

SUPPLEMENTAL INFORMATION

Supplemental figure 1 and 2 show data that support the main claims in the manuscript but cannot be included due to lack of space.

Supplemental table 1 lists AQUA peptides employed in this study.

Supplemental table 2 scheduled inclusion list of heavy and light irisin peptides.

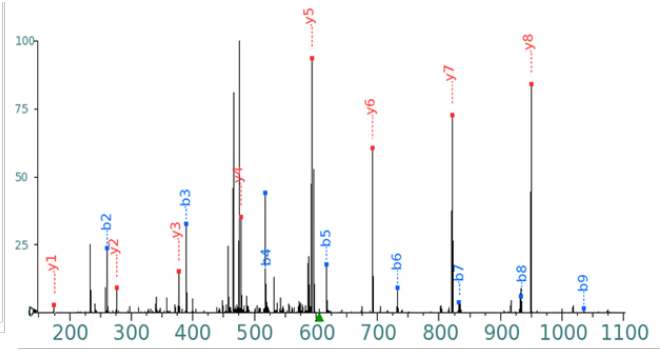
SUPPLEMENTAL FIGURES

Supplemental Figure 1

Deamidated

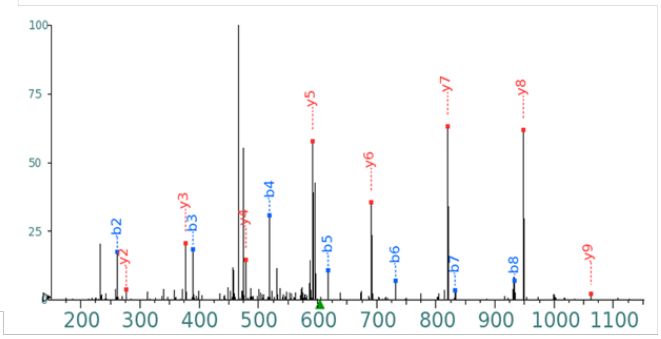
A

Sequence					
FIQEVNITTR					
Predicted Fragmentation Pattern					
Seq #	b: Δ Error	b	y	y: Δ Error	+1
F 1	---	148.076	---	---	10
I 2	349.410	261.160	1062.543	---	9
Q 3	-29.583	389.218	949.459	53.075	8
E 4	-84.799	518.261	821.400	3.746	7
V 5	-145.249	617.329	692.357	-18.667	6
N 6	-15.445	732.356	593.289	-75.296	5
T 7	-1110.426	833.404	478.262	-97.690	4
T 8	-888.679	934.452	377.214	60.633	3
T 9	-709.160	1035.499	276.167	1571.751	2
R 10	---	---	175.119	-1257.149	1



Normal asparagine

Sequence					
FIQEVNITTR					
Predicted Fragmentation Pattern					
Seq #	b: Δ Error	b	y	y: Δ Error	+1
F 1	---	148.076	---	---	10
I 2	753.746	261.160	1061.559	134.780	9
Q 3	-84.474	389.218	948.474	124.876	8
E 4	-155.125	518.261	820.416	129.322	7
V 5	205.421	617.329	691.373	-50.202	6
N 6	167.362	731.372	592.305	-82.211	5
T 7	126.878	832.420	478.262	-45.614	4
T 8	-874.086	933.468	377.214	-5.379	3
T 9	---	1034.515	276.167	1039.959	2
R 10	---	---	175.119	---	1



DSPSAPVNTVR

B

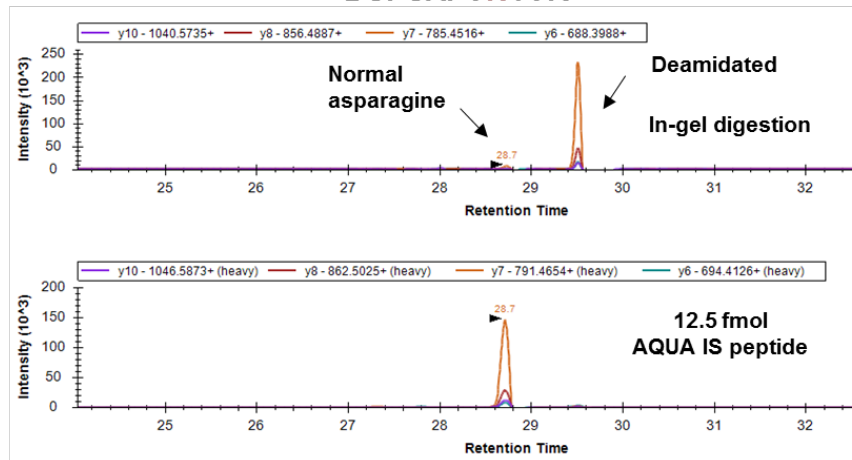


Figure S1, Related to Figure 1. Deamidated recombinant Irisin peptide spectra and n-terminal irisin PRM

(A) MS2 spectra acquired using an Orbitrap Elite mass spectrometer (CID fragmentation) and their b-, y-ion series m/z values. Mass accuracy values are given in PPMs and “@” denotes deamidation on the asparagine residue.

(B) PRM elution profile for n-terminal irisin peptide (DSPSPVNTVR) using Skyline software as found in sedentary subject 1. Top panel is the deamidated asparagine form of the peptide and the unmodified peptide found in the plasma. The bottom panel is 12.5 femtomoles of heavy internal standard (IS) AQUA peptide.

Supplemental Figure 2

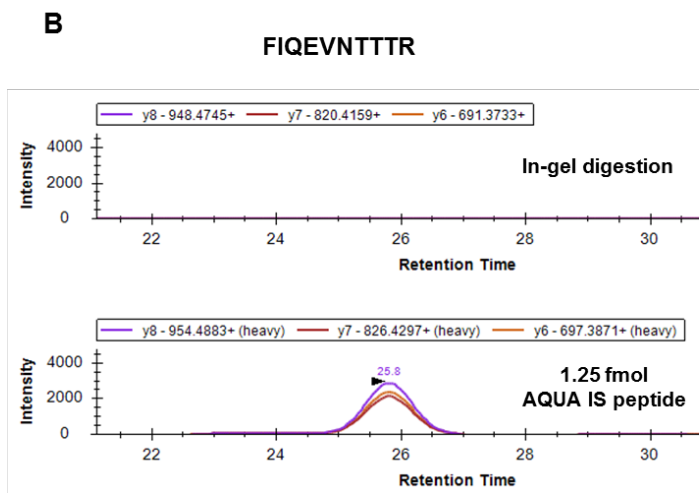
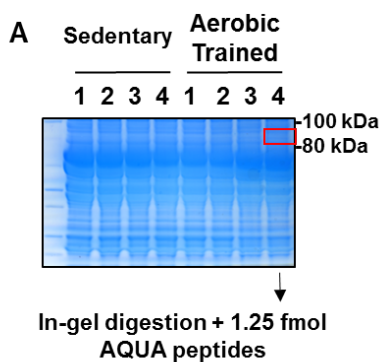


Figure S2, Related to Figure 2. PRM of irisin from the region 80-100 kDa

(A) SDS PAGE separation of 50 μ g of plasma from aerobically trained subject visualized by coomassie staining. Molecular mass regions from 80–100 kDa was excised from six separate gels (300 μ g from the original 100 μ l plasma) and digested in-gel in the presence of 1.25 femtomoles of each internal standard AQUA peptide.

(B) PRM elution profile for internal tryptic irisin peptide (FIQEVNTTTR) using Skyline software found in sedentary subject 1. Top panel is the PRM trace for the unmodified peptide and deamidated form of the internal irisin peptide found in the plasma and the bottom panel is 1.25 femtomoles of heavy internal standard (IS) AQUA peptide. No visible signal for irisin in the plasma in this region.

SUPPLEMENTAL TABLES

Table S1, Related to Experimental Procedure. AQUA peptides employed in this study

AQUA Peptide	Sequence	Mass (Da)	
		Light	Heavy
FNDC5 32-43	DSPSAPVNVTVR	1240.631	1246.655
FNDC5 79-88	FIQEVNTTTR	1207.609	1213.634

Table S2, Related to Experimental Procedure. Scheduled inclusion list of heavy and light irisin peptides

Mass [m/z]	CS [z]	Start [min]	End [min]	Peptide Sequence
604.817	2	23.99	27.99	FIQEVNTTTR (light)
607.824	2	23.99	27.99	FIQEVNTTTR (heavy)
605.309	2	24.17	28.17	FIQEVN[+1.0]TTTR (light)
608.316	2	24.17	28.17	FIQEVN[+1.0]TTTR (heavy)
621.327	2	27.82	31.82	DSPSAPVNVTVR (light)
624.334	2	27.82	31.82	DSPSAPVNVTVR (heavy)
621.819	2	27.13	31.13	DSPSAPVN[+1.0]VTVR (light)
624.826	2	27.13	31.13	DSPSAPVN[+1.0]VTVR (heavy)

CS Charge state