Cordeiro et al.

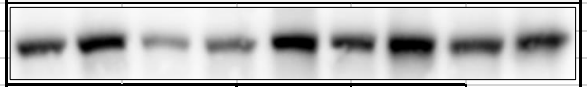
High-Intensity Exercise Training Induces Mitonuclear Imbalance and Activates the Mitochondrial Unfolded Protein Response (UPRmt) in Skeletal Muscle of Aged Mice

WESTERN BLOT SUPPLEMENTARY MATERIAL

Chronic High-Intensity Interval Training (HIIT)

Targed Protein

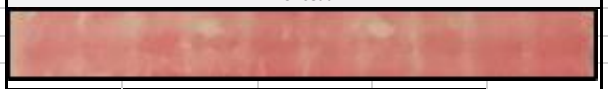
SDHA



Groups	Old CTL	Old HIIT
Absoluted Data	211153	301646
	274698	211630
	94900	330570
	155908	246219
		258599
Average	184164,75	269732,8

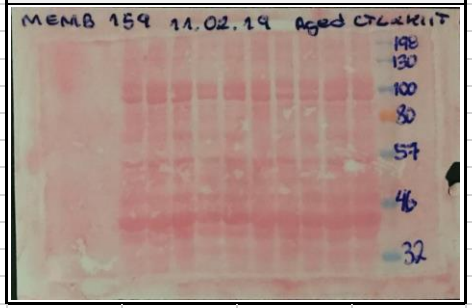
Load Control

Ponceau



Groups	Old CTL	Old HIIT
Absoluted Data	39258	46285
	47632	45915
	36831	45612
	46105	48492
		52374
Average	42456,5	47735,6

Ponceau



Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	5,378597993	6,517143783
	5,767089352	4,609169117
	2,576633814	7,247434886
	3,381585511	5,077517941
		4,937545347
Average	4,275976667	5,677762215

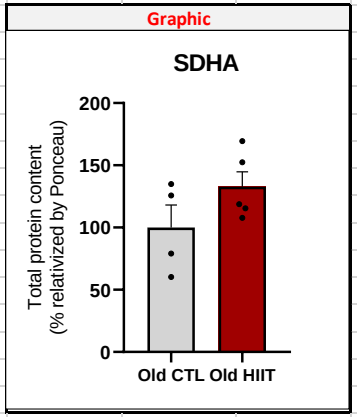
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	125,7864205	152,4129875
	134,871862	107,7921952
	60,25836936	169,4919184
	79,08334807	118,7452209
		115,4717561
Average	100	132,7828156

UNISCAN Software (Blot Quantification

SDHA		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	211153	1	39258
2	274698	2	47632
3	94900	3	36831
4	155908	4	46105
5	301646	5	46285
6	211630	6	45915
7	330570	7	45612
8	246219	8	48492
9	258599	9	52374

Graphic



Unpaired T Test

Table Analyzed	Mitonuclear Imbalance
Column B	Aged HIIT
vs.	vs,
Column A	Aged CTL
Unpaired t test	
P value	0,0466
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2,413, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	263,3
Difference between means (B - A) ± SEM	163,3 ± 67,67
95% confidence interval	3,253 to 323,3
R squared (eta squared)	0,454
F test to compare variances	
F, DFn, Dfd	37,01, 4, 3
P value	0,0138
P value summary	*
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

MTCO1

Groups	Old CTL	Old HIIT
Absoluted Data	16680	43908
	38285	63588
	11561	78618
	22124	123695
		158378
Average	22162,5	93637,4

Load Control

Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	50960	54615
	56324	54623
	50958	56155
	55728	55553
		56378
Average	53492,5	55464,8

Ponceau

Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	0,327315542	0,803954957
	0,679728002	1,164125002
	0,226873111	1,400017808
	0,396999713	2,226612424
		2,809216361
Average	0,407729092	1,680785311

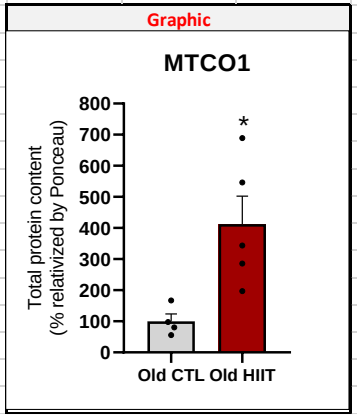
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	80,27770106	197,1787084
	166,7106948	285,5143342
	55,64310118	343,3696136
	97,36850293	546,1009449
		688,990905
Average	100	412,2309012

UNISCAN Software (Blot Quantification

MTCO1		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	16680	1	50960
2	38285	2	56324
3	11561	3	50958
4	22124	4	55728
5	43908	5	54615
6	63588	6	54623
7	78618	7	56155
8	123695	8	55553
9	158378	9	56378

Graphic



Unpaired T Test

Table Analyzed	MTCO1
Column B	Old HIIT
vs.	vs,
Column A	Old CTL
Unpaired t test	
P value	0,0199
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3,001, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	412,2
Difference between means (B - A) ± SEM	312,2 ± 104,0
95% confidence interval	66,22 to 558,2
R squared (eta squared)	0,5627
F test to compare variances	
F, DFn, Dfd	17,78, 4, 3
P value	0,0397
P value summary	*
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Mitochondrial-encoded Protein

MTCO1

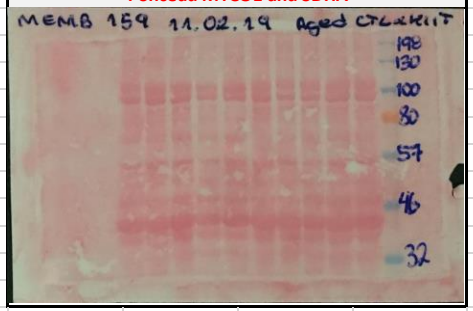
Groups	Old CTL	Old HIIT
Absoluted Data	0,327315542	0,803954957
	0,679728002	1,164125002
	0,226873111	1,400017808
	0,396999713	2,226612424
		2,809216361
Average	0,407729092	1,680785311

Nuclear-encoded Protein

SDHA

Groups	Old CTL	Old HIIT
Absoluted Data	5,378597993	6,517143783
	5,767089352	4,609169117
	2,576633814	7,247434886
	3,381585511	5,077517941
		4,937545347
Average	4,275976667	5,677762215

Ponceau MTCO1 and SDHA



Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	0,060855179	0,123360015
	0,117863269	0,252567214
	0,088050196	0,193174251
	0,117400465	0,438523793
		0,568949987
Average	0,096042277	0,315315052

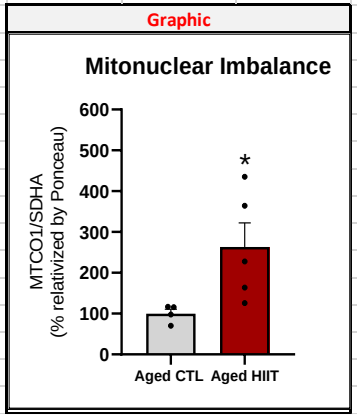
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	63,36290691	128,4434513
	122,720194	262,9750372
	91,67857971	201,1346015
	122,2383194	456,5945392
		592,3953536
Average	100	328,3085966

UNISCAN Software (Blot Quantification

MTCO1		SDHA	
Segment	Relativized	Segment	Relativized
1	0,327315542	1	5,378597993
2	0,679728002	2	5,767089352
3	0,226873111	3	2,576633814
4	0,396999713	4	3,381585511
5	0,803954957	5	6,517143783
6	1,164125002	6	4,609169117
7	1,400017808	7	7,247434886
8	2,226612424	8	5,077517941
9	2,809216361	9	4,937545347

Graphic



Unpaired T Test

Table Analyzed	Mitonuclear Imbalance
Column B	Aged HIIT
vs.	vs,
Column A	Aged CTL
Unpaired t test	
P value	0,0466
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2,413, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	263,3
Difference between means (B - A) ± SEM	163,3 ± 67,67
95% confidence interval	3,253 to 323,3
R squared (eta squared)	0,454
F test to compare variances	
F, DFn, Dfd	37,01, 4, 3
P value	0,0138
P value summary	*
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

Yme1L1

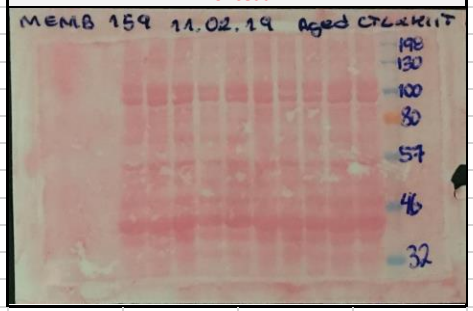
Groups	Old CTL	Old HIIT
Absoluted Data	33464	67849
	47412	49283
	49398	65337
	57689	53521
		35589
Average	46990,75	54315,8

Load Control

Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	65581	64076
	62178	54744
	56048	58816
	62372	66356
		59081
Average	61544,75	60614,6

Ponceau



Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	0,510269743	1,058883201
	0,762520506	0,900244776
	0,881351699	1,110871192
	0,924918233	0,806573633
		0,602376399
Average	0,769765045	0,89578984

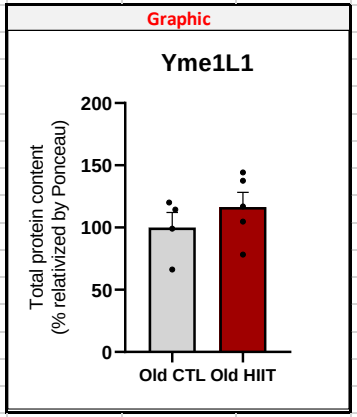
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	66,28902496	137,5592732
	99,05886357	116,9505918
	114,4961965	144,3130211
	120,1559149	104,7817952
		78,25457944
Average	100	116,3718522

UNISCAN Software (Blot Quantification

Yme1L1		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	33464	1	65581
2	47412	2	62178
3	49398	3	56048
4	57689	4	62372
5	67849	5	64076
6	49283	6	54744
7	65337	7	58816
8	53521	8	66356
9	35589	9	59081

Graphic



Unpaired T Test

Table Analyzed	Yme1L1
Column B	Old HIIT
vs.	vs,
Column A	Old CTL
Unpaired t test	
P value	0,3713
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0,9551, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	116,4
Difference between means (B - A) ± SEM	16,37 ± 17,14
95% confidence interval	-24,16 to 56,91
R squared (eta squared)	0,1153
F test to compare variances	
F, DFn, Dfd	1,205, 4, 3
P value	0,9147
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

Lonp1

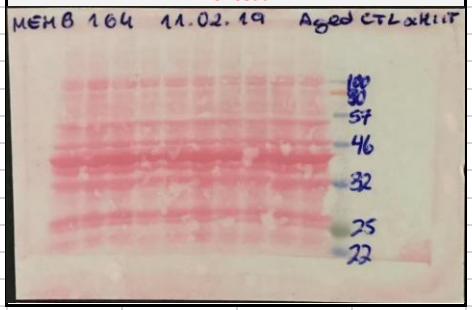
Groups	Old CTL	Old HIIT
Absoluted Data	34536	41163
	22532	36438
	23689	41128
	33876	38543
		39506
Average	28658,25	39355,6

Load Control

Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	48209	46662
	42289	39735
	38575	48035
	45648	46575
		38299
Average	43680,25	43861,2

Ponceau



Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	0,716380759	0,882152501
	0,532809951	0,917025293
	0,614102398	0,856209014
	0,742113565	0,827546967
		1,031515183
Average	0,651351668	0,902889792

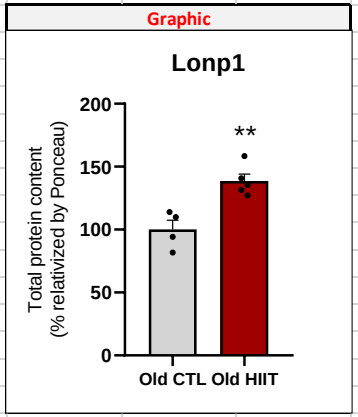
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	109,9837145	135,4341356
	81,80065804	140,788047
	94,28123518	131,4511126
	113,9343923	127,0507174
		158,3653246
Average	100	138,6178674

UNISCAN Software (Blot Quantification

Lonp1		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	34536	1	48209
2	22532	2	42289
3	23689	3	38575
4	33876	4	45648
5	41163	5	46662
6	36438	6	39735
7	41128	7	48035
8	38543	8	46575
9	39506	9	38299

Graphic



Unpaired T Test

Table Analyzed	Lonp1
Column B	Old HIIT
vs.	vs,
Column A	Old CTL
Unpaired t test	
P value	0,0035
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=4,312, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	138,6
Difference between means (B - A) ± SEM	38,62 ± 8,956
95% confidence interval	17,44 to 59,80
R squared (eta squared)	0,7265
F test to compare variances	
F, DFn, Dfd	1,487, 3, 4
P value	0,6915
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

CLpP

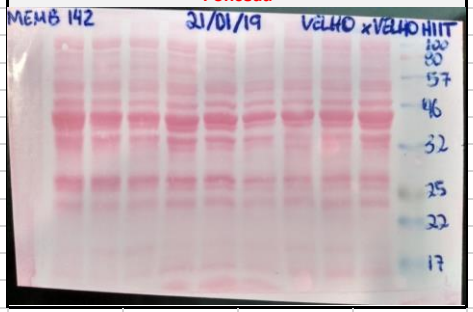
Groups	Old CTL	Old HIIT
Absoluted Data	39150	41674
	11261	12342
	12572	25526
	48748	62954
		61993
Average	27932,75	40897,8

Load Control

Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	53778	62429
	64174	51332
	59954	52019
	62883	55125
		49574
Average	60197,25	54095,8

Ponceau



Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	0,72799286	0,667542328
	0,175476049	0,240434816
	0,209694099	0,490705319
	0,775217467	1,142022676
		1,250514383
Average	0,472095119	0,758243904

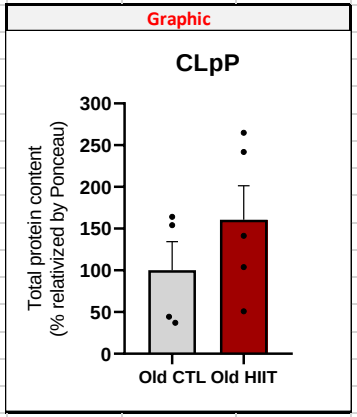
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	154,2046996	141,399964
	37,1696386	50,92931634
	44,41776465	103,9420447
	164,2078972	241,9052073
		264,8861072
Average	100	160,6125279

UNISCAN Software (Blot Quantification

CLpP		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	39150	1	53778
2	11261	2	64174
3	12572	3	59954
4	48748	4	62883
5	41674	5	62429
6	12342	6	51332
7	25526	7	52019
8	62954	8	55125
9	61993	9	49574

Graphic



Unpaired T Test

Table Analyzed	CLpP
Column B	Old HIIT
vs.	vs,
Column A	Old CTL
Unpaired t test	
P value	0,3075
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1,100, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	160,6
Difference between means (B - A) ± SEM	60,61 ± 55,08
95% confidence interval	-69,63 to 190,9
R squared (eta squared)	0,1475
F test to compare variances	
F, DFn, Dfd	1,761, 4, 3
P value	0,6701
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

GAPDH

Groups	Old CTL	Old HIIT
Absoluted Data	74759	84143
	97171	75566
	71666	69509
	73473	105461
		97696
Average	79267,25	86475

Load Control

Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	47150	51747
	57088	61337
	54027	57149
	56392	54583
		55276
Average	53664,25	56018,4

Ponceau

Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	1,585556734	1,626045954
	1,702126541	1,231980697
	1,326484906	1,21627675
	1,302897574	1,932121723
		1,767421666
Average	1,479266439	1,554769358

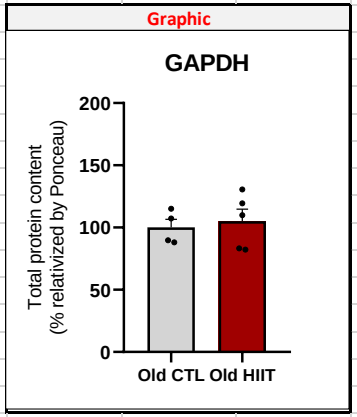
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	107,1853381	109,9224529
	115,0655823	83,28321826
	89,67180428	82,2216146
	88,07727533	130,6135036
		119,4796028
Average	100	105,1040784

UNISCAN Software (Blot Quantification

GAPDH		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	74759	1	47150
2	97171	2	57088
3	71666	3	54027
4	73473	4	56392
5	84143	5	51747
6	75566	6	61337
7	69509	7	57149
8	105461	8	54583
9	97696	9	55276

Graphic

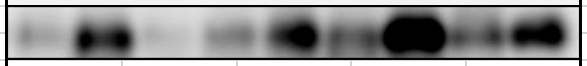


Unpaired T Test

Table Analyzed	GAPDH
Column B	Old HIIT
vs.	vs,
Column A	Old CTL
Unpaired t test	
P value	0,6939
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0,4103, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	105,1
Difference between means (B - A) ± SEM	5,104 ± 12,44
95% confidence interval	-24,31 to 34,52
R squared (eta squared)	0,02348
F test to compare variances	
F, DFn, Dfd	2,674, 4, 3
P value	0,4452
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

NRF-1



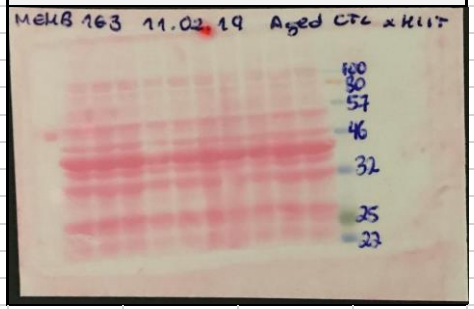
Groups	Old CTL	Old HIIT
Absoluted Data	71573	199028
	194802	160169
	51302	266432
	96508	154436
		204925
Average	103546,25	196998

Load Control

Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	55484	58800
	56305	57189
	49734	54910
	52165	58757
		52886
Average	53422	56508,4

Ponceau



Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	1,289975488	3,384829932
	3,459763787	2,800695938
	1,031527728	4,852158077
	1,850052717	2,628384703
		3,874844004
Average	1,90782993	3,508182531

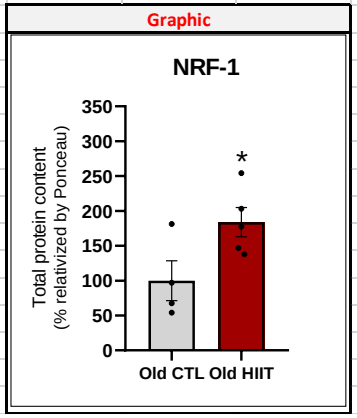
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	67,61480508	177,4178022
	181,3455032	146,8000839
	54,06811746	254,3286485
	96,97157426	137,7682917
		203,1021709
Average	100	183,8833994

UNISCAN Software (Blot Quantification

NRF-1		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	71573	1	55484
2	194802	2	56305
3	51302	3	49734
4	96508	4	52165
5	199028	5	58800
6	160169	6	57189
7	266432	7	54910
8	154436	8	58757
9	204925	9	52886

Graphic

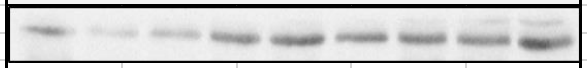


Unpaired T Test

Table Analyzed	NRF-1
Column B	Old HIIT
vs.	vs,
Column A	Old CTL
Unpaired t test	
P value	0,046
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2,422, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	183,9
Difference between means (B - A) ± SEM	83,88 ± 34,64
95% confidence interval	1,984 to 165,8
R squared (eta squared)	0,4559
F test to compare variances	
F, DFn, Dfd	1,470, 3, 4
P value	0,6988
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

TFAM



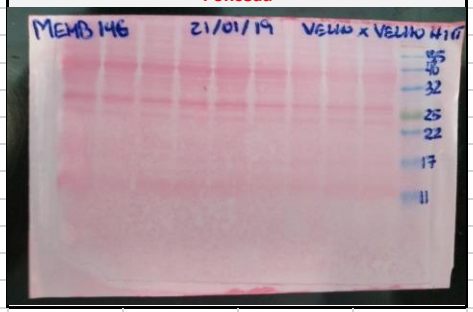
Groups	Old CTL	Old HIIT
Absoluted Data	18761	37643
	6061	27474
	12049	30981
	27282	31792
		44360
Average	16038,25	34450

Load Control

Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	81970	72395
	77555	62663
	73927	59194
	72325	60147
		59893
Average	76444,25	62858,4

Ponceau



Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	0,228876418	0,519966849
	0,07815099	0,438440547
	0,162985107	0,523380748
	0,377213965	0,528571666
		0,740654167
Average	0,21180662	0,550202795

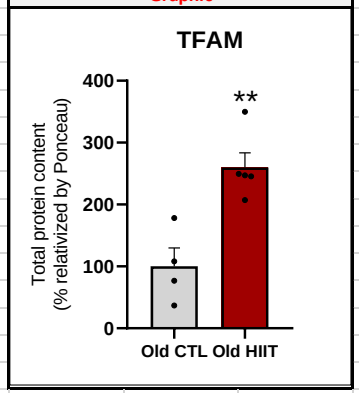
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	108,059143	245,4913113
	36,897331	207,0003984
	76,9499589	247,1031115
	178,0935671	249,5538933
		349,6841445
Average	100	259,7665718

UNISCAN Software (Blot Quantification

TFAM		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	18761	1	81970
2	6061	2	77555
3	12049	3	73927
4	27282	4	72325
5	37643	5	72395
6	27474	6	62663
7	30981	7	59194
8	31792	8	60147
9	44360	9	59893

Graphic



Unpaired T Test

Table Analyzed	TFAM
Column B	Old HIIT
vs.	vs,
Column A	Old CTL
Unpaired t test	
P value	0,0038
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=4,247, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	259,8
Difference between means (B - A) ± SEM	159,8 ± 37,62
95% confidence interval	70,81 to 248,7
R squared (eta squared)	0,7204
F test to compare variances	
F, DFn, Dfd	1,256, 3, 4
P value	0,8027
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

VDAC

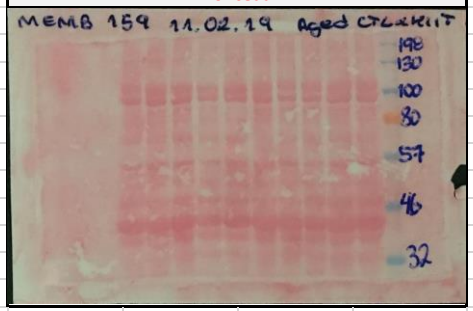
Groups	Old CTL	Old HIIT
Absoluted Data	95027	163457
	124672	197310
	129346	187688
	145956	160611
		161007
Average	123750,25	174014,6

Load Control

Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	25556	35875
	35940	40511
	30597	39344
	35266	40983
		35839
Average	31839,75	38510,4

Ponceau



Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	3,718383159	4,556292683
	3,468892599	4,870528992
	4,227407916	4,770435136
	4,138717178	3,918966401
		4,492508162
Average	3,888350213	4,521746275

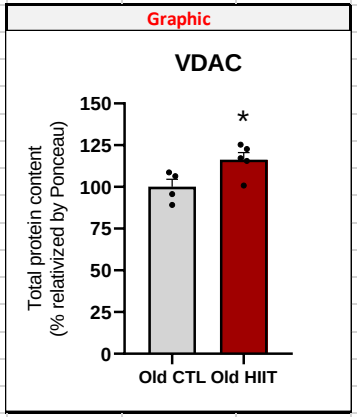
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	95,62881312	117,178043
	89,21245281	125,2595246
	108,7198345	122,685326
	106,4388996	100,7873825
		115,5376423
Average	100	116,2895837

UNISCAN Software (Blot Quantification

VDAC		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	95027	1	25556
2	124672	2	35940
3	129346	3	30597
4	145956	4	35266
5	163457	5	35875
6	197310	6	40511
7	187688	7	39344
8	160611	8	40983
9	161007	9	35839

Graphic



Unpaired T Test

Table Analyzed	VDAC
Column B	Old HIIT
vs.	vs,
Column A	Old CTL
Unpaired t test	
P value	0,036
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2,589, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	116,3
Difference between means (B - A) ± SEM	16,29 ± 6,293
95% confidence interval	1,409 to 31,17
R squared (eta squared)	0,4891
F test to compare variances	
F, DFn, Dfd	1,076, 4, 3
P value	0,9916
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

TUBULIN

Groups	Old CTL	Old HIIT
Absoluted Data	78863	63001
	81998	38207
	36297	55327
	57418	65814
		73571
Average	63644	59184

Load Control

Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	51533	59398
	58222	60790
	51326	64448
	57167	64056
		58251
Average	54562	61388,6

Ponceau

Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	1,530339782	1,060658608
	1,408367971	0,628507978
	0,707185442	0,85847505
	1,004390645	1,027444736
		1,262999777
Average	1,16257096	0,96761723

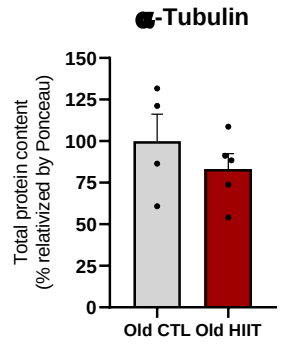
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	131,6340967	91,23388116
	121,1425383	54,06190245
	60,82944322	73,84280867
	86,3939217	88,37694826
		108,6385107
Average	100	83,23081025

UNISCAN Software (Blot Quantification

TUBULIN		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	78863	1	51533
2	81998	2	58222
3	36297	3	51326
4	57418	4	57167
5	63001	5	59398
6	38207	6	60790
7	55327	7	64448
8	65814	8	64056
9	73571	9	58251

Graphic



Unpaired T Test

Table Analyzed	alpha-Tubulin
Column B	Old HIIT
vs.	vs,
Column A	Old CTL
Unpaired t test	
P value	0,3735
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0,9505, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	83,23
Difference between means (B - A) ± SEM	-16,77 ± 17,64
95% confidence interval	-58,49 to 24,95
R squared (eta squared)	0,1143
F test to compare variances	
F, DFn, Dfd	2,521, 3, 4
P value	0,3931
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Cordeiro et al.

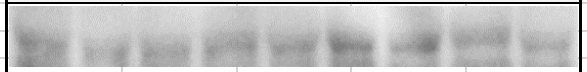
High-Intensity Exercise Training Induces Mitonuclear Imbalance and Activates the Mitochondrial Unfolded Protein Response (UPRmt) in Skeletal Muscle of Aged Mice

WESTERN BLOT SUPPLEMENTARY MATERIAL

Acute High-Intensity Interval Exercise (HIIE)

Targed Protein

OXPHOS CIV - MTCO1



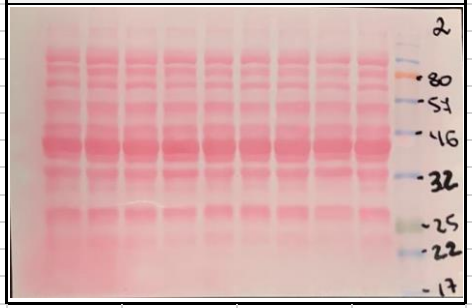
Groups	Old CTL	Old HIIT
Absoluted Data	89260	80967
	60384	86318
	68784	77535
	82196	77442
		59104
Average	75156	76273,2

Load Control

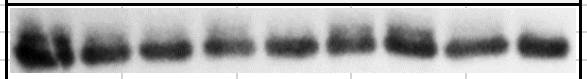
Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	20944	1942
	10618	1153
	3503	1380
	5688	2441
		848
Average	10188,25	1552,8

Ponceau



Quantitative Relativization (Target / Control)



Groups	Old CTL	Old HIIT
Relativization by the Load Control	4,2618411	41,69258496
	5,686946694	74,86383348
	19,63574079	56,18478261
	14,45077356	31,72552233
		69,69811321
Average	11,00882554	54,83296732

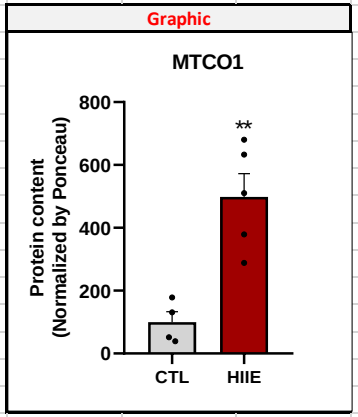
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	38,71294977	378,7196448
	51,65806902	680,0346979
	178,3636295	510,3612772
	131,2653517	288,1826242
		633,1112522
Average	100	498,0818992

UNISCAN Software (Blot Quantification

OXPHOS CIV - MTCO1		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	89260	1	20944
2	60384	2	10618
3	68784	3	3503
4	82196	4	5688
5	80967	5	1942
6	86318	6	1153
7	77535	7	1380
8	77442	8	2441
9	59104	9	848

Graphic

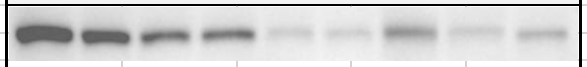


Unpaired T Test

Table Analyzed	MTCO1 SuperECL
Column B	HIIE
vs.	vs,
Column A	CTL
Unpaired t test	
P value	0,0029
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=4,477, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	498,1
Difference between means (B - A) ± SEM	398,1 ± 88,92
95% confidence interval	187,8 to 608,4
R squared (eta squared)	0,7411
F test to compare variances	
F, DFn, Dfd	6,233, 4, 3
P value	0,1647
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

OXPHOS (CV) - ATP5a



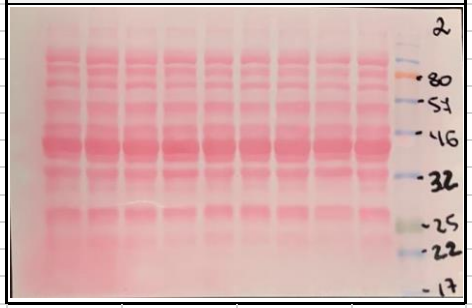
Groups	Old CTL	Old HIIT
Absoluted Data	159653	18078
	117090	14323
	81701	67810
	76271	24625
		35427
Average	108678,75	32052,6

Load Control

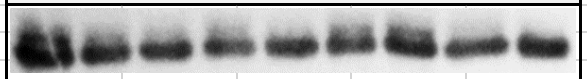
Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	23153	2780
	13307	339
	5042	1700
	4517	957
		1296
Average	11504,75	1414,4

Ponceau



Quantitative Relativization (Target / Control)



Groups	Old CTL	Old HIIT
Relativization by the Load Control	6,89556429	6,502877698
	8,799128278	42,25073746
	16,20408568	39,88823529
	16,88532212	25,73145246
		27,33564815
Average	12,19602509	28,34179021

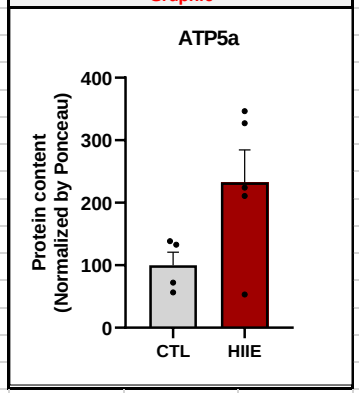
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	56,53944001	53,3196484
	72,14750882	346,4303914
	132,863663	327,0593082
	138,4493881	210,9822853
		224,1357159
Average	100	232,3854699

UNISCAN Software (Blot Quantification

OXPHOS (CV) - ATP5a		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	159653	1	23153
2	117090	2	13307
3	81701	3	5042
4	76271	4	4517
5	18078	5	2780
6	14323	6	339
7	67810	7	1700
8	24625	8	957
9	35427	9	1296

Graphic

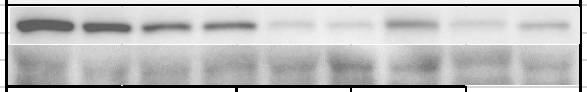


Unpaired T Test

Table Analyzed	ATP5a SuperECL
Column B	HIIE
vs.	vs,
Column A	CTL
Unpaired t test	
P value	0,0701
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=2,135, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	232,4
Difference between means (B - A) ± SEM	132,4 ± 61,99
95% confidence interval	-14,21 to 279,0
R squared (eta squared)	0,3945
F test to compare variances	
F, DFn, Dfd	7,835, 4, 3
P value	0,1223
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

Mitonuclear Imbalance



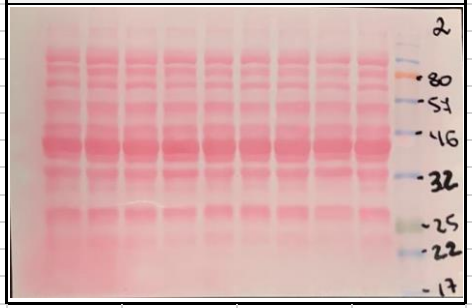
Groups	Old CTL	Old HIIT
Absoluted Data	89260	80967
	60384	86318
	68784	77535
	82196	77442
		59104
Average	75156	76273,2

Load Control

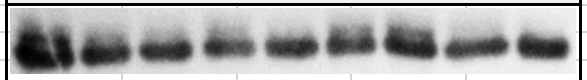
Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	159653	18078
	117090	14323
	81701	67810
	76271	24625
		35427
Average	108678,75	32052,6

Ponceau



Quantitative Relativization (Target / Control)



Groups	Old CTL	Old HIIT
Relativization by the Load Control	0,559087521	4,478758712
	0,515705867	6,026530755
	0,84189912	1,143415425
	1,077683523	3,144852792
		1,668332063
Average	0,748594008	3,292377949

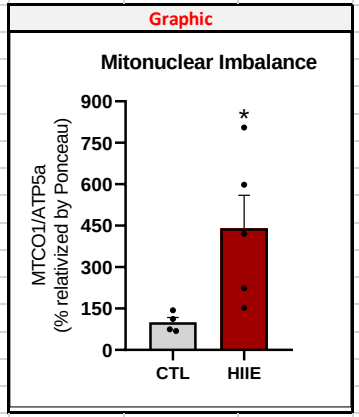
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	74,68501152	598,2894152
	68,88992723	805,0466196
	112,4640474	152,7417283
	143,9610138	420,101251
		222,8620648
Average	100	439,8082158

UNISCAN Software (Blot Quantification

MTCO1		ATP5a	
Segment	Pixel Total	Segment	Pixel Total
1	89260	1	159653
2	60384	2	117090
3	68784	3	81701
4	82196	4	76271
5	80967	5	18078
6	86318	6	14323
7	77535	7	67810
8	77442	8	24625
9	59104	9	35427

Graphic

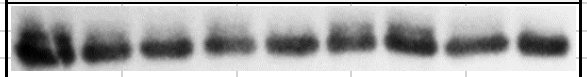


Unpaired T Test

Table Analyzed	Mitonuclear Imbalance SuperECL
Column B	HIIE
vs.	vs,
Column A	CTL
Unpaired t test	
P value	0,0422
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2,480, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	439,8
Difference between means (B - A) ± SEM	339,8 ± 137,0
95% confidence interval	15,79 to 663,8
R squared (eta squared)	0,4677
F test to compare variances	
F, DFn, Dfd	58,51, 4, 3
P value	0,0071
P value summary	**
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

GAPDH



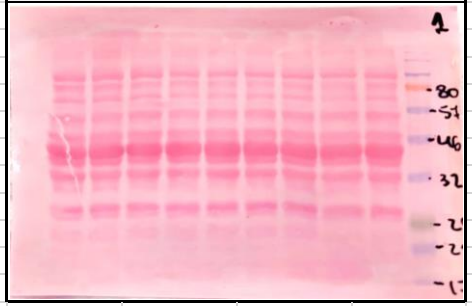
Groups	Old CTL	Old HIIT
Absoluted Data	146296	55823
	74486	49634
	63310	73577
	45173	55659
		66760
Average	82316,25	60290,6

Load Control

Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	52497	53827
	45203	54584
	49561	61506
	45310	57712
		55282
Average	48142,75	56582,2

Ponceau



Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	2,786749719	1,037081762
	1,647810986	0,909314085
	1,27741571	1,196257276
	0,996976385	0,964426809
		1,207626352
Average	1,6772382	1,062941257

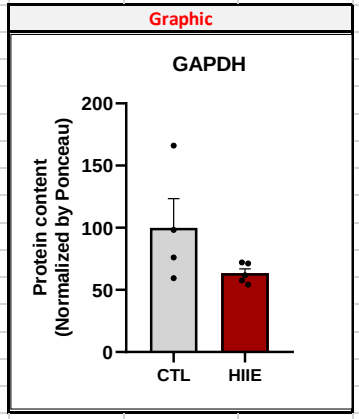
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	166,1511	61,83270581
	98,24549584	54,21496391
	76,16185405	71,32304021
	59,4415501	57,50088503
		72,00088528
Average	100	63,37449604

UNISCAN Software (Blot Quantification

GAPDH		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	146296	1	52497
2	74486	2	45203
3	63310	3	49561
4	45173	4	45310
5	55823	5	53827
6	49634	6	54584
7	73577	7	61506
8	55659	8	57712
9	66760	9	55282

Graphic

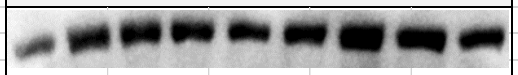


Unpaired T Test

Table Analyzed	GAPDH Ba
Column B	HIIE
vs.	vs,
Column A	CTL
Unpaired t test	
P value	0,1245
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1,745, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	63,37
Difference between means (B - A) ± SEM	-36,63 ± 20,99
95% confidence interval	-86,25 to 13,00
R squared (eta squared)	0,3032
F test to compare variances	
F, DFn, Dfd	34,02, 3, 4
P value	0,0053
P value summary	**
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

LONP1



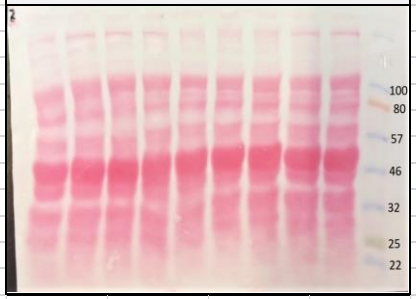
Groups	Old CTL	Old HIIT
Absoluted Data	44033	90291
	93706	110270
	102106	154173
	104571	134814
		100069
Average	86104	117923,4

Load Control

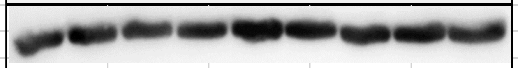
Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	73204	89345
	94358	95185
	103528	100019
	102904	129859
		162814
Average	93498,5	115444,4

Ponceau



Quantitative Relativization (Target / Control)



Groups	Old CTL	Old HIIT
Relativization by the Load Control	0,601510846	1,010588169
	0,993090146	1,158480853
	0,986264585	1,541437127
	1,016199565	1,03815677
		0,614621593
Average	0,899266286	1,072656902

Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	66,88906901	112,3791902
	110,4333791	128,8251179
	109,6743647	171,4105323
	113,0031873	115,4448673
		68,34700717
Average	100	119,281343

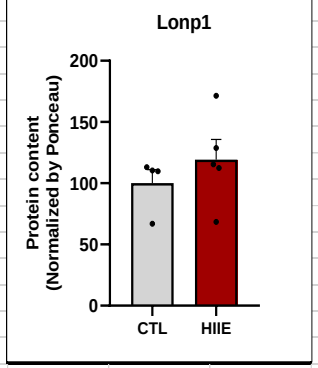
UNISCAN Software (Blot Quantification

LONP1		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	44033	1	73204
2	93706	2	94358
3	102106	3	103528
4	104571	4	102904
5	90291	5	89345
6	110270	6	95185
7	154173	7	100019
8	134814	8	129859
9	100069	9	162814

Unpaired T Test

Table Analyzed	Lonp1
Column B	HIIE
vs.	vs,
Column A	CTL
Unpaired t test	
P value	0,3915
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0,9133, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	119,3
Difference between means (B - A) ± SEM	19,28 ± 21,11
95% confidence interval	-30,64 to 69,20
R squared (eta squared)	0,1065
F test to compare variances	
F, DFn, Dfd	2,792, 4, 3
P value	0,4252
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5

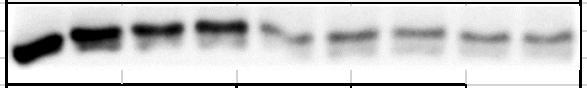
Graphic



	Significant?	P value	Mean of CTL	Mean of HIIE	Difference	SE of difference	t ratio	df	Adjusted P Value
Lonp1	No	0,388107	100	119,3	-19,28	21,88	0,8813	21	0,388107
CLpP	Yes	0,002857	100	26,15	73,85	21,88	3,376	21	0,008546
Yme1L1	Yes	0,005365	100	167,9	-67,92	21,88	3,105	21	0,0107

Targed Protein

CLPP



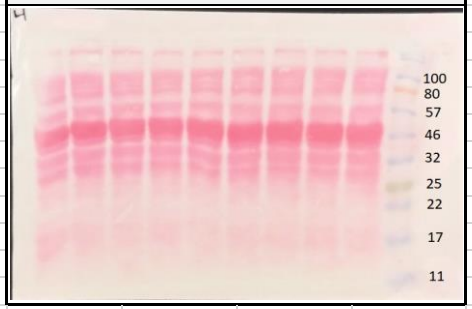
Groups	Old CTL	Old HIIT
Absoluted Data	317318	63142
	298963	89943
	188474	75482
	186889	63529
		66127
Average	247911	71644,6

Load Control

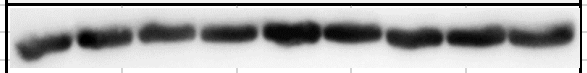
Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	16535	23757
	25247	25567
	23825	25449
	22730	21399
		19996
Average	22084,25	23233,6

Ponceau



Quantitative Relativization (Target / Control)



Groups	Old CTL	Old HIIT
Relativization by the Load Control	19,19068642	2,657827167
	11,84152573	3,517933273
	7,910766002	2,966010452
	8,222129344	2,968783588
		3,307011402
Average	11,79127687	3,083513177

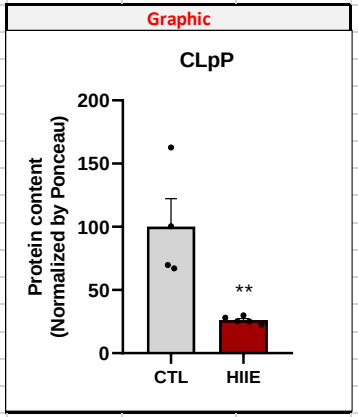
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	162,7532508	22,54062215
	100,4261528	29,83504934
	67,08998599	25,15427705
	69,73061045	25,17779558
		28,04625349
Average	100	26,15079952

UNISCAN Software (Blot Quantification

CLPP		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	317318	1	16535
2	298963	2	25247
3	188474	3	23825
4	186889	4	22730
5	63142	5	23757
6	89943	6	25567
7	75482	7	25449
8	63529	8	21399
9	66127	9	19996

Graphic

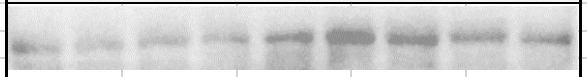


Unpaired T Test

Table Analyzed	CLpP
Column B	HIIE
vs.	vs,
Column A	CTL
Unpaired t test	
P value	0,007
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3,770, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	26,15
Difference between means (B - A) ± SEM	-73,85 ± 19,59
95% confidence interval	-120,2 to -27,53
R squared (eta squared)	0,67
F test to compare variances	
F, DFn, Dfd	246,3, 3, 4
P value	0,0001
P value summary	***
Significantly different (P < 0.05)?	Yes
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

YME1L1



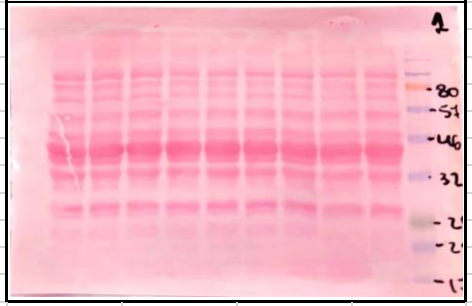
Groups	Old CTL	Old HIIT
Absoluted Data	56035	71090
	39839	85806
	46563	81761
	47341	71502
		66518
Average	47444,5	75335,4

Load Control

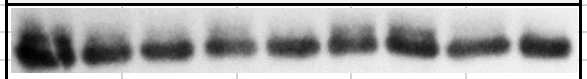
Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	92321	62207
	91905	65384
	82249	88984
	69009	96254
		95660
Average	83871	81697,8

Ponceau



Quantitative Relativization (Target / Control)



Groups	Old CTL	Old HIIT
Relativization by the Load Control	0,60695833	1,142797434
	0,433480224	1,31233941
	0,566122384	0,918828104
	0,686011969	0,742847051
		0,695358562
Average	0,573143227	0,962434112

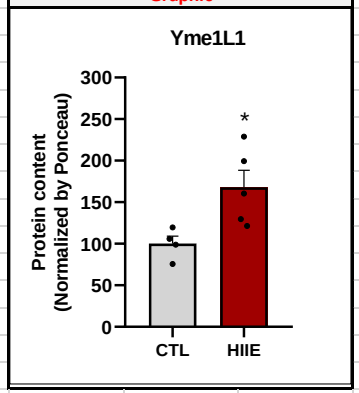
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	105,8999394	199,3912482
	75,63209398	228,9723316
	98,77502825	160,3138728
	119,6929384	129,609322
		121,3236986
Average	100	167,9220946

UNISCAN Software (Blot Quantification

YME1L1		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	56035	1	92321
2	39839	2	91905
3	46563	3	82249
4	47341	4	69009
5	71090	5	62207
6	85806	6	65384
7	81761	7	88984
8	71502	8	96254
9	66518	9	95660

Graphic

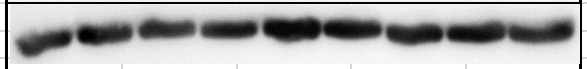


Unpaired T Test

Table Analyzed	Yme1L1 Ba
Column B	HIIE
vs.	vs,
Column A	CTL
Unpaired t test	
P value	0,0282
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2,758, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	167,9
Difference between means (B - A) ± SEM	67,92 ± 24,62
95% confidence interval	9,694 to 126,2
R squared (eta squared)	0,5208
F test to compare variances	
F, DFn, Dfd	6,200, 4, 3
P value	0,1658
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5

Targed Protein

GAPDH



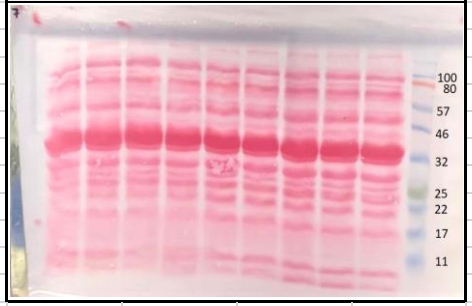
Groups	Old CTL	Old HIIT
Absoluted Data	390715	507954
	422262	430136
	359453	403913
	378362	441140
		417843
Average	387698	440197,2

Load Control

Ponceau

Groups	Old CTL	Old HIIT
Absoluted Data	104769	166834
	136230	169032
	154369	171117
	160248	165588
		151419
Average	138904	164798

Ponceau



Quantitative Relativization (Target / Control)

Groups	Old CTL	Old HIIT
Relativization by the Load Control	3,729299697	3,044667154
	3,099625633	2,544701595
	2,328530987	2,360449283
	2,361102791	2,664081938
		2,759514988
Average	2,879639777	2,674682992

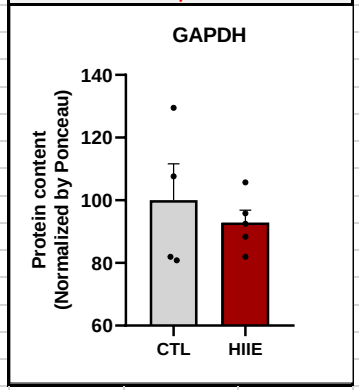
Quantitative normalization (Aged CTL = 100%)

Groups	Old CTL	Old HIIT
Normalization by the average from the Load Control (Sample/Average)x100	129,5057711	105,7308341
	107,6393533	88,3687472
	80,86188439	81,97029719
	81,99299126	92,51441654
		95,82847862
Average	100	92,88255472

UNISCAN Software (Blot Quantification

GAPDH		Ponceau	
Segment	Pixel Total	Segment	Pixel Total
1	390715	1	104769
2	422262	2	136230
3	359453	3	154369
4	378362	4	160248
5	507954	5	166834
6	430136	6	169032
7	403913	7	171117
8	441140	8	165588
9	417843	9	151419

Graphic



Unpaired T Test

Table Analyzed	GAPDH
Column B	HIIE
vs.	vs,
Column A	CTL
Unpaired t test	
P value	0,5435
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0,6385, df=7
How big is the difference?	
Mean of column A	100
Mean of column B	92,88
Difference between means (B - A) ± SEM	-7,117 ± 11,15
95% confidence interval	-33,48 to 19,24
R squared (eta squared)	0,05504
F test to compare variances	
F, DFn, Dfd	6,891, 3, 4
P value	0,0931
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	4
Sample size, column B	5