

The Subcutaneous Lipolytic Response to Regional Neural Stimulation Is Reduced in Obese Women

Christoph Dodt, Peter Lönnroth, Horst Lorenz Fehm, and Mikael Elam

Disturbed fat tissue metabolism with a reduction of the lipolytic rate could be an important pathogenetic factor in obesity. Lipolysis of the subcutaneous tissue of the thigh is partly under neural control and can be increased by intraneural stimulation of the lateral cutaneous femoral nerve in lean women. In the present study, we tested whether the lipolytic response to intraneural stimulation is altered in vivo in obese subjects. Seven obese women were examined and the results were compared with those of seven age-matched lean women. After an overnight fast, the lateral cutaneous femoral nerve was intraneurally stimulated for 10 min, and the local subcutaneous lipolytic response to this procedure was evaluated with microdialytic measurements of interstitial glycerol concentrations in the receptive field of the stimulated nerve fascicle. To exclude unspecific effects of stimulation, lipolysis was also controlled in a corresponding area of the contralateral leg. Intraneural stimulation produced no significant change in subcutaneous lipolysis in obese women ($25.7 \pm 9.7\%$, NS). This finding is in sharp contrast with the marked regional lipolytic response in lean women in which the same stimulation procedure enhanced the regional interstitial glycerol levels by $72 \pm 17\%$ ($P < 0.05$) compared with the unstimulated corresponding area of the contralateral leg. These in vivo results suggest that human obesity is characterized by a profound unresponsiveness of the subcutaneous adipose tissue to neurally stimulated lipolysis. This could be an important factor in the development and treatment of obesity. *Diabetes* 49:1875–1879, 2000

The pathogenesis of obesity has been suggested to be intimately linked to the catecholaminergic regulation of lipolysis and the function of the sympathetic nervous system (1–3). Norepinephrine and epinephrine activate lipolysis via β_1 , β_2 , and β_3 -adrenoceptors and inhibit it via α_2 -adrenoceptors, and these neurotransmitters are the most important lipolytic substances in vivo (4). Defects of catecholamine-induced lipolysis have been observed in a number of obese subjects, and polymor-

phisms of the β_2 (5) and β_3 -receptors (6) were suggested as explanations for this observation.

The importance of sympathetic innervation of fat tissue is underlined by the observation that the recently described weight-regulating hormones, leptin (7), and the proopiomelanocortin-derivate α -MSH (8) not only reduce appetite but also induce sympathoexcitation via the central nervous system. The relevance of such effects is obvious for the metabolically highly active brown adipose tissue in rats, which is densely innervated by sympathetic nerves directly controlled by autonomic brain stem centers (9–11). By comparison, the innervation of white adipose tissue (WAT) is sparse (12), and its role in regulating fat tissue metabolism is ill defined in humans (9,13). Recent studies from our laboratory on subjects with spinal cord injuries suggested that the neural control of basal WAT lipolysis appears to be insignificant (13). However, neurally mediated sympathoexcitation seems to be an important mechanism to increase WAT lipolysis during maneuvers known to induce lipolysis (13–16). Consequently, changes in sympathetic control of WAT could play a role in the overall energy balance and the size of the body fat mass.

Several investigators have demonstrated a disturbed sympathetic function in human obesity in studies of heart rate variability (17), plasma catecholamine concentrations (18), pupillary latency period (2), and sympathetic nerve activity to the muscle vascular bed (1,19–21).

It is unclear whether these findings relate to the pathogenesis of obesity or whether they are primary or secondary consequences of obesity. For example, obstructive sleep apnea commonly observed in obese subjects may substantially contribute to the activation of muscle sympathetic nerve activity (22). Furthermore, all studies on sympathetic activity in obese humans examined branches of the sympathetic nervous system, which did not directly supply fat tissue and therefore could not address the question of whether or not the direct neuronal effects on fat tissue metabolism are altered in obese humans.

We recently developed an in vivo model for studies of neural control of human WAT using intraneural electrical stimulation of the lateral cutaneous femoral nerve and evaluation of local lipolytic activity within the defined innervation territory of the stimulated nerve fascicle through measurements of interstitial glycerol concentrations via subcutaneous microdialysis. The results showed a significant neurally mediated regional lipolysis in lean female subjects (16). In the present study, we used this in vivo model to compare the lipolytic response of the WAT of obese subjects with the recently observed lipolytic response of lean subjects. It was hypothesized that the neural control of subcutaneous WAT is altered in obese subjects.

From the Department of Internal Medicine I (C.D., H.L.F.), Medical University of Lübeck, Lübeck, Germany; the Department of Internal Medicine (P.L.), the Lundberg Laboratory for Diabetes Research; and the Department of Clinical Neurophysiology (M.E.), University of Göteborg, Göteborg, Sweden.

Address correspondence and reprint requests to Christoph Dodt, MD, Department of Internal Medicine, Medical University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany. E-mail: dodt@medinf.mu-luebeck.de.

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ANOVA, analysis of variance; WAT, white adipose tissue.

TABLE 1
Anthropometric data of the subjects

	Lean subjects	Overweight subjects	<i>P</i>
<i>n</i>	7	7	
Age (years)	28.3 ± 2.5	33.6 ± 1.7	NS
Body weight (kg)	63.0 ± 1.9	83.3 ± 4.2	<0.005
BMI (kg/m ²)	21.9 ± 0.5	30.6 ± 1.3	<0.001
Waist-to-hip ratio	0.84 ± 0.01	0.89 ± 0.02	<0.05
Plasma insulin (μU/mL)	3.9 ± 1.0	5.3 ± 1.1	NS

Data are means ± SEM, unless otherwise indicated.

METHODS

Subjects. Seven obese women, age-matched to seven previously investigated lean women (16), participated in the study (Table 1). All subjects were healthy and without medication, including hormonal contraception. They had been weight stable for at least three months before the study. Participants were fasted overnight and were asked not to smoke the day before the experiment, as well as to abstain from alcohol three days before the experiments. The study was approved by the ethical committee of the University of Göteborg, and all participants gave their written informed consent.

Experimental protocol. Experiments started at 8 A.M. and were conducted in the laboratory of the Department of Clinical Neurophysiology. Room temperature was kept at 24 ± 2°C. Subjects were investigated while in a supine position. An intravenous cannula was inserted into a dorsal hand vein for blood sampling, and the hand was warmed with heating pads wrapped in cotton blankets to increase blood flow and induce arterIALIZATION of the venous blood. By this approach, a 93 ± 1% oxygen saturation was achieved in sampled blood.

The lateral cutaneous femoral nerve was located at the outer third of the inguinal fossa. For determining its route, the area was stimulated transcutaneously using repetitive electric stimuli (40–70 V, 1 Hz, Grass S 48 Stimulator; Grass Electronics, Quincy, MA). Subjects were asked to report on the occurrence, intensity, and location of the paresthesia induced by the stimuli, and the nerve route was marked when maximal intensity was reported. Subsequently, a tungsten microelectrode with a shaft diameter of 0.2 mm and an uninsulated tip of a few micrometers was inserted towards the nerve, and a reference electrode with a larger uninsulated tip was placed a few centimeters from the stimulating electrode. The search for the nerve was guided by electric stimuli (3–7 V, 1 Hz) through the microelectrode. When subjects reported paresthesia in the innervation territory of the nerve at low stimulation intensities, stimulation was switched off and nerve traffic was recorded (amplified × 50,000, filter bandwidth 0.7–2 kHz, window discriminator for noise reduction). While the skin was mechanically stimulated by stroking to evoke mechanoreceptive afferent impulses, the electrode was slightly adjusted to impale a nerve fascicle that supplied a receptive field with a size of at least 10 × 10 cm.

When an electrode position representing a suitable innervation territory had been achieved, short ramps (<30 sec) of intraneural stimulation with increasing intensity were used to determine an optimal level of stimulus intensity, judged by the subjects to be bearable for a 10-min stimulation period. Subsequently, two microdialysis catheters were placed, at least 2 cm apart, in the subcutaneous tissue within the receptive field of the impaled nerve fascicle and one catheter was placed in the corresponding area of the contralateral leg to control for unspecific stress effects of the stimulation procedure.

After a microdialysis equilibration period of 45 min, a baseline period of 30 min was started and microdialysate samples were collected every 10 min, followed by an intraneural stimulation period of 10-min duration (2 Hz). During stimulation and a subsequent 30-min period, glycerol dialysates were sampled every 5 min. Blood for determination of plasma insulin was drawn before the stimulation period. Plasma glycerol was measured before and after the stimulation period.

Microdialysis was performed with subcutaneous catheters (30 × 0.3 mm, cuprophane, 3,000 MV cutoff), perfused with isotonic saline with 2.5 mmol/l glucose at a rate of 2.5 μl/min using a microinjection pump (Carnegie Medicine, Stockholm, Sweden). Relative recovery of glycerol was assessed with the internal reference technique previously described (23). [¹⁴C] glycerol (~3000 cpm/μl, Amersham, Arlington Heights, IL) was added to the perfusate. Relative recovery was calculated as dialysate concentration/interstitial concentration of the analyzed substance. The recovery of the probes within the innervation territory of the nerve and the control area of the contralateral leg did not differ significantly. Dialysate glucose was measured simultaneously to

ensure a constant recovery, and we once again found no significant change in recovery of the probes during the course of the experiment (data not shown). **Laboratory analysis.** Glycerol in dialysates and plasma were analyzed in a CMA 600 microdialysis analyzer (Carnegie Medicine, enzymatical method, CV 3%). To determine the radioactivity of the marked substances, samples were put in a liquid scintillation analyzer (1900CA TRI-CARB, Packard, Downers Grove, IL). Plasma insulin was measured with an enzyme-linked immunosorbent assay (Dakopatts, Älvsjö, Sweden) with an intra-assay coefficient of variation <7.5% and an interassay coefficient of variation <9.5%. For statistical analysis, the three baseline values for interstitial glycerol release were collapsed and their mean compared with the single values during and after stimulation. The glycerol values of the two microdialysis probes in the stimulated area were averaged. Effects of stimulation were assessed by analysis of variance (ANOVA) with the repeated measures factor time and the factors stimulation (stimulated versus unstimulated) and weight (lean versus obese). Statistical differences between the baseline period and the single values during and after the stimulation period were analyzed with the Duncan's post hoc test. The null hypothesis was rejected at *P* < 0.05. All results are presented as means ± SEM.

RESULTS

The intensity of the intraneural electrical stimulation did not differ significantly between our present obese study group (6.2 ± 1.9 mV) and our previous lean study group (4.7 ± 0.7 mV). Innervation territories of the impaled nerve fascicles were of similar size in both groups (obese 269.3 ± 34.1 cm² and lean 257.9 ± 34.2 cm²). As in lean subjects, sympathetic nerve activity was recorded from the impaled nerve fascicle in approximately half the subjects (in three of seven obese and four of seven lean subjects) and the results of intraneural stimulation were not affected by whether it was recorded or not. When present, spontaneous nerve activity demonstrated the typical characteristics of cutaneous sympathetic nerve fibers and was activated by talking, arousal stimuli, and mental stress (Fig. 1) (16).

Effects of electrical stimulation. Unilateral intraneural stimulation of the lateral cutaneous femoral nerve did not induce any significant change in subcutaneous lipolysis within the stimulated nerve territory in our obese subjects. In fact, no difference in lipolysis between the stimulated and contralateral unstimulated microdialysis region was observed

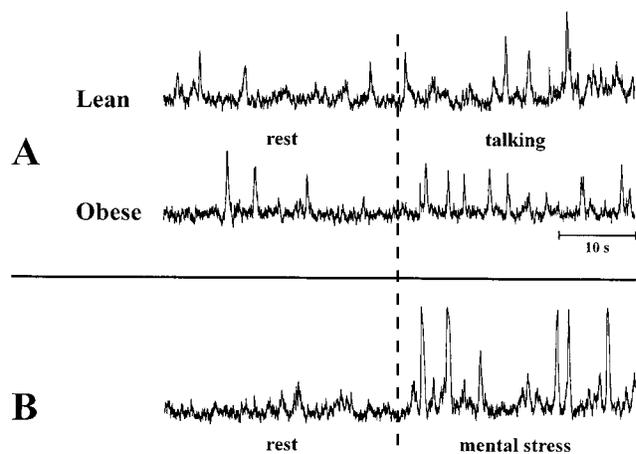


FIG. 1. Illustration of characteristics of sympathetic activity from the lateral cutaneous femoral nerve in a lean and an obese subject under conditions of complete relaxation and during a period of conversation (panel A). Both subjects had an equivalent sympathetic activity during rest and a similar activation during a period of relaxed conversation. An even stronger activation can be achieved by forced mental arithmetics, as shown in a lean subject in panel B of the figure.

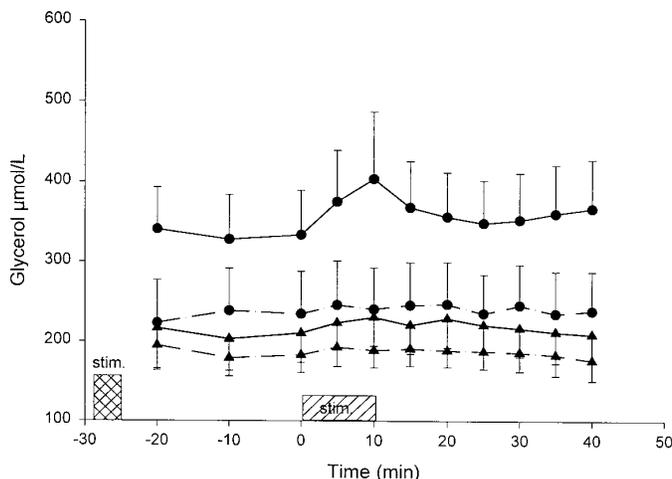


FIG. 2. Interstitial glycerol levels in the innervation territory of the lateral cutaneous femoral nerve in seven lean (●) and seven obese (▲) subjects (means \pm SE). — Represents the levels of the innervation area of the stimulated lateral cutaneous femoral nerve, whereas --- represents the levels of the unstimulated corresponding area of the contralateral leg. Before the insertion of the microdialysis probes, the nerve was localized with transcutaneous and intraneural stimulation (▨). After an equilibration period of 45 min and a baseline period of 30 min, the lateral cutaneous femoral nerve was again stimulated for 10 min (▨). Stimulation significantly enhanced glycerol release in lean subjects, whereas lipolysis was not significantly affected in the obese group (weight \times time interaction, $P < 0.05$). Stim, stimulation.

at any stage of the experiment. As illustrated in Fig. 2, this is in sharp contrast to findings in lean women of similar age in which the initial nerve searching procedure produced a significant elevation of glycerol release from the stimulated region as compared with the contralateral control region ($47 \pm 13\%$ higher before 10 min stimulation, $P < 0.05$). The elevation was further augmented ($P < 0.05$) by a 10-min intraneural stimulation ($72 \pm 17\%$ higher than unstimulated control region) (16). The lack of such a lipolytic response in our obese subjects resulted in a significant weight \times time interaction ($P < 0.05$) in an ANOVA, including both obese and lean subjects. No difference between the stimulated area and the unstimulated area of the contralateral leg or between the baseline period and the 10-min stimulation period in the stimulated area was found in our obese subjects.

There was no significant difference between the insulin levels of the lean and obese subjects ($P < 0.36$) (Table 1). Basal plasma glycerol levels directly before the stimulation were not significantly different between the groups (lean 47.1 ± 6.0 vs. obese 45.5 ± 6.4 $\mu\text{mol/l}$). The 10-min stimulation period increased the plasma glycerol levels significantly ($P < 0.05$) in both groups (lean 51.9 ± 8.9 and obese 56.8 ± 4.7 $\mu\text{mol/l}$), and this effect was not dependent on body weight.

DISCUSSION

The basis for the present study on obese young women was our recent development of a model for studies of neural control of regional lipolysis. With the model, we demonstrated that intraneural electrical stimulation of the lateral cutaneous femoral nerve induced a significant and long-standing increase in local glycerol release within the stimulated region in lean young women (16). The present study tested the hypothesis that neural control of WAT lipolysis may be

altered in obesity, and our findings demonstrate that the regional lipolytic response to intraneural stimulation is virtually abolished in obese young women.

The regional glycerol increase in lean subjects is likely the result of the lipolytic effect of norepinephrine released by electrically stimulated sympathetic neurons and bound to β_1 -, β_2 -, and β_3 -adrenoceptors in the innervation territory, which stimulate the fat cell (4,24,25). The lack of lipolytic response in the contralateral unstimulated microdialysis region argues against the assumption that unspecific stress effects, which slightly increased plasma glycerol levels, could explain the profound specific increase in local interstitial glycerol release in the stimulated area in lean subjects. Also, the effect on subcutaneous blood flow is unlikely to explain the present results. The intraneural stimulation procedure did not affect skin perfusion in the innervation territory of the impaled nerve fascicle in our previous study (16), and additionally, several previous experimental studies argue against blood flow changes mediating the regional increase in interstitial glycerol levels (26–28). Consequently, we consider the model to reflect neurally induced lipolysis.

In the obese subjects investigated, intraneural stimulation had no significant effect on subcutaneous lipolysis. This was not caused by a difference in the characteristics or size of the stimulated nerve fascicles and/or territories, because stimulation intensities and pain ratings were similar in obese and lean subjects, as were the size of the receptive fields. Hence, we regard the absence of a significant lipolytic response to intraneural stimulation as an indication of disturbed neural control of subcutaneous fat tissue in our obese subjects.

The present study does not give a clear explanation for the blunted lipolytic response found in the obese subjects. However, one explanation for the lipolytic resistance to neural stimulation could be a dysfunction of β -adrenoceptors on lipocytes and/or the hormone sensitive lipase (29). Using isolated adipocytes, several studies have reported a decreased β_2 -adrenoceptor expression (30), polymorphisms of β_2 - and β_3 -adrenoceptors (5,6), and reduced sensitivity of β -adrenoceptors on fat cells in different patient groups with obesity (31,32). However, because of the presence of sparse receptors, such receptor defects should result in reduced β -adrenergic sensitivity without alterations of the lipolytic capacity (33,34). Hence, our results in obese subjects could most likely be attributed to a postreceptor defect. Also, in vivo lipolysis is influenced by endocrine, paracrine, and autocrine factors, which modify the lipolytic response to catecholamines (35,36) underlining the importance of in vivo studies. The idea that receptor and postreceptor defects on isolated fat cells could be relevant for the pathogenesis of obesity was suggested in a recent in vivo study on obese children using epinephrine infusion (37). The present study using in situ stimulation of a cutaneous nerve and measurement of glycerol release within its innervation territory may support the concept of a reduction of adrenergically mediated lipolysis in obesity. However, other neurally released substances besides catecholamines could also be involved in the regulation of lipolysis in obesity. For example, neuropeptide Y, which inhibits lipolysis (38), may modulate the catecholamine effect. Whether changes in the release of this or other neuromodulators could be relevant for the lipolytic resistance in obesity remains to be elucidated.

Obesity is characterized by an increase in number and size of fat cells (39,40). One could imagine that a disproportional increase in fat cell mass in relation to efferent sympathetic nerve fibers and/or blood vessels may serve as an explanation for the present results. However, to the best of our knowledge, it is not known whether the efferent sympathetic fibers proliferate during the development of obesity or remain constant in size, thus leading to relatively less innervated adipose tissue in obesity. Decreased blood flow has been described in obese subjects in the abdominal subcutaneous fat but not for the femoral region in obese men (41). However, reduced blood flow would have induced an increase in interstitial glycerol levels and thus does not explain the present results.

Interestingly, no weight-related difference in interstitial glycerol levels was observed in the unstimulated fat tissue of the contralateral femoral control region, indicating that the reduced lipolytic responsiveness of obese subjects is unmasked by neural activation. This observation confirms recent results from subjects with decentralized sympathetic innervation due to spinal cord injury. In this patient group, the basal lipolytic rate in the femoral region was not altered, whereas centrally elicited sympathoexcitatory maneuvers failed to induce the normal increase in subcutaneous lipolysis in the decentralized region (13).

It's tempting to speculate on the putative importance of a reduced lipolytic response to sympathetic nervous stimulation in obesity. Provided that sympathetic activity and the fat cells' response to this activity is regulating fat cell size, the reduced lipolytic response demonstrated in the present study may be of pathogenic importance for the development of obesity and may impede weight reduction. Our data agree well with the observation of reduced energy expenditure in subjects with the predisposition to weight gain, which is suggested to be relevant for the increase in body weight (1,42). However, before arguing strongly in favor of impaired neural control of lipolysis being a pathogenic factor in obesity, similar direct measurements should be performed in prospective studies on pre- and postobese subjects. Also, it should be taken into consideration that the antilipolytic effect of insulin as well as the lipoprotein lipase activity may be equally as important as the adrenergic system for the regulation of fat cell size. Therefore, the impact of blunted responses to neural activation should be related to the importance of the reduced antilipolytic effect of insulin (41) and the altered lipoprotein lipase activity prevailing in obesity (43).

In summary, this *in vivo* study on neurally induced subcutaneous lipolysis in humans demonstrates that moderately obese women show a markedly reduced local lipolytic response to intraneural stimulation. Future prospective studies of the lipolytic response in subjects prone to develop obesity are warranted to evaluate the pathogenetic relevance of the present observation.

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