Supplement to:

"Impact of protocol-based physiotherapy on insulin sensitivity and peripheral glucose metabolism in critically ill patients"

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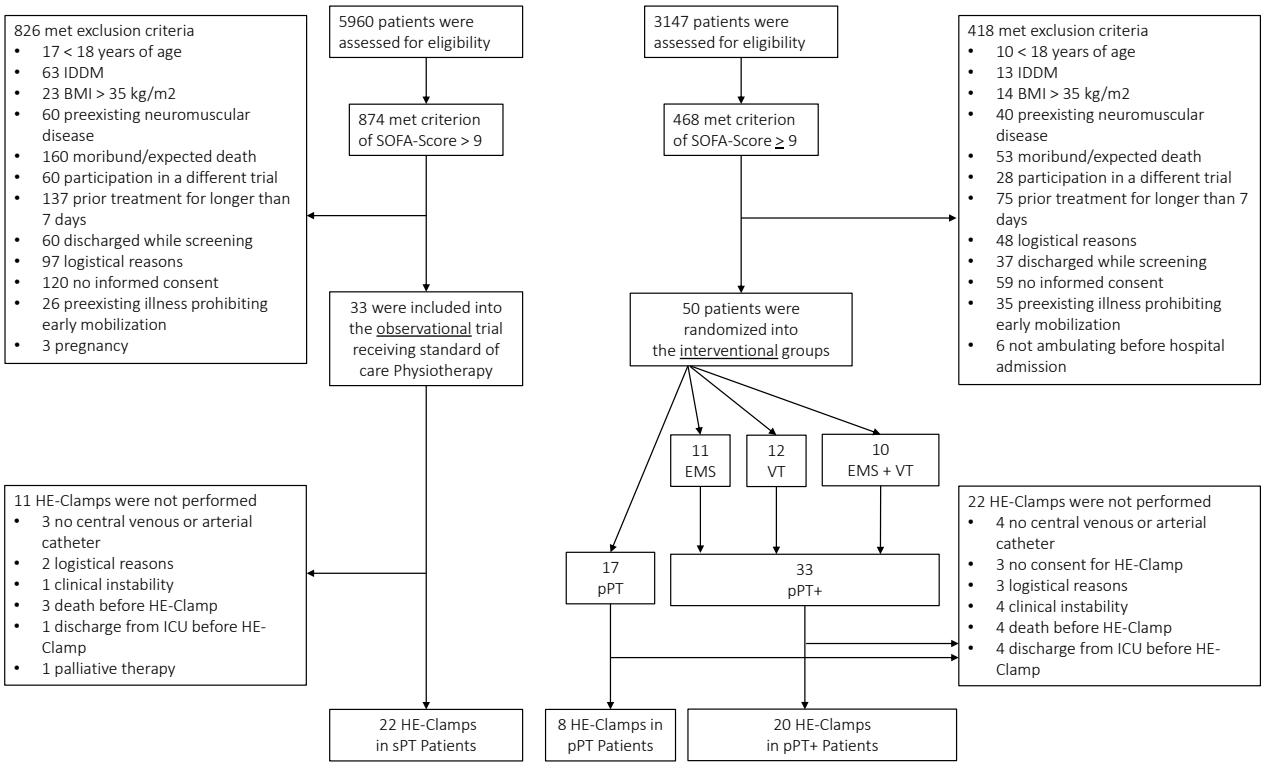

Figure S1: Study enrollment scheme for observational and interventional trials

"HE-clamp": Hyperinsulinemic Euglycemic Clamp; "EMS": electrical muscle stimulation; "VT": whole body vibration therapy; "sPT": standard physiotherapy; "pPT": protocol-based physiotherapy; "pPT+": protocol-based physiotherapy with additional muscle activating measures; "IDDM": insulin dependent diabetes mellitus; "SOFA-Score" sequential organ failure assessment score

Measurements

Hyperinsulinemic-Euglycemic Clamp (HE-Clamp)

According to de Fronzo et al. [10,11] the test is divided in three phases: 1) baseline, 2) titration and 3) steady state. During baseline patients received a basal glucose infusion of 2 mg/kg/min for 12 hours. Blood glucose level was kept in the target of 80-140 mg/dl by continuous insulin infusion due to local ICU standard.

During the titration phase, patients received an insulin bolus of 180 mIU/m²BSA/min over 30 min, followed by a continuous insulin infusion of 125 mIU/m²BSA/min. The glucose infusion rate was continuously adjusted until the blood glucose target (80-110 mg/dl) was stable for 30 min, without any further adjustment of the glucose infusion rate. This point is defined as the steady state in which glucose infusion corresponds to peripheral glucose uptake (DeFronzo 1979). Plasma insulin concentration was measured by laboratory from blood samples retrieved during the steady state.

Blood measurements were taken from arterial catheters. Glucose infusion was applied by central venous line. Catheters were established due to clinical indication in these severely ill ICU patients.

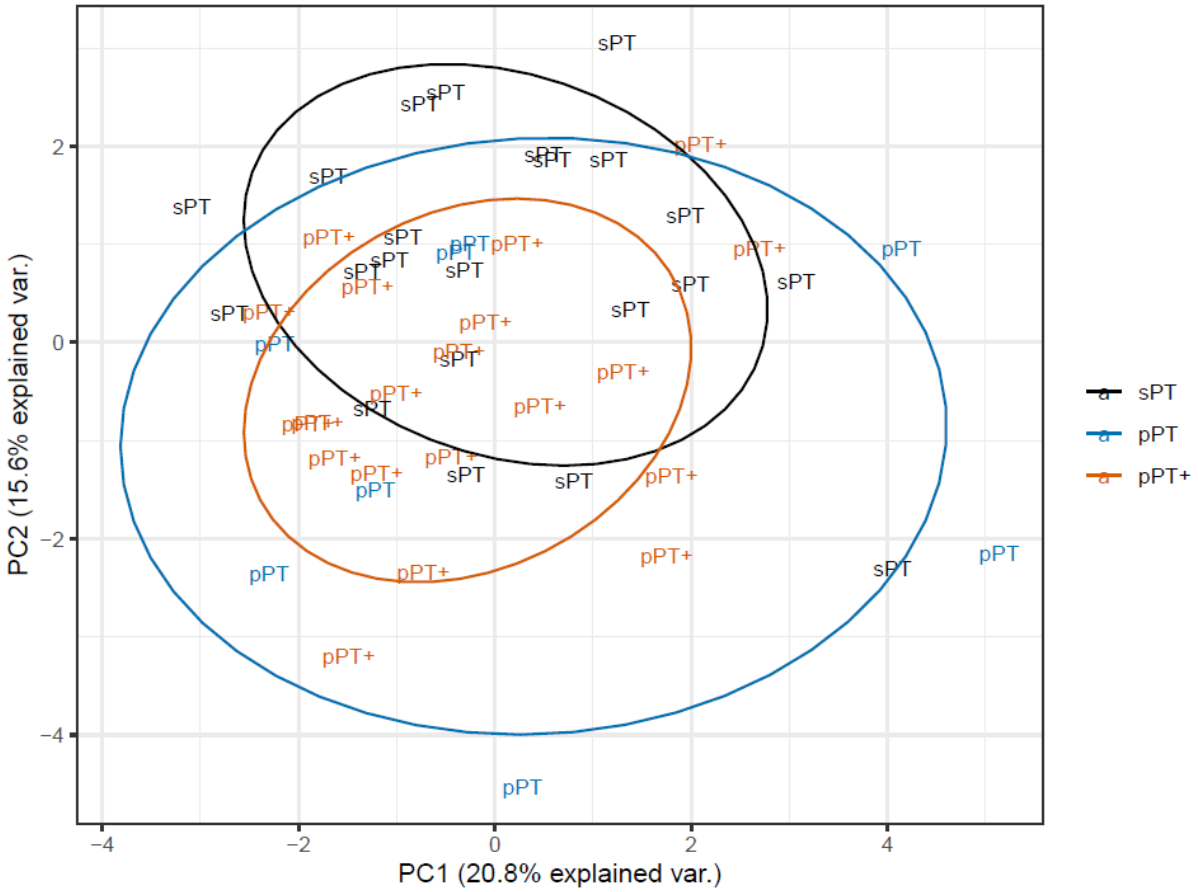

Figure S2: Principal Component Analysis

Principal Component Analysis including the following data: age, sex, weight, height, ICU stay before HE-Clamp, event leading to ICU admission, mean sedation level measured by Richmond Agitation and Sedation Scale, SOFA Score on admission, APACHE 2 Score on admission, mean norepinephrine dose before HE-Clamp, days in septic shock, mean dose of nutrition (kcal/kg PBW) before HE-Clamp, Mean insulin dose and fraction of days before HE-Clamp, mean blood glucose level before HE-Clamp. Plot shows good overlap of data, pooling of data is feasible.

Table S1: Baseline characteristics

		sPT	pPT	pPT+	p
		n=22	n=8	n=20	
Diagnosis					
	ARDS/Sepsis	15	4	11	
	Polytrauma	5	3	5	
	Neuro/others	2	1	4	
Sex					
	male	19	3	14	
	female	3	5	6	
age (years)		52 (36-69)	52.5 (33-64.5)	64 (51-69.5)	0.070
weight (kg)		83 (78-96)	80 (65-85)	84 (70-96)	0.218
height (m)		1.77 (1.72-1.85)	1.74 (1.68-1.75)	1.76 (1.68-1.80)	0.469
Illness severity scoring at ICU admission					
SOFA		12 (10-14)	14 (11-18)	13 (11-14)	0.438
SAPS2		39.5 (35.0-52.0)	55.5 (40.0-70,0)	59,5 (52.0-67.0)	0.784
APACHE II		18.5 (15.0-25.0)	27.5 (18.5-32.0)	25.5 (21.5-30.0)	0.940
ICU admission to HE-Clamp					
ICU stay before HE-Clamp (days)		16(13-18)	17(15.5-24.5)	20.5(15.5-22.5)	0.940
Fraction Days with septic shock (%)		14.8 (10.0-35.7)	41 (32.5-56.4)	20.9 (0.65-58.8)	0.087
median blood glucose before HE-Clamp		125(117-132)	133(108-139)	133(125-141)	0.566
Caloric intake* (kcal/kg PBW)		21.5 (17.8-24.5)	20.3 (13.0-28.5)	17.4 (1.0-25.2)	0.709
Insulin* (IU/d)		45.7 (30.4-64.6)	38.7 (14.4-56.9)	33.8 (18.6-61.5)	0.576
fraction of days receiving Insulin before HE-Clamp		0.90 (0.79-1.00)	0.91 (0.32-1.00)	0.95(0.54-1.00)	0.746
Norepinephrine* (µg/kg/min)		0.007 (0.000-0.072)	0.011 (0.005-0.043)	0.012 (0.003-0.066)	0.897

*Table showing baseline characteristics in the three therapeutic groups; categorical variables are presented as count (percentage), metric variables are presented as median (25th/75th percentile); *mean daily dose before HE-clamp; Shown baseline characteristics show no significant differences between observational and interventional trial;*
*Categorical variables are presented as count (percentage), metric variables are presented as median (25th-75th percentile); *mean daily dose before HE-Clamp;*
BSA: body surface area; PBW: predicted body weight; p-value determined by Kruskal Wallis

Table S2: Hyperinsulinemic Euglycemic Clamp Setup

		sPT	pPT	pPT+	
		n=22	n=8	n=20	p
Plasma Insulin concentration (mU/l)	baseline	24.6 (11.8-32.0)	18,4 (9,5; 32,6)	21.4 (10.9-35.6)	0.840
	steady state	158.8 (120.7-211.8)	215 (149/ 237,5)	200.6 (169.0-230.0)	0.214
Glucose infusion rate (mg/kg/min)	baseline	2 (2-2)	2 (2/ 2)	2 (2-2)	0.636
	steady state	5.3 (4.4-7.7)	6,4 (3,9/ 6,8)	6.4 (4.9-7.2)	0.747
Blood Glucose concentration (mg/dl)	baseline	124 (116-145)	138 (109/ 161)	129 (123-158)	0.691
	steady state	85 (81-96)	99 (90/ 102)	93 (87-98)	0.078
Blood Lactate concentration (mg/dl)	baseline	0.8 (0.6-1.6)	1.1 (0.9-1.7)	1.0 (0.8-1.5)	0.414
	steady state	0.9 (0.8-1.2)	1.2 (1.0-2.0)	1.3 (0.9-1.5)	0.195

Table showing hyperinsulinemic euglycemic Clamp setup in critically ill patients in the three therapeutic groups. No group difference could be observed. Setup and execution of the hyperinsulinemic euglycemic Clamp is consistent over all three patient groups of the two included studies; variables are presented as median (25th/75th percentile); p-value determined by Kruskal Wallis

When comparing the results of the ICU measurements to healthy controls the modification of the standard HE-clamp led to similar steady state glucose infusion rates or M values (median M value in pooled ICU patients 5.72 (4.37/ 7.20) mg/k1g/min vs all healthy controls 6.0 (5.07/ 6.35) mg/kg/min p=n.s.), representing a similar net glucose uptake into the insulin dependent tissue. On the other hand, the modification of the HE-clamp led to a higher baseline and steady state plasma insulin concentration (median baseline plasma insulin concentration in pooled ICU patients 21.08 (11.71/ 34.34) mU/l vs healthy controls 3.71 (3.39/ 4.55) p=0.002 and median steady state plasma insulin concentration in pooled ICU patients 192.08 (143.47/ 233.21) vs. all healthy controls 57.70 (54.67/ 63.92) mU/l p<0.001). The two distinct levels of relatively low and high insulin plasma concentrations could be achieved in all three ICU patient groups (baseline versus steady state: sPT p<0.001; pPT p=0.012; pPT+ p<0.001).

Table S3: Microdialysis of the m. vastus lateralis during HE-Clamp

		sPT n=17	pPT n=8	pPT+ n=20	Kruskal-Wallis (sPT, pPT, pPT+) p	healthy controls n=4
Glucose (mmol/l)	baseline	4.67 (3.8/5.52)	3.6 (2.52/4.52)	3.22 (2.06/4.03)	0.079	3.19 (3.06/3.4)
	steady state	2.68 (2.16/2.95)	2.06 (1.66/2.63)	1.71 (1.17/2.1)	0.019*	2.8 (1.88/3.22)
	relative change	-0.43 (-0.48/-0.28)	-0.4 (-0.45/-0.24)	-0.45 (-0.57/-0.22)	0.591	-0.13 (-0.41/-0.01)
	recovery rate	0.73 (0.52/0.92)	0.77 (0.6/0.82)	0.68 (0.62/0.84)	0.981	-
	steady state (corrected)	3,75 (2,99/4,81)	3,31 (2,64/4,11)	2,74 (1,77/3,48)	0.037*	-
Lactate (mmol/l)	baseline	2.35 (1.8/2.98)	2.05 (1.35/3.04)	2.25 (1.45/3.23)	0.864	1.75 (1.41/2.05)
	steady state	2.4 (2.02/2.51)	2.24 (1.62/2.81)	2.39 (1.88/3.69)	0.737	2.03 (1.89/2.45)
	relative change	0.07 (-0.13/0.31)	-0.06 (-0.22/0.59)	0.21 (-0.05/0.39)	0.669	0.26 (0.14/0.4)
	recovery rate	0.93 (0.78/1)	0.82 (0.77/1.16)	0.82 (0.73/1.09)	0.53	-
	steady state (corrected)	2.57 (2.27/3.22)	2.77 (1.73/3.38)	3.29 (2.21/4.36)	0.303	-
Pyruvate (µmol/l)	baseline	65.4 (57.7/115.5)	78 (56.8/127.3)	65.2 (45.7/101.5)	0.532	47 (41.5/54.5)
	steady state	67.8 (51.6/113.9)	81.5 (48.6/119.2)	82.3 (56.2/113.7)	0.924	117 (95/154.5)
	relative change	0.05 (-0.18/0.17)	-0.06 (-0.36/0.09)	0.06 (-0.17/0.61)	0.371	1.84 (1.05/2.15)
	recovery rate	0.85 (0.67/1.02)	0,77 (0,74/1,37)	0.82 (0.62/1)	0.964	-
	steady state (corrected)	100.9 (71.8/110)	64.2 (45.4/161.2)	102.7 (69.6/139)	0.814	-
Lactate/ Pyruvate Ratio	baseline	30 (22.8/36.4)	25.1 (23.2/33.2)	33.1 (22.6/49.1)	0.693	35.2 (33.8/37.8)
	steady state	32.5 (21.5/43)	25.9 (22.6/30.8)	30.7 (23.3/46.7)	0.755	16.1 (15.3/21.8)
	relative change	0.09 (-0.13/0.27)	0.07 (-0.16/0.31)	-0.06 (-0.19/0.16)	0.729	-0.52 (-0.56/-0.42)
Glycerol (µmol/l)	baseline	122 (28.6/172.8)	64.7 (37.3/120.3)	55.2 (35.4/76.1)	0.145	98 (72/141)
	steady state	34.7 (25/70)	57.1 (22/109.7)	42.6 (29.8/51.5)	0.822	25 (18.5/30)
	relative change	-0.39 (-0.8/-0.12)	-0.21 (-0.33/0.07)	-0.21 (-0.44/-0.03)	0.257	-0.74 (-0.87/-0.58)
	recovery rate	0.92 (0.61/1.12)	0.85 (0.68/1.08)	0.87 (0.74/0.95)	0.991	-
	steady state (corrected)	54 (32.1/76.8)	73.1 (34.5/118.3)	48 (30:3/65:8)	0.564	-

Table showing dialysate concentrations at baseline and steady state of the hyperinsulinemic euglycemic Clamp in critically ill patients divided by the three therapeutic groups. "sPT": standard physiotherapy; "pPT": protocol-based physiotherapy; "pPT+": protocol-based physiotherapy with additional muscle activating measures. Variables are presented as median (25th/75th percentile); p-value determined by Kruskal Wallis between sPT pPT and pPT+, results of four healthy individuals are shown as reference.

Table S4: Impact of predictors on Strength measured by MRC Score at discharge - Results of the linear Regression Analysis

Predictor	Unstandardized Regression Coefficient	Standard Error	Standardized Coefficient Beta	t	Significance
Constant	2.862	1.207	-	2.371	0.025
Age	-0.002	0.009	-0.04	-0.224	0.824
BMI	0.006	0.033	0.031	0.177	0.861
Sex	0.281	0.393	-0.132	-0.714	0.481
SOFA Score on admission	-0.012	0.048	-0.045	-0.247	0.807
Insulin Sensitivity Index	**18.671**	**8.368**	**0.484**	**2.231**	**0.034***

*Results of the linear regression. Dependent Variable: MRC at discharge. * indicates p<0.05. While all input variables correlate with muscle weakness measured by MRC Score at discharge, only Insulin sensitivity index has an independent impact.*