Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Petrone P, Pucci P, Niola M, et al. Heat-induced brain vitrification from the Vesuvius eruption in c.e. 79. N Engl J Med 2020;382:383-4. DOI: 10.1056/NEJMc1909867

Supplementary Appendix

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Background

In the mid-1960s, during the archaeological excavations directed by the superintendent of Herculaneum Amedeo Maiuri, in the *Collegium Augustalium* a small room was discovered containing a human victim lying on a wooden bed.[1] Figure S1 represents the victim as it was found in its original context, and is still now observable at the archaeological site: the individual is lying ventrally within the volcanic ash, face down, the skull and the postcranial skeleton completely charred and burst from being subjected to the intense heat of the pyroclastic ash surge, a high-speed turbulent cloud rich in hot gases, ash and steam.[2]

The present work is focused on the discovery of glassy black material within the cranial cavity of this 79 AD eruption victim, apparently derived from his brain. This glassy material was undetectable in other parts of the skeleton and in the volcanic ash, nor was it found elsewhere at the archaeological site. Cerebral tissues from archaeological human remains are rare finds, due to soft tissue decomposition and taphonomic conditions. Thus, very few preserved brain remains are known so far, which are mostly saponified, a process by which tissues have been converted to soap.[3,4]

Results

Analysis of glassy material from the skull

Proteomic analysis of the atypical glassy black material that fills the cranial cavity (Fig. S2) and encrusts the bone surface (Fig. S3) allowed identification of several proteins highly expressed in different human brain districts (Tab. S1, samples C236, C240). Adipic and margaric fatty acids, reported as components of human hair fat,[5] were detected by Gas Chromatography-Mass Spectrometry (Tab. S2, samples C236, C240) exclusively in this glassy material. Stearic, palmitic and myristic fatty acids which are typical of human triglycerides from the brain, although they can be found as well in vegetable and animal fats, were also detected. However, no plant or animal remains were found associated to the skeletal remains in the site of the victim's discovery, as reported by Maiuri in his excavation journal[1].

Analysis of bone from other areas of the victim’s body

The left tibia, whose trabecular bone is partly characterized by a glassy-like appearance (Fig. S6A), showed oleic, linoleic, palmitic, stearic and myristic fatty acids (Tab. S3, sample C241). Unlike what was seen in the case of the glassy atypical fragments found within the cranial cavity, the trabecular bone of tibia retained intact its original structure (Fig. S6A).

Further analysis of a rib fragment with partial glassy-like appearance from the spongy mass entrapping the chest bones (Fig. S4) showed presence of stearic, myristic, palmitic and other fatty acids (Tab. S3, sample C246).

Adipic and margaric fatty acids were not found.

Analysis of charred wood

A single wood fragment of a charred beam from a workshop[6] situated in the third *Cardo* nearby the *Collegium Augustalium* shows a partially glassy-like appearance, while retaining an intact structure (Fig. S6B), as was also seen for the tibia. Analysis of this charcoal (Tab. S3, sample C249) and charred wood from the victim's bed (Tab. S3, sample C252) showed preservation of elaidic and abietic fatty acids, distinctive of plants, together with fatty acids common to plants and humans. Adipic and margaric fatty acids were not found. No glassy appearance was detectable in the carbonized wood of the bed and other charcoal fragments from the archaeological site.

Analysis of temperature

Temperatures detected on charcoal fragments from the *Collegium Augustalium* were calibrated from experimental curves for *Picea mariana* (black spruce) and *Picea glauca* (white spruce)[7] (Fig. S5). The temperature from the mean of the three highest Ro % values[8] was preferred since carbonization in Herculaneum occurred in disequilibrium conditions.[9] Therefore, the process of carbonization being non-retrograde, only the highest value may provide evidence of the peak and duration of heat exposure.[10,11] Hence, we also report temperature from the average Ro % plus SD. The most reliable values are considered in the temperature range from 470 to 520 °C (analyses were performed by A. Pensa). The exposure to such radiant heat was followed by a rapid drop in temperature, as supported by our charcoal reflectance analysis.

Analysis of volcanic ash

Analysis of the ash substrate (volcanic ash deposit containing the victim's skeletal remains) close to the skull showed most of its components to be different from fatty acids (Tab. S2, sample C251). Adipic and margaric fatty acids were not found.

Further discussion

Vitrification in archaeology

Vitrification[12] rarely occurs in nature, a process that mostly involves charcoal.[13] As in our case, the term *vitrified* referring to archaeological material is applied to substances that have been converted to a glass-like appearance because of exposure to high temperature, and has been used to describe charcoal with a glassy appearance recovered in the archaeological record. Archaeological evidence shows that dry wood may undergo vitrification at 310-530°C.[13]

A report on the firestorm victims of the Second World War

Observations similar to those we made on the 79 AD victim were reported by an English survivor of the firestorms in Dresden and Hamburg during the Second World War bombing. In his account, he reports the finding of victims in bomb shelters who died from being subjected to intense heat: "There were no real complete bodies, only bones and scorched articles of clothing matted together on the floor and stuck together by a sort of jelly substance. There was no flesh visible, what had once been a congregation of people sheltering from the horror above them was now a glutinous mass of solidified fat and bones swimming around, inches thick, on the floor”.[14]

Conclusive considerations

The most significant outcome is the identification exclusively in the glassy fragments from the skull of several proteins expressed in human brain tissues along with adipic and margaric fatty acids attributable to the victim’s brain and to his hair, respectively.

The reported findings indicate that the vitrified black material is resulting from the victim's brain exposure to high temperature.

Future research

Considering the discovery of vitrified brain remains from a victim of the 79 AD Vesuvius eruption, it may be of some interest to the scientific community to open a discussion on the process of vitrification occurring in human remains.

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Additional Figures

Figure S1 - The corpse of a 79 AD eruption victim. The victim is lying on a wooden bed, filled by volcanic ash. The skull and the postcranial bones are exploded and charred. The chest bones are entrapped by a solidified porous mass. Note the different coloring of the ash in proximity of the body (adult male, *Collegium Augustalium*, Herculaneum).

Figure S2 - Atypical glassy black material filling the skull. Note the cracked margins of the bone (gray arrows), surrounded by a volcanic substratum made of small pumices and ash (bar scale 10 cm).

Figure S3 - Parietal bone fragments from the exploded skull. The inner bone surface is encrusted by glassy black material (gray arrows) (bar scale 5 cm).

Figure S4 - Spongy mass entrapping the chest bones. A. Inner periosteal surface of a rib fragment showing glassy-like appearance; B. Outer bone surface of rib encrusted by a solidified spongy mass incorporating small pumices (human victim's skeleton, *Collegium Augustalium*, bar scale 2 cm).

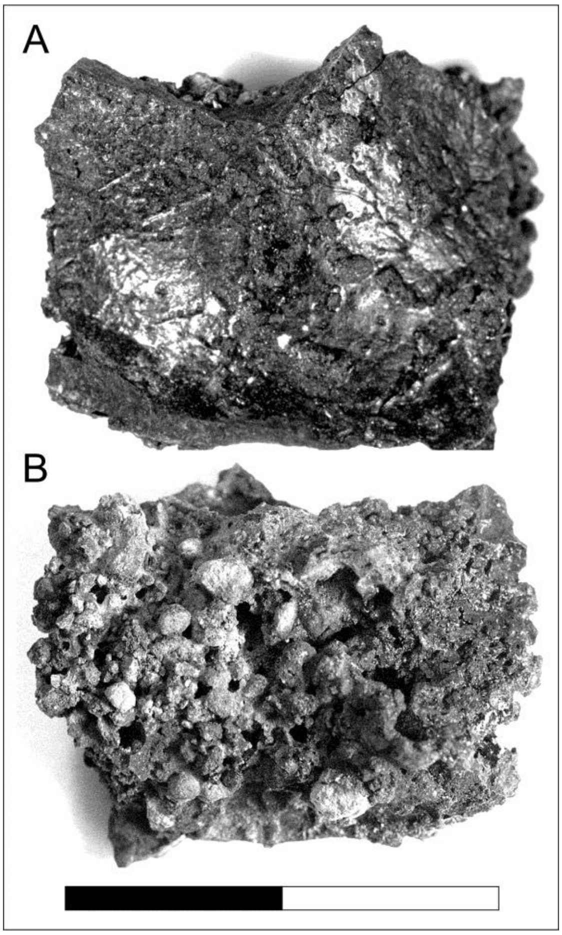


Figure S5 - Reflectance analysis of wood remains. Highest values of the Ro % mean charcoal reflectance for samples taken from carbonized wood (*Collegium Augustalium*, Herculaneum) coupled with microphotographs (ALBA Laboratory, University of Rome-Roma Tre, Rome, Italy).

Sample	Type	Ro%	sd	Ro+sd	Ro% mean three max	T°C	sd	T°C+ sd	T°C mean three max
C5	wooden pole	1,0818	0,1946	1,2764	1,4	445	±25	470	482
C6	wooden pole	1,168	0,3403	1,5083	1,85	455	±40	495	524

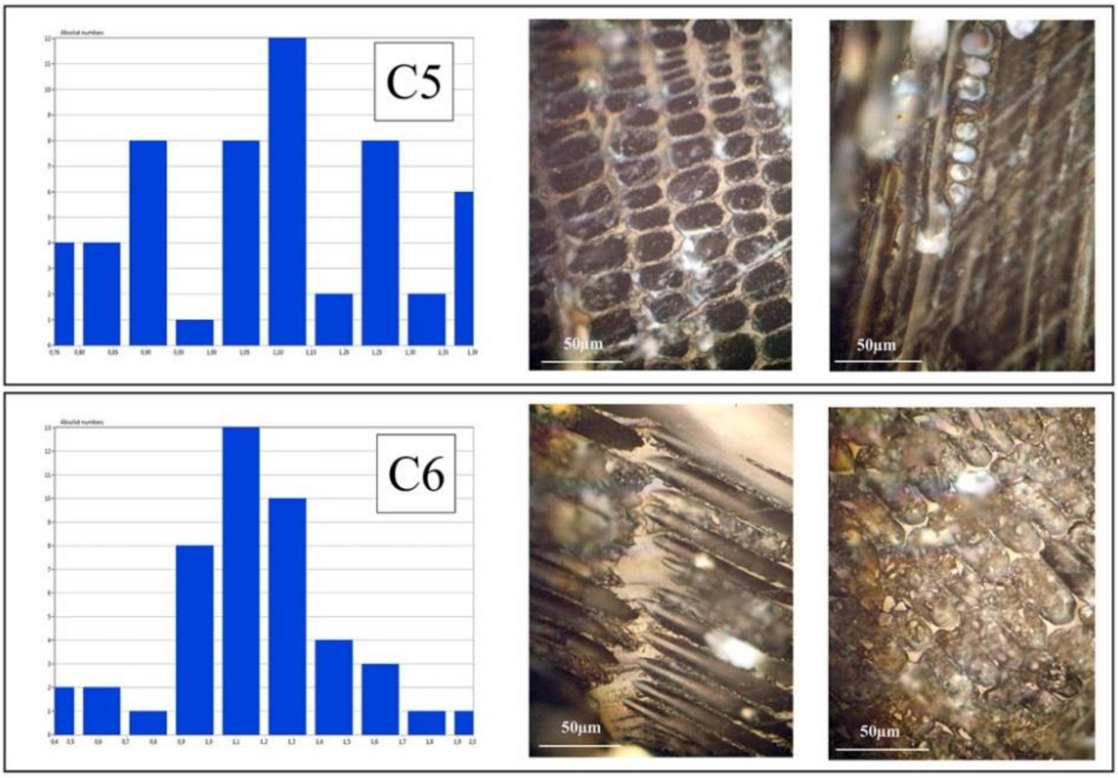
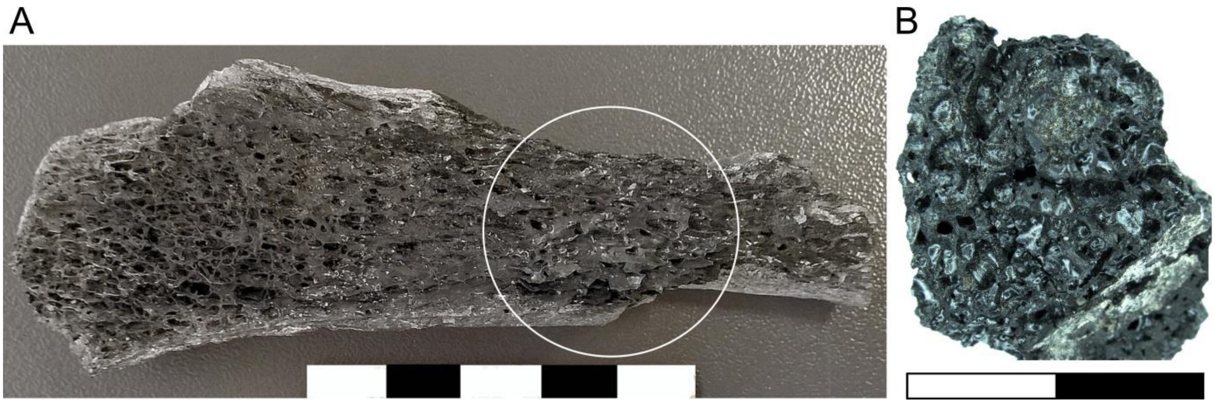


Figure S6 - Glassy appearance in bone and charcoal. A. Trabecular bone characterized by an intact structure with partial glassy appearance (white circle) (left tibia, human victim's skeleton, *Collegium Augustalium*) (bar scale 5 cm); B. Fragment of charcoal partially vitrified (bar scale 2 cm). Note the interspersed areas where a structure reminiscent of wood is still visible (holes of different size) whilst other areas appear smooth and glassy (house ceilings, workshop, III cardo, Herculaneum).



Additional Tables

Table S1 - Proteins identified in the glassy material expressed in human brain districts according to the Bgee Gene Expression database.

Sample	Gene	Protein	Organism	Expression
C236 *glassy material from the cranial cavity*	MED13L	Mediator of RNA polymerase II transcription sub 13-like Q71F56	*Homo sapiens* (human)	Forebrain, Cerebral cortex, Cerebellum
	HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase P04035	*Homo sapiens* (human)	Forebrain, Frontal cortex
C240 *glassy material encrusting the skull bone*	ATP6V1F	V-type proton ATPase subunit F Q16864	*Homo sapiens* (human)	Hypothalamus, Anterior cingulate cortex, Amygdala Dorsolateral prefrontal cortex
	WDR13	WD repeat-containing protein 13 Q9H1Z4	*Homo sapiens* (human)	Amygdala
	IWS1	Protein IWS1 homolog Q96ST2	*Homo sapiens* (human)	Frontal cortex, Substantia nigra
	KIF26B	Kinesin-like protein KIF26B Q2KJY2	*Homo sapiens* (human)	Neocortex, Forebrain, Frontal cortex, Substantia nigra
	RP617	40S ribosomal protein S17 P08708	*Homo sapiens* (human)	Cerebral cortex

Table S2 - GC-MS data from the glassy black material (values in percentage of total ion current).

Sample	Species	%	Sample	Species	%
C236	sugars	/	**C240**	sugars	/
glassy material	adipic acid	10,8	*glassy material*	adipic acid	10.49
from the	oleic acid	10,9	*encrusting the*	margaric acid	1.77
cranial cavity	linoleic acid	2,36	*skull bone*	oleic acid	38.32
(Fig. 1)	palmitic acid	19,1	(Fig. S3)	linoleic acid	2.44
	stearic acid	14,34		palmitic acid	22.15
	myristic acid	0,73		stearic acid	17.63
	12 methyl tridecanoic acid	2,7		myristic acid	1.39
	15 methyl hexadecanoic acid	19,9			
	vaccine acid	1,5			
	dehydroabietic acid	2,32			

Table S3 - GC-MS data from tibia, chest bones, charcoal and ash (values in percentage of total ion current).

Sample	Species	%	Sample	Species	%
C241	sugars	/	**C251**	sugars	/
left tibia	oleic acid	8,91	*volcanic ash*	malic acid	37,40
(Fig. S6A)	linoleic acid	0,40	*substrate*	2 malonic oxo	0,80
	palmitic acid	12,99	(Fig. S1)	methyl laurate	0,50
	stearic acid	17,29		valylvaline, allylamine	0,50
	myristic acid	19,02		4 hydroxyphenylacetic	0,60
	taiguic acid	0,43		methyl hydroxynaphthalate	0,70
	melissic acid	1,26		uracil derivative	1,80
	12 methyl tridecanoic acid	0,34		methyl pentadecanoate	0,80
C246	sugars	/		norleucine	2,50
rib from the	benzaldehyde	1,20		methyl stearate	4,80
solidified	butanthriol	1,20		methyl palmitate	12,40
spongy	methyl myristate	2,00		methyl elaidate	9,80
mass	methyl palmitate	1,60		dehydroabietic acid	0,80
(Fig. S4)	methyl elaidate	11,70	**C252**	sugars	/
	methyl vaccine	4,90	*charcoal*	methyl palmitate	15,20
	methyl stearate	14,70	*from the*	methyl elaidate	7,80
	bisphenol F	1,60	*wooden bed*	methyl stearate	14,80
	butyl palmitate	3,20	(Fig. S1)	dehydroabietic acid	2,40
	butyl stearate	3,50		hydrocarbons (wood comb.)	/
	dehydroabietic acid	1,10			
C249	sugars	/			
charcoal	butanthriol	0,70			
from the	methyl myristate	2,40			
workshop	methyl palmitate	11,10			
(Fig. S6B)	methyl elaidate	6,40			
	methyl stearate	11,70			
	docohexanedienoic	0,90			
	indacen	1,10			
	dehydroabietic acid	3,10			