Regulation of Lipolysis by the Sympathetic Nervous System: A Microdialysis Study in Normal and Spinal Cord–Injured Subjects

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To evaluate the regulation of lipolysis by the sympathetic nervous system, eight spinal cord–injured (SCI) subjects with a lesion above T5 resulting in a decentralization of the lower-body sympathetic nervous system and adrenal medulla (age, 36 ± 2 years; weight, 82 ± 5 kg; body fat mass, 26.8 ± 3.0 kg; all mean ± SE) and nine control subjects (age, 33 ± 2; weight, 80 ± 3; NS; body fat mass, 16.1 ± 1.5 kg; $P < .01$) were investigated after fasting overnight. Each subject was studied with subcutaneous microdialysis and ^{133}Xe-clearance adipose tissue blood flow (ATBF) in the umbilical and clavicular regions during postabsorptive rest and after sympathoexcitatory stimulation by means of mental stress and isometric handgrip exercise. SCI subjects had an increased body fat mass, hyperinsulinemia, and an elevated lipolytic rate at rest compared with control subjects. ATBF and lipolysis were activated to a normal extent following mental stress and isometric handgrip exercise in the umbilical region in control subjects. ATBF was increased in tissue above but not below the lesion level in SCI subjects following mental stress. Glycerol release was not different between groups in either tissue region despite significantly lower noradrenaline and adrenaline levels in SCI subjects. This finding argues against a significant adrenergic control of the lipolytic rate at rest. Furthermore, the small differences in stimulated glycerol release between groups, as well as the increased plasma glycerol levels in SCI subjects, cast doubt on the view that interruption of adrenergic activity below the lesion is the sole mechanism underlying the increased body fat mass in SCI subjects.

ADRENERGIC STIMULATION of the lipolytic rate is a key mechanism by which stored triglycerides are hydrolyzed and mobilized from adipose tissue.[1] Increasing interest has recently been focused on the mechanisms involved in the regulation of lipolysis, suggested to be one of the major mechanisms underlying insulin resistance.[2] An increased rate of lipolysis and the resulting increase in plasma free fatty acid (FFA) level results in inhibition of muscle glucose oxidation[3] and downregulation of insulin sensitivity in the liver.[4] In some insulin-resistant states such as obesity[5] and acute stress reactions,[6] increased activation of sympathetic nerves has been proposed as the cause of increased lipolysis and insulin resistance.[7,8] However, in insulin-resistant hypertensives, direct intraneural recordings have only shown a weak correlation between fasting insulin levels and muscle sympathetic nerve activity,[8-10] casting doubt on the notion that altered sympathetic outflow to the muscle vascular bed is of major importance for the development of insulin resistance. Furthermore, although FFA turnover and total-body lipolysis are increased in obese subjects,[11] the notion that this depends on autonomic nervous hyperactivity could be questioned, since microdialysis studies in adipose tissue have shown that the lipolytic rate per gram of tissue is normal in these subjects, and the increased rate of total-body lipolysis may instead be the result of the increased body fat mass.[12] In fact, even reduced lipolytic rates have been proposed as a mechanism underlying obesity, resulting from an inherent reduction in adrenergic sensitivity.[13-16] It may thus be concluded that the autonomic nervous regulation of lipolysis and its putative link to insulin resistance remains unclear.

Spinal cord–injured (SCI) subjects constitute a unique subject group for studies of the regulation of lipolysis, since they have a disrupted sympathetic nerve traffic below the spinal lesion level. With injuries above the T5 level, the functional block includes the adrenal medulla. SCI subjects show an increased prevalence of insulin resistance[17] and an increase in fat tissue mass.[18] Using a subcutaneous microdialysis technique, we recently demonstrated a normal rate of lipolysis in SCI subjects in the postabsorptive state and after oral glucose, arguing against a neural control of lipolysis in the basal state.[19] The aim of the present study was to further examine adrenergic control of the lipolytic rate during central stimulation of the sympathetic nervous system, to evaluate its tentative importance for the regulation of body fat mass. Microdialysis measurements of interstitial glycerol output were performed above and below the lesion level in SCI subjects and in healthy control subjects.

SUBJECTS AND METHODS

Subjects

The two groups consisted of eight SCI subjects (one woman and seven men) and nine weight-matched control subjects (one woman and eight men). Patient characteristics are listed in Table 1. The lesion level in the SCI group was T3-T4 in five subjects and C7 in three. Seven SCI subjects had complete lesions, and one subject had an incomplete sensory disruption. The duration of paresis was 13 ± 2 years (mean ± SE).

Both SCI and control subjects lacked ongoing drug treatment, except for one SCI subject who took anticholinergic drugs to prevent urinary leakage. All subjects were healthy, except for one in the SCI group who showed a minor pressure sore on his left foot. Two had a history of venous embolism during the acute postinjury phase, and one had a history of gestational hypertension.

Six SCI subjects underwent primary care in the Gothenburg region,

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and one subject was recruited from Stockholm and one from Copenhagen. Control subjects were recruited as volunteers.

To determine the segmental level of interrupted sympathetic output in the SCI group, we performed a forced-perspiration test.[20] The hands and wrists were placed in 40°C water, and the sweat droplets were visualized beneath a thin plastic film (Nobecutan; Astra Tech, Lund, Sweden). An absence of droplets was taken as an indicator of absent sympathetic innervation.[20] The level of sympathetic disruption was set within plus or minus one level of sensory disruption.

Body composition was evaluated in all subjects with dual-energy x-ray absorptiometry scanning (Lunar DPX L; Scanexport Medical, Melsingborg, Sweden).[21]

All subjects provided informed consent, and the study was approved by the Ethics Committee of the University of Gothenburg.

Protocol

Subjects arrived at the laboratory at 8 AM after an overnight fast and were investigated in the supine position. Room temperature was kept at 26°C to guarantee optimal microdialysis conditions.[22] A polyethylene catheter was placed in a left forearm vein for blood sampling. The arm was heated with electric pads (60° to 70°C) to arterialize the venous blood to an O_2 saturation of 93% ± 1%.[23] Heart rate and continuous noninvasive blood pressure were monitored with the volume clamp technique (Finapres; Ohmeda Monitoring Systems, Englewood, CO), and resting values were taken as mean values obtained during the last 5 minutes before stress. During stress stimulation, mean values were calculated per minute.

Microdialysis catheters (30 × 0.3 mm, Cuprophane, B4 AH, 3,000 MV cutoff; Cobe, Denver, CO) were placed in the subcutaneous adipose tissue 5 cm lateral to the umbilicus and 5 cm above the mamilla, corresponding to a level 5 cm proximal to the sympathetic disruption in the SCI group. The nylon tubing inlet of the microdialysis catheter was connected to a microinjection pump (Carnegie Medicine, Stockholm, Sweden) and perfused with isotonic saline with 2.5 mmol/L glucose at a rate of 2.5 μL/min. Samples were collected every 15 minutes. After a 30-minute equilibration, a calibration procedure was performed.[24] Four different concentrations of glycerol (0 to 200 μmol/L) were administered, and the net change in the metabolite concentration of the dialysate was recorded ($glycerol_{out} - glycerol_{in}$ = net change). A linear relationship was established between the added concentration of glycerol in the perfusate and the concentration change in the dialysate. The concentration of glycerol in the perfusate equilibrating with the interstitial glycerol concentration was calculated by regression analysis.[24]

Blood samples were immediately centrifuged, and the plasma was stored at −4°C. Plasma was analyzed for glucose, insulin, glycerol, FFA, lactate, adrenaline, and noradrenaline. Dialysates were analyzed for glycerol.

Adipose tissue blood flow (ATBF) was measured by the ^{133}Xe-washout method.[25] Briefly, 6 to 9 MBq ^{133}Xe (Mallinchrodt, Petten, The Netherlands) in 0.1 mL sterile saline was injected into the subcutaneous adipose tissue contralateral to the microdialysis sites, with one depot in the clavicular region and one in the umbilical region. The depot was injected slowly over a 2-minute period at least 5 mm under the skin at an angle of 45°. After a 60-minute decay period, radioactivity was monitored continuously with a registration every 30 seconds. ATBF was calculated for 15 minutes before and after every stimulation.

After calibration of microdialysis catheters, stimulation of the sympathetic nervous system was performed. Two different stressors were used. Mental stress was induced for 5 minutes by means of forced arithmetics, whereby the subject was forced to rapidly subtract 17 continuously from a number, starting at 100. Immediately after completion of the stimulation, blood samples were taken as indicated earlier. Isometric handgrip exercise was performed at 30% of maximal strength until exhaustion (3 to 6 minutes). The stimulations were made in randomized order, and each was followed by a 30-minute resting period.

Analyses

Glycerol content in the dialysates and plasma was determined according to the method reported by Laurell and Tibbling.[26] Blood glucose and plasma FFA concentrations were estimated enzymatically (Wako Chemicals, Neuss, Germany). Plasma insulin was measured with radioimmunoassays (Pharmacia, Uppsala, Sweden) and catecholamine levels were measured with high-performance liquid chromatography at the Department of Clinical Chemistry, University of Gothenburg.

Calculations

In microdialysis-calibration calculations, regression analyses were performed with the least-squares method and linear correlations were tested using Pearson's correlation coefficient. To estimate the regional rate of glycerol release, Fick's principle was used. Arterialized venous plasma (A) and venous plasma concentrations of glycerol (V) and plasma flow rate (Q) were entered into the formula, $(V - A) \times Q \times (1 - \text{hematocrit} \times 10^{-2})$. Recalculation of interstitial (I) to venous (V) glycerol levels was made with the equation, $V = (I - A) \times (1 - e^{-ps/Q}) + A$, where ps is the permeability surface area product (~5 mL/100 g/min).[27,28]

Student's two-tailed t test for unpaired and paired data was used. ANOVA for repeated measurements was used when appropriate.

RESULTS

The groups were age- and weight-matched. However, the data listed in Table 1 show that lean body mass was significantly lower in SCI subjects, and accordingly, the total fat tissue mass was increased in SCI subjects compared with controls.

Table 1. Subject Characteristics

Characteristic	SCI Subjects	Controls	*P*
No. of subjects	8	9	
Sex (F/M)	1/7	1/8	
Age (yr)	36 ± 2	33 ± 2	NS
Weight (kg)	82 ± 5	80 ± 3	NS
Lean body mass (kg)	51.2 ± 2.8	60.4 ± 2.2	.021
Fat tissue mass (kg)	26.8 ± 3.0	16.1 ± 1.5	.008
Total fat (%)	33.8 ± 2.6	20.1 ± 1.7	.001
Regional fat (%)			
Arms	29.9 ± 3.4	18.9 ± 3.1	.043
Legs	32.7 ± 2.7	19.1 ± 1.8	.001
Trunk	33.7 ± 2.5	21.2 ± 1.6	.001
Occupation (n)			
Employed			
Part-time	3	0	
Full-time	2	9	
Unemployed	2	0	
Disability pensioner	1	0	
Biochemistry			
Glycerol (μmol/L)	82 ± 7	61 ± 4	.019
Lactate (mmol/L)	0.71 ± 0.04	0.87 ± 0.05	.033
Insulin (pmol/L)	68.7 ± 15.4	35.8 ± 4.1	.046
Glucose (mmol/L)	4.85 ± 0.15	4.64 ± 0.16	NS
FFA (mmol/L)	0.64 ± 0.09	0.52 ± 0.11	NS
Adrenaline (nmol/L)	0.09 ± 0.04	0.15 ± 0.03	NS
Noradrenaline (nmol/L)	0.78 ± 0.16	1.23 ± 0.14	.049

NOTE. Values are the mean ± SE.

Table 2. ATBF and Interstitial and Plasma Glycerol Concentrations Following Mental Stress

Parameter	SCI Subjects		Control	
	Rest	MS	Rest	MS
Clavicular region				
ATBF (mL/min/100 g)	3.95 ± 0.8	12.02 ± 2.3†	8.26 ± 2.1	14.7 ± 2†
Interstitial glycerol (μmol/L)	127 ± 21‡	143 ± 20	105 ± 8‡	104 ± 8‡
Calculated glycerol release (nmol/min/100 g)	114 ± 31	186 ± 53	99 ± 16	106 ± 15
Umbilical region				
ATBF (mL/min/100 g)	5.62 ± 1.2	8.80 ± 1.2	4.46 ± 0.8	9.0 ± 1.8*
Interstitial glycerol (μmol/L)	126 ± 10‡	130 ± 12	106 ± 17‡	123 ± 11*‡§
Calculated glycerol release (nmol/min/100 g)	110 ± 30	155 ± 80	98 ± 41	167 ± 43*
Arterialized plasma				
Glycerol (μmol/L)	82 ± 7	92 ± 11	62 ± 3	75 ± 5*

NOTE. Values are the mean ± SE. Plasma levels of glycerol during stimulation are the mean of samples at 0, 5, and 10 minutes.
Abbreviation: MS, mental stress.
*$P < .05$, †$P < .01$: rest *v* MS.
‡$P < .05$, interstitial *v* plasma.
§$P < .05$, umbilical *v* clavicular.

Resting State

There was no difference in mean arterial pressure (MAP) between groups at rest, whereas heart rate was significantly higher in SCI subjects compared with controls (Fig 1). ATBF monitored in the clavicular and umbilical regions in both groups showed no regional or groupwise differences (Table 2). Interstitial glycerol concentrations were higher than those obtained in plasma, and there was no difference between regions or groups (Table 2). The calculated production of glycerol was similar in both regions in the two subject groups (Table 2).

At rest, glycerol and insulin concentrations in plasma were higher in SCI subjects than in controls, whereas plasma lactate was significantly lower in the SCI group (Table 1). There were no differences in plasma glucose, FFA, or adrenaline levels, but the noradrenaline concentration was significantly lower in SCI subjects (Table 1).

Mental Stress

Both groups showed an increase in MAP and heart rate during mental stress (Fig 1). ATBF increased significantly only in the clavicular region in the SCI group, whereas a significant increase was registered in both regions in the control group (Table 2).

Despite a similar increase in ATBF following mental stress in control subjects, the interstitial concentration of glycerol in the umbilical region was significantly higher than in the clavicular

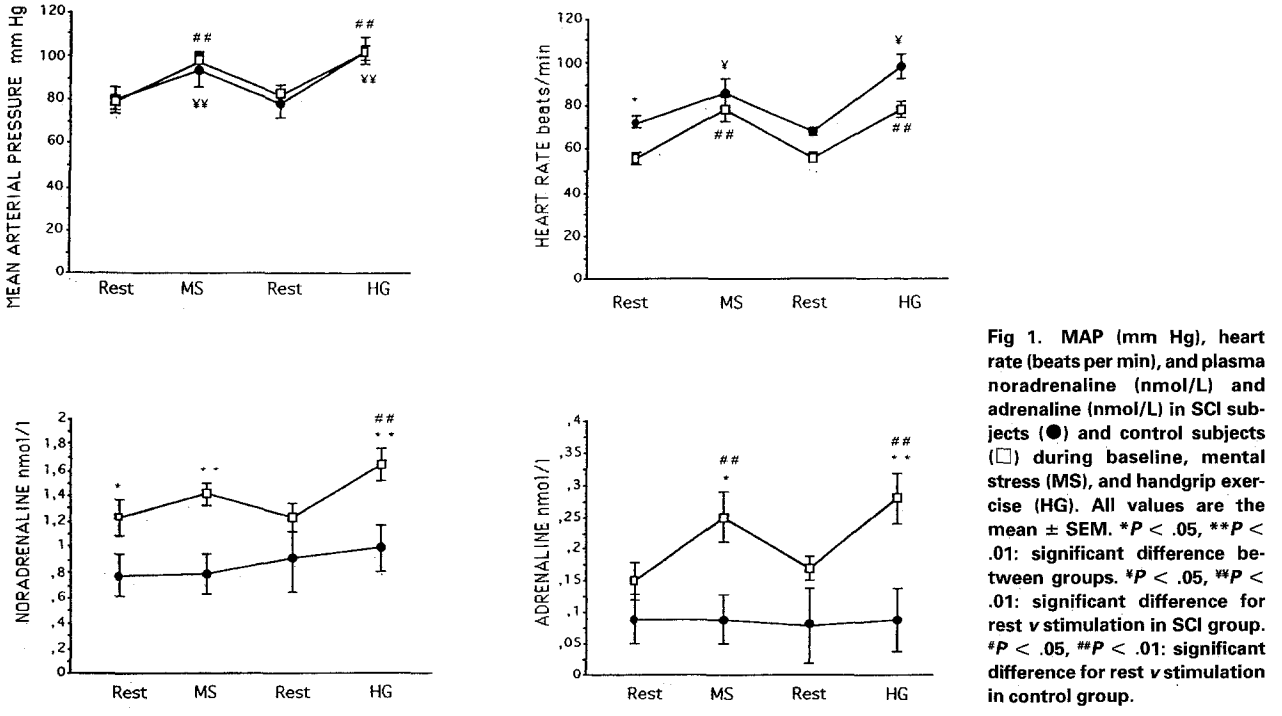

Fig 1. MAP (mm Hg), heart rate (beats per min), and plasma noradrenaline (nmol/L) and adrenaline (nmol/L) in SCI subjects (●) and control subjects (□) during baseline, mental stress (MS), and handgrip exercise (HG). All values are the mean ± SEM. *$P < .05$, **$P < .01$: significant difference between groups. ¥$P < .05$, ¥¥$P < .01$: significant difference for rest *v* stimulation in SCI group. #$P < .05$, ##$P < .01$: significant difference for rest *v* stimulation in control group.

region ($P = .049$; Table 2). Accordingly, the calculated release of glycerol increased significantly in the umbilical region in the control group, whereas no apparent increase was registered in the clavicular region (Table 2). SCI subjects did not show an increase of glycerol release in either region (Table 2).

Following mental stress, plasma glycerol, FFA, and insulin concentrations increased significantly in the control group, whereas lactate and glucose concentrations were unchanged (Fig 2). Adrenaline increased in control subjects, whereas plasma noradrenaline showed a tendency to increase but this did not reach statistical significance. However, plasma adrenaline and noradrenaline concentrations were significantly higher in control subjects compared with SCI subjects during stimulation (Fig 1). In SCI subjects, no significant increase in either of the above substrates or hormones was recorded following mental stress (Figs 1 and 2).

Handgrip Exercise

MAP and heart rate increased in both groups during handgrip exercise (Fig 1). ATBF increased significantly in the umbilical region in both groups (Table 3).

Interstitial glycerol tended to increase in both regions in the control group, and the calculated glycerol release increased significantly in the umbilical region (Table 3). No such increase was registered in the SCI group (Table 3).

After handgrip exercise, there was a significant increase in plasma glycerol in the control group (maximum concentration, 83 ± 6 μmol/L after 5 minutes; Fig 2). Plasma lactate increased approximately threefold in both groups (Fig 2). Plasma insulin, adrenaline, and noradrenaline levels increased significantly in the control group, the catecholamines reaching concentrations significantly higher than those in SCI subjects, in whom no significant increase in either plasma catecholamines or insulin appeared (Fig 1). Also, plasma glucose and FFA levels were unchanged in these subjects (Fig 2).

DISCUSSION

The present data obtained from microdialysis measurements clearly confirm[19] that the lipolytic rate in SCI subjects is normal in the postabsorptive state, whereas plasma glycerol and FFA levels are increased. They extend previous data by showing that despite the loss of stimulation of lipolysis in tissue lacking central sympathetic control, only small differences in glycerol output were recorded. This finding suggests that lipolysis was not a determining factor for the insulin resistance and increase of fat tissue mass seen in SCI subjects.

Measurements at Rest

SCI subjects in the present investigation were clearly insulin-resistant, since their fasting plasma insulin levels were significantly increased. The present data confirm that the interstitial glycerol concentration in subcutaneous tissue is normal in SCI subjects.[19] Also, the resting ATBF rate was equal in the two subject groups, and hence the calculated subcutaneous glycerol release was similar. However, plasma glycerol levels were elevated at rest in SCI subjects, as a marker of increased total-body lipolytic activity. This is in agreement with previous findings made in obese insulin-resistant individuals, ie, lipolysis per gram of tissue is normal, whereas glycerol release per fat cell increases as adipocytes expand, and consequently, total-body lipolysis correlates with body fat mass.[12,29] It might be argued that these measurements were made solely in subcutaneous fat and that the contribution from the visceral depot, which is metabolically more active, may have been overlooked.[30] However, visceral fat mass correlates with total body fat,[31] and furthermore, the subcutaneous fat depot contributes 80% to 90% of the total-body fat mass, and the lipolytic activity of visceral fat is not more than 10 times that of subcutaneous fat.[30] Interestingly, recent investigations in vivo suggest that an increasing visceral fat mass may even result in reduced portal FFA levels.[32] It may thus be concluded from the present data that the SCI subjects had an increased body fat mass and the lipolytic activity per gram of tissue was normal at rest, and therefore, total-body release of glycerol would have been increased. The data also confirm the previous notion that adrenergic activity seems not to significantly influence lipolytic activity after fasting overnight.[19]

Effects of Stress

Mental stress and isometric exercise resulted in a clear activation of the sympathetic nervous system in both subject groups, as judged by the simultaneous increase in heart rate and systemic blood pressure. However, as a marker of regionally disrupted sympathetic activity in SCI subjects, catecholamine levels in plasma did not increase after stress stimulations. When the blood flow rate and lipolytic activity were measured, the expected[33] increase in ATBF was recorded in both regions in control subjects and in the clavicular region in SCI subjects during mental stress, whereas increased glycerol release was detected only in the umbilical region in control subjects. A regional difference in lipolysis was present also in the controls, with increased interstitial glycerol levels in the umbilical region compared with the clavicular region following mental stress, confirming the previous notion that abdominal subcutaneous adipocytes are lipolytically more responsive to catecholamine stimulation than peripheral fat cells.[34]

However, the most striking finding after stress stimulation was the small difference in interstitial glycerol concentration and glycerol release between groups.

A weak tendency towards subnormal glycerol release in abdominal tissue during the stimulations was recorded also in SCI subjects, but this never reached statistical significance. Glycerol release was not stimulated in the abdominal region in these subjects, since the interstitial glycerol concentration was unchanged following stress stimulation, and moreover, plasma glycerol and FFA concentrations were not altered. It may be argued that small changes in lipolytic activity may have been masked in this study, since activation of lipolysis may occur rapidly and thus cannot be monitored by a 15-minute sampling microdialysis system. In a previous study, 5-minute samples were collected, but no registration of ATBF was performed.[35] The ^{133}Xe-clearance measurements presently used require a longer registration time,[25,33] and hence, glycerol output was calculated as the mean change occurring during a 15-minute period. Previous measurements under non–steady-state conditions have convincingly demonstrated that the technique presently used enables quantifying of lipolysis despite the duration of the current sampling time.[12,36]

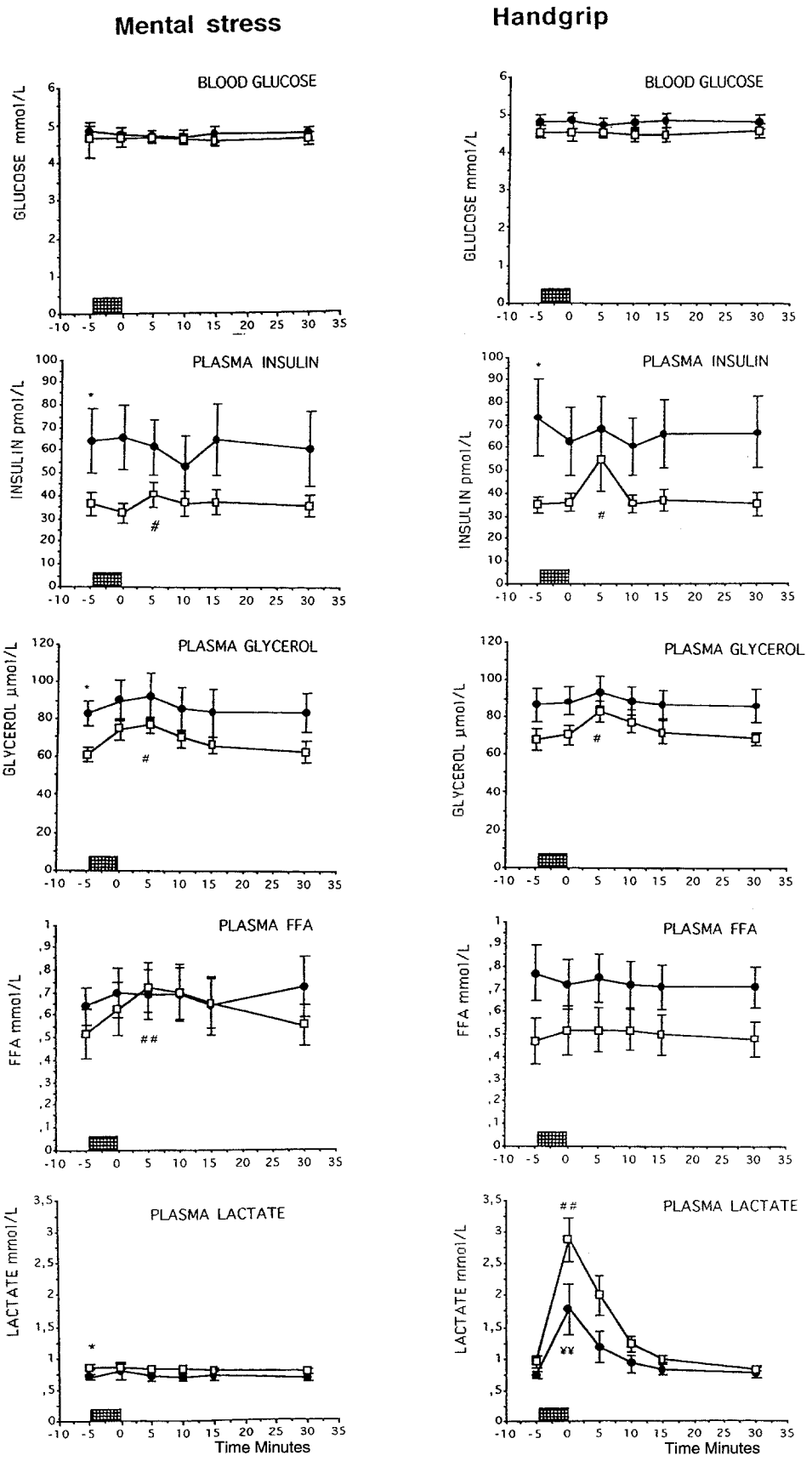

Fig 2. Biochemical profiles among SCI subjects (●) and control subjects (□) during baseline (−5 min), directly following mental stress and handgrip (0 min), and 5, 10, 15, and 30 minutes later. (▨) Stimulation period. All values are the mean ± SEM. *$P < .05$, **$P < .01$: significant difference between groups. ¥$P < .05$, ¥¥$P < .01$: significant difference for rest *v* stimulation in SCI group. #$P < .05$, ##$P < .01$: significant difference for rest *v* stimulation in control group.

Table 3. ATBF and Interstitial and Plasma Glycerol Concentration Following Handgrip Exercise

Parameter	SCI Subjects		Control	
	Rest	HG	Rest	HG
Clavicular region				
ATBF (mL/min/100 g)	4.65 ± 1.3	3.78 ± 1.0	6.64 ± 1.0	9.77 ± 2.3
Interstitial glycerol (μmol/L)	128 ± 30	135 ± 30	111 ± 10†	120 ± 14†
Calculated glycerol release (nmol/min/100 g)	101 ± 52	129 ± 75	125 ± 38	158 ± 55
Umbilical region				
ATBF (mL/min/100 g)	3.79 ± 1.0	6.84 ± 0.6*	4.02 ± 0.9	6.58 ± 1.5*
Interstitial glycerol (μmol/L)	118 ± 12	120 ± 12†	112 ± 14†	121 ± 12†
Calculated glycerol release (nmol/min/100 g)	79 ± 26	107 ± 39	94 ± 35	157 ± 49*
Arterialized plasma				
Glycerol (μmol/L)	86 ± 9	88 ± 7	68 ± 7	73 ± 5

NOTE. Values are the mean ± SE. Plasma levels of glycerol during stimulation are the mean of samples at 0, 5, and 10 minutes.
Abbreviation: HG, handgrip exercise.
*$P < .05$, rest *v* HG.
†$P < .05$, interstitial *v* plasma.

Regulation of Lipolysis in SCI Subjects

The present data show that sympathoexcitatory stress induced the expected lipolytical responses, but due to the limited response and the presence of inherent regional differences also in the controls and differences in body fat mass, the impact on total-body glycerol turnover may not have been large. It is notable that plasma insulin levels also increased following stress stimulation in control subjects, and that the glycerol output might be counteracted by the antilipolytic effect of insulin. Another reason for the lack of difference between SCI subjects and controls may be that sympathetic nervous activation can be exerted by peripheral stimulation below the lesion level in SCI subjects, especially by manipulating the tension of the urinary bladder wall,[37] and this may also stimulate lipolysis. Interestingly, peripheral reflex activation below the lesion in SCI subjects by tapping over the urinary bladder may stimulate lipolysis to the same extent as in normal tissue (unpublished observation in our laboratory, September 1993 to May 1994).

The present data enable us to speculate on the possible mechanisms underlying the increased fat tissue mass in SCI subjects. It may be hypothesized that a tentative lack of β-adrenergic stimulation leads to accumulation of fat depots, but we doubt that this is the only possible explanation for the increased fat cell mass in SCI subjects, since (1) fat cell size was demonstrated to be unchanged in denervated tissue,[19] (2) no regional difference in lipolytic activity was recorded in the present study, and (3) regional distribution of fat tissue was not different between the subject groups. Furthermore, basal lipolytic activity, which is probably the most important determinant of body glycerol and FFA turnover, was normal despite the decentralization of the sympathetic nervous system[19] (and the present study). Instead, additive mechanisms may include those operating on FFA uptake and/or reesterification. In this context, further investigation is warranted to study the tentative importance of underlying regulatory factors such as an increase of the preadipocyte mass due to sympathetic denervation[38] or altered energy expenditure.[39]

In summary, in SCI subjects with an increased body fat mass, basal lipolysis per gram of fat was normal but decentralized adipose tissue depots failed to activate lipolysis following central sympathetic nervous stimulation. The small difference in overall lipolytic activity between SCI and control subjects both in the resting state and during stimulation indicates that altered lipolysis may not be the sole mechanism behind the increased fat mass in SCI subjects.

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