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Tyramine in the assessment of regional adrenergic function

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ABSTRACT

Regional adrenergic function is difficult to assess in humans. Tyramine given through a microdialysis probe may be a useful tool in this regard. However, tyramine data is hard to interpret given the drug's complex mode of action. We characterized the response to tyramine, isoproterenol, and dopamine in adipose tissue with microdialysis probes in normal subjects. We measured glycerol concentrations to follow changes in lipolysis and monitored tissue perfusion with ethanol dilution. During perfusion with tyramine, dialysate glycerol concentration increased dose-dependently from 83 ± 8 μM at baseline to 181 ± 18 μM at 3.5 mM tyramine ($p < 0.001$) followed by a fall down to 121 ± 9 μM at 35 mM tyramine ($p < 0.001$). Propranolol almost completely blocked this response. A similar lipolytic response was not observed in isolated human adipocytes. Dopamine <35 μM did not replicate the tyramine-induced lipolysis; however, dopamine >35 μM potently inhibited lipolysis. We conclude that tyramine-induced lipolysis is explained by a pre-synaptic mechanism. Tyramine applied through a microdialysis probe in concentrations up to 3.5 mM can be used to assess pre- and post-synaptic mechanisms regulating lipid mobilization.

1. Introduction

In conditions, such as the postural tachycardia syndrome and diabetic neuropathy, sympathetic nervous system dysfunction is not evenly distributed throughout the body [1]. However, regional sympathetic adrenergic function is difficult to assess. Tyramine applied through a microdialysis probe could be useful in this regard. Tyramine is taken up through the norepinephrine transporter and induces non-exocytotic norepinephrine release [2]. Impaired norepinephrine uptake or adrenergic degeneration causes tyramine resistance [3]. Tyramine applied via microdialysis elicits local norepinephrine release [4]. The quantity of local tyramine-induced norepinephrine release could be used as a marker for peripheral sympathetic function. The low in vivo recovery of norepinephrine and the small sample volume restrict the use of the method to very few research facilities. Instead, one could monitor the physiological response to the released norepinephrine. Norepinephrine augments adipose tissue lipolysis through post-synaptic β-adrenoreceptor stimulation [5]. Changes in lipolysis can be detected by measuring microdialysate glycerol concentrations [5]. We tested the response to indirect adrenoreceptor stimulation with tyramine and direct β-adrenoreceptor stimulation with isoproterenol on tissue lipolysis in healthy subjects. Tyramine preparations are contaminated with dopamine. Therefore, we also tested the response to dopamine [6]. Finally, we excluded a direct lipolytic effect of tyramine.

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2. Methods

2.1. Subjects and protocol

We studied 26 healthy, young, non-smokers (14 men, 12 women, 26 ± 0.5 years, BMI 22.2 ± 0.3 kg/m^2). All studies were approved by the institutional review board. We obtained written informed consent before study entry.

We conducted four separate experiments. In the first study, we applied tyramine (14 men, 12 women) and the β-adrenoreceptor agonist isoproterenol (eight women, eight men) and measured tissue perfusion and metabolism in subcutaneous adipose tissue. In the second study, we characterized the effect of (1) local β-adrenoreceptor blockade on responses to tyramine (three women, two men) and isoproterenol (two women, three men) and (2) local α-adrenoreceptor blockade on responses to tyramine (seven women, seven men) in adipose tissue. In the third study, we tested dopamine in adipose tissue (four women, four men). In the fourth study, we characterized finally the effect of tyramine (seven women) and dopamine (five women) on isolated adipocytes.

2.2. Microdialysis

After an overnight fast, microdialysis probes were inserted into abdominal subcutaneous adipose tissue at the level of the umbilicus [7–9]. Probes were perfused at a flow rate of 2 μl/min, with lactate-free Ringer's solution (E156, Serumwerk Bernburg AG, Bernburg, Germany) supplemented with 50 mM ethanol (Alkohol-Konzentrat 95%, B. Braun Melsungen AG, Melsungen, Germany). Both microdialysis probes (CMA/60) and pumps (CMA/102) were from Microdialysis AB, Solna, Sweden. After 60 min tissue recovery and baseline calibration, we collected two 15-min dialysate fractions for baseline measurements. Then, we perfused probes with incremental concentrations of either tyramine (Merck Biosciences AG, Laeufelfingen, Germany; 0.35, 3.5, and 35 mM), isoproterenol (ISOPREL®, Abbot, Ottignies, France; 0.01, 0.1, and 1 μM), or dopamine (DOPAMINE SOLVAY®, Solvay Arzneimittel GmbH, Hannover, Germany; 3.5, 35, and 350 μM). Dopamine concentrations corresponded to the contamination expected in the tyramine preparation [6]. In the second study (adrenoreceptor blocker experiment), one of the two probes was perfused without, the other one with the unspecific β-adrenoreceptor blocker propranolol (OBSIDAN®, Alpharma-Isis GmbH & Co. KG, Langenfeld, Germany; 10 μM) or with the unspecific α-adrenoreceptor blocker phentolamine (REGITIN®, Novartis Pharma Schweiz AG, Bern, Switzerland; 100 nM). The blockers were added to the perfusate of 60 min before starting the perfusion of both probes with incremental doses of either tyramine or isoproterenol. Three 15-min dialysate fractions were collected during each perfusion step.

2.3. Measurement of skin-fold thickness

We used Lange's skin-fold caliper (Beta Technology Incorporated, Cambridge, MD, USA) to determine skin-fold thickness in a subgroup of our subjects [9]. We obtained our measurements in the middle of the line between the umbilicus and the anterior superior iliac crest.

2.4. Isolation and incubation of adipocytes

We isolated human adipocytes from subcutaneous adipose tissue obtained during breast reduction surgery healthy women (20–50 years, BMI 22–35 kg/m^2) by collagenase digestion as described previously [10]. Briefly, adipocytes (100 μl of packed volume) were incubated in vials containing 400 μl Krebs Ringer Hepes buffer (25 mM HEPES, pH 7.4; 120 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$) supplemented with BSA (1%, w/v), glucose (5 mM), and ascorbate (5 μM). Each experiment was conducted in triplicate or quadruplicate at 37 °C in a shaking incubator (50 rpm) for 2 h in the absence (control) and presence of isoproterenol (1 μM), tyramine (0.035, 0.35, 3.5, 35 and 350 mM), or dopamine (3.5, 35, 350 and 3500 μM). We terminated incubations by putting the vials on ice and measured glycerol concentration in medium with a CMA/600 analyzer. We expressed lipolytic activity as micromolar of glycerol released per 100 μl of cells per 2 h.

2.5. Analytical methods and calculations

We measured dialysate glycerol concentrations using the CMA/600 analyzer (CMA Microdialysis AB, Solna, Sweden). We determined ethanol concentrations in the perfusate (inflow) and dialysate (outflow) using a standard enzymatic assay [11]. Blood flow changes were estimated using the ethanol dilution technique. The technique is based on Fick's principle [12]. A decrease in the ratio between ethanol in the dialysate and perfusate ($[ethanol]_{dialysate}/[ethanol]_{perfusate}$) corresponds to an increase in blood flow and *vice versa*.

2.6. Data analysis

All data are expressed as mean ± S.E.M. Intraindividual differences were compared by one-way ANOVA for repeated measurements followed by Dunnett's multiple comparison test. Two-way ANOVA for repeated measurements was used for multiple comparisons (control versus β-adrenoreceptor blockade) followed by Bonferroni post-test. A value for $p < 0.05$ was considered statistically significant. Differences between groups (lower versus higher skin-fold thickness) were compared by unpaired *t*-test.

3. Results

3.1. Adipose tissue responses

Perfusion with lower tyramine concentrations (0.35–3.5 mM) had no effect on ethanol ratio (Fig. 1A). The highest tyramine concentration (35 mM) decreased ethanol ratio to a minimum of 0.34 ± 0.04 ($p < 0.001$). Basal glycerol concentration was 83 ± 8 μM (Fig. 1B). Perfusion with tyramine up to 3.5 mM elicited increased dialysate glycerol to 181 ± 18 μM ($p < 0.001$) followed by a decrease to 121 ± 9 μM at 35 mM tyramine ($p < 0.001$ versus baseline).

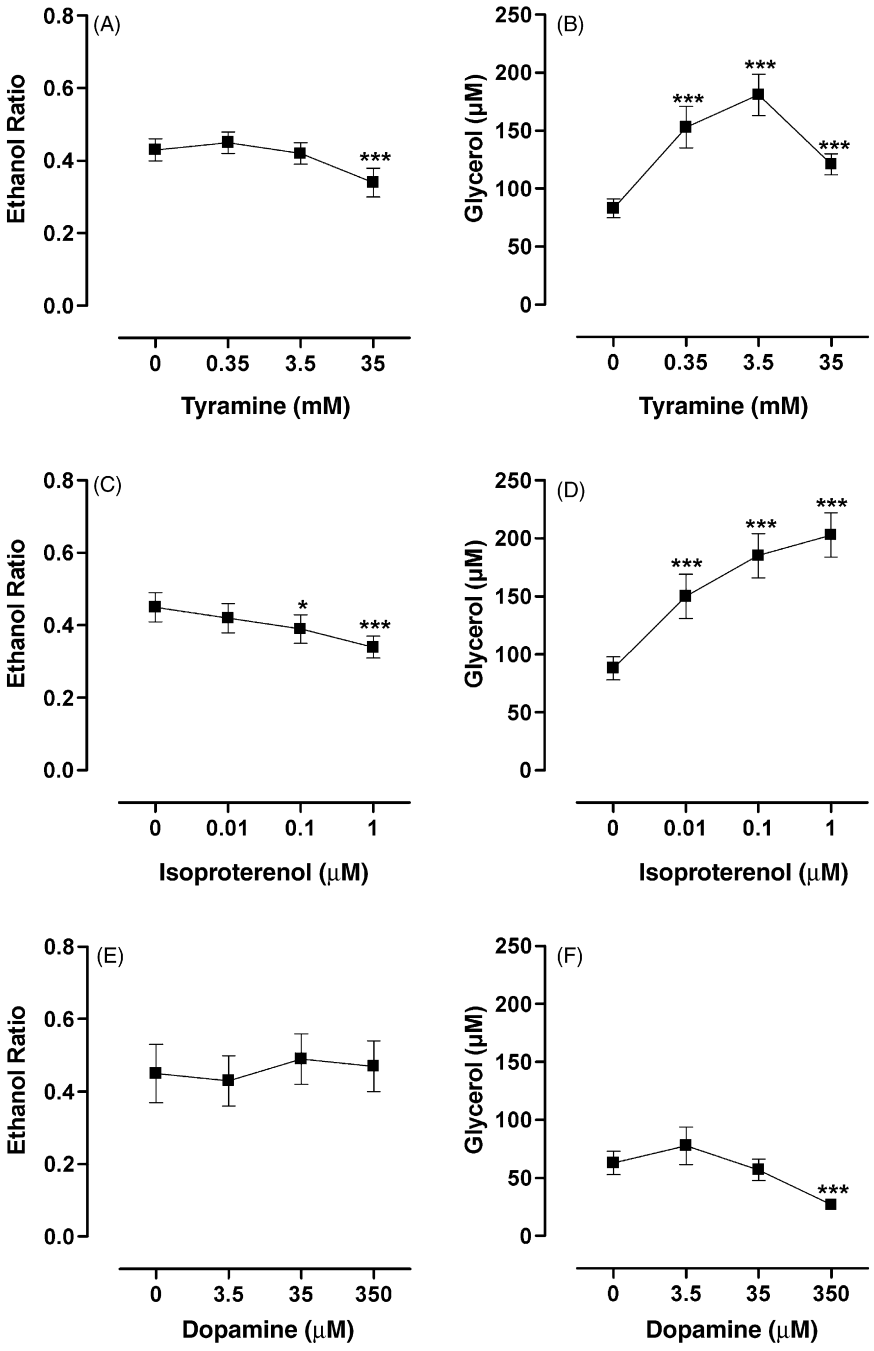

Fig. 1 – Changes in ethanol ratio and dialysate glycerol concentrations in abdominal subcutaneous adipose tissue of normal weight women and men during local perfusion with increasing doses of tyramine (A and B; $n = 26$), isoproterenol (C and D; $n = 16$), and dopamine (E and F; $n = 8$). Data are given as means ± S.E.M., $^{***}p < 0.001$, $^{*}p < 0.05$; baseline vs. drugs, one-way ANOVA and Dunnett's multiple comparison test.

With isoproterenol, ethanol ratio decreased moderately ($p < 0.001$, Fig. 1C). Dialysate glycerol concentration increased from 88 ± 10 μM at baseline to 203 ± 19 μM at 1 μM isoproterenol ($p < 0.001$, Fig. 1D). Propranolol had no effect on basal dialysate glycerol concentration but attenuated the lipolytic response to tyramine and to isoproterenol (Fig. 2A and B). Glycerol concentrations during tyramine administration were similar in the presence and in the absence of phentolamine (data not shown).

Baseline ethanol ratio was 0.31 ± 0.04 in men and 0.42 ± 0.06 in women (n.s.). The change in the ethanol ratio with either tyramine or isoproterenol was similar in men and in women. Baseline glycerol dialysate concentration was 83 ± 16 μM in men and 94 ± 13 μM in women (n.s.). During stimulation with 1 μM isoproterenol, dialysate glycerol was 163 ± 27 μM in men and 242 ± 15 μM in women ($p < 0.05$). During perfusion with 3.5 mM tyramine, dialysate glycerol increased to 149 ± 34 μM in men and 226 ± 29 μM in women

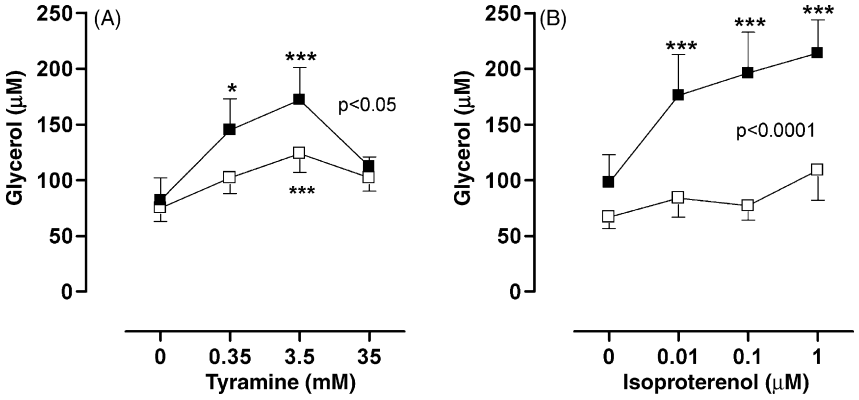

Fig. 2 – Changes dialysate glycerol concentrations during perfusion with increasing concentrations of tyramine (A) and isoproterenol (B) in the absence (■) and presence (□) of 10 μM propranolol, respectively, in abdominal subcutaneous adipose tissue of normal weight subjects. Data are given as means ± S.E.M., $n = 5$; $^{***}p < 0.001$, $^{*}p < 0.05$; baseline vs. tyramine and isoproterenol, respectively, one-way ANOVA and Dunnett's multiple comparison test. Absence vs. presence of propranolol, two-way ANOVA and Bonferroni post-tests.

(n.s.). The ratio between tyramine and isoproterenol responses was similar in both groups.

Ethanol ratio did not change with increasing dopamine concentrations (Fig. 1E). Basal dialysate glycerol concentration was 63 ± 10 μM (Fig. 1F). With lower dopamine concentrations (3.5 and 35 μM), dialysate glycerol did not change. The highest dopamine concentration (350 μM) decreased dialysate glycerol to 27 ± 4 μM ($p < 0.001$).

3.2. *Tyramine, dopamine, and isoproterenol in isolated adipocytes*

At first, we characterized the effect of tyramine on adipocytes (Fig. 3A). Baseline glycerol release was 31 ± 6 μM per 100 μl of cells per 2 h. When 1 μM isoproterenol was added to the incubation medium, glycerol release increased to 80 ± 16 μM per 100 μl of cells per 2 h ($p < 0.001$). With tyramine, the highest concentration (350 mM) decreased glycerol release to 15 ± 5 μM per 100 μl of cells per 2 h ($p < 0.05$). No changes were seen with lower tyramine concentrations. Secondly, we characterized the effect of dopamine on adipocytes (Fig. 3B). Baseline glycerol release was 31 ± 4 μM per 100 μl of cells per 2 h. With isoproterenol, glycerol release increased to 69 ± 10 μM per 100 μl of cells per 2 h ($p < 0.001$). With dopamine, the highest concentration only (3500 μM) decreased glycerol release to 14 ± 2 μM per 100 μl of cells per 2 h ($p < 0.001$).

3.3. *Lipolytic response and skin-fold thickness*

Subjects were separated into two groups of equal size according to skin-fold thickness. The average skin-fold thickness was 16 ± 1 mm in the lower and 30 ± 3 mm in the group with higher thickness. Body mass index was similar in both groups. Ethanol ratio with 3.5 mM tyramine and 1.0 isoproterenol perfusion was significantly higher in the thicker (0.46 ± 0.04 and 0.36 ± 0.04) versus the thinner skin-fold group (0.30 ± 0.03 and 0.16 ± 0.03; $p < 0.01$; data not shown). The lipolytic response to 3.5 mM tyramine was substantially

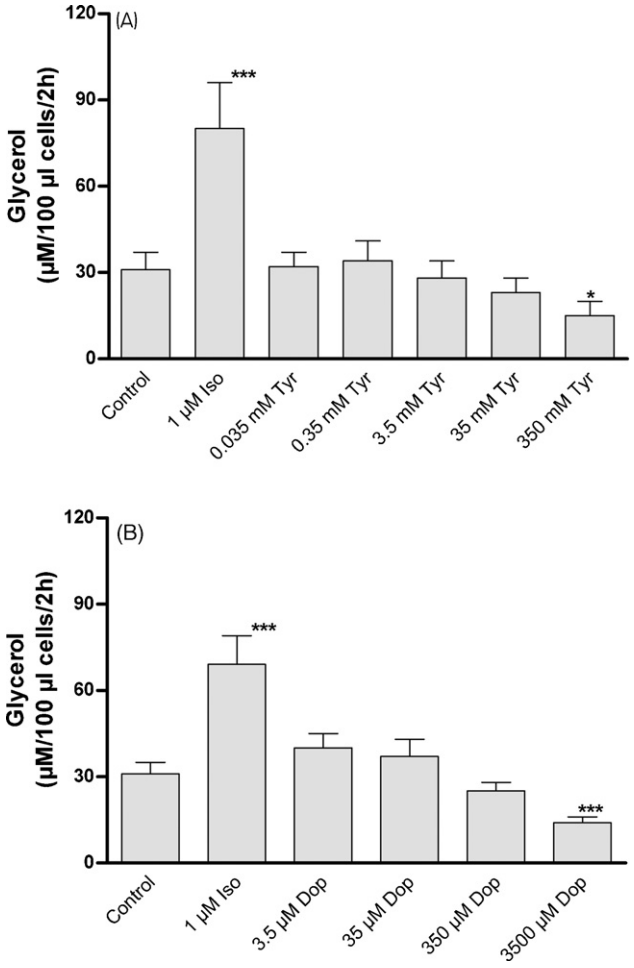

Fig. 3 – Lipolytic response of isolated adipocytes incubated in the absence (control) and presence of either isoproterenol or tyramine (A; $n = 7$) and isoproterenol or dopamine (B; $n = 5$). Data are given as means ± S.E.M.; $^{***}p < 0.001$, $^{*}p < 0.05$; baseline vs. isoproterenol, tyramine, and, dopamine, respectively, one-way ANOVA and Dunnett's multiple comparison test.

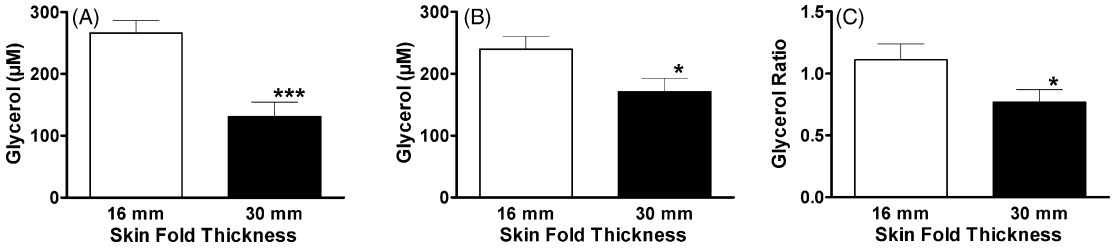

Fig. 4 – Tyramine (3.5 mM) induced lipolysis (A) or isoproterenol (1.0 μM) induced lipolysis (B) and glycerol ratio (tyramine-induced lipolysis/isoproterenol-induced lipolysis, C) in subcutaneous abdominal adipose tissue of subjects with a lower skin-fold thickness (16 mm; $n = 10$) and a higher skin-fold thickness (30 mm; $n = 10$), respectively. Data are given as means ± S.E.M., $^{***}p < 0.001$, $^{*}p < 0.05$; lower vs. higher skin-fold thickness, unpaired *t*-test.

higher in the thinner versus the thicker skin-fold group ($p < 0.001$, Fig. 4A). The lipolytic response to 1 μM isoproterenol was only moderately, but also significantly higher in the thinner versus the thicker skin-fold group ($p < 0.05$, Fig. 4B). Thus, the ratio between the responses to tyramine and isoproterenol was also higher in the thinner versus the thicker skin-fold group ($p < 0.05$, Fig. 4C).

4. Discussion

We showed that tyramine at low doses stimulated adipose tissue lipolysis blood flow-independently. The effect at lower doses resembled the response to direct β-adrenoreceptor stimulation with isoproterenol. Tyramine and isoproterenol-induced lipolysis was eliminated by β-adrenergic blockade. Tyramine did not stimulate lipolysis in isolated adipocytes. Thus, tyramine-induced norepinephrine release influenced metabolism and blood flow in a concentration-dependent fashion. Tyramine at high concentrations decreased lipolysis, as did dopamine. We believe that dopamine contamination, which is unavoidable in tyramine preparations, confounds the results but only at high doses. Our results underscore the utility of tyramine as a pharmacological probe.

In isolated human adipocytes, tyramine did not stimulate in vitro lipolysis. In contrast, isoproterenol elicited a robust lipolytic response. Thus, metabolic and hemodynamic responses to lower tyramine concentrations are related to tyramine-induced norepinephrine release rather than a direct post-synaptic mechanism.

We applied the ethanol dilution technique to monitor local blood flow changes. With lower tyramine concentrations, adipose tissue blood flow did not change. Thus, any change in dialysate glycerol with lower tyramine concentrations is explained by a change in glycerol production rather than a change in blood flow. Tyramine increased adipose tissue blood flow at concentrations above 3.5 mM.

Tyramine had a concentration-dependent effect on adipose tissue lipolysis. Lower tyramine concentrations stimulated lipolysis. Higher tyramine concentrations inhibited lipolysis. Propranolol attenuated tyramine's lipolytic effect. Thus, tyramine-induced lipolysis in adipose tissue is explained by norepinephrine release and subsequent β-adrenoreceptor stimulation [5].

The tyramine preparation applied in the present study has been shown to contain approximately 0.01% dopamine [6]. Assuming a 0.01% dopamine contamination, dopamine concentration may have been 35 μM at the highest tyramine concentration applied in our experiments. Dopamine concentrations below 3.5 mM did not inhibit in vitro lipolysis. α-2 adrenoreceptor stimulation strongly inhibits adipose tissue lipolysis [13]. Tyramine-induced antilipolysis could not be blocked with the α-adrenoreceptor antagonist phentolamine. Therefore, the antilipolytic response to tyramine cannot fully be explained through either dopamine contamination or α-2 adrenoreceptor stimulation. Instead, we suggest that tyramine's antilipolytic action may be related to its degradation by monoamine-oxidases (MAO A and B) and the semi-carbazide-sensitive oxidase (SSAO). MAO and SSAO are highly expressed in plasma membrane of adipocytes [14]. Oxidation of tyramine through these enzymes generates H_2O_2 which inhibits lipolysis in rodent adipocytes [14,15].

We validated our approach in a relatively simple physiological model, namely hypertrophic adipose tissue. Mildly increased body fat content, which can be easily quantified through skin-fold thickness measurements, is associated with adipocyte hypertrophy. The increased adipocyte mass exceeds blood vessel and adrenergic nerve terminal growth. Similarly, adrenergic nerve terminal rarefication occurs in cardiac hypertrophy [16]. With adrenergic nerve terminal rarefication, one would expect to observe decreased tyramine responsiveness. Indeed, individuals with a thicker skin-fold featured a profoundly attenuated tyramine-induced lipolysis, whereas isoproterenol responsiveness was only slightly decreased. Tyramine- and isoproterenol-stimulated blood flow was lower in probands with thicker than in probands with thinner skin-folds. A lower tissue perfusion results also in a reduced clearance of metabolites such as glycerol. Taking such a product accumulation into account, the actual difference in the lipolytic response to either tyramine or isoproterenol between the thicker and thinner skin-fold group is much greater than displayed by the glycerol concentrations measured in the dialysates.

We also compared tyramine and isoproterenol responses in women and in men. Women tended to be more tyramine sensitive. Isoproterenol-stimulated lipolysis was significantly more pronounced in women compared with men. Therefore, the gender difference in tyramine responsiveness should

originate from differences in adrenoreceptor distribution in adipose tissue [17].

Thus, tyramine testing through a microdialysis probe may not only be useful to demonstrate near complete adrenergic degeneration. The methodology may also be useful in demonstrating incomplete adrenergic degeneration in a graded fashion and to distinguish between pre- and post-synaptic adrenergic dysfunction at the tissue level.

Our data suggest that lower tyramine concentrations applied through a microdialysis probe can be used to probe the functional integrity of adrenergic neurons in human adipose tissue. In this tissue, glycerol production is a useful physiological read out. Because adipose tissue blood flow does not change at lower tyramine concentrations, blood flow monitoring may not be required. Higher tyramine concentrations inhibit lipolysis and cause a ''paradoxical'' vasodilation. The paradoxical vasodilation is presumably explained by dopamine contamination [6,18]. However, dopamine contamination cannot fully explain the antilipolytic response to tyramine. We suggest that tyramine responses can be compared with responses to direct adrenergic agonists to account for possible differences in post-synaptic sensitivity. The methodology may be particularly useful to assess adrenergic regulation in disorders in which adrenergic dysfunction is localized or patchy, such as diabetic autonomic neuropathy or POTS [1,19].

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