4.1). The largest variation in δ^{15}N

values was observed in glycine of cattle (6.6 ± 0.5‰), followed by those of sheep (4.6±0.7‰) and those of goats (2.1±1.2‰); this pattern is similar to that observed in bulk collagen values. I conclude that the $\delta^{15}N$ values of glycine are significantly different among cattle, sheep, and goats (χ^2 = 6.25, p = 0.044). In contrast, the $\delta^{15}N$ values of other amino acids, such as glutamic acid and phenylalanine, exhibit low variability (7.4‰−9.4‰ and 9.6‰−10.1‰, respectively). Proline and hydroxyproline show overlapping $\delta^{15}N$ ranges in the different herbivores, suggesting no significant differences in the $\delta^{15}N$ values of these animals. Thus, not all amino acids exhibit large differences in $\delta^{15}N$ values in the different herbivores.

Despite the large differences in the $\delta^{15}N$ values of bulk collagen in different herbivores, the TP_{ter} values of the herbivores were similar, and were estimated to be 2.1±0.0 for cattle, 2.0±0.1 for sheep, and 2.1±0.1 for goats (Fig. 4.1); this pattern is consistent with the expected trophic position of these herbivores as certain primary consumers (i.e., TP = 2.0). Moreover, the $\delta^{15}N$ values of phenylalanine were similar amongst these herbivores (Table 4.1). Because the $\delta^{15}N$ values of phenylalanine show little variation with respect to the trophic position of animals (Chikaraishi et al., 2010), the results indicate that these herbivores likely consumed feeds with similar $\delta^{15}N$ values. This finding is consistent with the observation that these herbivores were domestic animals raised around a settlement. Thus, small variations observed in the $\delta^{15}N$ values of phenylalanine among cattle, sheep, and goats suggest that large differences in the $\delta^{15}N$ values of bulk collagen among herbivores are not caused by isotopically different feed sources.

The relative abundances of glycine, proline, glutamic acid, phenylalanine, and hydroxyproline were 26.2, 10.1, 5.9, 1.2, and 8.2 wt%, respectively, and these amino acids account for more than a half of the nitrogen in bone collagen (Eastoe, 1955). The difference between the $\delta^{15}N$ value of bulk collagen and that of the weighted mean of the five amino acids was 0.7±0.7‰ (Table 3.1), and these values showed a positive correlation (slope = 0.86, R^2 = 0.84). However, among these five amino acids, the strongest positive correlation was observed between the $\delta^{15}N$ values of bulk collagen and glycine (slope = 1.2, R^2 = 0.87; Fig. 3.2a). On the other hand, the $\delta^{15}N$ values of proline and hydroxyproline were only weakly correlated with that of bulk collagen (R^2 = 0.27 for proline and R^2 = 0.46 for hydroxy-proline; Fig. 4.2b). The $\delta^{15}N$ values of glutamic acid were weakly correlated with those of bulk collagen (R^2 = 0.40; Fig. 4.2c), whereas the $\delta^{15}N$ values of phenylalanine showed no correlation with those

of bulk collagen ($R^2 = 0.07$; Fig 4.2c) and those of glycine ($R^2 = 0.00$). These patterns explain the peculiar differences in the $\delta^{15}N$ values of bulk bone collagen among the herbivores.

4.4. Discussion

The $\delta^{15}N$ value of bulk bone collagen could be strongly influenced by that of glycine, because glycine is the most abundant amino acid, normally accounting for 1/4 to 1/3 of the amino acids in bone collagen (e.g., Eastoe, 1955). Additionally, because the difference of the $\delta^{15}N$ value in bulk collagen among herbivores is larger than that in the weighted mean of the five amino acids, there may be the other amino acid which shows the larger difference of the $\delta^{15}N$ value.

In contrast, because the $\delta^{15}N$ of phenylalanine little changes (0.4‰) in connection with prey and assimilation, these ruminants showing the similar $\delta^{15}N_{Phe}$ and also $\delta^{15}N_{Glu}$ seem to have consumed forages which had a similar $\delta^{15}N$ on average at Tell Ain el-Kerkh, despite the large difference of $\delta^{15}N_{col}$. Therefore, the fact that ruminants assimilate rumen bacterium seems to cause no elevation of trophic position for ruminant, contradicting a deduction of Hristov (2002). Also, the difference of $\delta^{15}N$ among plants consumed by each ruminant according to the environments was not the main reason of the variations of $\delta^{15}N_{col}$ among herbivores in this site, in contrary to the result of Hartman (2011). Though the difference of $\delta^{15}N$ among plants according to the environments might be a potential cause of the difference of herbivore $\delta^{15}N_{col}$ among sites, it is not a cause of intra-site difference among species. As these herbivores were identified to livestock, the environment of the pasture area of each species might have been similar.

Although specific mechanisms controlling the $\delta^{15}N$ values of glycine from animals are unclear (e.g. tolerance of aridity, protein quantity of preferred plants) at this stage, I consider that the $\delta^{15}N$ value of glycine may mirror differences in the physiological pathways of glycine among these herbivores. According to previous studies (Ambrose and DeNiro, 1986; Ambrose, 1991; Gröcke et al., 1997; Sponheimer et al., 2003b), the difference of urea excretion among herbivores may be a suspected cause of the inter-species difference in $\delta^{15}N$ of glycine and collagen. As other hypotheses, the inter-species difference in synthesis of hippuric acid may be one of causes for the difference in $\delta^{15}N$ of glycine, because benzoic acid is drained in the form of hippuric acid through glycine

conjugation from the mammal body (Bender, 2014).

Besides, the $\delta^{15}N$ of glycine may become the index to identify human diets. Because the muscle tissue differs in amino acid composition from collagen, the difference of bulk $\delta^{15}N$ of muscle tissue among herbivores may be not as large as that of collagen. By contrast, it is thought that the $\delta^{15}N$ of glycine in muscle tissue is little differentiated from that in collagen, though the turnover of collagen takes a different amount of time to that of muscle tissue as edible part. Therefore, the difference in $\delta^{15}N$ of glycine among herbivores as livestock is possible cause of the $\delta^{15}N_{col}$ variations in humans. In addition, despite non-essential amino acid, glutamic acid shows a constant $\Delta^{15}N_{diet-predator}$ among whole organism (Chikaraishi et al., 2010). Hence, *salvage* pathway may be more dominant than *de novo* pathway as amino acid metabolism of glutamic acid *in vivo*. This situation may be same with amino acid metabolism of glycine.

4.5. Conclusion

At Tell Ain el-Kerkh, the $\delta^{15}N$ values of bulk bone collagen from archaeological herbivores probably reflect differences in the ^{15}N-enrichment factor among the herbivores rather than $\delta^{15}N$ variations of feed plants in the foraging area. The isotopic signatures of feed plants in several growth areas for the herbivores are well preserved in the $\delta^{15}N$ values of phenylalanine, even if the $\delta^{15}N$ values of bulk collagen and glycine are highly variable.

Studies of the diets of ancient herbivores have revealed differences in the $\delta^{15}N$ values of bulk bone collagen among different herbivore species, especially in animals consuming mainly terrestrial resources. Although further studies need for a large number of sample sets from different sites, the observed data at Tell Ain el-Kerkh demonstrated that a peculiar difference in the $\delta^{15}N$ values of bulk collagen among archaeological herbivores can be ascribed mainly to variations in the ^{15}N-enrichment factors of several specific amino acids, such as glycine, rather than to isotopic variations of feed plants in the growth environment. In contrast, the $\delta^{15}N$ values of relatively minor amino acids, such as glutamic acid and phenylalanine, well preserved the information of the trophic position of the animals. Thus, the $\delta^{15}N$ values of bone collagen are influenced by at least three different factors: physiological, environmental (i.e., by feed plant $\delta^{15}N$ values), and ecological setting (i.e., the trophic position of the animals). Moreover, the nitrogen isotopic composition of the different amino acids can resolve the

relative contributions of each of these factors. The nitrogen isotopic composition of individual amino acids enhances the resolution and reduces the uncertainty in assessing the diets and life styles of ancient humans and animals. Furthermore, the differences in the $\delta^{15}N$ values of bulk collagen and the ^{15}N-enrichment factors among mammal herbivores may be useful as potential tracers in the terrestrial food web.

5. Combination of Isotopic Analyses to Estimate Carnivory and Identify Mainly Consumed Species at Tell Ain-el Kerkh

5.1. Introduction

By recent research, a Pottery Neolithic (PN) cemetery has been excavated adjacent a habitation area of a north Levantine site, Tell Ain el-Kerkh (Tsuneki, 2011). Interestingly, these burials were not located evenly in the area of cemetery, but were concentrating to several locations within the cemetery (Fig. 5.1). Probably, the buried locations of each individual seem to have been decided for some reason, such as household, labor and status, in this cemetery. On the other hand, all ages from pre-birth to 50s and both sexes were buried to the same locations (Tsuneki, 2011). It was considered these lumped skeletons were not buried at the same time, but intermittently to the same place, as a large number of primary burials at different timing were included in a close location. And because articulated bones were found among secondary buried bones, interments to the same place were repeated in a short span of time.

The characteristics of PN cemetery at Tell Ain el-Kerkh were quite different from PPN graves within the house, and perhaps indicated the shift of burial place from private house to public space. On the other hand, many stamp seals as personal property were found with most members of the cemetery, and hence it is indicated that the concept of proprietorship had become common among the later Neolithic people (Tsuneki, 2011). The ownerships would have become less sharing by the entire community, and economic independence of each person and/or household perhaps had become strong during the late PPNB and PN (Byrd et al., 2000; 2005; Kadowaki, 2012). Thus, this communal cemetery had features of public and even private space. Accordingly, it should determine whether the advent of communal cemetery in the Neolithic society was an accommodation to demand unification of a large community, or a result by divide of the community.

As mentioned above, I report carbon and nitrogen stable isotopic compositions of humans buried in the PN cemetery of Tell Ain el-Kerkh. Individuals of layer 4 were showed wide distribution of carbon and nitrogen isotopic compositions of collagen, and this might be caused by the difference of consumption of species and quantities of meats among individuals. However the $\delta^{15}N_{col}$ values do not exhibit significant differences by gender, and by graves with or without burial goods. The difference of those characteristics and diet did not show

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any correlation in Pottery Neolithic Tell Ain el-Kerkh.

However, only by carbon and nitrogen isotopic compositions it may be insufficient to elucidate about dietary bias within the cemetery and discuss about food sharing in the community, because consumable food resources around this interior Neolithic settlement were limited to inland resources. Hence, I try to both clarify the quantity of animal protein in the diets and identify main source animal from terrestrial resources such as livestock in inland communities such as Neolithic southwest Asia. Then, the resolution of the food distribution and social complexity within the cemetery of late Neolithic community, Tell Ain el-Kerkh by isotopic analysis of individual amino acids. I predict that the decrease of economic interdependence of whole community might be expressed as the difference of isotopic compositions among buried locations associated with households, even within the communal cemetery.

5.2. Material

In the cemetery of Tell Ain el-Kerkh, especially in layer 4, 33 human burials could be divided into 7 groups based on buried locations (Table 5.1). Additionally in this section, I measure also the nitrogen isotopic compositions of glutamic acid and phenylalanine for 14 individuals belong to 4 groups of different locations in layer 4 (Fig. 5.1). Besides, I analyze 12 animal bones including cattle, pigs, sheep, goats, and freshwater fishes (Cyprinidae) for ^{15}N of amino acids.

5.3. Results

5.3.1. Carbon and nitrogen isotopic compositions of bulk collagen

The $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ values of these humans and animals were reported in previous section. The summary of human groups and animals are shown in Table 5.1 and Fig. 5.2. The individuals of layer 4, an upper layer of PN layers, ranged wide variation for both carbon and nitrogen isotopic compositions. This is because each individual of layer 4 had various diets in similar time.

To interpret the wide range distribution of isotopic compositions of adults in layer 4, I considered that individuals from layer 4 are grouped on locations of burial. Human individuals of Tell Ain el-Kerkh are

separated into 7 groups as mentioned (Fig. 5.1). A part of individuals in Area 1, 2, 3, 4 and 5 were measured the radiocarbon age, and their dates were contemporary with each other (Table 5.1). The buried places of these groups were considered to have been used concurrently around the same time.

The $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ of adults had the similar isotopic signatures to each other within the each group almost individuals of other groups (Fig. 5.2), even though the ranges of $\delta^{15}N_{col}$ of each group are overlapped (Fig. 5.3). So as to validate the uniformity of the diets within the group, I compare the Euclidean distance of $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ between two individuals within each group from that among all individuals, based on the following calculation (Table 5.1, Fig. 5.4).

$$\text{Distance[A-B]} = \sqrt{(\delta^{13}C_{col}[A] - \delta^{13}C_{col}[B])^2 + (\delta^{15}N_{col}[A] - \delta^{15}N_{col}[B])^2}$$

In the result, the Euclidean distances of $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ between two individuals within each group are significant shorter (p-value < 0.05) than that among all individuals (two tailed Welch's t test: df = 51, $t = -2.9$, p = 0.0056). This result may indicate that individuals buried in close locations consumed foods having more similar isotopic compositions within the limited isotopic variations in inland environment. For that matter, it may suggest that they lived in a close relationship and had meals together regardless of sex, age and property. In addition, each animal resource perhaps consumed in this site was not divided equally for whole site, but varied quantity and/or species by each group.

Meanwhile, as breast-fed child being high trophic position relative to one's mother, young child $\delta^{15}N_{col}$ should be higher than that of its mother (Fuller et al., 2006, Tsutaya et al., 2013, Reynard and Tuross, 2015). Also $\delta^{13}C_{col}$ values show a similar change, approximately +1−2‰ (Richards et al., 2003). In layer 4, each young child (perinatal, infant, young-juvenile) has higher $\delta^{15}N_{col}$ relative to the mean $\delta^{15}N_{col}$ of adult of the same group (Table 5.1, Fig 5.3), regardless of hierarchy within entire layer 4. This result suggests that each young child was maintained breast-feeding from an individual having $\delta^{15}N_{col}$ similar to the adult in the same group.

5.3.2. Nitrogen isotopic composition of amino acids from fauna

At Tell Ain el-Kerkh, freshwater fish and terrestrial herbivores can be separate by nitrogen isotopic compositions of phenylalanine and glutamic acid (Fig. 5.5, Table 5.2). The $\delta^{15}N_{Phe}$ values of pigs at Tell Ain

el-Kerkh range from 8.5 to 12.4‰, and the $\delta^{15}N_{Glu}$ values ranging from 9.7 to 14.2‰. In contrast, the $\delta^{15}N_{Phe}$ values of other ruminants are similar and ranging from 8.9 to 9.7‰, and the $\delta^{15}N_{Glu}$ values are ranging from 7.4 to 9.7‰. The trophic positions estimates based on terrestrial equation of the ruminants were very similar (cattle 2.1, sheep 2.1, goats 2.0; Fig. 4.1). Despite of varied $\delta^{15}N$ values of bulk bone collagen among terrestrial animal, this pattern is consistent with the expected trophic position of these ruminants as certain primary consumers. As pig is omnivorous animal, the trophic positions of pigs 2.2 was reasonable.

By contrast, two fishes were identified as Cyprinidae, and there $\delta^{15}N$ of amino acids ($\delta^{15}N_{Phe}$ = 3.1‰, $\delta^{15}N_{Glu}$ = 21.0‰) are completely different from terrestrial animals. The mean trophic positions of these freshwater fishes estimated based on aquatic equation is 2.9, suggesting primary carnivores.

5.3.3. Nitrogen isotopic composition of amino acids from humans

The $\delta^{15}N_{Phe}$ values of individuals at Tell Ain el-Kerkh range from 9.2 to 14.3‰, and the $\delta^{15}N_{Glu}$ values range from 11.7 to 17.1‰ (Fig. 5.5, Table 5.3).

Based on the $\delta^{13}C$ of collagens, these individuals may not have consumed C_4 plants but mainly C_3 plants at the studied period. Hence I evaluate their tropic level by calculation of TP_{ter}. The trophic positions of individuals based on terrestrial ecosystem are range from 2.22 to 2.58. Their $\delta^{15}N_{Phe}$ and TP_{ter} could be explained by only the terrestrial resources, both some C_3 plants and meats of terrestrial animals. If any, the contributions of freshwater resources were very limited for the diets of inhabitants at Tell Ain el-Kerkh at PN period. Although, it was little known about how many freshwater fish they ate by conventional isotopic analysis of collagen. Yet these early farmers seem to have depended largely on terrestrial food resource, perhaps raised livestock by themselves according to amino acids analysis.

Interestingly, the young-juvenile at 1.5 years old shows the higher $\delta^{15}N_{Phe}$ relative to others like isotopic compositions of collagen, in contrast the TP_{ter} is similar to others. The mother of this young-juvenile may have had lower meat consumption than other adults. Then, infant and perinatal show almost similar $\delta^{15}N_{Phe}$ from adults. However, perinatal show similar TP_{ter} to others, whereas infant have TP_{ter} lower than adults. In dietary reconstruction by the $\delta^{15}N$ of phenylalanine and glutamic acid, it would appear that newborn child show a different tendency, alike as that by collagen analysis.

5.4. Discussions

5.4.1. Estimation of carnivory

So as to elucidate the carnivory of inhabitants at Tell Ain el-Kerkh, I estimate the fraction animal protein in total terrestrial protein based on $\delta^{15}N$ of amino acids (Fa_{AA}) of individual by following calculation of TP_{ter} (see Naito et al., 2013; Styring et al., 2015). Because the TP_{ter}[animals] at Tell Ain el-Kerkh range from 2.0 of goats and 2.2 of pigs, the estimated Fa_{AA} of each human at Tell Ain el-Kerkh is showed range.

$$Fa_{AA} (\%) = (TP_{ter}[human] - 2)/(TP_{ter}[animals] - 1) \times 100$$

Because ruminants consumed by humans as animal protein show similar TP_{ter} despite large difference of the $\delta^{15}N_{col}$ (Fig. 4.1), this calculation allows precise Fa_{AA} available in this site (Table 5.3). By nitrogen isotopic analysis of individual amino acids, the accurate proportion of terrestrial animal protein to total protein even of the Neolithic southwest Asian is able to be estimated.

Meanwhile, bulk bone collagen $\delta^{15}N$ value is averaged the $\delta^{15}N$ values of each amino acid, but the value is affected mainly by that of glycine (Fig. 4.2a; Table 4.2). Hence, the $\delta^{15}N_{col}$ has information different from both $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$. Thus, to compare with Fa_{AA}, I estimate the Fa by $\delta^{15}N_{col}$ (Fa_{col}) (Minagawa and Wada, 1984; Post et al., 2002; Hedges and Reynard, 2007; Phillips, 2012; Styring et al., 2015). I use two calculations from the $\delta^{15}N_{col}$; (i) according to the $\delta^{15}N_{col}$ values of terrestrial animals and (ii) according to the $\delta^{15}N$ values of both collagen of terrestrial animals and cereal grains. Then I considered that the isotopic enrichment factor from diet to consumer ($\Delta^{15}N_{consumer–diet}$) is +3.4‰ for bulk collagen (Minagawa and Wada, 1984).

$$Fa_{col} (\%) = \frac{(\delta^{15}N_{col}[human] - \delta^{15}N_{col}[herbivore])}{\Delta^{15}N_{consumer-diet}} \quad \text{(i)}$$

$$Fa_{col} (\%) = \frac{(\delta^{15}N_{col}[human] - \Delta^{15}N_{consumer-diet}) - \delta^{15}N[cereal]}{\delta^{15}N_{col}[herbivore] - \delta^{15}N[cereal]} \quad \text{(ii)}$$

If the $\delta^{15}N$ values of plants consumed by human were similar to that by herbivore, the result of calculation (ii) is accorded with the result of calculation (i). In this study, I use the $\delta^{15}N$ values of wheat grains excavated from Tell Ain el-Kerkh (2.2‰) as the $\delta^{15}N$ [cereal] (Table. 2.2) for calculation (ii). As a result, the individual's Fa_{col} of calculation (i) and (ii) are different each other (Table 5.4). This may be because the estimated $\delta^{15}N$ of animal's forage ($\delta^{15}N_{col}$ [animal] – 3.4‰) were higher than measured $\delta^{15}N$ of cereal consumed by human (2.2‰). At

Çatalhöyük, as the $\delta^{15}N$ of cereals consumed by humans were higher than that of the animal's forage, cereals were considered to be manured (Styring et al., 2015). By contrast, the $\delta^{15}N$ of cereals at Syrian farmer sites such as Tell Ain el-Kerkh became lower than Tell Qaramel, hunter-gatherer site neighbor upon Kerkh and this change was caused maybe by continuous cropping (Araus et al., 2014).

In addition, the Fa_{col} of a human individual who have similar $\delta^{15}N_{col}$ values is estimated different percentage based on $\delta^{15}N_{col}$[herbivore] in each calculation (Fig. 1.5). The $\delta^{15}N_{col}$ of human seem to include the difference of $\delta^{15}N_{col}$ among herbivores as a factor in addition to trophic position. Thus in the case a Fa_{col} according to an animal species is most well accorded with Fa_{AA}, this species may be most contributed meat for each individual's diet. Though the results of Fa_{cal} by calculation (i) and (ii) are different and have a large margin of error, the combination of Fa_{AA} and Fa_{col} would help identification of animal consumed as meat. As a result, for many individuals, cattle and pigs are most contributing animals as food. By calculation (i), Fa_{col} based on pig are well accorded to Fa_{AA} for many individuals (Fig.5.6a). By contrast, Fa_{col} based on cattle are well accorded to Fa_{AA} by calculation (ii) (Fig.5.6b). Incidentally, from zooachaeological research pigs were important livestock next to cattle, the cattle was 35% and the pigs were 25% of the animal remains from the PN layers (Yano, 2000). The result combined of both Fa_{AA} and Fa_{col} is consistent with zooachaeological knowledge. By comparing the isotopic compositions especially Nitrogen of amino acids and collagen, species mainly consumed perhaps is able to be loosely identified in each site.

5.4.2. Social structure

Human individuals of Tell Ain el-Kerkh are separated into 9 groups according to locations buried in cemetery (Figs. 5.1, 5.2). In this study, I compared the isotopic composition of amino acids of 4 groups; Areas 1, 2, 3 and 4. The results of isotopic analysis for amino acids are biased according to groups of buried locations (Table 5.3), as with that for collagen (Table 5.1).

The individuals without infants from Area 1 have both bulk $\delta^{15}N_{col}$ and TP_{ter} (2.52±0.05) higher than individuals of other groups. By contrast, because an adult of Area 3 has similar $\delta^{15}N_{col}$ to those from Area 2 and 4, and lowest TP_{ter} in this site, their high $\delta^{15}N_{col}$ perhaps may be caused by the small consumption of herbivore

having high $\delta^{15}N_{col}$. And so the $\delta^{15}N_{Phe}$ is higher than other humans and herbivores, individual of Area 3 might have consumed food resources different from that consumed at others. In Area 4, the $\delta^{13}C_{col}$ of individuals show wide distributions, which is different from no other groups. However their $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ are similar to each other. Accordingly, their meat consumption rate might be less different at Area 4.

At Tell Ain el-Kerkh, the estimated TP_{ter} are inconsistent with relative position estimated by the $\delta^{15}N_{col}$ among groups. The $\delta^{15}N_{col}$ of individuals may be strongly influenced by the difference of animals mainly consumed. So, I estimate the mean of the $\delta^{15}N$ of terrestrial animal protein consumed by human on the basis of Fa_{AA}, the $\delta^{15}N_{col}$ of human and the $\delta^{15}N$ of cereal.

$$\delta^{15}N[\text{animal protein}] = Fa_{AA} \times (\delta^{15}N_{col}[\text{human}] - \Delta^{15}N_{\text{consumer-diet}}) - \delta^{15}N[\text{cereal}] \times (1 - Fa_{AA})$$

Even though estimated ^{15}N [animal protein] consistently shows the value mixing the $\delta^{15}N$ values of all protein sources, it is considered that estimated ^{15}N [animal protein] is strongly affected by the dominantly contributory animal protein. In the result, species having most optimum $\delta^{15}N_{col}$ are biased depending on the groups of location (Fig. 5.7), than the variations of Fa_{AA} within the each group. It perhaps indicate that individual of Area 3 mainly ate species with higher $\delta^{15}N_{col}$ than cattle, whereas pigs were precious food in addition to cattle for individuals of Area 1 and 2. However, infant of Area 3 is also estimated to ^{15}N [animal protein] higher than adult of Area 3 by 2.2‰. Probably not only an adult individual, individuals of Area 3 consumed food resources different from herbivores measured in this study. On the other hand, individuals of Area 4 might mainly have consumed sheep and pigs. Even though their Fa_{AA} are different within the group, mainly consumed animal might be similar.

This result indicates that the buried location was determined by kinship and/or household sharing subsistence. Furthermore if species mainly consumed as food resources in feeding community was differ by households, it may have been caused by the difference of species bred each household and flesh was perhaps not shared equally in whole community.

The bias of distribution of resources among groups at Tell Ain el-Kerkh might have derived from social behavior of individuals and/or sexual difference. According to archaeological aspects, it is thought that there was not hereditary social stratum established in the Pottery Neolithic period. However the division of subsistence among groups within the community had perhaps begun in the Neolithic period already, and it might have

developed successional social stratum in the following periods.

5.5. Conclusion

The nitrogen isotopic composition of the different amino acids allow us to resolve each of these factors previously indistinguishable, such as the quantity and the main species of animal protein at Neolithic Tell Ain el-Kerkh. Therefore the diets and life styles of ancient communities are reduced the uncertainty in assessing, more than ever can be assessed. In the cemetery at Tell Ain el-Kerkh, buried people had no difference in burial rituals among located groups, while they had diverse diets by the groups. This aspect might indicate the consuming activity of inhabitants was divided to several groups of equal status within the late Pottery Neolithic site. Through uniting personal information such as the burial rituals and the diet by isotopic analysis of archaeological remains, I may acquire ability to access early phase of social development ambiguous yet.

6. Amino Acid Analysis to Estimate Freshwater Fish Contribution at Hasankeyf Höyük and Hakemi Use

6.1. Introduction

In this section, I analyze humans and fauna from Hasankeyf Höyük, an early Neolithic hunter-gatherer site and Hakemi Use, a late Neolithic farmer site. These sites are situated beside the upper of Tigris in southeastern Turkey (Fig. 6.1). The interest here was the potential consumption of freshwater foods, as well as terrestrial food resources, by Neolithic community at this riverside environment in upper Tigris region before and after food production.

At Hasankeyf Höyük, the $\delta^{15}N_{col}$ of humans are not so sufficiently high to indicate a significant contribution of freshwater resources to their diet. Similarly, at Körtik Tepe, a neighbor Neolithic site from Hasankeyf Höyük, the $\delta^{15}N_{col}$ values of human did not indicate significant contribution of freshwater resources in diet, despite of the large quantity of fish bones from this site (Coşkun et al., 2010). Furthermore, at Aktopraklık in northwest Anatolia, human $\delta^{15}N_{col}$ evidence (~9.4‰) did not indicate significant consumption of freshwater resources during the late Neolithic and Chalcolithic periods, despite that this site was near by both freshwater and marine environments (Budd et al., 2013). By contrast, two individuals at Pınarbaşı, an Epipaleolithic site in Central Anatolia, showed high $\delta^{15}N$ values of collagen ~15‰, and were estimated that they consumed substantial amount of freshwater resource (Baird et al., 2013). Individual with +15‰ of $\delta^{15}N_{col}$ was estimated that they consumed 80% of protein from aquatic resources (Bonsall et al., 2004). If freshwater resources did not contribute considerably to human diet, it may be difficult to distinguish consumption of aquatic resource from terrestrial diets only by carbon and nitrogen isotopic analyses of collagen in Neolithic southwest Asia (Coşkun et al., 2010).

Therefore, I distinguish terrestrial and aquatic foods and estimate terrestrial animal protein intake ratio by analyzing individual amino acids. I aim at the resolution about the contribution of freshwater resources and food allocation in Neolithic communities at river side, Hasankeyf Höyük and Hakemi Use. The features of hunter-gatherers at Hasankeyf Höyük and early farmer at Hakemi Use are important as information of primary subsistence in Neolithic society before and after food production.

6.2. Materials

6.2.1. Hasankeyf Höyük

Hasankeyf Höyük, PPNA site, is located in the upper Tigris valley in Batman, Turkey (Fig. 1.1). The details about Hasankeyf Höyük were mentioned above in section 3.2. In this study, 34 human bones were treated by gelatinization, but only 15 specimens showed C/N ratios in the biological range of 2.9–3.6 (Table 6.1), as a known range of modern animal collagens measured by DeNiro (1985). These 15 individuals are separated to 5 groups according to structures (str.) and its layers buried in, such as newer pit on str. 3 (Group 1), str. 3 upper floor (Group 2), str. 3 middle floor (Group 3), str. 30 (Group 4) and str. 54 (Group 5) (Fig. 6.2). Str. 3 might have been particular ceremonial building, because it was rectangular and larger than other round residences. In str. 3, Group 1 was earlier than Group 2 and also 3, as having been buried to above layer. Then str. 30 was surely used before building str. 3, because str. 30 was broken to build str. 3. Therefore, Groups 1, 2, 3 and 4 were probably living at different times. And str. 54 was thought contemporary structure of str. 30 by the situation.

6.2.2. Hakemi Use

Hakemi Use, PN site, is located in the upper Tigris valley in Bismil, Turkey (Fig. 1.1). The details about Hakemi Use were mentioned above in section 3.2.In this section, 19 human bones were analyzed for isotopic compositions of individual amino acids (Table 6.1).

Because I have not collected animal bones of Hakemi Use yet, I compared the human data of Hakemi Use with those of animals excavated from Hasankeyf Höyük, as reference of terrestrial and freshwater animals living in the same region.

6.3. Results

6.3.1. Carbon and nitrogen isotopic compositions of bulk collagen

The $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ values of these humans and animals were reported in previous section (Fig. 3.2). The summary of human groups and animals at Hasankeyf Höyük are shown in Tables 6.1, 6.2 and Figure 6.3. And the summary of humans at Hakemi Use is shown in Table 6.3 and Figure 3.2.

6.3.2. Nitrogen isotopic compositions of amino acids from fauna

Naito et al. (2010) suggested that the $\delta^{15}N$ of both phenylalanine ($\delta^{15}N_{Phe}$) and glutamic acid ($\delta^{15}N_{Glu}$) are no significant difference between the collagens with biological C/N ratios (2.9–3.6) and the collagens with altered C/N ratios (upward of 3.6) of the same species. The individual amino acids of this method (Chikaraishi et al., 2007) were isolated from other amino acids and materials in pretreatment and gas chromatographic separation as compared to the bulk collagen. Also because the diagenetic change of amino acid composition in prehistoric collagens is one of the cause for postmortem alteration in C/N ratio of bone collagen (DeNiro and Weiner, 1988), it is thought that the isotopic compositions of individual amino acids remain original values even so the C/N ratio of the collagen is altered. Thus even samples with the C/N ratio outside the biological range for bone collagen (2.9–3.6) could be available dietary analysis by the $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$. Actually in this study also, TP_{ter} showed no difference between Caprine with biological C/N ratios, 2.0±0.0 (±1 s.d.) and with altered C/N ratio, 2.0±0.0 in this study, showing expected values as herbivore. Although some animal collagens have altered C/N ratios, I adopted these faunal samples for amino acids analysis.

At Hasankeyf Höyük, freshwater fish and terrestrial animals have different nitrogen isotopic compositions for both phenylalanine and glutamic acid (Fig. 6.4). The $\delta^{15}N_{Phe}$ values of terrestrial herbivores at Hasankeyf Höyük range from 7.6 to 14.5‰, whereas the $\delta^{15}N_{Glu}$ from 7.8 to 14.5‰. In addition, the $\delta^{15}N_{Phe}$ values of herbivores show a mean of 10.9‰, whereas the $\delta^{15}N_{Glu}$ values of that showed similar positions around for all terrestrial mammals including pig (TP_{ter}) of the terrestrial mammals containing pigs were similar, and were estimated to be 2.0. Despite of varied $\delta^{15}N$ values of bulk bone collagen among terrestrial animals, these similar TP_{ter} are consistent with the expected trophic position of these mammals as primary consumers. The trophic position of fox is as high as 2.8, suggesting that the fox was high carnivorous–omnivore. A chelonian shows $\delta^{15}N_{Phe}$ value similar to terrestrial mammals but different from freshwater fishes, further suggesting them to be tortoise. When terrestrial equation is applied, the trophic position of the chelonian is estimated to be 1.8.

In contrast, the trophic positions assured for aquatic ecosystems (TP_{aqua}) of freshwater fishes were estimated to 2.4 to 3.1 as secondary consumers. These fishes were all identified as Cyprinidae, with a specimen being identified as *Barbus* ($\delta^{15}N_{Phe}$ = 1.8‰, $\delta^{15}N_{Glu}$ = 16.2‰). These TP_{aqua} values give close agreement with the

expected TP of Cyprinidae, 2.5, based on the described diet (Vander Zanden et al., 1997). Although the $\delta^{15}N_{Phe}$ values of freshwater fishes split into two groups; three ranges from −0.2 to 1.8‰ and two are 7.8 and 7.9‰. However, two specimens showing higher $\delta^{15}N_{Phe}$ were estimated to be a body length of 50–100cm and larger than other freshwater fishes. Therefore the difference of $\delta^{15}N_{Phe}$ among freshwater fishes might be caused by a difference of foraging activity according to growing.

6.3.3. Isotopic compositions of amino acids from human individuals

The $\delta^{15}N_{Phe}$ values of human individuals at Hasankeyf Höyük range from 5.3 to 12.0‰ and a mean of 8.5±2.0‰ (mean ± s.d.), whereas the $\delta^{15}N_{Glu}$ from 12.0 to 16.1‰ and a mean of 13.5±1.0‰ (Fig. 6.4, Table 6.1).

The TP_{ter} for many individuals with $\delta^{15}N_{Phe}$ higher than 7.6‰ (inside a range of two standard deviation of terrestrial herbivore) are estimated 2.65 (Fig. 6.5). They seem to have depended on the terrestrial resources. By contrast, some individuals of lower $\delta^{15}N_{Phe}$ than 7.6‰ are estimated high TP_{ter} (2.8–3.2). Thus, individuals showing lower $\delta^{15}N_{Phe}$ are considered ad they had different diets from others showing higher $\delta^{15}N_{Phe}$.

Besides, the $\delta^{15}N_{Phe}$ values of human individuals at Hakemi Use range from 5.6 to 11.4‰, whereas the $\delta^{15}N_{Glu}$ from 9.4 to 14.9‰ (Fig. 6.6, Table 6.3). The $\delta^{15}N_{Phe}$ of almost individuals shows higher than 7.6‰ (inside a range of two standard deviation of terrestrial herbivore at Hasankeyf Höyük) (Fig. 6.7).

According to the nitrogen isotopic compositions of amino acids, individuals of Hakemi Use seem to have mainly depended on the terrestrial resources. However, one juvenile have a $\delta^{15}N_{Phe,}$ 5.6‰, distinctly lower than others. By contrast to Hasankeyf Höyük, all individuals are estimated to similar TP_{ter}, around 2.5±0.07 (Fig. 6.7).

6.4. Discussions

6.4.1. Estimations of carnivory

I estimate the fraction animal protein in total terrestrial protein (Fa) estimated by amino acids of Hasankeyf Höyük. The estimation by amino acid isotopic analysis provides the Fa of individual which is constant regardless of the variability in $\delta^{15}N_{col}$ among terrestrial herbivores (see Naito et al., 2013; Styring et al., 2015).

$$Fa_{AA} (\%) = (TP_{ter}[human] - 2)/(TP_{ter}[herbivore] - 1) \times 100$$

Thus, if they depended only on the terrestrial food resources, the Fa_{AA} of mean among all individuals at Hasankeyf Höyük is approximately estimated to 77%. But provided that individuals of low $\delta^{15}N_{Phe}$ were somewhat influenced by freshwater resources, actual Fa of inhabitants at Hasankey Höyük might be lower. The Fa_{AA} of individuals with $\delta^{15}N_{Phe}$ of higher than 7.6‰ are estimated to be 63.7±6.9% regardless of sex and age.

Besides, I estimate the Fa_{AA} of Hakemi Use. Thus, if they depended only on the terrestrial food resources, the Fa_{AA} of mean among all individuals at Hakemi Use is estimated to 50.3±6.9% regardless of sex and age.

6.4.2. Estimations of dependence to freshwater resources

The result of isotopic analysis for amino acids suggests that individuals with lower $\delta^{15}N_{Phe}$ and higher TP_{ter} seem to have consumed a significant amount of aquatic (i.e. freshwater) resources at Hasankeyf Höyük (Fig. 6.5). Only high TP_{ter} more than 3.0 is able to be explained by a consumption of carnivorous animal such as fox in terrestrial ecosystem. However, because their $\delta^{15}N_{Phe}$ values are lower than a range of two standard deviation of terrestrial herbivore, the diets of these individuals with the $\delta^{15}N_{Phe}$ values lower than 7.6‰ are difficult to explain by only the usual consumption of terrestrial resources (Fig. 6.5). Actually, the $\delta^{15}N_{Phe}$ of the fox, probably consuming mainly terrestrial animals, shows 11.0‰. To explain the diets of low $\delta^{15}N_{Phe}$ individuals only by terrestrial resources, they had to have strong carnivorous diet depending selectively on terrestrial animals which had the $\delta^{15}N_{Phe}$ lower than the range of terrestrial animals from Hasankeyf Höyük. Therefore, the individuals of Hasankeyf Höyük are supposedly considered as the lower $\delta^{15}N_{Phe}$ they have, the higher rate they consumed freshwater resources with. Though, the diets of individuals with $\delta^{15}N_{Phe}$ values higher than 7.6‰ are able to be explained according to the consumption of terrestrial resources only (Fig. 6.5). If they have consumed plants which were higher $\delta^{15}N_{Phe}$ than estimated based on the terrestrial herbivores in addition to very few terrestrial animal proteins, there was the potential of the contribution of freshwater resource to their diets.

Whereby a model to address the quantitative estimate of aquatic resource consumption *vs.* terrestrial resource (Qa) by humans using distances between TP_{ter} line and TP_{aqua} line was proposed by previous study (Naito et al., 2013).

$$Qa = \frac{\text{distance between } \delta^{15}N_{\text{Glu-Phe}}[\text{human}] \text{ and } TP_{\text{ter}}[\text{Terrestrial diet}]}{\text{distance between } TP_{\text{aqua}}[\text{freshwater fish}] \text{ and } TP_{\text{ter}}[\text{Terrestrial diet}]}$$

However, the estimated Qa is varied based on animal protein fraction in terrestrial protein (Fig. 6.8). Therefore, provided that individual potentially consumed both terrestrial and freshwater resources, it is difficult to accurately decide the Qa in the diet unless defining the animal protein fraction in terrestrial protein (Fa$_{AA}$).

Nevertheless, because individuals with $\delta^{15}N_{\text{Phe}}$ within two standard deviation range of herbivore have constant and low TP$_{\text{ter}}$, they are suspected of having consumed rarely freshwater resources. If the Fa of individuals with the $\delta^{15}N_{\text{Phe}}$ of lower than 7.6‰ is defined as similar to those of individuals with higher $\delta^{15}N_{\text{Phe}}$, 63.6%, their freshwater resources are estimated to have accounted for 8–17% of all protein in their diets (Fig. 6.8). On the other hand, if individuals with lower $\delta^{15}N_{\text{Phe}}$ have consumed a less amount of terrestrial animal proteins than others, their estimated consumption rate of fish protein is higher than 20%. In addition, if the Fa of individuals with higher $\delta^{15}N_{\text{Phe}}$ is 0%, their freshwater resources are estimated to have accounted for 17–20% of all protein in their diets, though unlikely according to previous knowledge. However, this interpretation requires the assumption that these individuals consumed undiscovered plants of higher $\delta^{15}N_{\text{Phe}}$ than herbivores feed. In either case, freshwater fishes probably were one of the major protein resources for hunter-gatherer in upper Tigris.

Similar to Hasankeyf Höyük, the $\delta^{15}N_{\text{col}}$ of humans at Hakemi Use are not so sufficiently high to indicate a significant contribution of freshwater resources to their diet (see Fig. 3.2). Also, because most individuals at Hakemi Use show $\delta^{15}N_{\text{Phe}}$ values higher than 7.6‰, their diets are able to be explained according to the consumption of terrestrial resources only (Fig. 6.7). If the Fa of individuals at Hakemi Use is 50.3% on average, the Qa of mean among all individuals at Hakemi Use is estimated to be approximately 0.0% on less (Fig. 6.8).

Although one juvenile (5.6‰) have a low $\delta^{15}N_{\text{Phe}}$, the TP do not indicate clear consumption of aquatic animals (TP$_{\text{ter}}$ = 2.6 and TP$_{\text{aqua}}$ = 1.1). Provided that this individual did not habitually consume aquatic first producer such as waterweed in aquatic resources, the $\delta^{15}N_{\text{Glu}}$ and TP$_{\text{ter}}$ should not show these few values (Fig. 6.7). Thus, individual who have $\delta^{15}N_{\text{Phe}}$ lower than 7.6‰ probably consumed very few freshwater resources, especially freshwater animals in the diet, because the $\delta^{15}N_{\text{Glu}}$ of this individual is too low to be explained by a consumption of freshwater fish from Hasankeyf Höyük. By contrast with hunter-gatherer in upper Tigris, freshwater fishes

probably were not important food resources for early farmer at Hakemi Use after the beginning of food production.

6.4.3. Individual variation

The individuals at Hasankeyf Höyük are divisible into 5 groups according to the structures and the layers buried in (Fig. 6.2). So as to validate the uniformity of the diets within the group, I compare the Euclidean distance of isotopic compositions of bulk collagen and amino acids between two individuals within each group from that among all individuals (Figs. 6.9 and 6.10). In the result, the Euclidean distances of $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ between two individuals within each group are significant shorter (p-value < 0.05) than that among all individuals (two tailed Welch's t test: df = 27, t = −8.6, p = 3.4 × 10^{-9}; Fig. 6.9). In a similar way, the Euclidean distances of $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ between two individuals within each group are significant shorter (p-value < 0.05) than that among all individuals (two tailed Welch's t test: df = 24, t = −6.2, p = 1.9 × 10^{-6}; Fig. 6.10). Therefore, the isotopic compositions of both bulk collagen and amino acids of individuals are biased according to buried locations at Hasankeyf Höyük (Table 6.2 and Figs. 6.3 and 6.5).

As compared to each group, the collagens from individuals from Groups 2, 3 and 5 are enriched in $^{15}N_{col}$ relative to those from other groups. Because individuals of Group 2 have lower $\delta^{15}N_{Phe}$, their high $\delta^{15}N_{col}$ are dovetailed suggesting consumption of freshwater resources by amino acid analysis. By contrast, as individuals of Groups 3 and 5 have highest $\delta^{15}N_{Phe}$ and lowest TP_{ter} of this site. Because Groups 1, 3 and 5 show similar TP_{ter}, their high $\delta^{15}N_{col}$ perhaps are caused by consumption of not freshwater resources but terrestrial resource which had higher ^{15}N than Group 1. And the individuals with the lower $\delta^{15}N_{col}$ of the Groups 1 and 4, have similar $\delta^{15}N_{col}$ to red deer and badger. Despite lower $\delta^{15}N_{col}$, these individuals from Group 4 are indicated the consumption of freshwater resources by $\delta^{15}N$ of phenylalanine. These aspects might suggest that animal with lower $\delta^{15}N_{col}$ accounted for the majority of the animal protein in terrestrial resources for these groups with. Furthermore, within each Group 2 and 4 the contributions of freshwater resources to diets were varied by each individual. On the other hand, all individuals from Groups 1, 3 and 5 who are strongly depend on terrestrial proteins indicate similar quantity of meat consumptions.

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At Hasankeyf Höyük, the diets are various among groups based on buried building (Fig. 6.2). Furthermore, they were different among layers in Structure 3. To consume freshwater resources or not of people buried to Structure 3 seems to depend on times. Similarly, at nearly the same time people buried to Structure 30 more consumed freshwater resources than those buried to Structure 54. These results indicate that individuals buried at the similar time applied similar kind of food resources, though a deciding factor of buried location should be revealed by additional research. However, the contributions of freshwater resources were varied among individuals in the same groups. At Hasankeyf Höyük, later hunter-gatherer site on upper Tigris River, perhaps, they might not have spread the food resources, especially freshwater resources, equally in the community.

6.5. Conclusion

The diets of inhabitants at Hasankeyf Höyük suggest even if hunter societies were able to access to freshwater resources, they did not always consume large quantity of freshwater resources. The members in each group might have not consumed freshwater resources equally. Even if the diets of a hunter-gatherer community seem to widely range within a whole site, the diets might include several closed groups. On the one hand, the diets of inhabitants at Hakemi Use suggest uniform meat consumption rate and dependence on the terrestrial resources among the members. Furthermore, the early farmer might have had the meat consumption rate lower than the late hunter-gatherer in southeast Anatolia.

The isotopic analysis of glutamic acid and phenylalanine is able to isolate human who consumed freshwater resources in combination with terrestrial resources, not just only human dependent exclusively on freshwater resources. The nitrogen isotopic composition of amino acids is useful for resolving each of dietary factors with higher resolution, such as distinguishing terrestrial *vs* freshwater resources, and enhances the resolution and reduces the uncertainty in assessing the structure of ancient societies, more than ever.

7. General Conclusion

7.1. Comparisons of the diets with previous studies

In previous studies (Richards et al., 2003a; Lösch et al., 2006; Pearson et al., 2010; 2013; Budd et al., 2013) and this study, the collagens of Neolithic humans excavated from each site were measured the carbon and nitrogen isotopic compositions for the dietary reconstruction. According to these, the isotopic compositions, especially nitrogen, showed various values according to each site and region (see Fig. 3.5). However, only by carbon and nitrogen isotopic compositions it was insufficient to elucidate dietary bias among Neolithic sites and discuss the causes, such as the differences of quantity and/or composition, because consumable food resources around this inland Neolithic settlement were limited to inland resources. Hence, I try to both clarify the quantity of terrestrial animal protein in the diets and identify source of food resources by isotopic analysis of individual amino acids. Accordingly, I review anew about dietary changes occurring in the process of "Neolithisation" in the southwest Asia on the basis of the nitrogen isotopic compositions of individual amino acids of collagen in addition to carbon and nitrogen isotopic compositions of bulk collagen.

In this section, I compare humans of 4 Neolithic sites measured the $\delta^{15}N_{col}$ and the fraction animal protein in total terrestrial protein (Fa) estimated by amino acids at Tell Ain el-Kerkh, Hakemi Use, Hasankeyf Höyük and Çatalhöyük reported in previous studies (Richards et al., 2003; Styring et al., 2015), because these sites were measured the isotopic compositions of both collagen by EA–IRMS and amino acids by GC–C–IRMS (Table 7.1). The detail of results about the $\delta^{15}N_{Phe}$, $\delta^{15}N_{Glu}$ and TP_{ter} were reported in previous sections (Table 5.3, 6.1, 6.3) and previous study (Styring et al., 2015). Also, the discussion about isotopic compositions of archaeological collagens of these 4 sites and other Neolithic sites, Tell Qaramel, Çayönü Tepesi, Nevalı Çori, Aşıklı Höyük and Aktopraklık, were reported in previous section (Table 5.3, 6.1, 6.3), and the detail of the $\delta^{15}N_{col}$ were reported in previous studies (Richards et al., 2003a; Lösch et al., 2006; Pearson et al., 2010; Pearson et al., 2013; Budd et al., 2013).

In these Pottery Neolithic sites, the mean of human $\delta^{15}N_{col}$ at Hakemi Use (7.4‰) is lower than those at Çatalhöyük (10.9‰; Richards et al., 2003) and Tell Ain el-Kerkh (8.3‰) (Fig. 3.5). In addition, the mean of human $\delta^{15}N_{col}$ at Hasankeyf Höyük (8.6‰) is as high as that at Tell Ain el-Kerkh. Therefore, inhabitants of

Hasankeyf Höyük seem to have had similar carnivory with PN farmer in north Levant, according to analysis of collagen simply.

On the contrary, the $\delta^{15}N$ of each food resource, such as animals and cereals, were varied according to each site (see Fig. 1.4). For this reason, I evaluate $\delta^{15}N_{col}$ of humans in each site by the difference from that of a particular food resource, goat in this case (Fig. 7.1). Thus, the difference of $\delta^{15}N_{col}$ between humans and goats ($\Delta^{15}N_{human-goat}$) at Tell Ain el-Kerkh (4.0‰) is similar to that of Çatalhöyük (4.1‰) and larger than that of Hakemi Use (1.5‰), because each herbivore such as goats showed different $\delta^{15}N_{col}$ among Tell Ain el-Kerkh (4.3‰), Çatalhöyük (6.8‰) and Hasankeyf Höyük (6.0‰), the neighbor Neolithic site from Hakemi Use. Therefore, inhabitants of Hasankeyf Höyük seem to have had lower carnivory than inhabitants of PN site, according to analysis of collagen.

Then I compare the fraction animal protein in total protein by amino acids of Tell Ain el-Kerkh than Anatolian Neolithic sites, Hasankeyf Höyük at PPNA, Hakemi Use and Çatalhöyük at PN (Styring et al., 2015). The mean Fa_{AA} of north Levantine farmers at Tell Ain el-Kerkh (45%) is lower than those in central Anatolian farmers at Çatalhöyük (53%; Styring et al., 2015) and southeast Anatolian farmers at Hakemi Use (50%). By contrast, the Fa_{AA} of hunter-gatherer at Hasankeyf Höyük (66%) is higher than those in three PN farmer sites. Thus isotopic analysis of the individual amino acids indicates that the PPNA hunter-gatherers at upper Tigris had more carnivorous diets than farmers of Pottery-Neolithic period. It is important finding that the inhabitants of Hasankeyf Höyük showed lower $\delta^{15}N_{col}$, despite the much consumption of both terrestrial meat and freshwater resources than PN individuals in central Anatolia and north Levant.

7.2. New information obtained by combination of isotopic analyses

The nitrogen isotopic analysis of amino acids allows us to resolve the quantity of dietary animal protein in total terrestrial protein at inland communities in southwest Asia. Then, these carnivory of inhabitants in Neolithic southwest Asia should be assessed for significance within the human history.

Incidentally, Hedges and Reynard (2007) estimated the fraction of animal protein in total protein contribution of modern humans into developed countries (57%) and developing countries (30%) based on the report of the

Food and Agriculture Organization (FAO, 2014). Furthermore, they estimated of a 60% of the fraction of animal protein in total protein is equivalent to 35% of energy from animals in total energy, insofar as a typical terrestrial meat and cereal diet (Hedges and Reynard, 2007). On the other hand, European Neolithic humans in several sites having agricultural economy were summarized the Fa_{col} in 60–80% (Hedges and Reynard, 2007). Altogether, according to isotopic analysis of collagen, pre-historic European post-agriculture had carnivory much higher than inhabitants in modern developing countries. If this calculation is correct, it might be consistent with the hypothesis that European Neolithic economy was first introduced from southwest Asia as a complete package (Arbuckle, 2013). When comparing Fa_{AA} of this study to Fa_{col} of previous study about Neolithic European (Hedges and Reynard, 2007), early agriculture invented in southwest Asia seems to have provided a consumption rate of animal resources to southwest inhabitants (approximately 50%) lower than European early farmer (60–80%).

Whereas Styring et al. (2015) reported that inhabitants of two European Neolithic sites showed low Fa_{AA} (Vaihingen in Germany, 30% and Makriyalos in Macedonia, 28%), despite high Fa_{col} (Vaihingen in Germany, 58% and Makriyalos in Macedonia, 63%). If comparing Fa_{AA} of this study to Fa_{AA} of the previous study about Neolithic European (Styring et al., 2015), early farmers in southwest Asia seems carnivorous more than the European early farmers. Also, the gap between two methods was interpreted as due to the difference of $\delta^{15}N$ between cereals consumed by human and forage consumed by herbivore, because the cereal $\delta^{15}N$ values were higher than estimated forage $\delta^{15}N$ values in these sites (Styring et al., 2015).

Similarly, by comparison between nitrogen isotopic analyses of collagen and amino acids in this study, it is reaffirmed that $\delta^{15}N_{col}$ is not necessarily correlated with Fa_{AA}. On the other hand, the $\delta^{15}N$ value of cereal (2.2‰) falls within estimated $\delta^{15}N$ value of forage according to herbivore species (1.2–4.3‰) at Tell Ain el-Kerkh (Tables 3.1 and 3.2). Thus, the difference of $\delta^{15}N$ between cereal and forage seems to not a major reason of the gap between Fa_{AA} and Fa_{col} at Tell Ain el-Kerkh. Hence, the un-correlation between Fa_{AA} and Fa_{col} among these Neolithic sites may indicate that the collagen nitrogen isotopic composition is decided by not only the fraction animal protein in total protein, but the difference of animal mainly consumed.

However, because the site variation of Fa_{AA} is smaller than that of Fa_{col} based on each animal $\delta^{15}N_{col,}$

accomplished farmer societies in PN period seem to have had similar consumption rates of animal protein despite the variations in composition of animal species among sites (Fig. 7.2). For instance, though Tell Ain el-Kerkh has the lower % of animal protein and larger $\Delta^{15}N_{Human–Goat}$. Therefore, inhabitants of Tell Ain el-Kerkh at PN period may have consumed a small amount of cattle meats preferentially as animal protein. Actually, it is thought that cattle were important livestock at Tell Ain el-Kerkh according to the composition of animal remains (Yano, 2000). In contrast, caprine include sheep and goats, showing $\delta^{15}N_{col}$ lower than cattle, dominated to the composition of animal remains in southeast Anatolian sites, Hakemi Use (Omar, 2013) and Hasankeyf Höyük. At other southeast Anatolian site, for instance Çayönü Tepesi, the mainstay of the animal protein in the diet was pigs, not cattle (Hongo et al., 2009). Conceivably, the low $\delta^{15}N_{col}$ of humans at southeast Anatolian sites might be caused by absence of cattle, not the smaller consumption of meat. However, to draw a conclusion of this argument, other communities such as Çayönü Tepesi and Nevalı Çori also need to be measured for isotopic compositions of individual amino acids by GC–C–IRMS. Neolithic farmers at PN sites seem to have consumed comparable ratio of animal protein among regions. On the other hand, the main animal species as source of supply seem to have been different according to the regions. Also, Neolithic hunter-gatherer probably had carnivorous diet more than the early farmer at late Neolithic period despite low $\delta^{15}N_{col}$.

The gap between isotopic analyses of collagen and amino acids is able to be interpreted as the difference of main resource as animal protein, because it coincides to the compositions of animal remains in each site. Thus, the combination of two isotopic analyses enables to identify and quantitate main protein source for human individuals, not only relative comparison of isotopic compositions among communities like the conventional approach. Then the precise individual diets provide new information on prehistoric societies and the gradual development of food acquisition.

7.3. Impact of Neolithisation

According to the combination of isotopic analyses, hypotheses about adaptive significance of Neolithisation to human food acquisitions are verified in this study.

Previous hypothesis suggested based on researches of subsistence activity and demographics, predicts that the

division of labor between males and females played a clear role in early Neolithic hunter-gatherer communities (Driver, 1995; Eshed et al., 2004). On the basis of this hypothesis, males of hunter-gatherer might have gotten more opportunity to eat animal proteins than females. However, the difference of diets between male and female according to the isotopic compositions had already been small at early Neolithic hunter-gatherer than previously thought based on sexual division (Driver, 1995). Therefore the results of this study do not support previous hypothesis.

On the other hand, though the species constitutions of animal remains among early farmers in PN period were varied from region to region, the variation in contributions of terrestrial animal proteins has been uncertain among each region and/or site yet. In the result of isotopic analyses, accomplished farmer societies in the late Neolithic period seem to have had similar consumption rates of animal protein despite the variations in composition of animal species among sites. Furthermore, in southeast Anatolia the late hunter-gatherer in Neolithic period seems to have had the meat consumption rate higher than the early farmer.

Besides, freshwater resources as food have been indicated the importance for river side hunter-gatherer based on excavated aquatic animal remains and fishing tools, whereas the quantification of the contributions was indistinct (Coşkun et al., 2010; Miyake et al., 2012). In the result of amino acid isotopic analysis, freshwater fishes probably were one of the major protein resources for Neolithic hunter-gatherer in upper Tigris, though the contribution to human diets was smaller than hunter-gatherers in Upper Paleolithic. However, according to a disaggregated group within the site, the diets of inhabitants at Hasankeyf Höyük suggest even if hunter societies were able to access to freshwater resources, they did not always consume large quantity of freshwater resources. In contrast, freshwater fishes probably were not important food resources for early farmer at Hakemi Use despite river side site, as contrasted to hunter-gatherer in same upper Tigris. Along with the growth of importance of produced food resources, the relative importance of freshwater resources as part of foraged food resources might have been diminished during Neolithic period.

Therefore, food production as context of Neolithisation seems to have produced standardization of food consumption to Neolithic hunter-gatherers utilizing a huge variety of food resource. Furthermore social structure change thought to occur as a consequence of food production from nomadic hunter-gatherer might have been

started by settled hunter-gatherer in early Neolithic periods already. The some distinctions of post-Neolithisation, such as social complexity and change of sexual division of labor, may date back to early Neolithic period and perhaps before then, by the dietary information based on the combination of isotopic analyses in addition to the conventional archaeological evidences.

7.4. Conclusion

By comparing the isotopic compositions especially Nitrogen of amino acids and collagen, possibly mainly consumed species is able to be roughly identified in each site. The nitrogen isotopic composition of the different amino acids allow to resolve each of these factors previously indistinguishable, such as both the quantity and the source species of animal protein in Neolithic sites in southwest Asia. Therefore the diets and life styles of prehistoric communities are reduced the uncertainty, especially about the estimated consumption rate of animal protein, can be assessed by these methods more than ever. The $\delta^{15}N$ values of glutamic acid and phenylalanine are able to extract the information of the trophic position, free of influence from both the distribution of bulk $\delta^{15}N$ and the difference of ecosystem among food resources. However, also the important information by individual amino acids lost the difference of $\delta^{15}N_{col}$ among herbivores living in similar place, despite the potent influence to human $\delta^{15}N_{col}$. In contrast, by correcting based on the TP_{ter} of human, the information of human $\delta^{15}N_{col}$ reduces the uncertainty caused by the fraction of animal protein in total terrestrial protein. This concept of combined isotopic analyses is own unique yet, although the isotopic analysis of individual amino acids for archaeological remains is thought as an exceptional method to decide precise tropic position of prehistoric human by reducing any noise (e.g. Naito et al., 2013; Styring et al., 2015). As further suggestion, a question which species of livestock, cattle, pig, goat and sheep, was important food resources for prehistoric individual, especially post-Neolithic, is one of central propositions in human history, because each animal had different values as luxury food in many cultures. For future study, the combination of isotopic analyses will be able to provide an answer to this question.

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Table 3.1. Isotopic results of collagen for faunal skeletal remains from Tell Ain el-Kerkh and Hasankeyf Höyük.

Period	Site	Species	N	$\delta^{13}C_{col}$ (‰)		$\delta^{15}N_{col}$ (‰)	
PPNB	Tell Ain el-Kerkh	Cattle	2	-20.6	±0.7	7.8	±1.0
		Sheep	1	-21.6	-	5.8	-
		Goat	1	-20.5	-	5.4	-
		Pig	1	-20.5	-	4.3	-
PN	Tell Ain el-Kerkh	Cattle	4	-20.8	±0.5	7.7	±0.6
		Sheep	2	-21.0	±0.1	6.3	±0.8
		Goat	3	-21.3	±0.5	4.3	±1.2
		Pig	5	-20.3	±0.4	6.9	±1.6
		Fox	1	-18.7	-	9.6	-
		Cyprinidae	1	-20.5	-	11.0	-
PPNA	Hasankeyf Höyük	Cattle	1	-20.5	-	10.5	-
		Goat	3	-19.8	±0.3	6.0	±0.3
		Pig	1	-20.2	-	6.8	-
		Red deer	3	-20.5	±0.3	7.4	±0.3
		Badger	1	-19.5	-	7.5	-
		Fox	1	-19.1	-	9.5	-

Table 3.2. Isotopic results for cereal grains from Tell Qaramel (Araus *et al.* 2014) and Tell Ain el-Kerkh. %N is

the percentage of nitrogen in the charred samples.

Period	Site	Species	N	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)		%N	
PPNA	Tell Qaramel	*Triticum boeticum*	17	-23.2	±0.7	6.1	±2.0	4.3	±0.8
PN	Tell Ain el-Kerkh	*T. turgidum* ssp. *dicoccum*	10	-22.4	±0.4	2.2	±0.6	4.1	±0.5

Table 3.3. Isotopic results of collagen for human skeletal remains from Tell Qaramel, Tell Ain el-Kekrh, Hasankeyf Höyük and Hakemi Use.

Period	Site	N	$\delta^{13}C_{col}$ (‰)		$\delta^{15}N_{col}$ (‰)		Region
PPNA	Tell Qaramel	6	-19.8	±0.3	8.7	±1.2	north Levant
Bronze Age	Tell Qaramel	6	-20.5	±0.1	8.2	±0.6	north Levant
PPNB	Tell Ain el-Kerkh	6	-20.0	±0.2	8.7	±0.8	north Levant
PN	Tell Ain el-Kerkh	47	-20.1	±0.5	7.9	±0.8	north Levant
PPNA	Hasankeyf Höyük	15	-19.5	±0.3	8.6	±0.8	southeast Anatolia
Iron Age	Hasankeyf Höyük	5	-20.0	±0.5	6.7	±0.5	southeast Anatolia
PN	Hakemi Use	17	-19.7	±0.3	7.4	±1.3	southeast Anatolia

Table 4.1. $\delta^{15}N$ values (mean ± 1σ ‰) of bulk bone collagen, glycine, glutamic acid, phenylalanine, poline, hydroxyproline, the weighted mean of the five amino acids (occupying 51.6% nitrogen of collagen by weight), and the estimated trophic positions (TP_{ter}) for the cattle (n = 3), sheep (n = 2), and goats (n = 3).

Table	Cattle		Sheep		Goats	
Bulk collagen	7.5	± 0.7	6.3	± 0.8	4.3	± 1.2
Glycine	6.6	± 0.5	4.6	± 0.7	2.1	± 1.2
Glutamic acid	9.5	± 0.1	8.7	± 1.5	8.4	± 1.0
Phenylalanine	9.5	± 0.3	9.0	± 0.4	9.5	± 0.6
Proline	10.5	± 0.6	10.1	± 2.8	9.9	± 1.8
Hydroxyproline	9.3	± 1.0	8.6	± 1.3	7.4	± 0.9
Weighted mean	8.2	± 0.4	7.3	± 1.3	5.4	± 1.5
TP_{ter}	2.1	± 0.0	2.1	± 0.1	2.0	± 0.1

Table 5.1. Summary of isotopic results for human groups of Tell Ain el-Kerkh. Intra-group distance shows the mean of Euclidean distance of $\delta^{13}C$ and $\delta^{15}N$ between two individuals within the group. Total of distance shows the mean of Euclidean distance of $\delta^{13}C$ and $\delta^{15}N$ between two individuals in all individuals.

Location	Age	N	$\delta^{13}C_{col}$		$\delta^{15}N_{col}$		Intra-group distance	Total of distance	^{14}C age (BC)
All	except child	28	-20.1	±0.5	8.5	±0.9	1.00	1.34	
	young child	*9*	*-19.6*	*±0.5*	*9.4*	*±1.4*			
Area 1	except child	4	-19.9	±0.3	8.5	±0.3	0.60	1.06	5460–5400
	young child	*0*	*-*		*-*				
Area 2	except child	3	-19.7	±0.3	7.8	±0.6	0.56	1.26	5530–5475
	young child	*4*	*-19.8*	*±0.5*	*8.6*	*±0.8*			
Area 3	except child	3	-20.3	±0.3	9.2	±1.1	0.98	1.45	5505–5450
	young child	*1*	*-19.1*		*11.7*				
Area 4	except child	4	-19.4	±0.6	7.9	±0.6	1.03	1.45	5525–5365
	young child	*1*	*-20.0*		*8.1*				
Area 5	except child	3	-20.5	±0.3	8.3	±0.8	0.76	1.21	5475–5425
	young child	*1*	*-18.9*		*11.0*				
Area 6	except child	5	-20.4	±0.4	8.9	±1.3	1.72	1.50	
	young child	*0*	*-*		*-*				
Area 7	except child	2	-20.7	±0.2	8.3	±0.7	1.04	1.20	
	young child	*0*	*-*		*-*				
other	except child	4	-20.5	±0.5	8.5	±1.3			
	young child	*0*	*-19.3*		*10.1*				

Table 5.2. Isotopic results for faunal remains from Tell Ain el-Kekrh. C/N is the atomic ratio of carbon to nitrogen.

Sample	$\delta^{13}C_{col}$	$\delta^{15}N_{col}$	C/N	$\delta^{15}N_{Glu}$	$\delta^{15}N_{Phe}$	Layer	Species
KP17	-20.8	7.2	3.3	9.4	9.6	PN	Cattle
KP21	-20.9	7.1	3.5	9.5	9.2	PN	Cattle
KP22	-21.3	8.3	3.4	9.5	9.7	PN	Cattle
KP02	-21.0	6.8	3.4	9.7	9.3	PN	Sheep
KP04	-20.9	5.7	3.3	7.6	8.7	PN	Sheep
KP06	-21.2	3.4	3.6	7.4	8.9	PN	Goat
KP08	-20.9	5.6	3.2	9.3	9.6	PN	Goat
KP27	-21.8	3.9	3.4	8.6	10.1	PN	Goat
KP01	-20.0	5.1	3.3	9.7	8.5	PN	Pig
KP14	-20.0	6.8	3.3	10.6	10.5	PN	Pig
KP19	-19.9	8.8	3.4	14.2	12.4	PN	Pig
KP39	-21.4	9.9	3.8	21.4	3.0	PN	Cyprinidae
KP41	-20.5	11.0	3.0	20.7	3.2	PN	Cyprinidae

Table 5.3. Isotopic results for human skeletal remains of Area 1–4 from Tell Ain el-Kekrh. C/N is the atomic

ratio of carbon to nitrogen. TP_{ter} is the trophic positions estimates based on terrestrial equation.

Sample	Location	$\delta^{13}C_{col}$	$\delta^{15}N_{col}$	C/N	$\delta^{15}N_{Glu}$	$\delta^{15}N_{Phe}$	TL_{C3}	Age	Sex	Burial Type
STR507	Area1	-20.2	8.2	3.4	11.9	9.3	2.45	8-10 years		with goods
STR1044	Area1	-20.1	8.6	3.4	12.3	9.2	2.51	middle Adult	Male	
STR1047	Area1	-19.8	8.8	3.3	13.3	9.7	2.58	yong Adult	Male	
STR1050	Area1	-19.5	8.2	3.3	13.0	9.7	2.54	yong Adult	Male	left femur and fibula
STR513	Area2	-20.4	8.1	3.6	12.8	10.5	2.41	Perinatal		
STR521	Area2	-19.4	7.3	3.3	12.6	10.1	2.43	12-15 years		
STR524	Area2	-19.6	7.6	3.4	12.6	9.9	2.46	middle adult	Female	
STR527	Area2	-19.3	7.7	3.3	11.7	10.8	2.22	infant		
STR533	Area2	-20.0	8.4	3.5	12.3	9.0	2.54	40-50　years	Female	
STR710	Area3	-20.1	8.1	3.2	13.4	12.4	2.24	about 20 years	Male	skull and legs
STR726	Area3	-19.1	11.7	3.2	17.1	14.3	2.47	1.5 years		
STR926	Area4	-19.8	7.3	3.2	13.4	10.9	2.43	Adult	Female	Secondary, with goods
STR927y	Area4	-18.7	8.3	3.2	12.9	9.8	2.51	yong Adult	Female	with goods
STR930	Area4	-19.9	7.4	3.3	12.5	10.4	2.38	Adult	Female	Secondary

Table 5.4. Estimated fraction of animal protein in total terrestrial protein of human from Tell Ain e-Kerkh, calculated using the 3 methods, assuming 3.4‰ as $\Delta^{15}N_{human\text{-}diet}$.

Calculation	All		Group 1		Group 2		Group 3	Group 4	
Fa_{AA} (%)	45.8	±9.4	51.6	±5.0	47.6	±5.9	23.6	43.8	±6.4
Fa_{col} (i) (%)									
from Cattle	14.7	±13.5	22.1	±8.5	21.6	±3.4	11.8	-0.9	±15.4
from Pig	50.0	±13.5	57.4	±8.5	56.9	±3.4	47.1	34.4	±15.4
from Sheep	61.8	±13.5	69.1	±8.5	68.6	±3.4	58.8	46.2	±15.4
from Goat	105.9	±13.5	113.2	±8.5	112.7	±3.4	102.9	90.3	±15.4
Fa_{col} (ii) (%)									
from Cattle	47.3	±8.3	51.8	±5.3	51.5	±2.1	45.5	37.6	±9.5
from Pig	60.5	±10.6	66.3	±6.7	65.9	±2.7	58.1	48.1	±12.2
from Sheep	66.7	±11.7	73.1	±7.4	72.6	±3.0	64.1	53.1	±13.4
from Goat	108.4	±19.1	118.8	±12.1	118.1	±4.8	104.2	86.2	±21.8

Table 6.1. Isotopic results for human skeletal remains from Hasankeyf Höyük. C/N is the atomic ratio of carbon to nitrogen. The Qa are values calculated in case of Fa = 63.6%.

Sample	$\delta^{13}C_{col}$	$\delta^{15}N_{col}$	$\delta^{15}N_{Glu}$	$\delta^{15}N_{Phe}$	TL_{C3}	TL_{aqua}	Fa	Qa	Structure	Group	Age	Sex
16	-19.0	7.5	12.1	8.1	2.6	1.1	63.0%	-0.4%	newer pit on str.3	Group 1	young adult	
26	-19.4	8.2	12.8	8.6	2.7	1.1	65.8%	0.7%	newer pit on str.3	Group 1	adult	M
27	-19.6	7.5	13.6	9.0	2.7	1.2	71.6%	2.8%	newer pit on str.3	Group 1	adult	M
28	-19.1	9.7	14.0	6.4	3.1	1.6	110.8%	17.2%	str.3 upper floor	Group 2	adult	
39b	-19.4	9.3	12.2	8.2	2.6	1.1	63.1%	-0.4%	str.3 upper floor	Group 2	adult	
39c	-19.2	9.6	13.3	5.3	3.2	1.6	115.7%	19.1%	str.3 upper floor	Group 2	adult	
50	-19.6	8.2	12.0	6.9	2.8	1.2	77.9%	5.1%	str.30	Group 4	juv-subadult,	
52	-19.2	9.5	13.4	7.4	2.9	1.3	88.9%	9.2%	str.3 upper floor	Group 3	adult	
67	-19.9	8.7	13.3	9.7	2.6	1.0	57.2%	-2.5%	str.54 upper floor	Group 5	15-20years	
92	-19.9	7.7	12.8	8.4	2.7	1.1	68.3%	1.6%	str.30	Group 4	3-5years	
101	-19.5	8.8	15.7	12.0	2.6	1.0	59.4%	-1.7%	str.3 middle floor	Group 3	adult	F
103	-19.6	8.3	14.3	10.6	2.6	1.0	59.3%	-1.7%	str.3 middle floor	Group 3	adult	
125	-20.2	9.4	14.1	10.2	2.6	1.1	60.9%	-1.1%	str.3 middle floor	Group 3	adult	
131	-19.9	7.4	13.4	5.7	3.1	1.6	110.7%	17.2%	str.30	Group 4	6-8year	
110b	-19.7	9.3	14.8	10.5	2.7	1.1	67.8%	1.4%	str.3 middle floor	Group 3	adult	

Table 6.2. Summaries of isotopic results for human and fauna from Hasankeyf Höyük. The Qa are values calculated in case of Fa = 63.6%.

Structure	N	$\delta^{13}C_{col}$		$\delta^{15}N_{col}$		N	$\delta^{15}N_{Glu}$		$\delta^{15}N_{Phe}$		TL_{C3}		TL_{aqua}		Qa	
All humans	15	-19.5	±0.3	8.6	±0.8	15	13.5	±1.0	8.5	±1.9	2.8	±0.2	1.2	±0.3	4.4%	±7.3
newer pit on str.3 (Group 1)	3	-19.3	±0.3	7.7	±0.4	3	12.8	±0.8	8.6	±0.5	2.7	±0.0	1.1	±0.0	1.0%	±1.2
str.3 upper floor (Group 2)	4	-19.2	±0.1	9.5	±0.2	4	13.2	±0.8	6.8	±1.3	2.9	±0.2	1.4	±0.2	11.3%	±7.7
str.3 middle floor (Group 3)	4	-19.8	±0.3	9.0	±0.5	4	14.7	±0.7	10.8	±0.8	2.6	±0.0	1.1	±0.0	-0.8%	±1.3
str.30 (Group 4)	3	-19.8	±0.2	7.8	±0.4	2	12.7	±0.7	7.0	±1.4	2.9	±0.2	1.3	±0.2	11.2%	±6.1
str.54 upper floor (Group 5)	1	-19.9		8.7		1	13.3		9.7		2.6		1.1		-2.5%	
Terrestrial herbivore	9	-20.1	±0.5	7.2	±1.4	16	10.9	±2.0	11.6	±2.0	2.0	±0.1	0.5	±0.1		
Cattle	1	-20.5		10.5		1	14.2		14.0		2.1		0.6			
Goat	3	-19.8	±0.3	6.0	±0.3	4	9.8	±1.3	10.7	±1.6	2.0	±0.1	0.4	±0.0		
Sheep						3	11.4	±0.7	12.5	±0.4	2.0	±0.1	0.4	±0.0		
Pig	1	-20.2		6.8		3	9.9	±1.9	10.2	±2.4	2.1	±0.1	0.5	±0.1		
Red deer	3	-20.5	±0.3	7.4	±0.3	3	11.1	±2.9	11.6	±2.7	2.0	±0.2	0.5	±0.2		
Badger	1	-19.5		7.5		1	14.4		14.5		2.1		0.5			
Tortoise						1	9.6		11.9		1.8		0.3			
Fox	1	-19.1		9.5		1	15.9		11.0		2.8		1.2			
FW Fish (All)						5	20.5	±6.0	3.8	±3.8	4.3	±0.3	2.8	±0.3		
FW Fish (a)						3	16.5	±3.0	1.1	±1.2	4.1	±0.3	2.6	±0.3		
FW Fish (b)						2	26.6	±1.0	7.9	±0.1	4.6	±0.1	3.0	±0.1		

Table 6.3. Isotopic results for human skeletal remains from Hakemi Use. C/N is the atomic ratio of carbon to nitrogen. The Qa are values calculated in case of Fa = 50.3%.

Sample	$\delta^{13}C_{col}$	$\delta^{15}N_{col}$	C/N	$\delta^{15}N_{Glu}$	$\delta^{15}N_{Phe}$	TL_{C3}	TL_{aqua}	Fa	Qa	Age	Sex
M7	-19.2	11.2	3.4	14.9	11.7	2.5	1.0	52.1%	0.5%	Child	
M51	-20.0	6.6	3.2	13.0	9.1	2.6	1.1	62.0%	3.9%	Adult	Female
M52a	-19.4	7.6	3.3	10.4	7.5	2.5	0.9	48.0%	-1.0%	Adult	Male
M53	-20.0	6.5	3.3	9.4	5.6	2.6	1.0	60.0%	3.2%	Child	
M141	-19.5	6.9	3.2	12.3	8.7	2.6	1.0	58.7%	2.8%	Adult	Female
M144	-20.5	7.3	4.1	12.4	10.1	2.4	0.9	41.0%	-3.3%	Adult	Female
M145A	-20.3	6.4	3.8	11.4	8.6	2.5	0.9	47.4%	-1.2%	Adult	Female
M146A	-19.2	6.6	3.3	12.7	9.6	2.5	1.0	51.1%	0.1%	Child	
M152	-20.0	7.3	3.3	11.4	8.9	2.4	0.9	44.2%	-2.4%	Adult	Female
M153	-19.9	5.8	3.4	11.1	8.0	2.5	1.0	51.8%	0.2%	Adult	Female
M155	-19.7	6.9	3.4	11.5	9.6	2.4	0.8	35.8%	-5.2%	Child	
M158	-19.5	9.6	3.4	13.3	10.9	2.4	0.9	42.0%	-3.2%	Adult	Female
M165	-19.7	7.4	3.3	12.8	9.7	2.5	1.0	52.0%	0.2%	Adult	Female
M174	-19.8	7.0	3.3	12.1	8.8	2.5	1.0	55.2%	1.4%	Child	
M177	-19.6	6.7	3.3	11.1	8.0	2.5	1.0	52.3%	0.5%	Child	
M182	-19.6	7.7	3.3	12.0	9.1	2.5	0.9	48.6%	-0.8%	Adolescent	
M183	-20.2	6.9	3.4	13.6	10.9	2.4	0.9	44.9%	-2.0%	Old Adult	Male
M189	-19.7	7.5	3.2	13.2	9.6	2.6	1.0	58.4%	2.7%	Old Adult	Female
M192	-19.7	6.9	3.5	12.0	9.0	2.5	1.0	50.4%	-0.1%	Young Adult	Female

Table 7.1. Isotopic results of collagen and individual amino acids for humans from Tell Ain el-Kekrh, Hasankeyf Höyük, Hakemi Use and Çatalhöyük (Richards et al., 2003; Styring et al., 2015).

Site		N	$\delta^{15}N_{col}$		$\Delta^{15}N_{human-goat}$	N	Fa_{AA}	Period	Region
Tell Ain el-Kerkh		47	7.9	±0.8	4.0	11	45.8%	PN	north Levant
Hasankeyf Höyük	all	15	8.6	±0.8	2.6	15	79.5%	PPNA	southeast Anatolia
	$7.6<\delta^{15}N_{Phe}$	10	8.5	±0.7	2.5	10	63.6%		
Hakemi Use		17	7.4	±1.3	1.5	19	50.3%	PN	southeast Anatolia
Çatalhöyük		67	10.9	±1.2	4.1	5	53.0%	PN	Central Anatolia
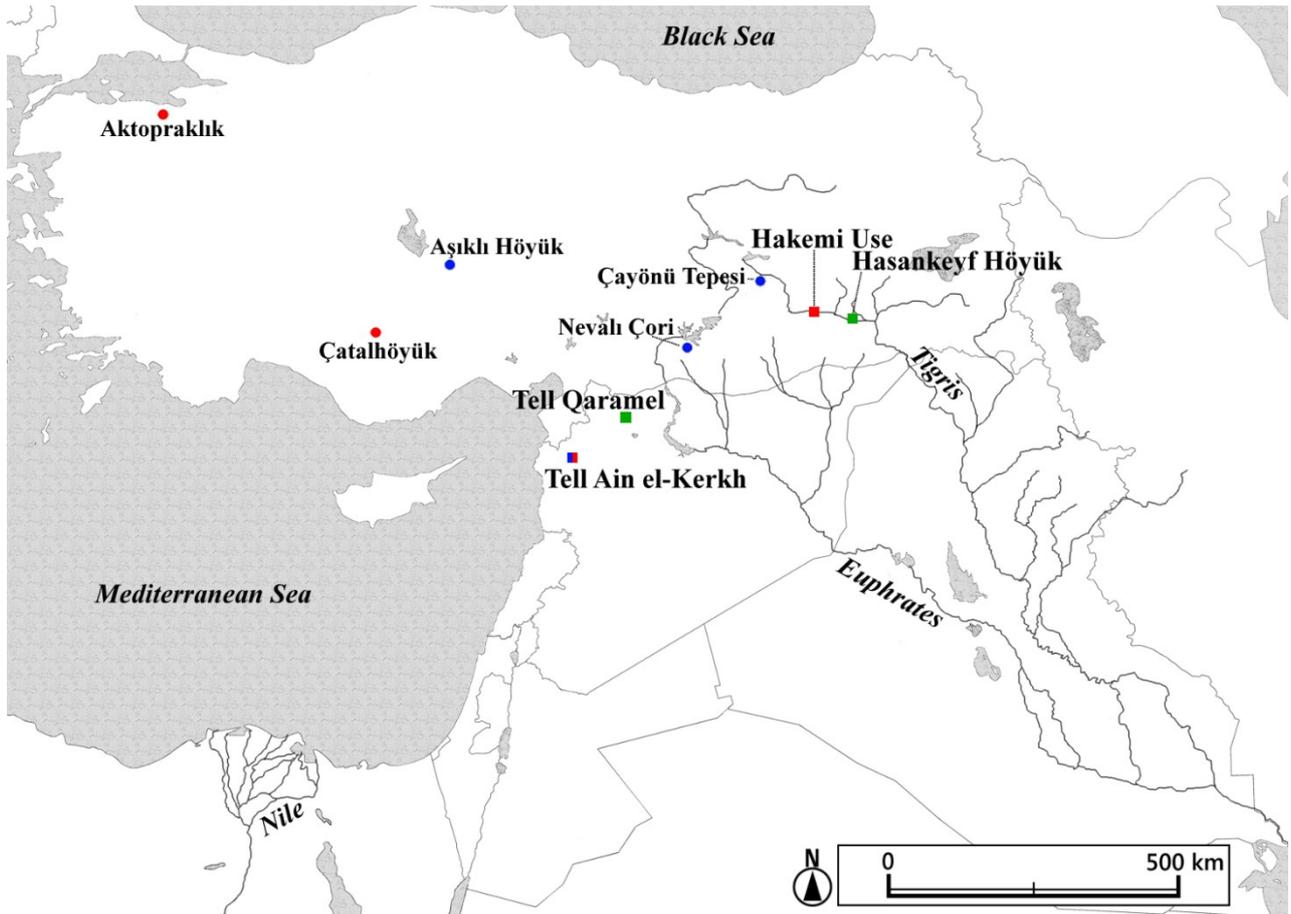
Fig. 1.1. Map of the southwest Asia showing location of Neolithic sites mentioned in the study. Square indicates

site analyzed by this study and circle indicates site analyzed in previous studies. Markers of each color indicate

PPNA site (green), PPNB site (blue) and PN site (red).
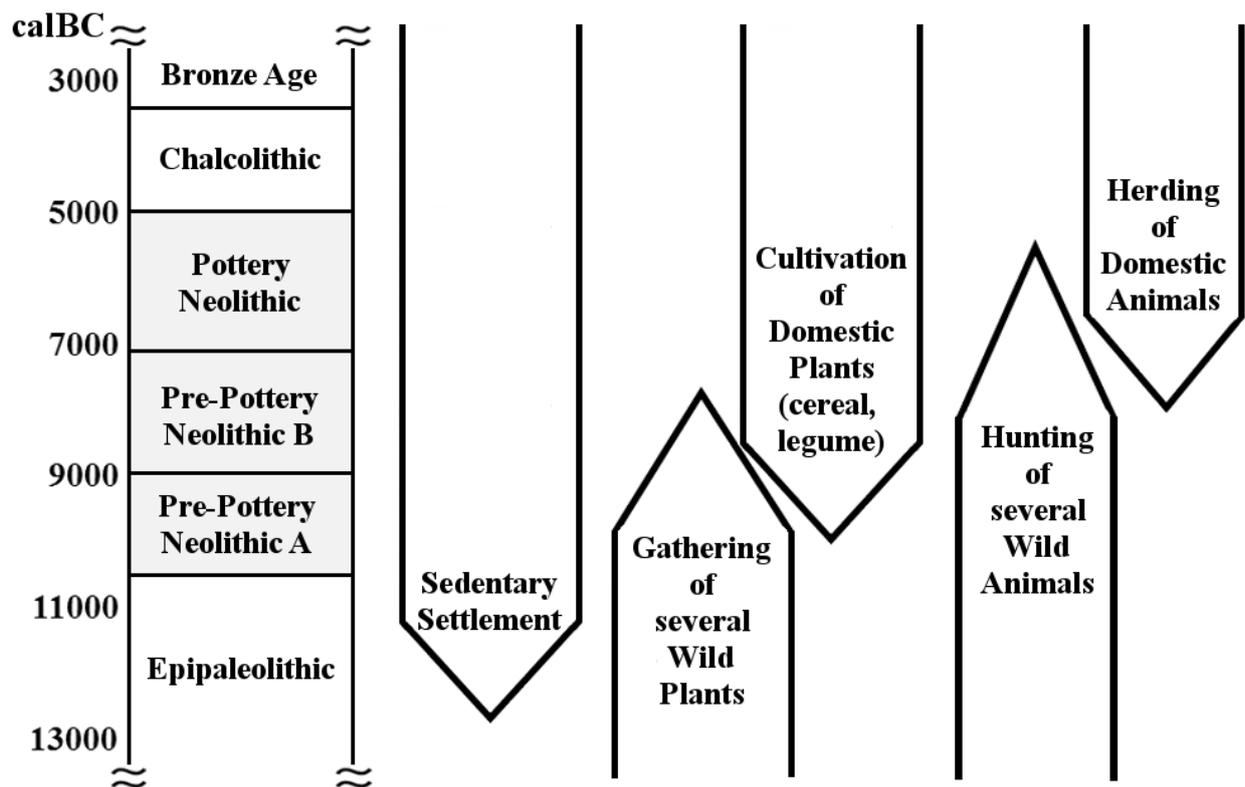
Fig. 1.2. Gradual development of "Neolithisation" associated with period.
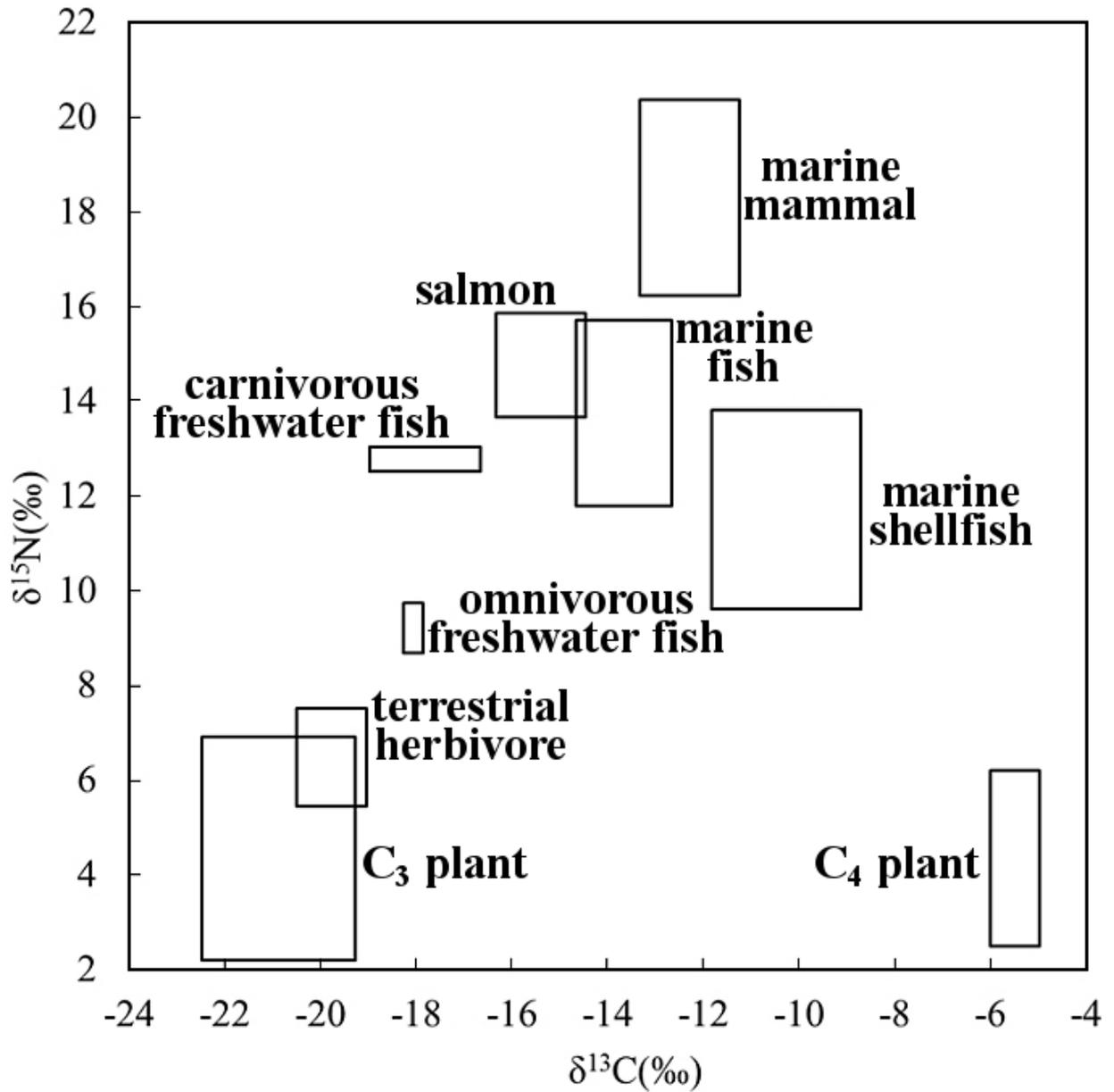
Fig. 1.3. Bulk isotopic data of the food resources for human in Japan. Squares are showing one standard deviation of composed of Japanese food groups (Yoneda et al., 2004).
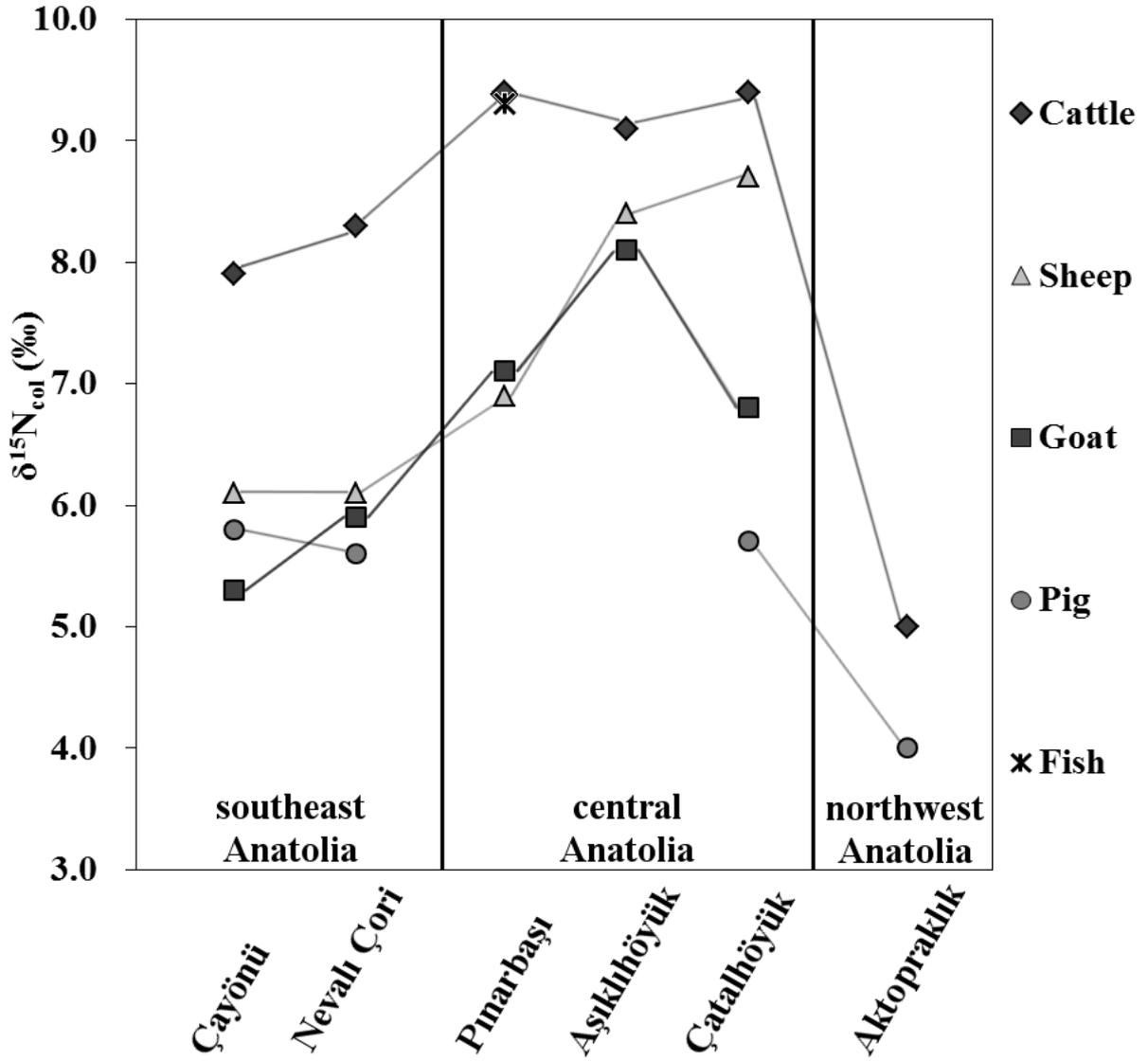
Fig. 1.4. Variation of animal δ^{15}N$_{col}$ from Çayönü Tepesi (Pearson et al., 2013), Nevalı Çori (Lösch et al., 2006), Pınarbaşı (Baird et al., 2013), Aşıklıhöyük (Pearson et al., 2010), Çatalhöyük (Richards et al., 2003a), and Aktopraklık (Budd et al., 2013).
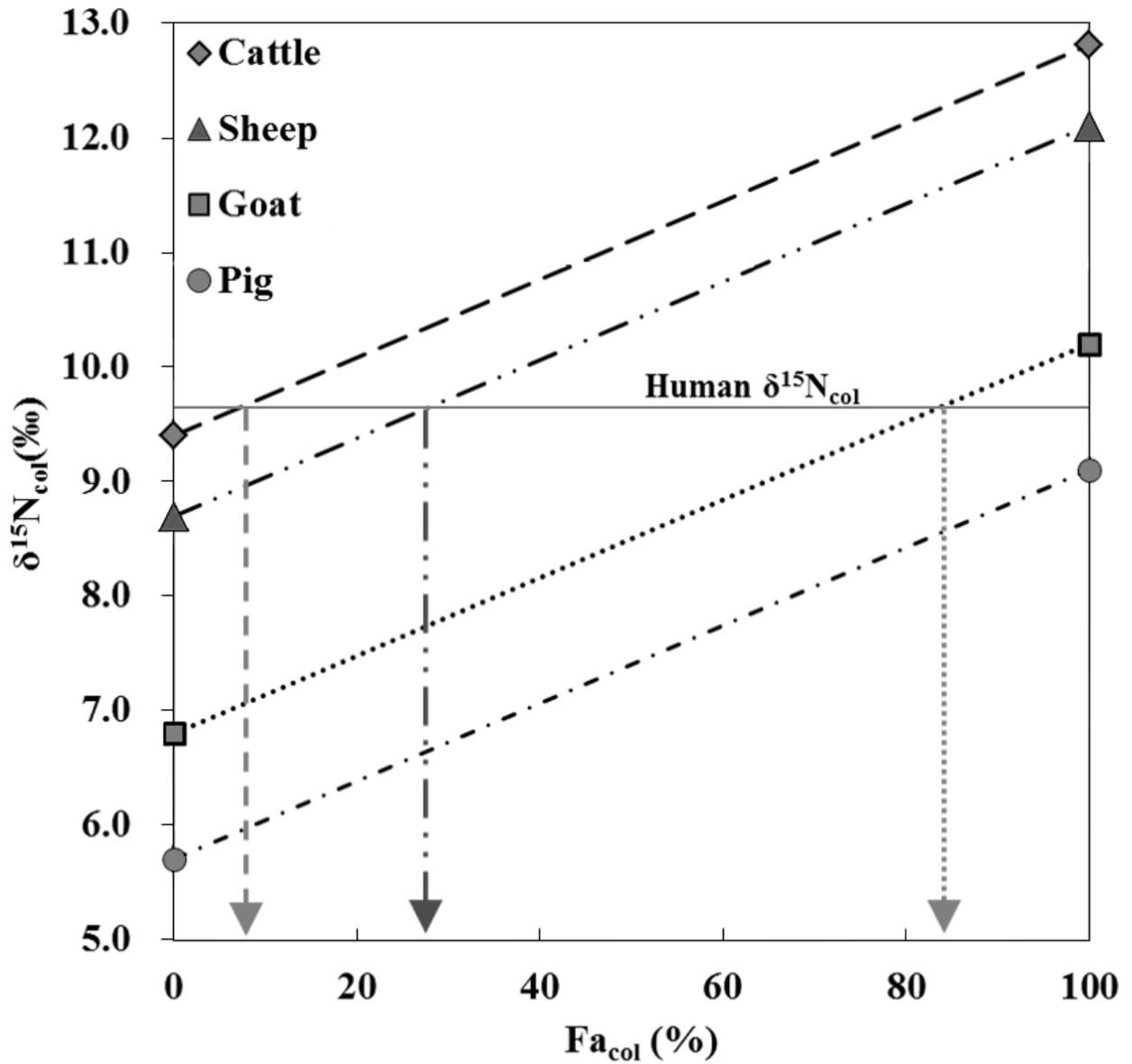
Fig. 1.5. Variation of fraction of animal protein in the diet based on the each animal $\delta^{15}N_{col}$ (Fa_{col}) of Çatalhöyük (Richards et al., 2003a) assuming a $\delta^{15}N_{collagen-diet}$ value of 3.4‰ (Minagawa and Wada, 1984), with a human $\delta^{15}N_{col}$ value.

(i) Aquatic ecosystems
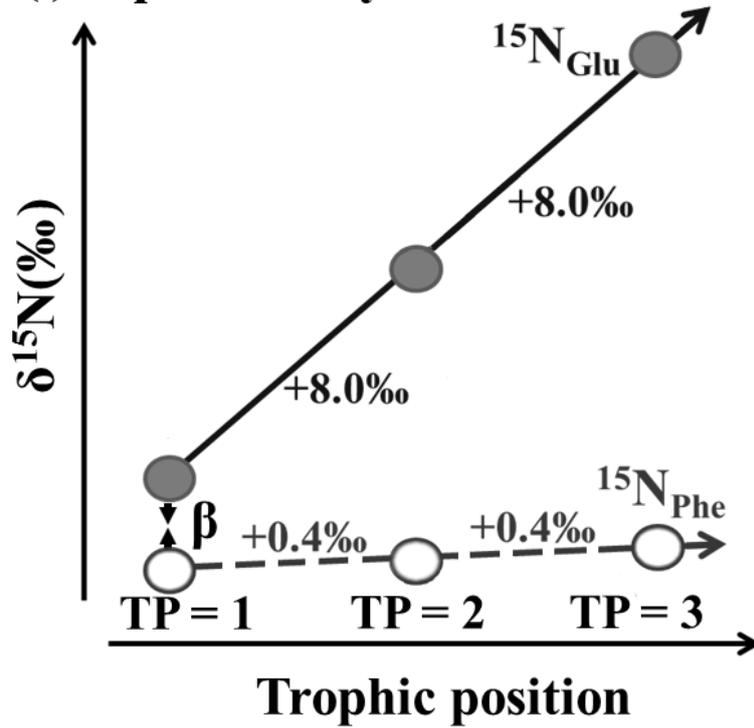
(ii) Terrestrial plant ecosystems
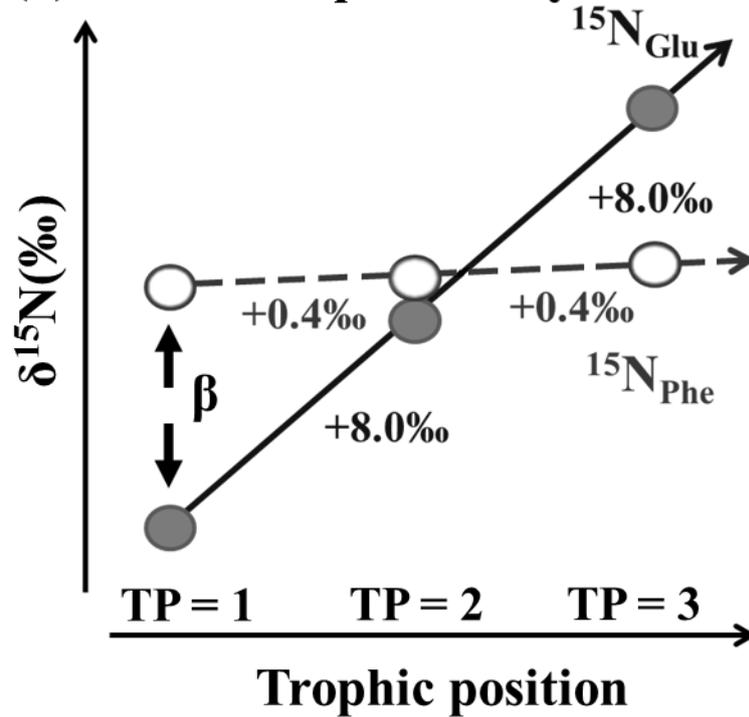
Fig. 1.6. Schematic illustration of the relationship between the nitrogen isotopic composition of amino acids (glutamic acid and phenylalanine) and trophic position in each ecosystem. β shows the difference of between $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ in primary producers (−3.4‰ for (i)aquatic ecosystem, +8.4‰ for (ii)terrestrial ecosystem).
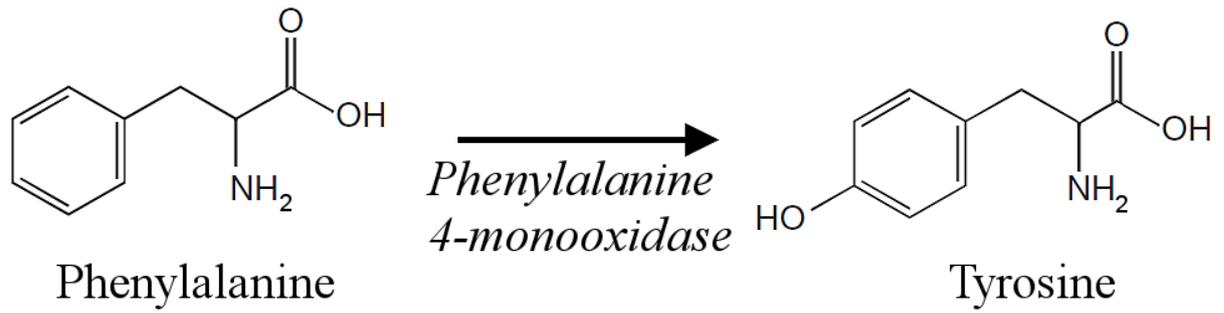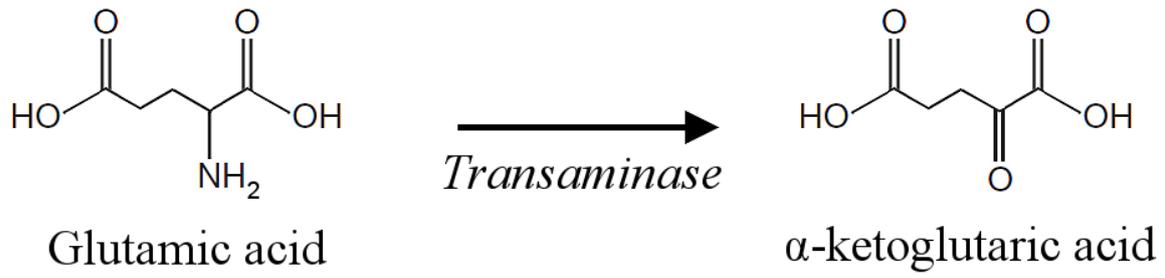
Fig. 2.1. Dominant initial steps of amino acid metabolic processes in animals (Bender, 2014).
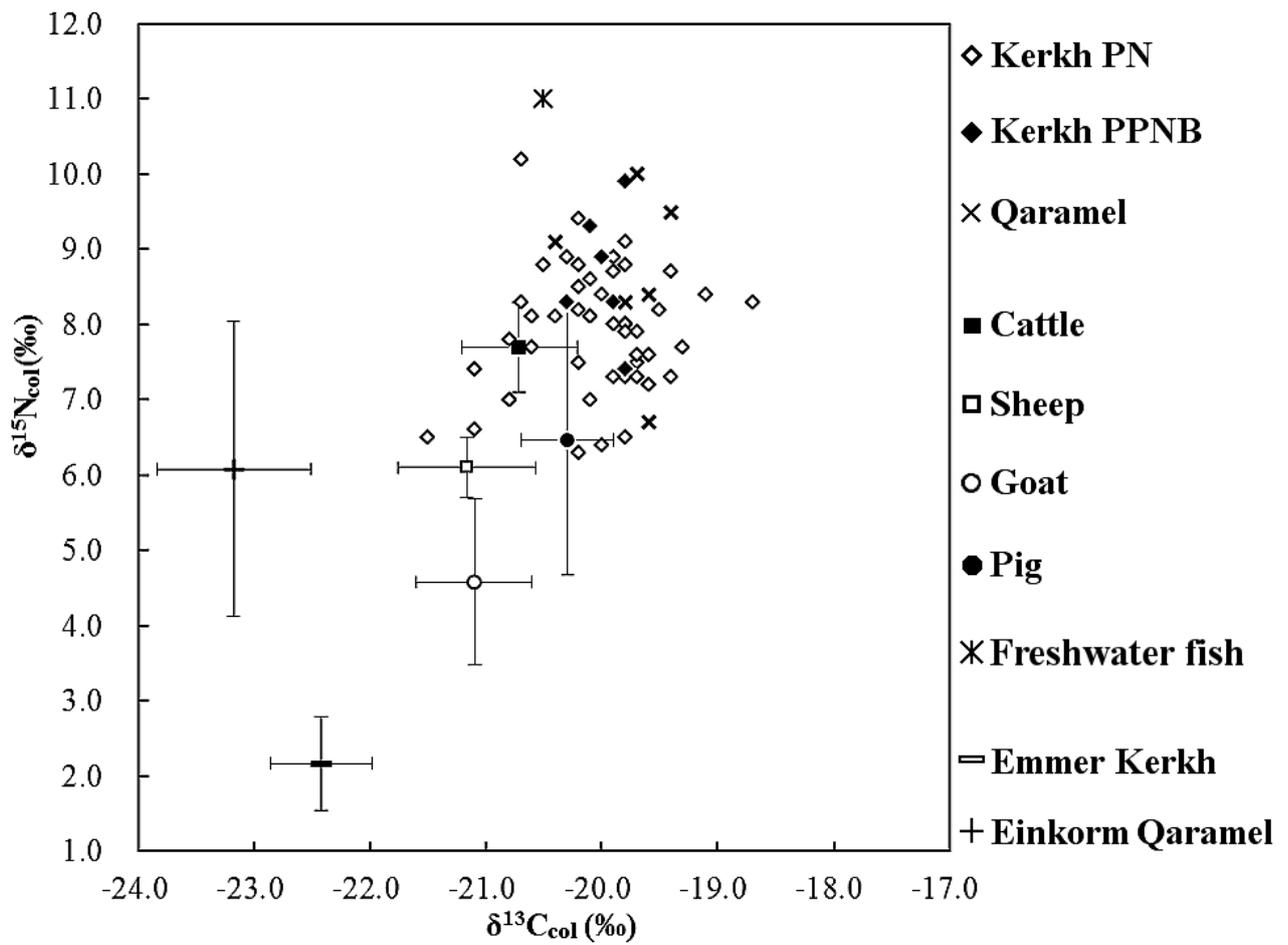
Fig. 3.1. Human individual $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ values in north Levant (Kerkh PN, Kerkh PPNB and Qaramel) plotted with mean isotope values for fauna (cattle, sheep, goat, pig and freshwater fish) and wheats (einkorn at Tell Qaramel (Araus et al., 2014) and emmer at Tell Ain el-Kerkh) (bars represent 1σ values).
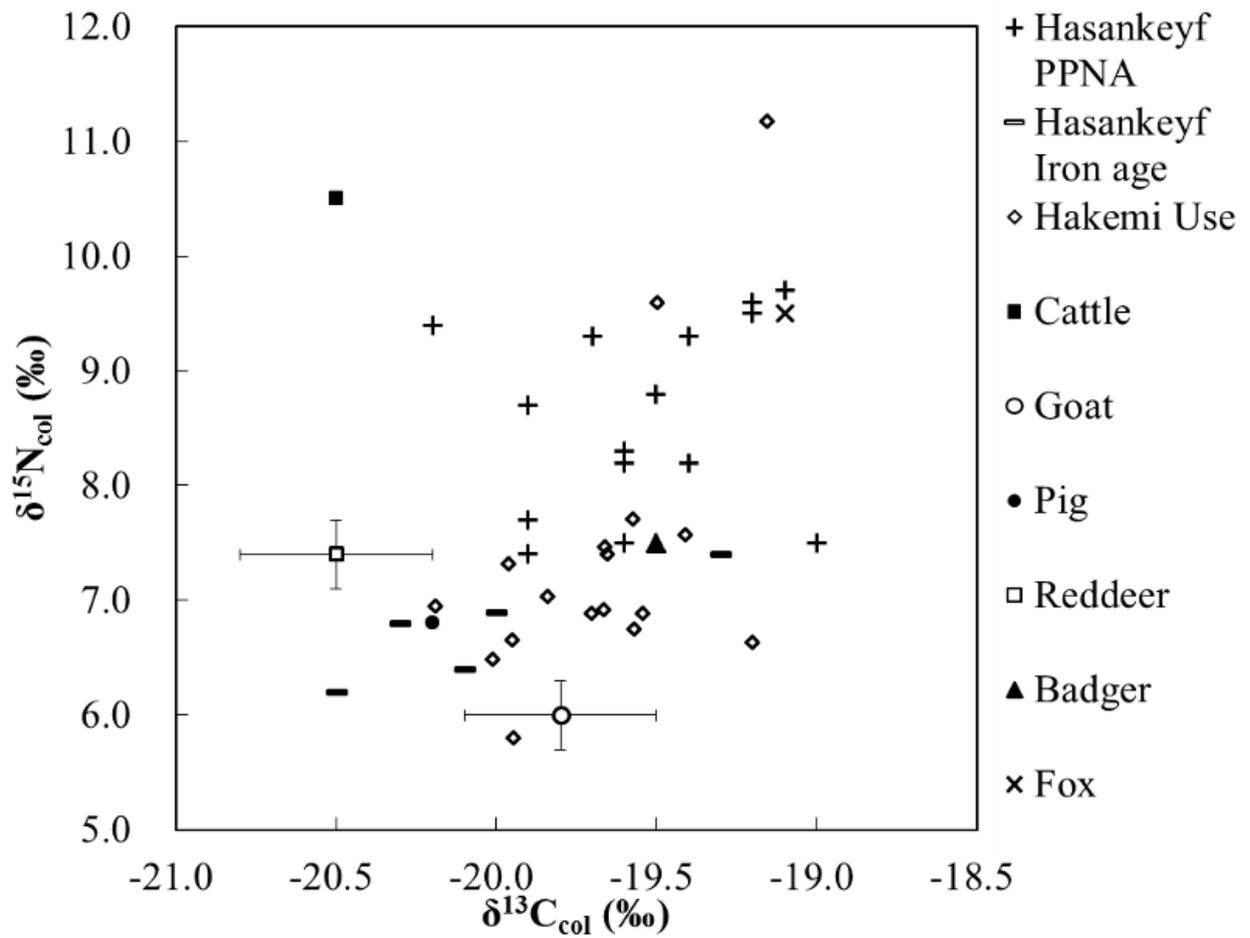
Fig. 3.2. Human individual $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ values in southeast Anatolia (Hasankeyf PPNA, Hasankeyf Iron age and Hakemi Use) plotted with mean isotope values for fauna (cattle, sheep, goat, pig, red deer, badger and fox) (bars represent 1σ values) at Hasankeyf Höyük.
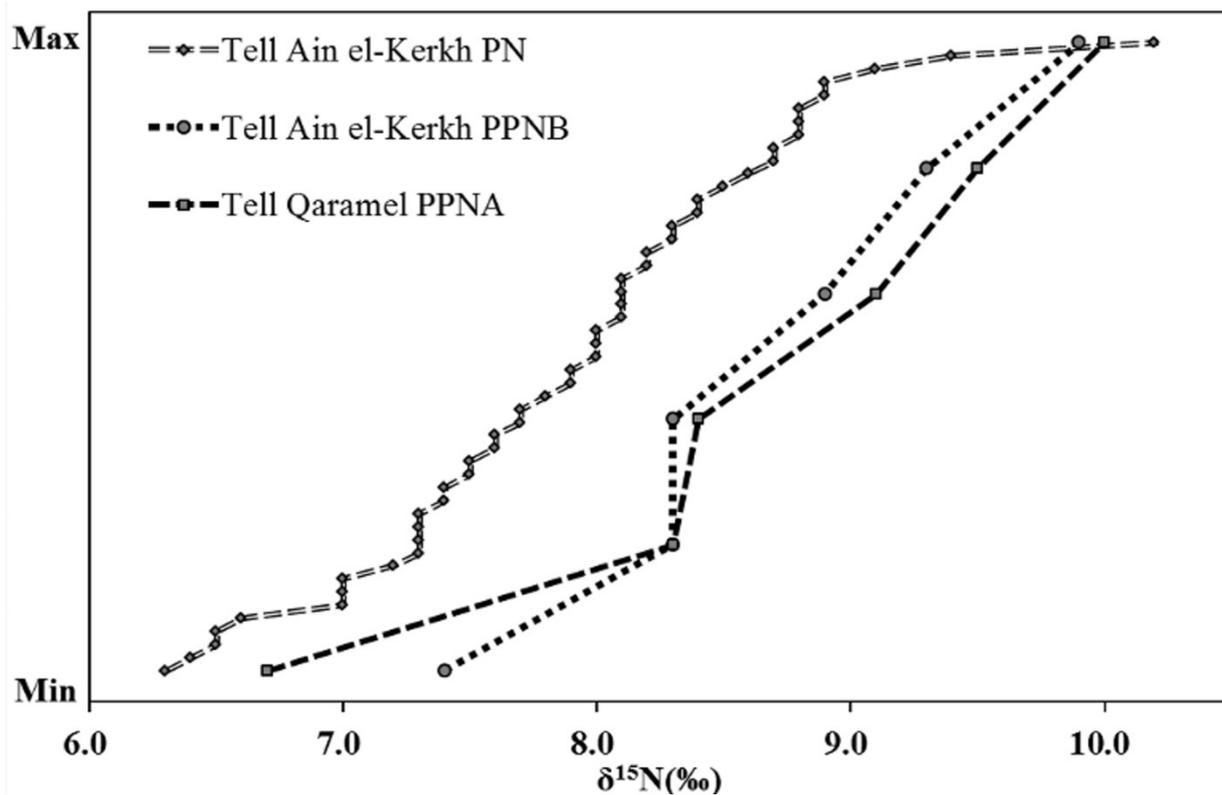
Fig. 3.3. The $\delta^{15}N$ values of humans in the Tell Qaramel PPNA layer and the Tell Ain el-Kerkh PPNB and PN

layers (plotted on the x-axis), with individual samples seriated from minimum to maximum $\delta^{15}N$ on the y-axis.
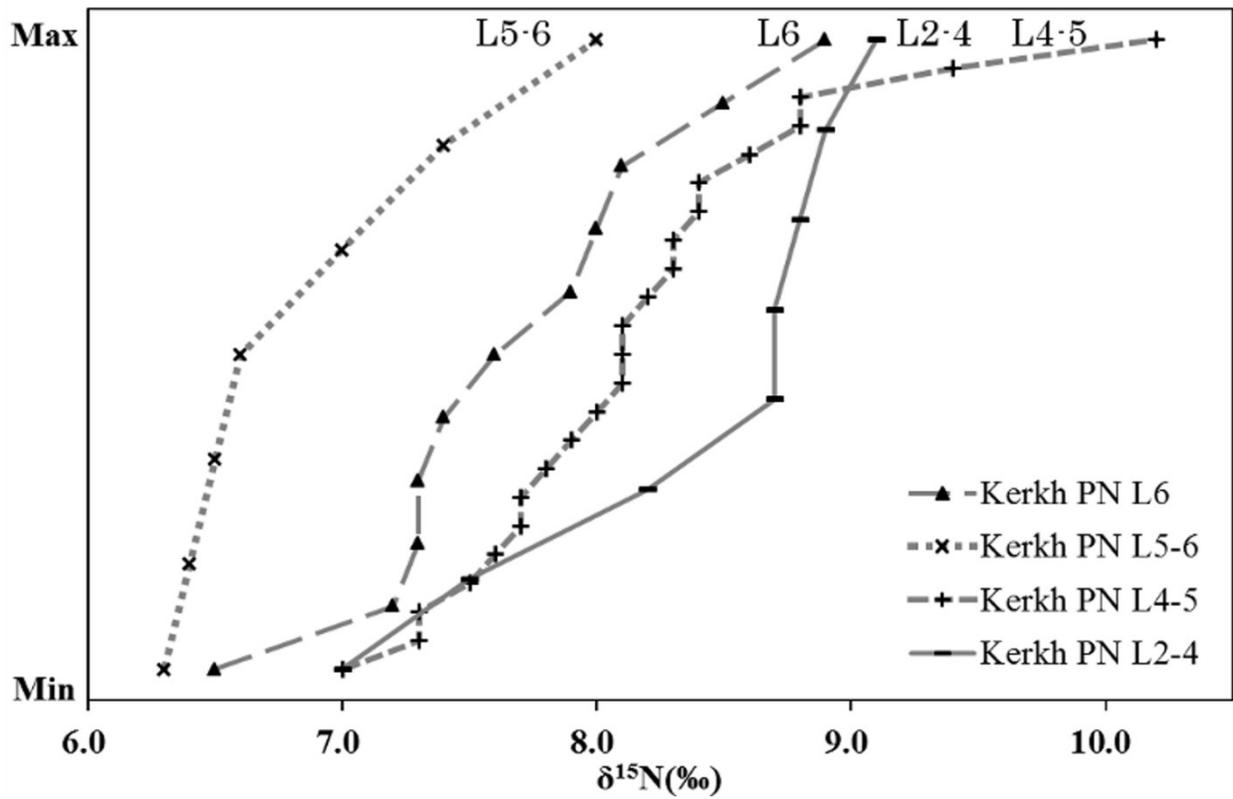
Fig. 3.4. The δ^{15}N values of humans from several sub-layers of the PN at Tell Ain el-Kerkh (x-axis), with individual samples seriated from minimum to maximum δ^{15}N on the y-axis; data are for each sub-layer.
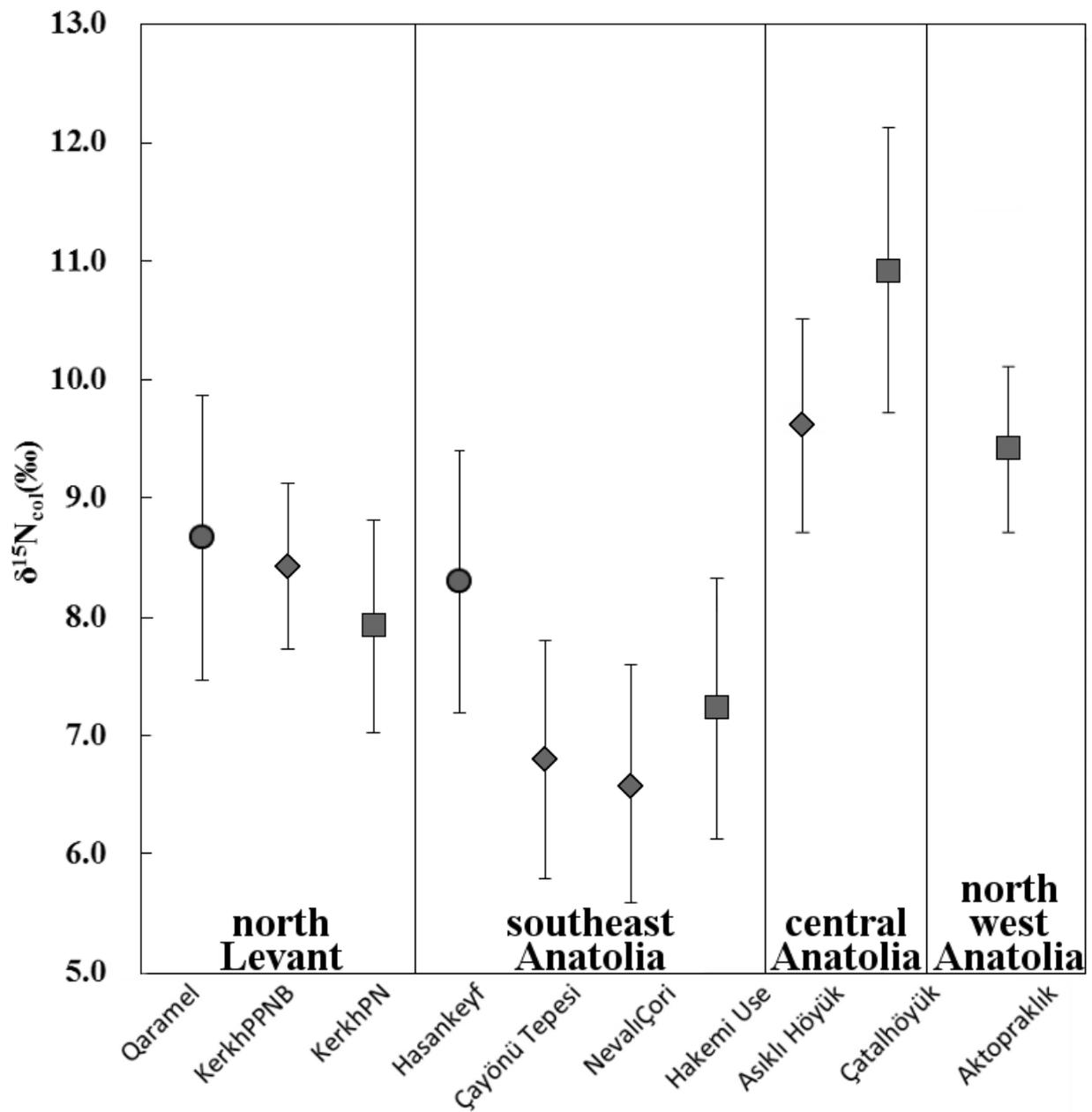
Fig. 3.5. The mean of $\delta^{15}N_{col}$ values of human from different Neolithic southwest Asia sites (with bars representing 1σ standard deviations). The human $\delta^{15}N$ values (and references) are from Çayönü Tepesi (Pearson et al., 2013), Nevalı Çori (Lösch et al., 2006), Aşıklıhöyük (Pearson et al., 2010), Çatalhöyük (Richards et al., 2003a), and Aktopraklık (Budd et al., 2013). Each icon indicates PPNA site (●), PPNB site (◆) and PN site (■).
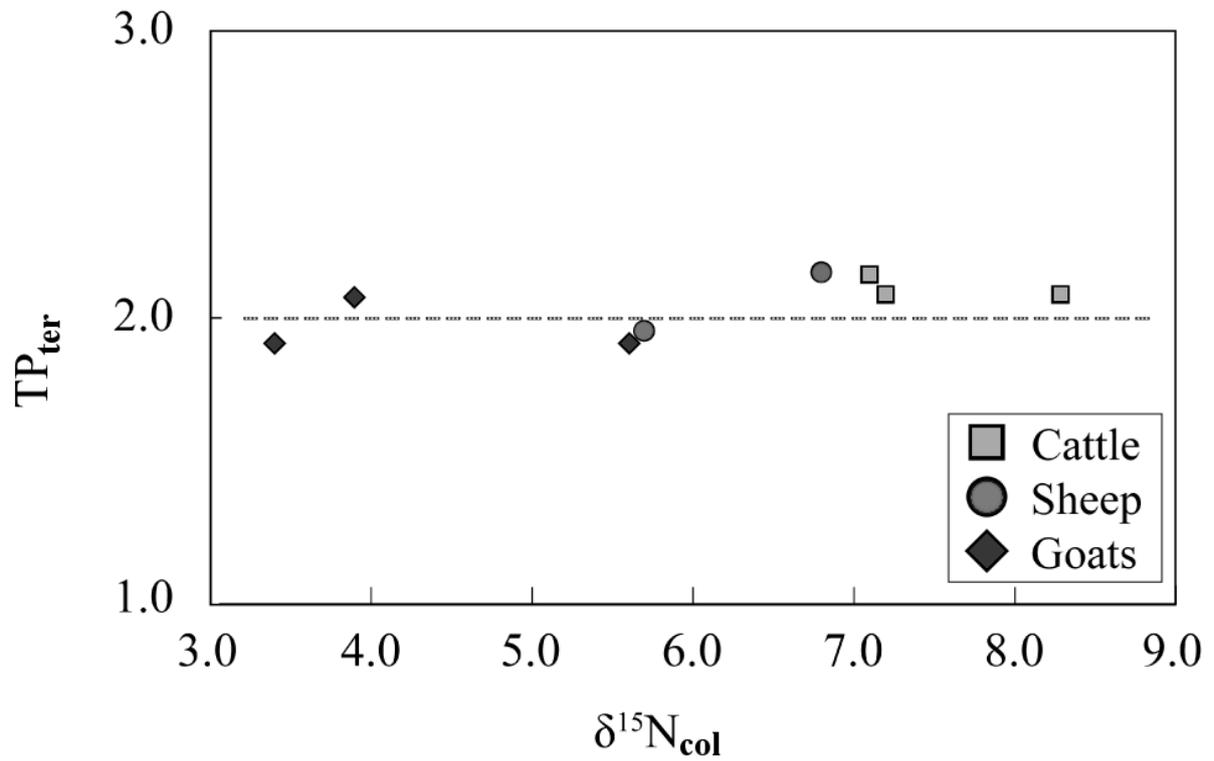
Fig. 4.1. Relationship between the $\delta^{15}N$ values of bulk bone collagen and the estimated trophic positions (TP$_{Glu/Phe}$) of the three herbivores. The dashed line indicates a TP$_{Glu/Phe}$ value of 2.0.
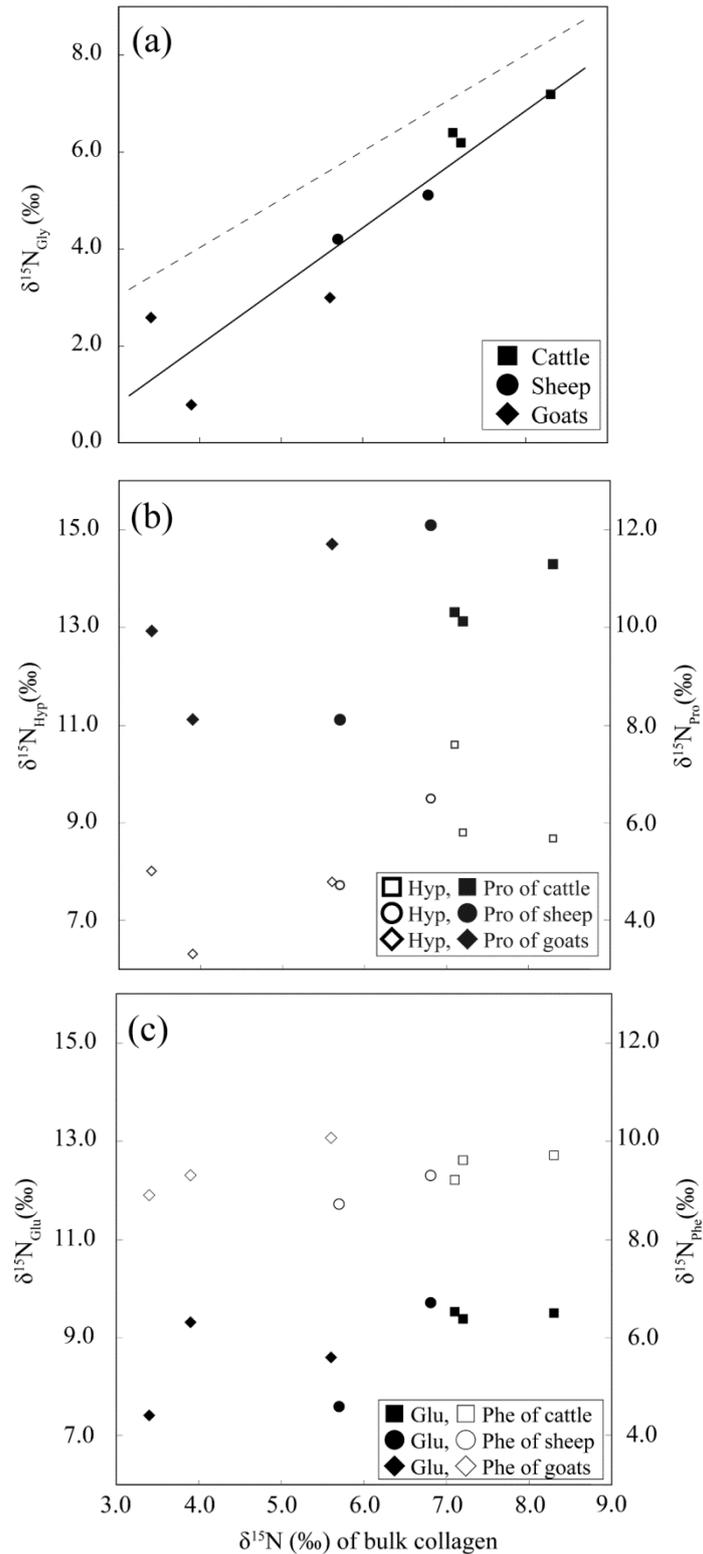
Fig. 4.2. Relationships between the $\delta^{15}N$ values of bulk bone collagen and (a) glycine (the solid line shows the result of a linear regression of $\delta^{15}N_{bulk}$ *vs* $\delta^{15}N_{Gly}$, and the dashed line indicates a 1:1 relationship between the two variables), (b) hydroxyproline (Hyp) and proline (Pro), and (c) Glutamic acid (Glu) and phenylalanine (Phe) of the three herbivores.
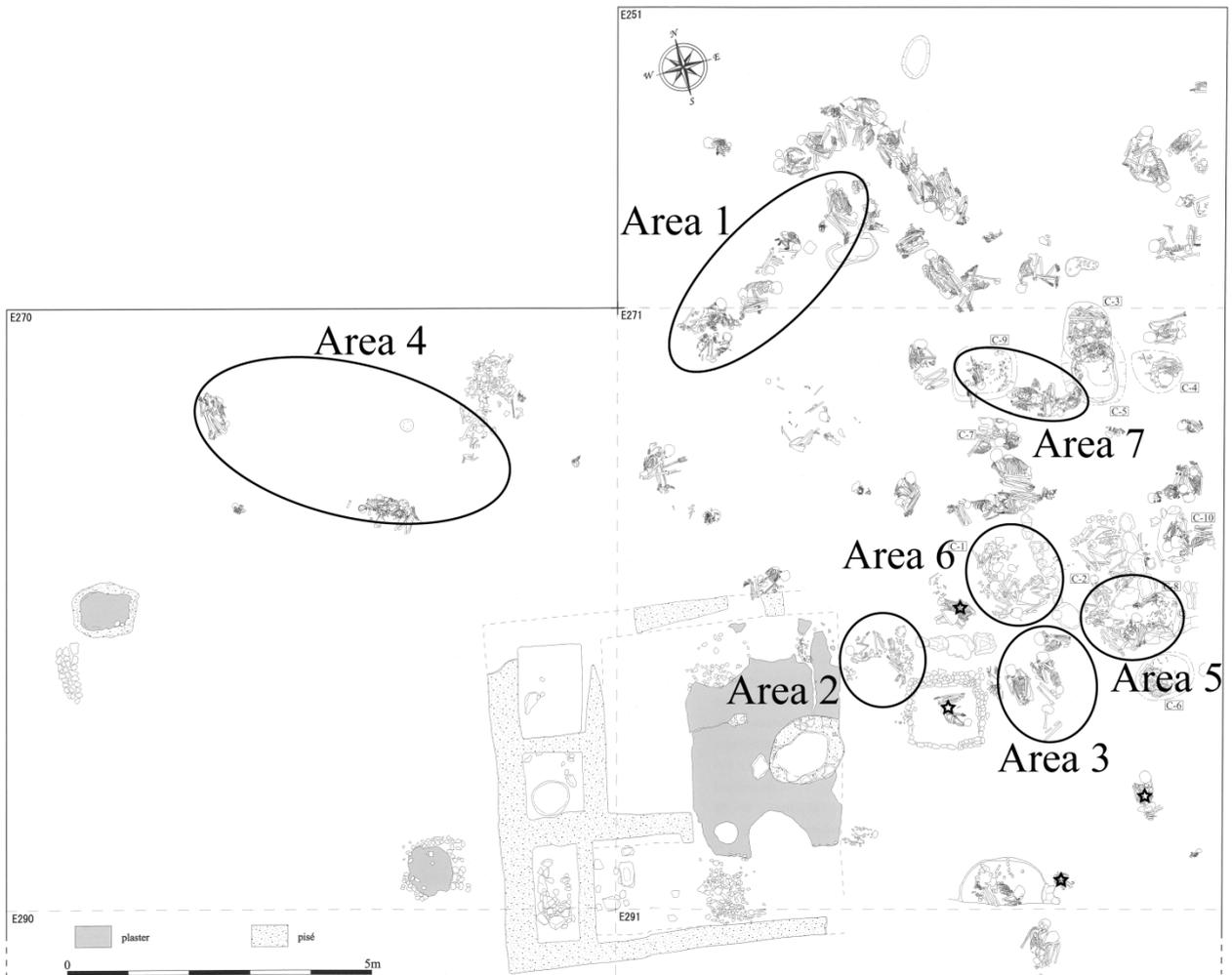
Fig. 5.1. Plan of cemetery at Tell Ain el-Kerkh and burial groups (Area 1–7). "☆" indicates individuals who were not comprised in the each group (Area 1–7).
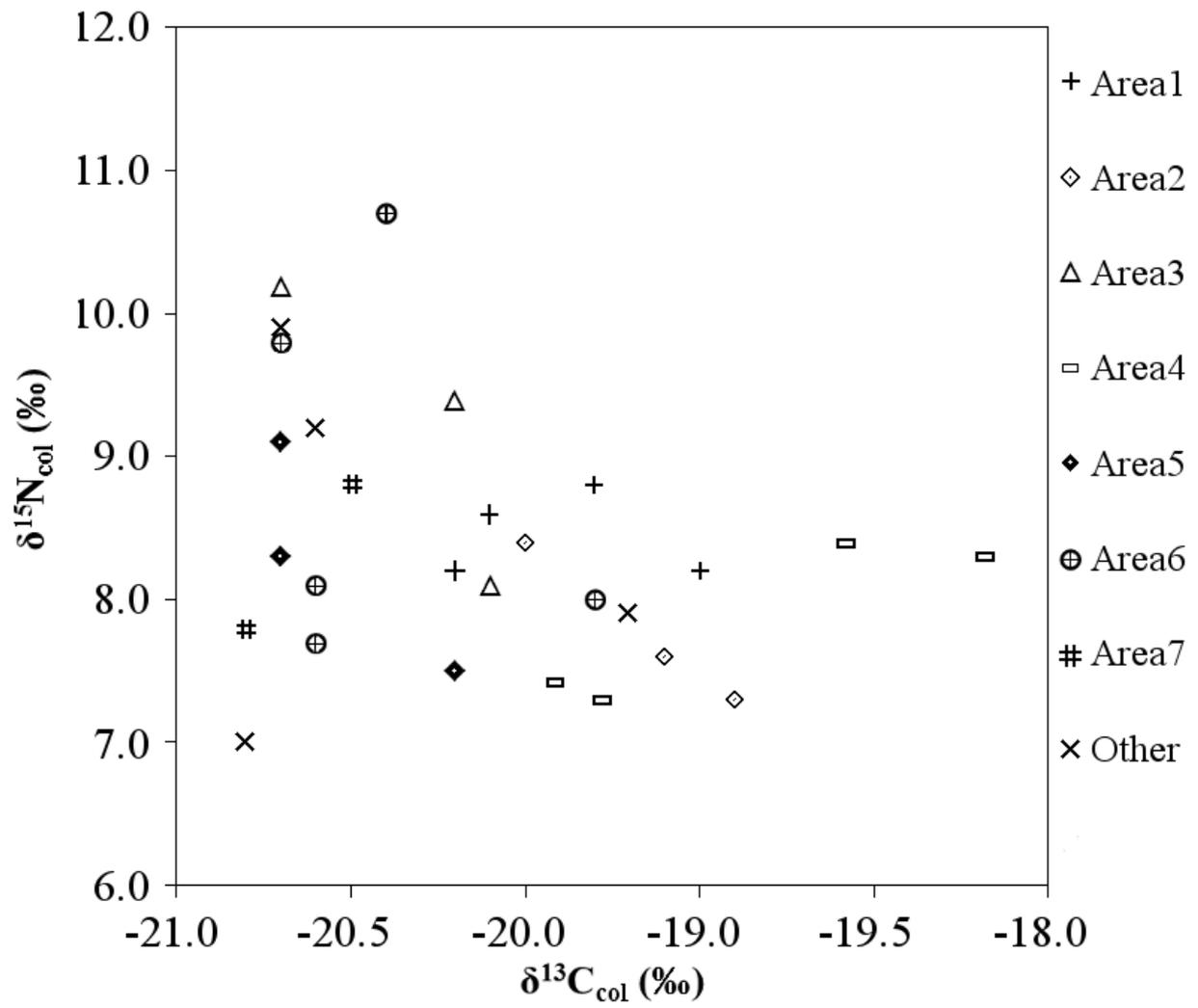
Fig. 5.2. Human $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ along each group (Area 1–7) and others are plotted at Tell Ain el-Kerkh.
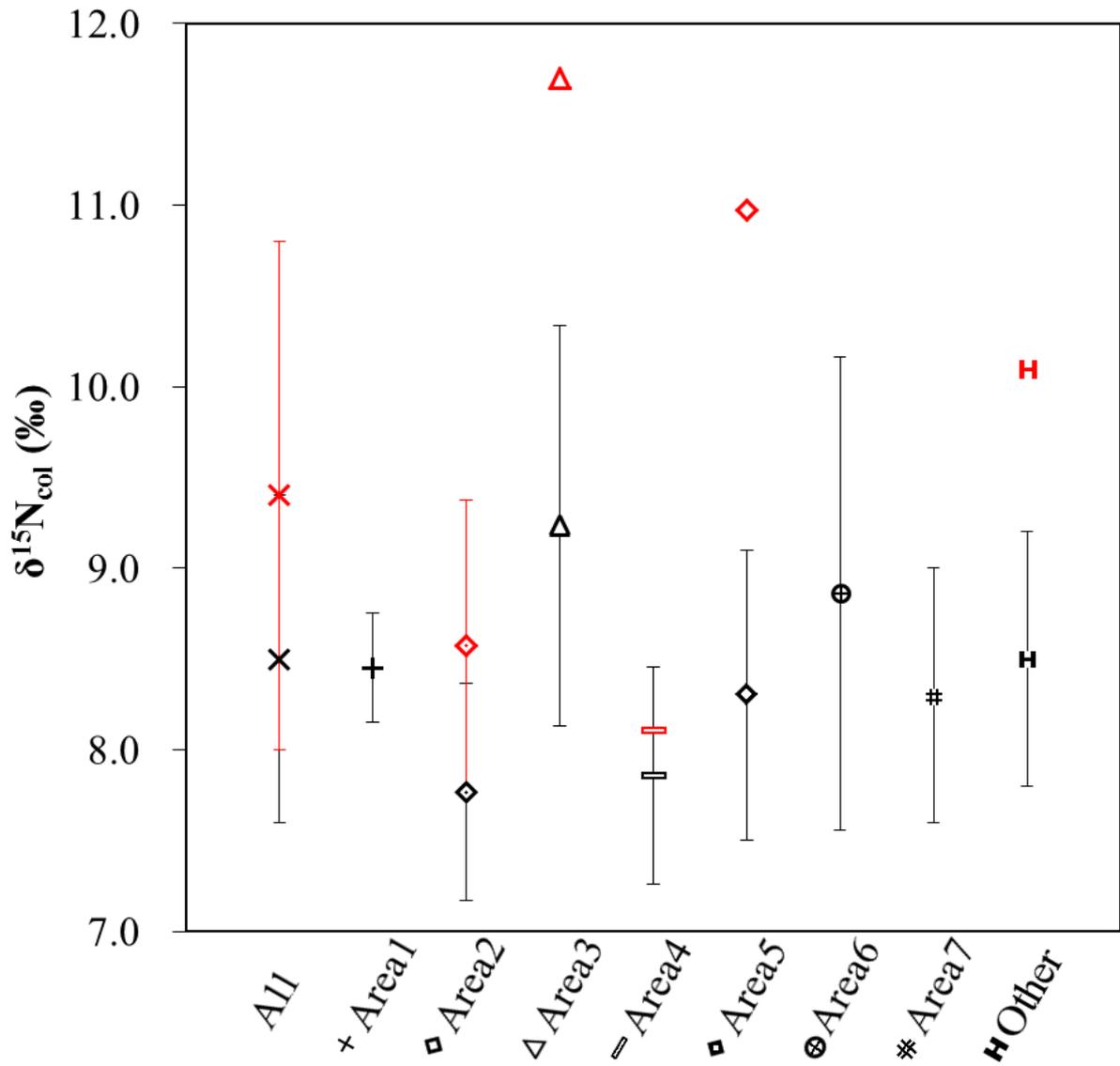
Fig. 5.3. Mean of $\delta^{15}N_{col}$ along each group (Area 1–7) and other are plotted (with bars representing 1σ standard deviations and with red marker show infants under 4yrs) at Tell Ain el-Kerkh.
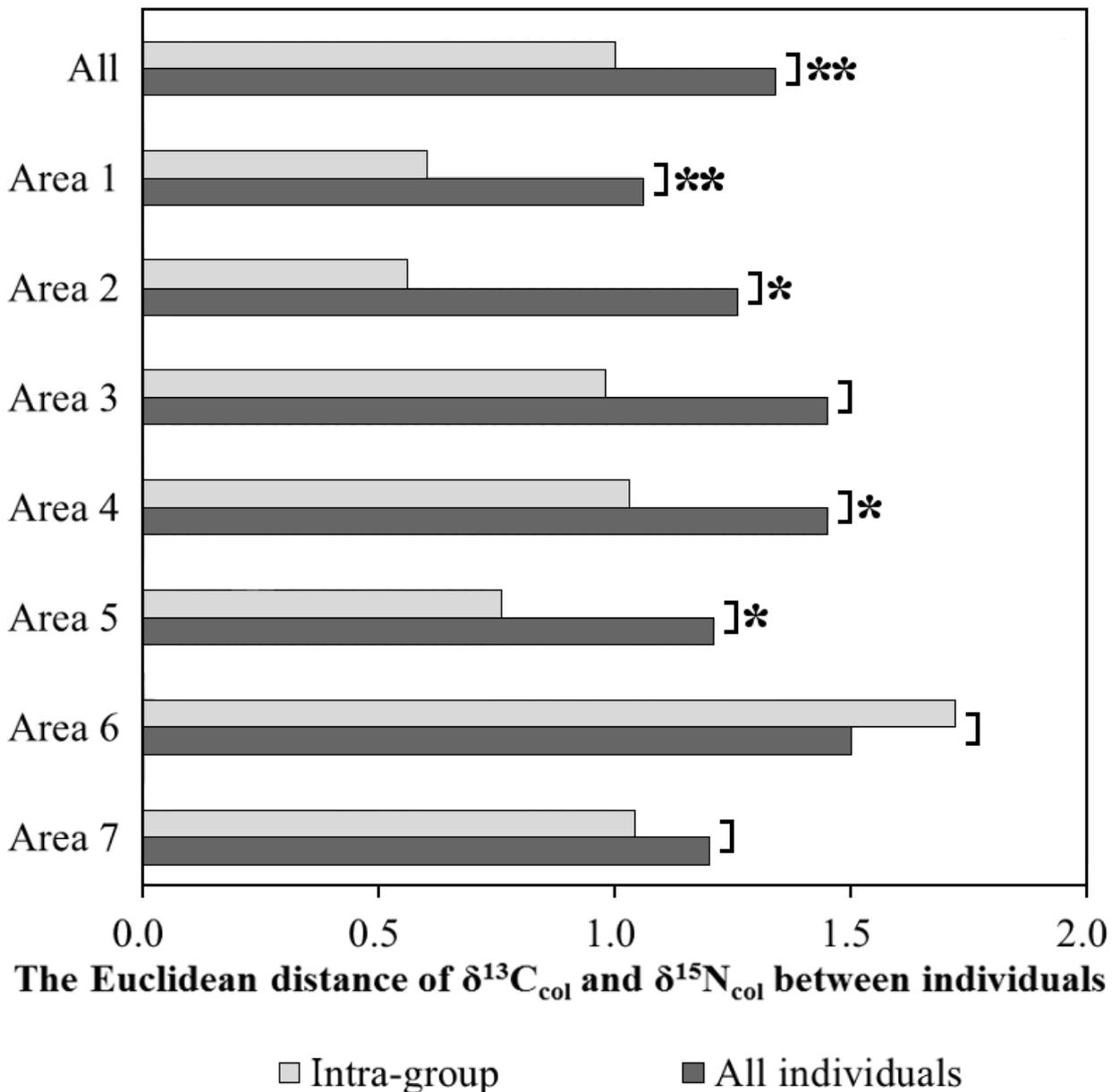
Fig. 5.4. Mean of $\delta^{15}N_{col}$ along each group (Area 1–7) are plotted at Tell Ain el-Kerkh. "Intra-group" shows the mean of Euclidean distance of $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ between two individuals within the each group. "All individuals" shows the mean of Euclidean distance of $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ between two individuals in all individuals. "*" indicates significant difference ($p < 0.05$) and "**" indicates significant difference ($p < 0.01$), by two tailed Welch's t test.
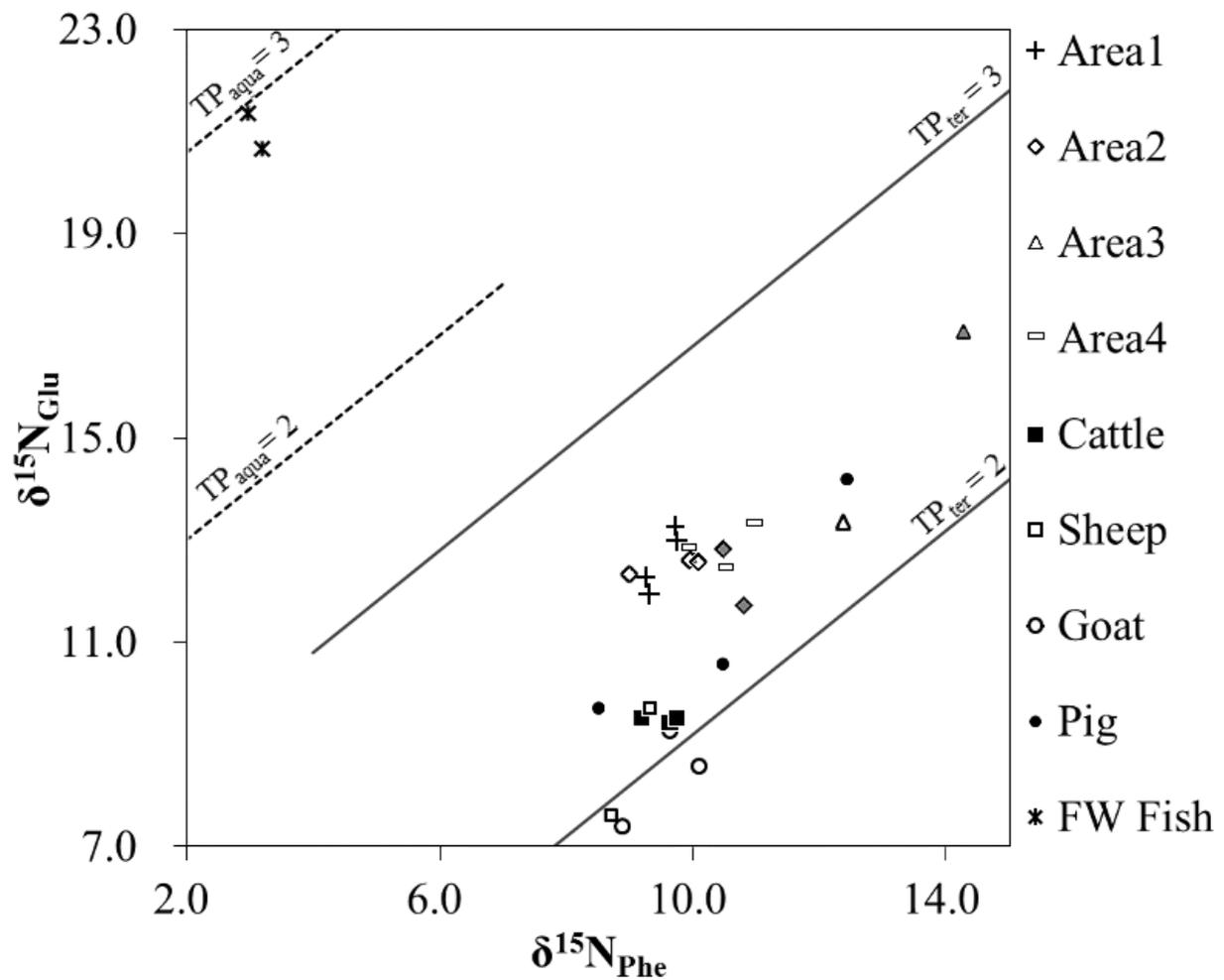
Fig. 5.5. Comparison between nitrogen isotopic composition of amino acids ($\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$) for humans of each group and faunal remains at Tell Ain el-Kerkh. Solid lines represent expected $\delta^{15}N$ values with $TP_{ter} = 2$ (primary consumer) or 3 (secondary consumer), and dashed lines represent expected $\delta^{15}N$ values with $TP_{aqua} = 2$ (primary consumer) or 3 (secondary consumer).
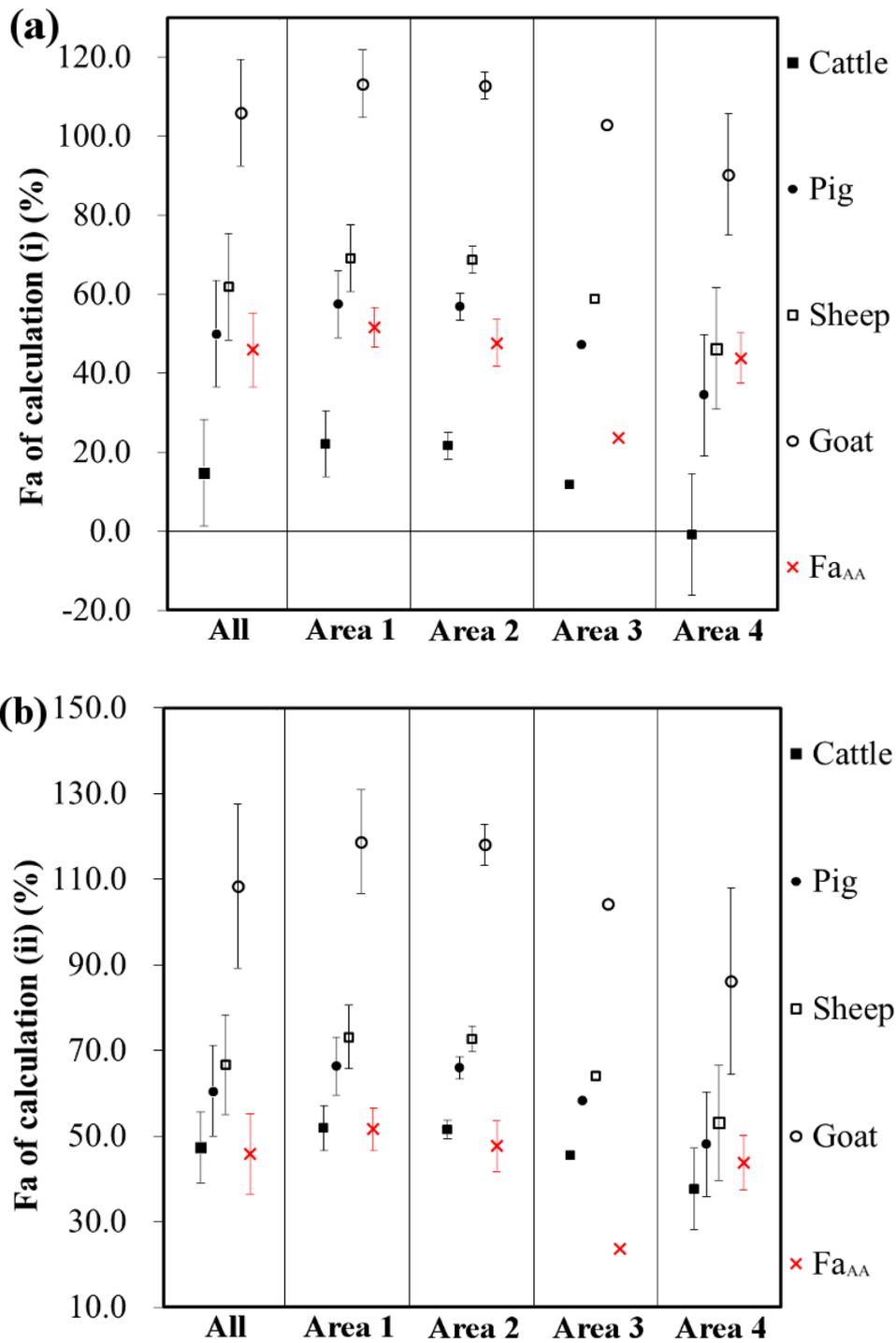
Fig. 5.6. The estimated fraction animal protein in total terrestrial protein by amino acids (Fa_{AA}) and collagen (Fa_{col}) calculated (i) according to the $\delta^{15}N$ values of terrestrial animals and (ii) according to the $\delta^{15}N$ values of both terrestrial animals and cereal grains. Each kind of markers indicates Fa_{AA} (with red marker) and Fa_{col} according to each animal (cattle■, sheep□, goat○, pig●).
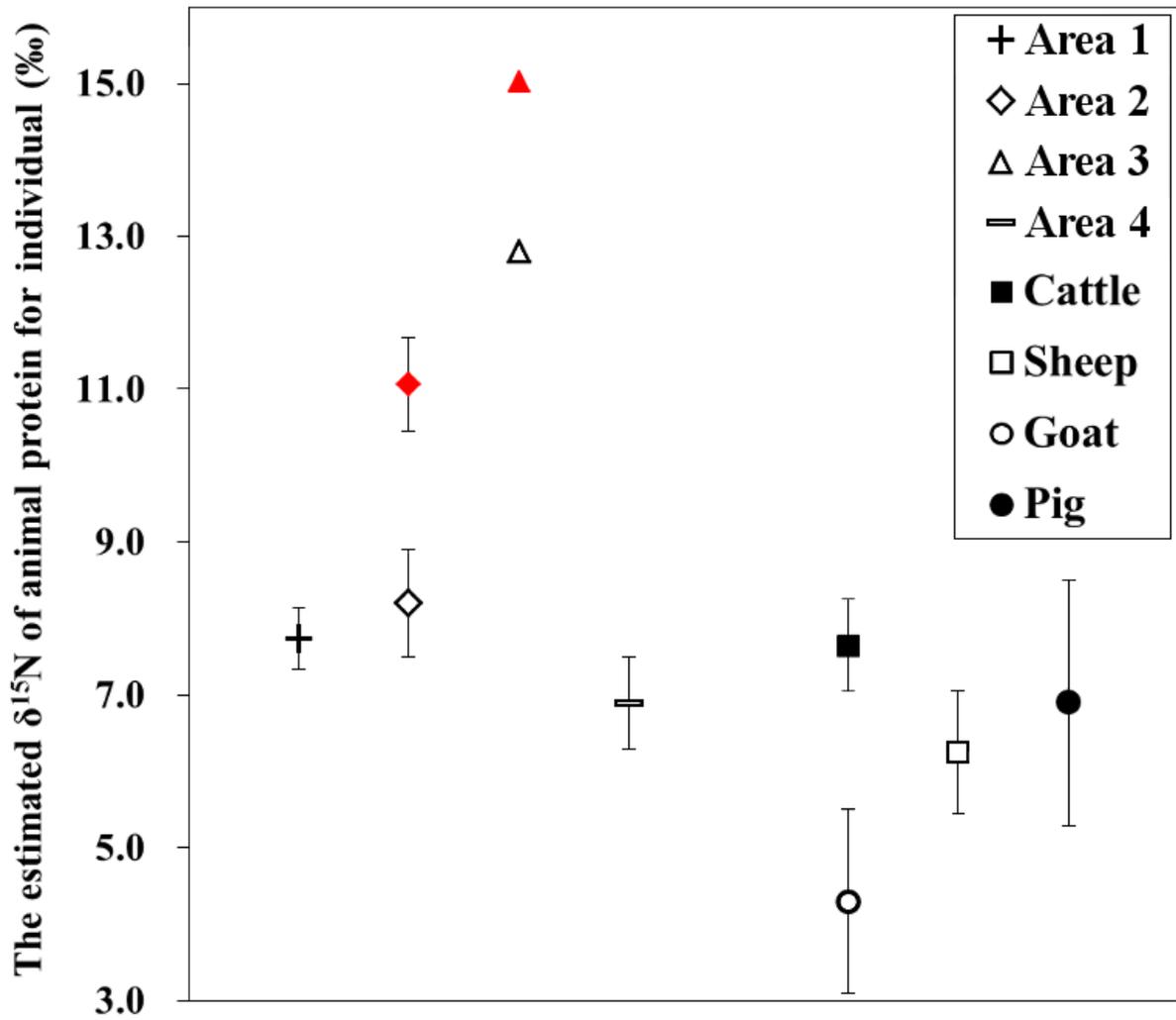
Fig. 5.7. The estimated $\delta^{15}N$ of animal protein for each burial group (with red marker show infants under 4yrs) with the $\delta^{15}N_{col}$ values of each terrestrial animals (cattle■, sheep□, goat○, pig●).
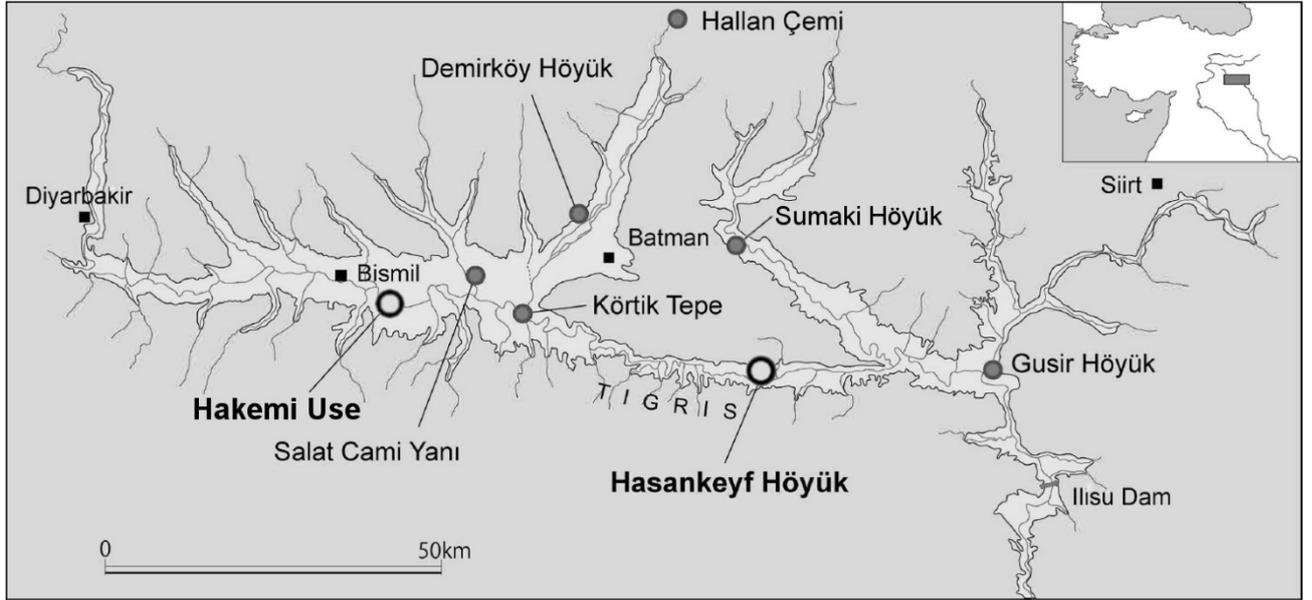
Fig. 6.1. Location of Hasankeyf Höyük and Hakemi Use in Upper Tigris (Miyake et al., 2012).
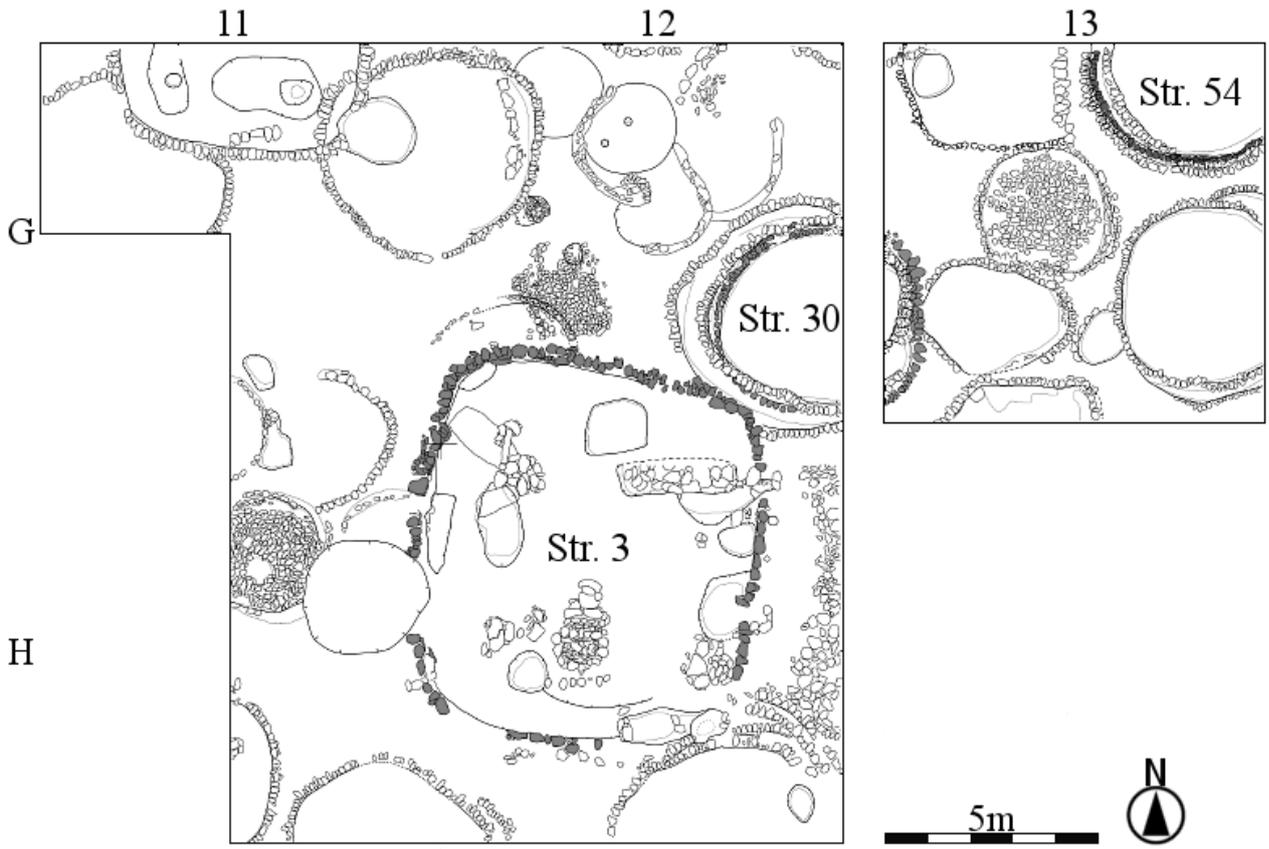
Fig. 6.2. Plan of structures at Hasankeyf Höyük.
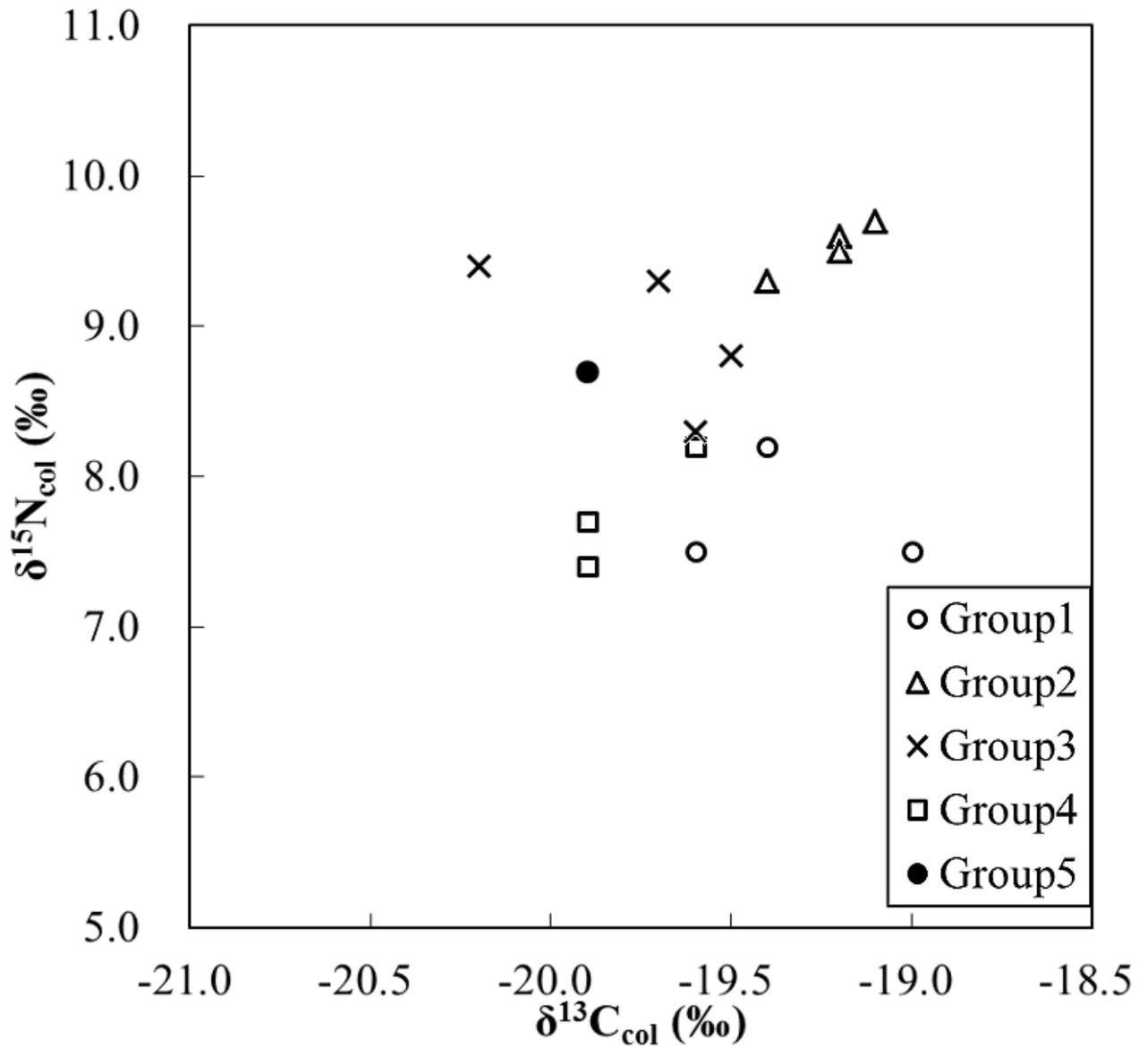
Fig. 6.3. The $\delta^{13}C$ and $\delta^{15}N$ values of humans at Hasankeyf Höyük are plotted. The markers of each individual

are separated to groups according to buried locations.
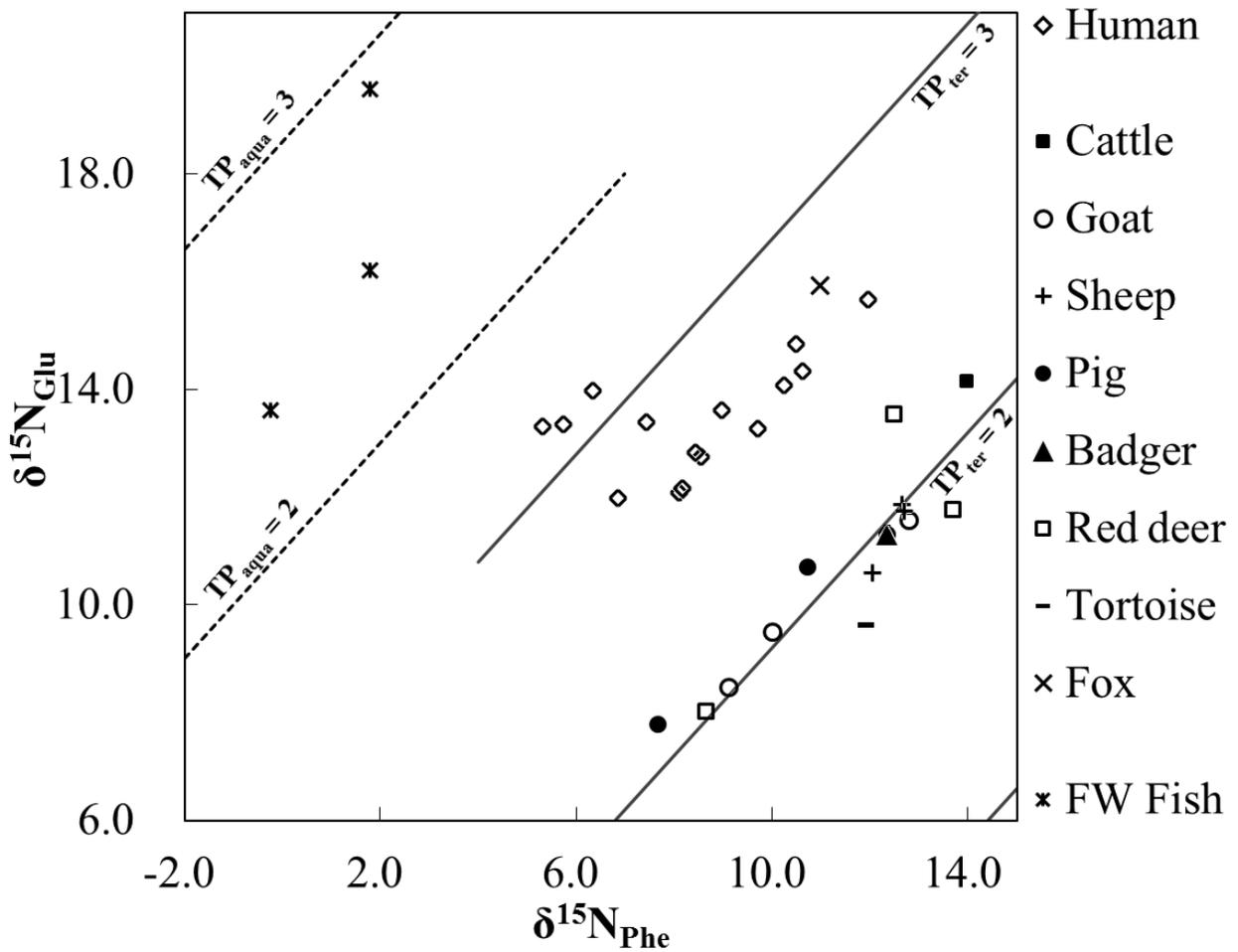
Fig. 6.4. Comparison between nitrogen isotopic composition of amino acids ($\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$) for human and faunal remains at Hasankeyf Höyük. Solid lines represent expected $\delta^{15}N$ values with TP_{ter} = 2 (primary consumer) or 3 (secondary consumer), and dashed lines represent expected $\delta^{15}N$ values with TP_{aqua} = 2 (primary consumer) or 3 (secondary consumer).
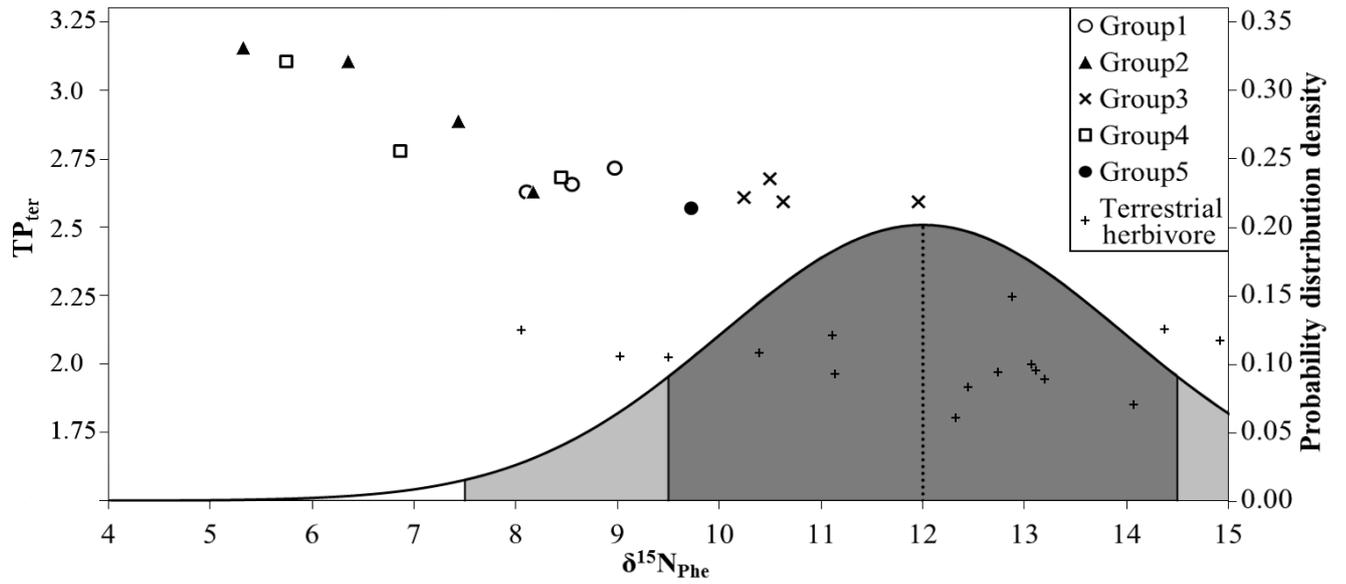
Fig. 6.5. The $\delta^{15}N_{Phe}$ and TP_{ter} of Human at Hasankeyf Höyük buried in each residence plotted. The normal distributions and standard deviations for probability distribution density along $\delta^{15}N_{Phe}$ of terrestrial herbivores (12.0±2.0‰) were shown in the graph. The $\delta^{15}N_{Phe}$ values of food resources are corrected +0.4‰ from observed values.
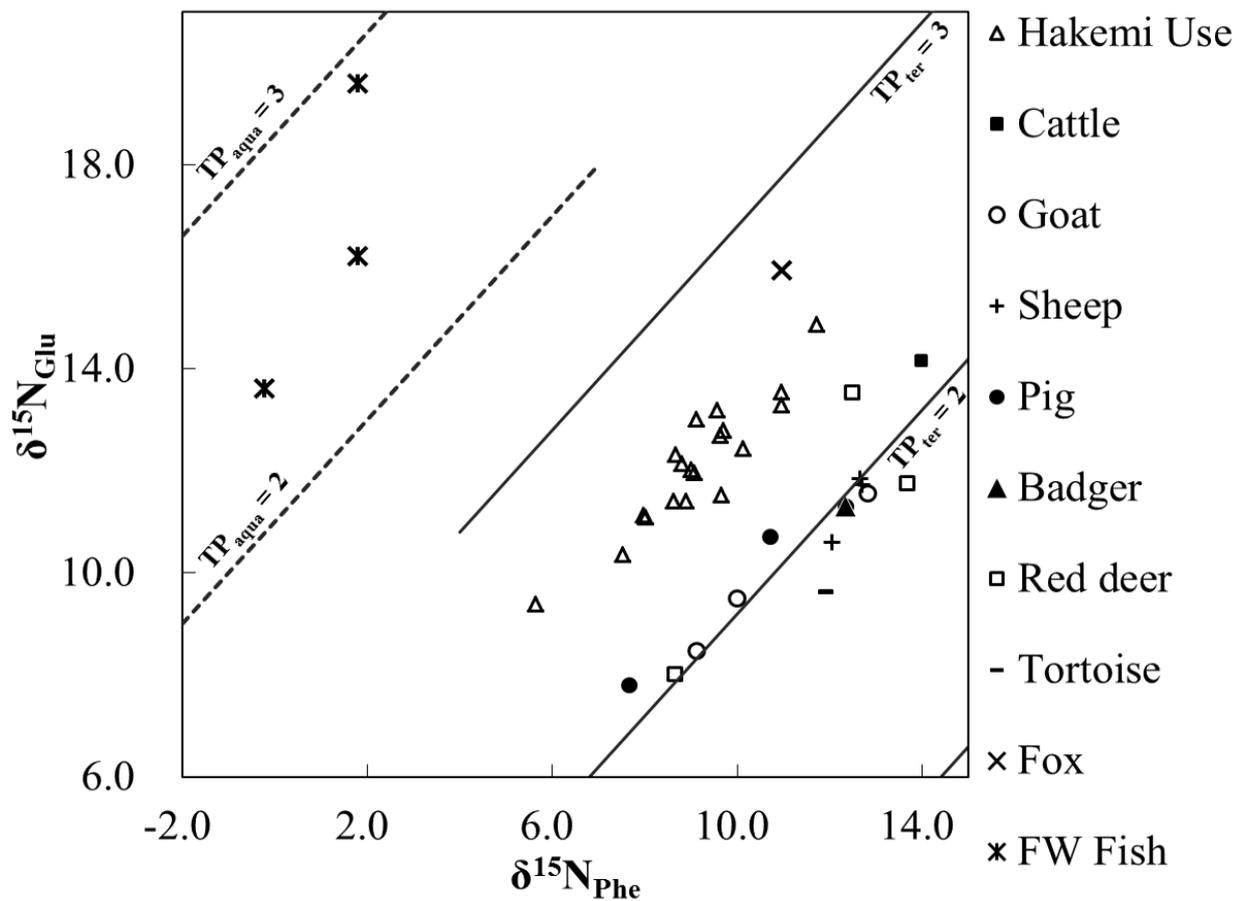
Fig. 6.6. Comparison between nitrogen isotopic composition of amino acids ($\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$) for human at Hakemi Use and faunal remains at Hasankeyf Höyük. Solid lines represent expected $\delta^{15}N$ values with $TP_{ter} = 2$ (primary consumer) or 3 (secondary consumer), and dashed lines represent expected $\delta^{15}N$ values with $TP_{aqua} = 2$ (primary consumer) or 3 (secondary consumer).
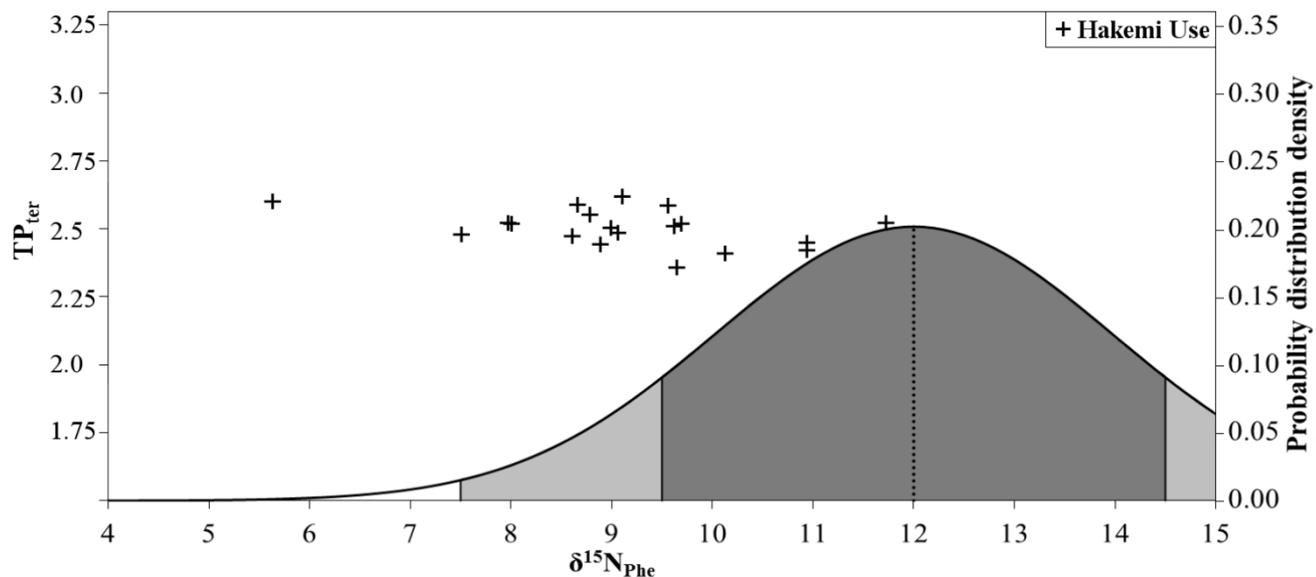
Fig. 6.7. The $\delta^{15}N_{Phe}$ and TP_{ter} of Human at Hakemi Use plotted. The normal distributions and standard deviations for probability distribution density along $\delta^{15}N_{Phe}$ of terrestrial herbivores (12.0±2.0‰) were shown in the graph. The $\delta^{15}N_{Phe}$ values of food resources are corrected +0.4‰ from observed values.
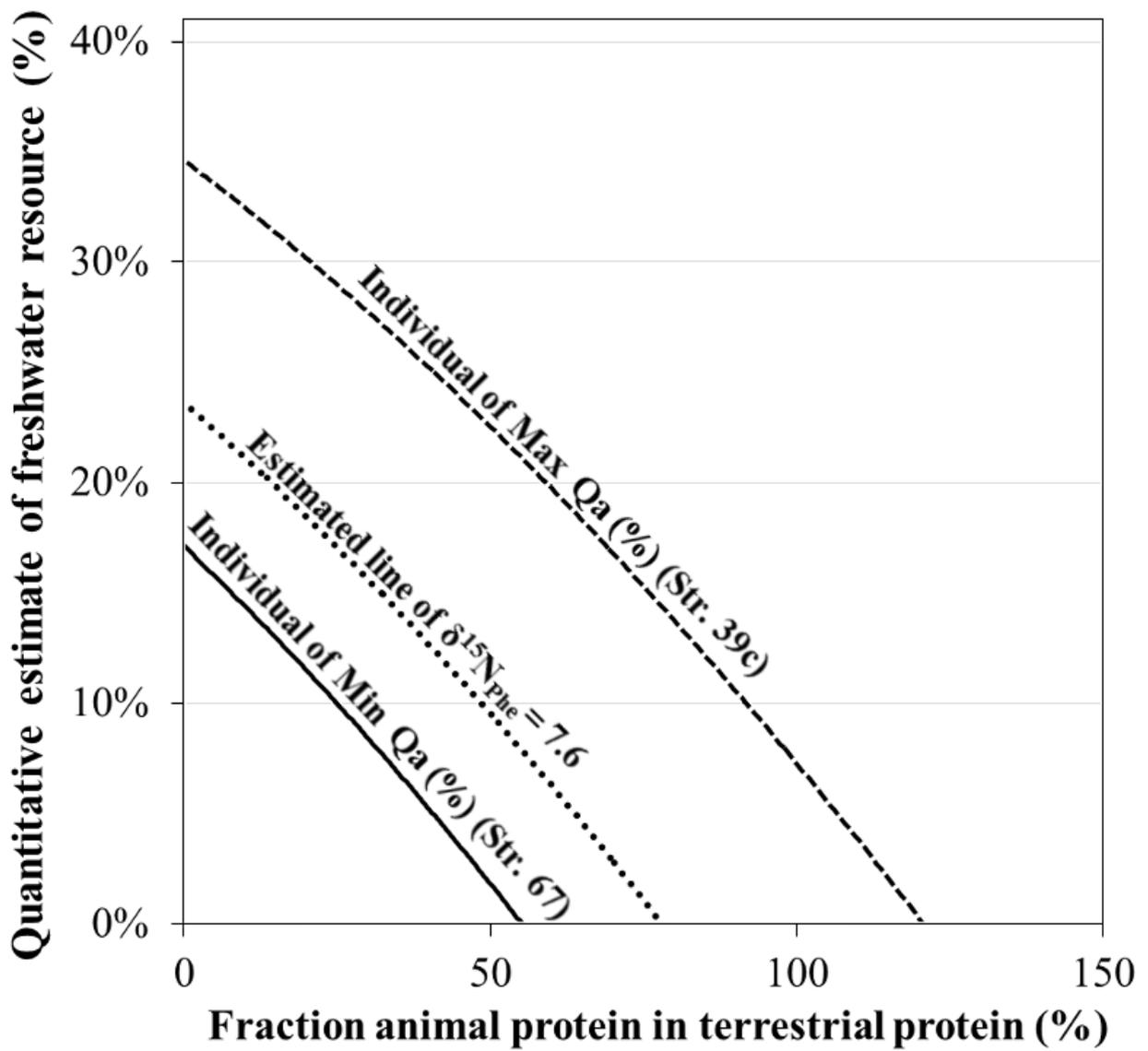
Fig. 6.8. Quantitative estimate of freshwater resource with fraction of animal protein in the terrestrial resources based on $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Glu}$ at Hasankeyf Höyük.
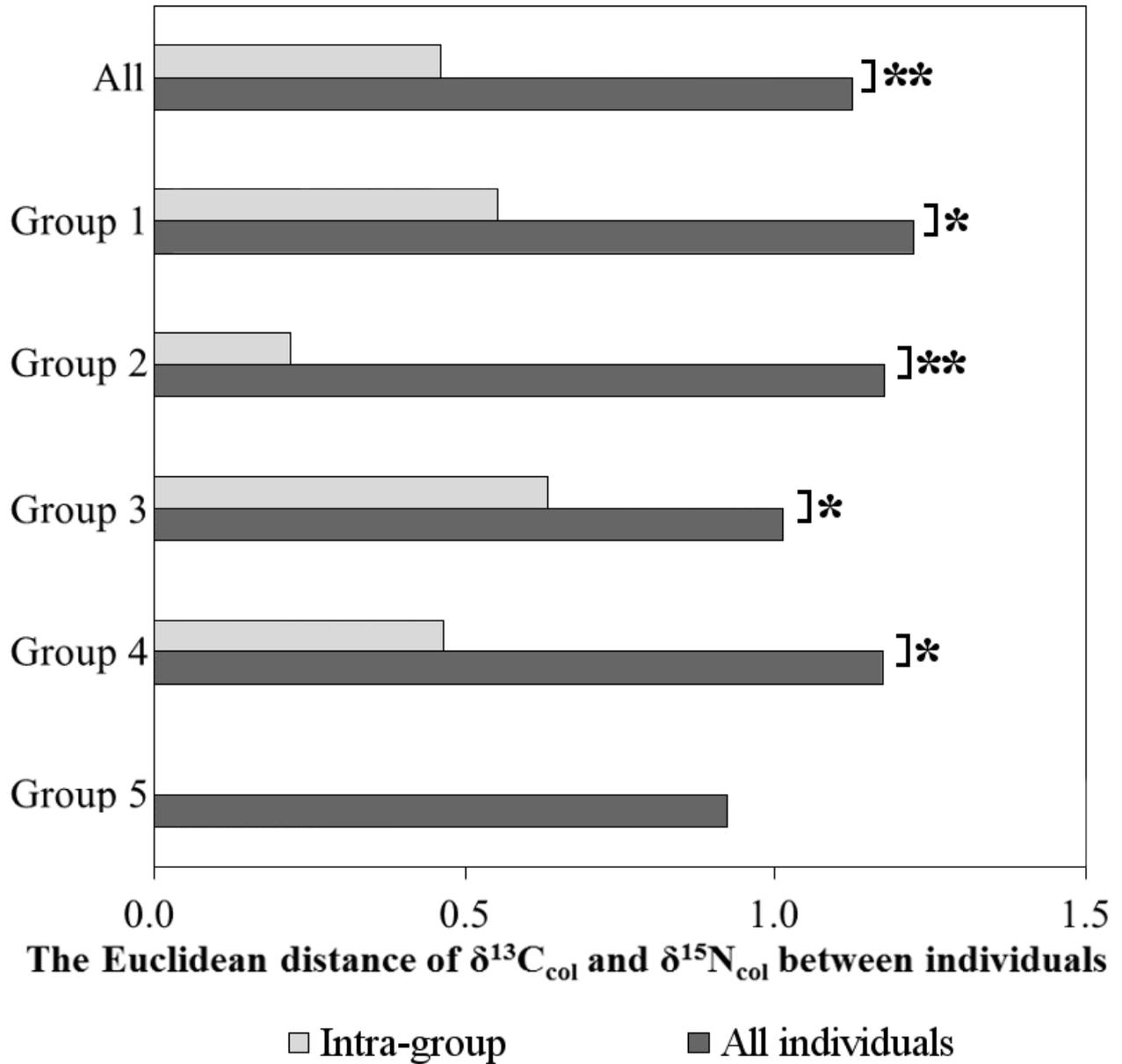
Fig. 6.9. Mean of Euclidean distance of $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ along each group (Group 1–5) are plotted at Hasankeyf Höyük. "Intra-group" shows the mean of Euclidean distance of $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ between two individuals within the each group. "All individuals" shows the mean of Euclidean distance of $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ between two individuals in all individuals. "*" indicates significant difference ($p < 0.05$) and "**" indicates significant difference ($p < 0.01$), by two tailed Welch's t test.
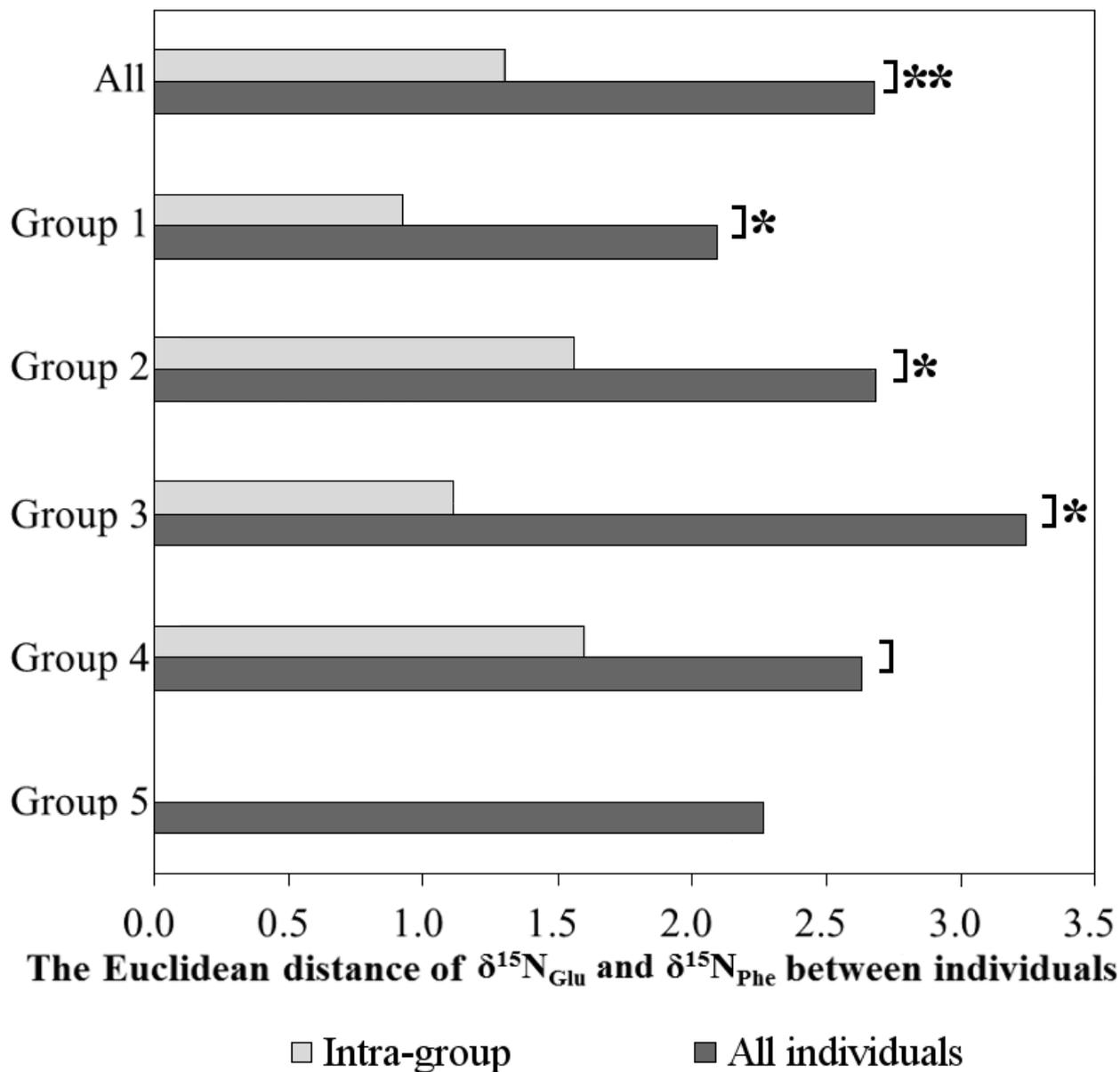
Fig. 6.10. Mean of Euclidean distance of $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ along each group (Group 1–5) are plotted at Hasankeyf Höyük. "Intra-group" shows the mean of Euclidean distance of $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ between two individuals within the each group. "All individuals" shows the mean of Euclidean distance of $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ between two individuals in all individuals. "*" indicates significant difference ($p < 0.05$) and "**" indicates significant difference ($p < 0.01$), by two tailed Welch's t test.
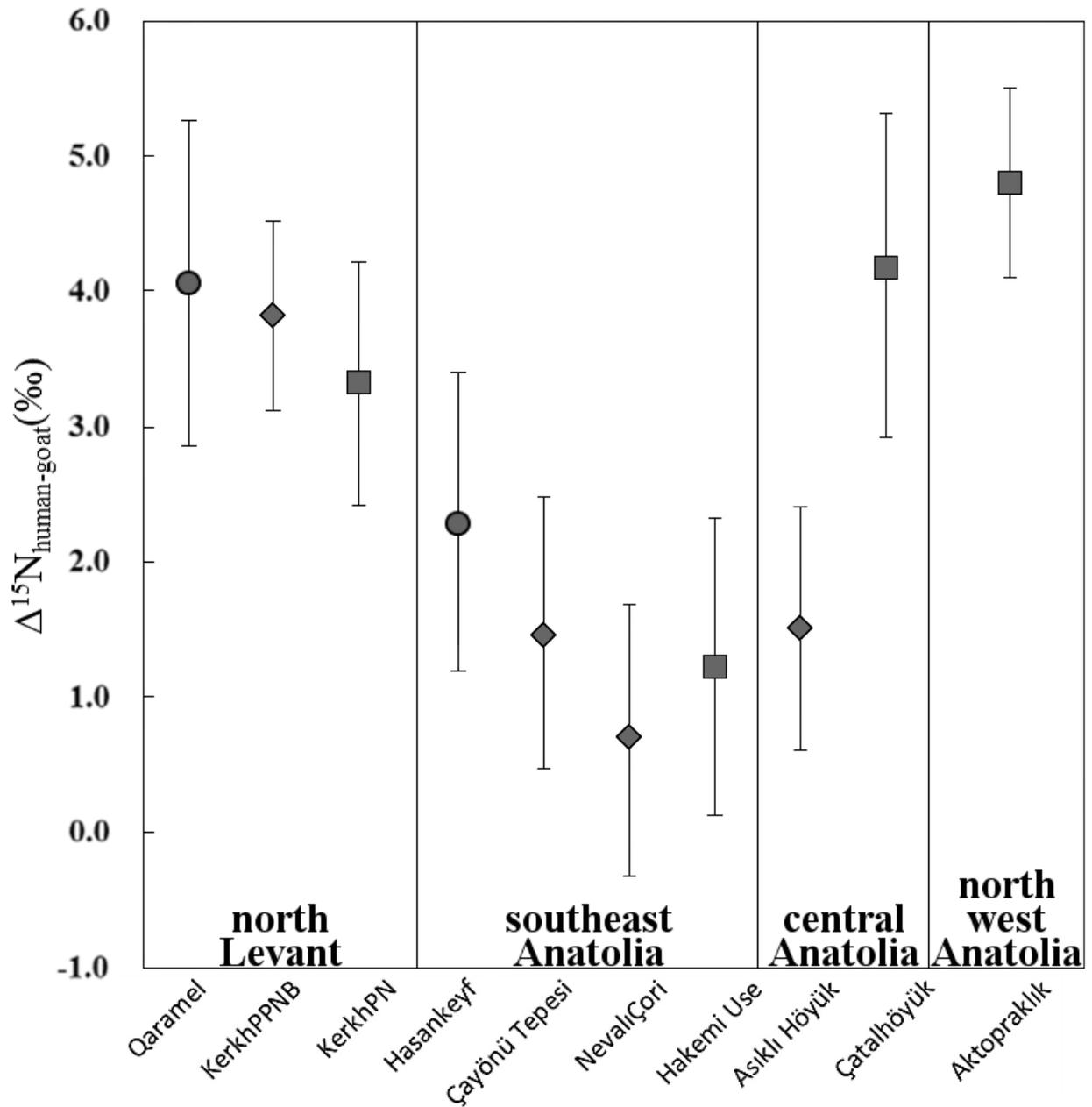
Fig. 7.1. The difference of $\delta^{15}N_{col}$ between human and goat ($\Delta^{15}N_{human-goat}$) from each Neolithic southwest Asia site (with bars representing 1σ standard deviations). The $\delta^{15}N$ values of human and goat (and references) are from Çayönü Tepesi (Pearson et al., 2013), Nevalı Çori (Lösch et al., 2006), Aşıklıhöyük (Pearson et al., 2010), Çatalhöyük (Richards et al., 2003a), and Aktopraklık (Budd et al., 2013). Each icon indicates PPNA site (●), PPNB site (◆) and PN site (■).
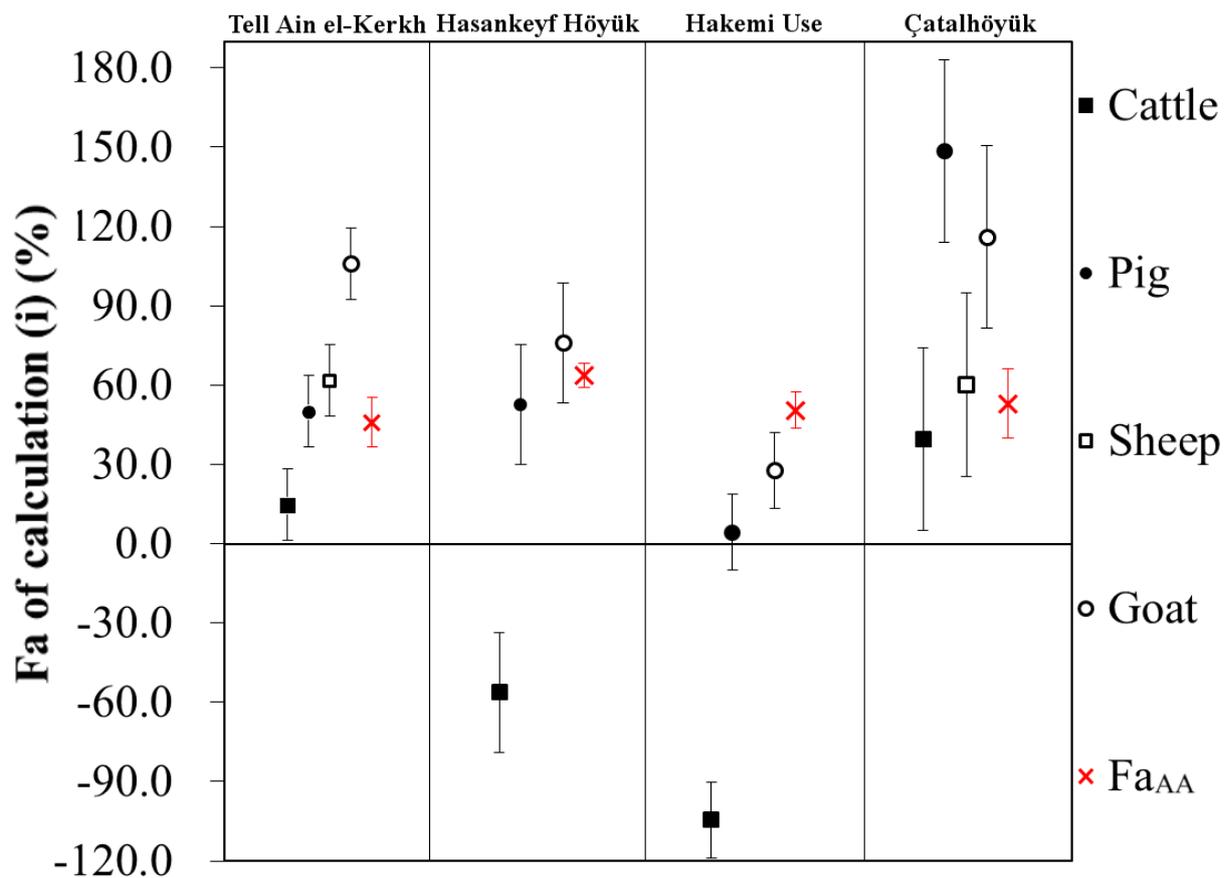
Fig. 7.2. The estimated fraction animal protein in total terrestrial protein by amino acids (Fa_{AA}) and collagen (Fa_{col}) calculated (i) according to the $\delta^{15}N$ values of terrestrial animals for each site. Each kind of markers indicates Fa_{AA} (with red marker) and Fa_{col} according to each animal (cattle■, sheep□, goat○, pig●).