Research article

Tyramine activates lipid accumulation in rat adipocytes: influences of *in vitro* and *in vivo* administration

Francisco Les[1,2,3], Zsuzsa Iffiú-Soltész[1,2], Josep Mercarder[1,4], and Christian Carpéné[1,2,*]

[1] Institut des Maladies Métaboliques et Cardiovasculaires, Institut National de la Santé et de la Recherche Médicale (INSERM U1048), I2MC, 31432 Toulouse Cedex 4, France
[2] Université Paul Sabatier, I2MC-UPS, CHU Rangueil, Toulouse, France
[3] present address: Health Sciences, Universidad San Jorge, Villanueva de Gállego, Zaragoza, Spain
[4] present address: LBNB, Alimentomica, Universitat de les Illes Balears, Palma de Mallorca, Spain

* Correspondence: Email: christian.carpene@inserm.fr.

Abstract: Tyramine is naturally occurring in foods. This biogenic amine is known to induce hypertension, especially when ingested by individuals treated by irreversible inhibitors of monoamine oxidases (MAO). However, in animals, tyramine is also an agonist for trace amine-associated receptors and substrate of semicarbazide-sensitive amine oxidases (SSAO). Though tyramine can alter aminergic transmission by various mechanisms, it was recently reported that prolonged tyramine supplementation does not deteriorate glucose tolerance and does not cause adverse cardiovascular effects in mice. Since previous studies have described insulin-like effects of tyramine in fat cells from diabetic rats, we have further tested tyramine *in vitro* and *in vivo* actions on fattening. To this aim, antilipolytic and lipogenic responses to insulin and tyramine were first compared in rat adipocytes while tyramine oxidation was compared in adipose and intestinal tissues. Then, effects of repeated intraperitoneal injections of tyramine (17 µmol/kg/day) on adiposity and on adipocyte functions were studied in young and mature rats. Millimolar doses of tyramine inhibited glycerol release by adipocytes with a maximal antilipolytic effect comparable to that of insulin. Repeated tyramine administration enhanced fat deposition in epididymal white adipose tissue, irrespective of the age of treated rats. This effect was not accompanied by desensitization to lipogenic activation by insulin, tyramine or hydrogen peroxide. Lastly, tyramine was more readily oxidized in adipose than in intestinal tissues when enzymatic activity was expressed per mg of protein. Moreoever, MAO mostly contributed to oxidative deamination in gut while SSAO was predominant in fat. Thus, both short-term and prolonged effects of tyramine were evidencing somewhat insulin-like actions in adipose tissue and led to reconsider the risk/benefit ratio of the dietary intake of this amine.

1. Introduction

Tyramine is a naturally occurring molecule belonging to the families of dietary amines and trace amines. This amine is a metabolite of the amino acid tyrosine and is mainly known to be involved in the "cheese effect", i.e. a fatal hypertensive crisis induced by dietary amines in depressive patients treated with irreversible inhibitors of monoamine oxidase (MAO). In mammals, *p*-tyramine is also an agonist at trace amine receptors [1] and an agent displacing neurotransmitters from adrenergic terminals, both resulting in an increase in systolic blood pressure. As a consequence, the pressor response to tyramine is now a widely accepted screening method for quantifying the potential hypertensive complications triggered by drugs suspected to inhibit monoamine oxidases or to alter catecholaminergic transmission [2]. Such pressor test is based on the determination of the tyramine dose producing a ≥ 30 mm Hg increase in systolic blood pressure in subjects previously treated by studied agents or placebo [3-6], considering that MAO irreversible inhibitors dramatically reduce such required tyramine dose. The adverse cardiovascular effects of drugs inhibiting MAO [7] and the tyramine-induced cardiac arrhythmias [8], led the scientific community to consider the dietary tyramine content as a matter of toxicological safety. Since tyramine is found at high concentrations in cheese, dry sausage, red wine, beer and various other food items [9,10], their dietary intake has been consequently restricted to avoid fatal hypertensive crisis during the therapeutic use of MAO inhibitors. Such initial recommendations for low-tyramine diets have been extended to dietary restrictions that are nowadays recognized almost unnecessary [11]. Indeed, for unmedicated adults, ingestion of more than 200 or 800 mg of tyramine is needed to induce a modest rise in blood pressure (~30 mm Hg) [12].

Tyramine does not act solely on nervous and cardiovascular systems because it is oxidized by MAO A and B (E.C. 1.4.3.4), but also by semicarbazide-sensitive amine oxidase (SSAO, or primary amine oxidase recently re-classified E.C. 1.4.3.21), which is highly expressed in white adipose tissue (WAT) [13]. Whatever the amine oxidase, tyramine generates hydrogen peroxide when oxidized. Hydrogen peroxide is involved in the tyramine-induced activation of glucose transport in fat cells, which requires oxidation by MAO and SSAO and which is potentiated by vanadium [14,15]. Tyramine also stimulates adipogenesis in preadipocytes [16], and therefore exhibits various "insulin-like" actions that are in line with reported *in vivo* effects such as improvement of glucose tolerance, when injected in combination with vanadium [17], when delivered by osmotic minipumps [18], or when orally given to type 1 diabetic rats [19]. Recently, we have reported that sustained tyramine administration in mice influences glucose handling without promoting cardiovascular injury [20].

Our aim was therefore to study whether tyramine could influence WAT functions in non-diabetic rodents by focusing on two essential pathways in the management of triacylglycerol storage: lipolysis and lipogenesis. Experiments were first performed under short-term incubations with rat adipocytes, thereafter under prolonged treatments in young and slim rats and therefore in older and fatter animals to verify whether WAT constitutes a target for *in vitro* and *in vivo* tyramine actions.

2. Materials and Methods

2.1. Chemicals

Tyramine hydrochloride, sodium orthovanadate, (−)-isoprenaline hydrochloride, hydrogen peroxide (H_2O_2), collagenase type II (C-6885), bovine insulin, bovine albumin, amine oxidase inhibitors, and other reagents were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). [14C]-tyramine was from Sigma-Aldrich and [3H]-3-glucose was from Perkin Elmer Life Science (Boston, MA, USA).

2.2. Animals and treatments

Twenty-five Wistar rats of both sexes (Harlan, Gannat, France) were used for preliminary lipolytic determinations and for amine oxidase assays in adipose and intestinal tissue preparations, after sacrifice according to INSERM guidelines. Adipose tissue removal and preparation of freshly isolated adipocytes were performed as already detailed [21]. Two-month old male Wistar rats were separated in two weight-matched groups before daily intraperitoneal (i.p.) administration of tyramine at 17 μmol/kg/day for 10 days (tyramine-treated group, n = 4), while control group (n = 4) received saline vehicle. Nine-month aged rats underwent the same distribution and treatment. At sacrifice performed between 08:00 and 10:00 a.m. under fed conditions, to avoid any reduction of the fat stores by overnight fasting, lipogenic responses were determined on freshly isolated adipocytes, while plasma was prepared and stored at −80 °C for further glucose and insulin measurements as previously reported [20].

2.3. Adipose cell functional explorations

Minced pieces of adipose tissue were digested by 1 mg/mL collagenase type II for approximately 45 min under agitation in Krebs-Ringer salt solution pH 7.5 containing 15 mM sodium bicarbonate, 10 mM HEPES and 3.5% of fat-depleted bovine serum albumin (KRBHA). The isolation, washing and distribution steps of the procedure were performed as previously reported [22]. Lipolytic activity was assessed by determining the glycerol released in the medium after 90-min incubation of adipocytes with the tested agents according to [22]. Lipogenic activities were determined on adipocytes by measuring the radioactivity incorporated into cellular lipids from [3-3H]-glucose as described [23].

2.4. Assessment of amine oxidase activity

Amine oxidase activity was measured on rat tissue homogenates with 0.5 mM [14C]-tyramine, as in [24]. Homogenates were prepared with thawed samples by using homogenizer Tissue Master-125 (Omni International, Kennesaw, GA, USA). Amine oxidation was then determined on 30-min incubation at 37 °C after 15-min preincubation in the absence or the presence of reference inhibitors, as already described [25]. MAO was defined as the fraction of activity inhibited by 0.1 mM pargyline preincubation, while SSAO was abolished by 1 mM semicarbazide [26]. Protein quantification was performed using DC Protein Assay kit (BioRad, Hercules, CA, USA).

Results are presented as mean ± standard error of the mean (SEM). Statistical analysis for comparisons between treated and respective control used Student's *t* test. ns: non-significantly different from control.

3. Results and Discussion

3.1. Further insights on the tyramine antilipolytic effect in adipocytes

We have already reported that tyramine activates glucose uptake and inhibits lipolysis in rat adipocytes in a manner that is: maximal at 1 mM, dependent on amine oxidation by MAO and SSAO [14], mediated by subsequent hydrogen peroxide production and potentiated by vanadium [15]. Thus, only further comparisons between the antilipolytic actions of tyramine and insulin are reported thereafter.

Between 0.1 and 1mM, tyramine exhibited an increasing capacity to impair the stimulation of glycerol release by the β-adrenergic agonist of reference isoprenaline (Table 1), and thus confirmed its already reported antilipolytic properties [14]. An inhibition of one-half of the isoprenaline-induced lipolysis was also observed with 100 nM insulin or 0.1 mM hydrogen peroxide. However, the inhibition induced by 1 mM tyramine alone was greater than those induced by the other antilipolytic agents and could not be increased further by 0.1 mM sodium orthovanadate (Table 1). Though vanadate was not antilipolytic on its own when tested at submillimolar dose, it induced some synergism with the other antilipolytic agents tested. Although the molecular mechanisms by which vanadate potentiates inhibition of triglyceride breakdown in rat fat cells remain to be determined, it appeared likely that there were mediated by peroxovanadate, a well-recognized phosphatase irreversible inhibitor, which was generated by a chemical interaction between vanadate and the hydrogen peroxide (either added in the milieu or produced during amine oxidation) as already argued for the stimulation of glucose uptake by SSAO substrates [27]. Whatsoever, the presence of the transition metal was not considered mandatory for detecting tyramine antilipolytic effects.

This consideration was further supported by the fact that 1 mM tyramine provoked a $47 \pm 8\%$ inhibition of the lipolytic response to adenosine deaminase 2 IU/ml, confirming that its antilipolytic properties could be detected without vanadium. Indeed, the removal by adenosine deaminase of adenosine that exerts a continuous negative feedback on glycerol release is a lipolytic stimulus that was impaired by tyramine. Importantly, such tyramine inhibitory action was prevented by 1 mM phenelzine: only $21 \pm 4\%$ inhibition remained detectable in the presence of this MAO- and SSAO-inhibitor (n = 4, $p < 0.05$ *vs* tyramine, not shown). In contrast, the insulin-induced antilipolysis was unaltered by phenelzine (51 ± 11 and $51 \pm 8\%$ inhibition, for insulin with and without inhibitor, respectively, n = 4, ns, not shown). Thus, amine oxidation by MAO and/or SSAO activity was involved in tyramine - but not in insulin - antilipolytic effect. In addition, the hypothesis that tyramine may behave as a partial agonist or an antagonist at β-adrenergic receptors could be ruled out, when one considers that tyramine impaired adenosine deaminase-promoted lipolysis, which does not result from β-adrenoceptor activation. Lastly, tyramine was not lipolytic on its own, even at 1 mM (not shown), discarding a putative activation of Gs-coupled trace amine-associated receptors in rat adipocytes.

Table 1. Inhibition of triglyceride breakdown by tyramine and other antilipolytic agents in rat adipocytes

Incubation condition % inhibition of Iso-induced lipolysis	Control	+ Vanadate 0.1 mM
Isoprenaline 10 nM	0	14.2 ± 3.3
Iso + insulin 100 nM	50.0 ± 5.9	80.5 ± 1.9 **
Iso + hydrogen peroxide 0.1 mM	51.4 ± 8.9	80.4 ± 5.4 **
Iso + tyramine 0.1 mM	31.3 ± 4.1	57.3 ± 5.6 ***
Iso + tyramine 1 mM	71.5 ± 3.3	76.0 ± 7.4

Glycerol release was determined after 90-min incubation of rat adipocytes in the presence of 10 nM isoprenaline (Iso) alone or with the indicated concentrations of the tested agents. Data are expressed as percentage of inhibition on isoprenaline-induced lipolysis, with 10 nM isoprenaline-stimulation set at 0% and maximal inhibition returning to basal lipolysis set at 100% in each experiment, and being equivalent to 1.4 ± 0.1 and 0.4 ± 0.1 µmol glycerol released/100 mg lipid/90 min, respectively. Mean $\pm$ SEM of 20−25 experiments. Different from corresponding antilipolysis without vanadate (Control) at: ** $p < 0.02$, *** $p < 0.01$.

These descriptive aspects of acute *in vitro* effects of tyramine on triacylglycerol breakdown further confirmed the potential of this biogenic molecule to mimic insulin antilipolytic action, and prompted us to investigate the influence of *in vivo* tyramine treatment on adiposity.

3.2. Influence of tyramine treatment on adiposity and lipogenic responses in rats

In vivo prolonged treatment consisted in daily i.p. injections of tyramine in: i) young, normoglycemic and highly insulin-responsive rats weighing less than 200 g, and ii) older, fatter and less insulin sensitive males weighing almost 400 g. After 10 days of treatment, rats that received tyramine hydrochloride 3 mg/kg body weight/day (corresponding to 17 µmol/kg/day) showed slightly higher body weight gain and/or fattening (Table 2). As tyramine exhibits catecholamine releasing properties [28], it is not so surprising that its repeated administration affects food consumption, body weight gain and adiposity. Indeed, most of the pharmacological inhibitors of monoamine reuptake transporters have been reported to reduce food intake and body weight in obese subjects [29]. However, tyramine treatment improved body mass gain in young rats when compared to their age- and weight-matched controls. The increase of body weight gain occurred without noticeable change of food intake that averaged 23 g/rat/day in young rats. An unexplained higher variability in the body mass gain of the control group hampered the detection of such effect in older rats. Nevertheless, epididymal white adipose tissue (EPIWAT) was always heavier in the groups receiving tyramine than in control receiving saline vehicle irrespective of the age, suggesting a somewhat obesogenic effect of tyramine administration. However, no such increase was observed for the subcutaneous inguinal WAT (not shown).

The larger epididymal fat pads of tyramine-treated rats were in line with the *in vitro* antilipolytic effect of tyramine, but were not in agreement with previous reports on chronic tyramine treatment of other animal models, which did not evidence any clear increase of body weight gain in polyuric and insulinopenic diabetic rats treated with tyramine by osmotic minipumps (116 µmol/kg/day for two weeks) [18] or in mice drinking tyramine (1100 µmol/kg/day for twelve weeks) [20].

Here, young rats exhibited a greater body weight gain and a lower fed blood glucose than the older ones. This was expected and likely traducing a period of more intense growth and higher

insulin sensitivity in young males than in the older and fatter group. Nevertheless, non-fasting blood glucose was not lowered after tyramine treatment, irrespective of age or adiposity (Table 2). It was also the case for plasma insulin (not shown). The lack of decrease in non fasting blood glucose observed in the present study could not be considered as in total disagreement with previous works since normoponderal and normoglycemic rats were challenged with tyramine and it can be easily conceived that increasing their fat deposition could occur without decreasing their normally regulated glycaemia.

**Table 2.** Effect of ten-day treatment with tyramine on adiposity in young and mature rats

	young rats		mature rats	
in vivo treatment	control	tyr-treated	control	tyr-treated
final body weight (g)	177 ± 8	205 ± 14	386 ± 7	400 ± 4
body weight gain (g)	23.0 ± 1.4	33.3 ± 2.7 **	16.3 ± 7.3	26.7 ± 2.6
EPIWAT mass (g)	0.5 ± 0.1	0.8 ± 0.1 *	4.3 ± 0.1	5.6 ± 0.2 ***
blood glucose (mM)	5.29 ± 1.07	5.33 ± 0.53	7.68 ± 0.28	7.96 ± 0.14

EPIWAT: epididymal white adipose tissue. Means $\pm$ SEM of four rats.

Difference between group receiving i.p. injections of tyramine (17 µmol/kg body weight/day; tyr-treated) and corresponding control at: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

In an attempt to decipher the somewhat "insulin-like" obesogenic action of tyramine treatment in such non-diabetic rats, *ex vivo* tests of the capacity of the amine to mimic insulin activation of lipid deposition were performed in their adipocytes by measuring lipogenic responses to various activators. The well-known stimulation of glucose incorporation into lipids by insulin was equivalent to a tenfold activation of baseline in fat cells from young rats (Figure 1A). Such high lipogenic activation obtained with 100 nM insulin was half-reproduced by 1 mM tyramine plus 0.1 mM vanadate. Vanadate was almost ineffective in modifying basal or insulin-stimulated lipogenesis, while it improved the lipogenic effect of hydrogen peroxide (which, when present alone increased baseline glucose incorporation into lipids by 2.7 ± 0.4 and 2.8 ± 0.3 fold factor in control and tyramine-treated rats, respectively). Of note, *in vivo* tyramine treatment did not modify basal lipogenesis in adipocytes from young rats (38 ± 3 and 40 ± 12 nmol of [³H]-glucose incorporated/100 mg cellular lipids/90 min, ns) and did not hamper its activation by insulin, tyramine or hydrogen peroxide (Figure 1A).

In older rats, the maximal insulin stimulation of [³H]-glucose incorporation into lipids was more limited, reaching hardly fourfold increase (Figure 1B). In such expected conditions of age-related decrease in insulin responsiveness, the combination of 1 mM tyramine with 0.1 mM vanadate was still able to reproduce more than one-half of the insulin lipogenic action, as did hydrogen peroxide plus vanadium. Again, tyramine treatment did not significantly affect basal or stimulated lipogenesis.
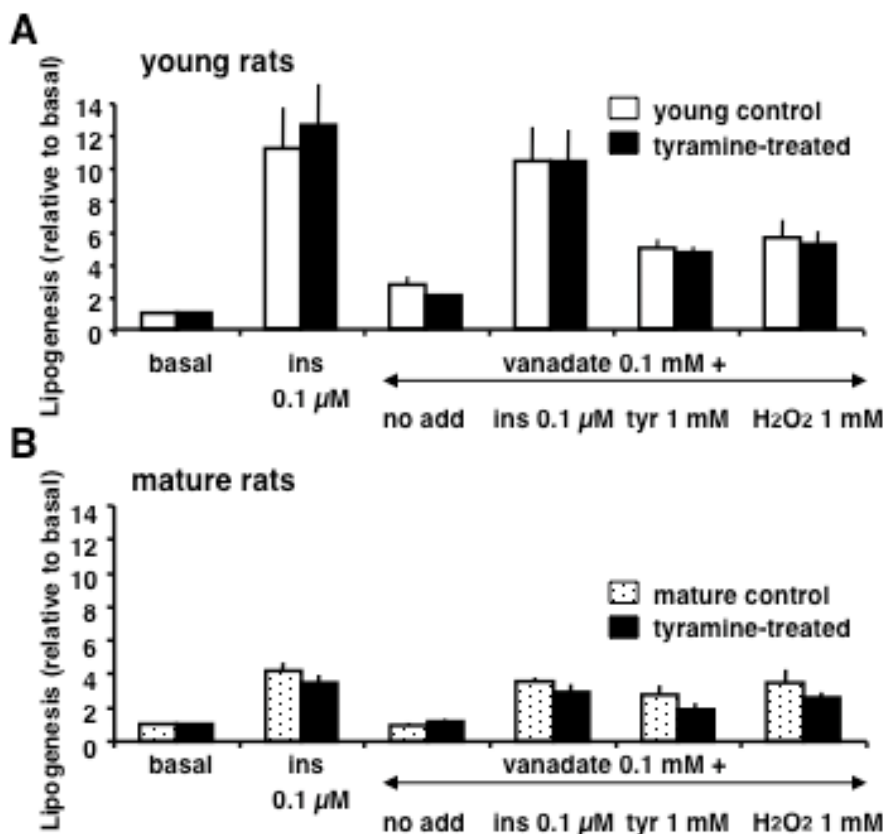
Figure 1. Effect of tyramine chronic treatment on adipocyte lipogenic activity White adipocytes isolated from control or tyramine-treated rats were incubated for 90 min with 0.5 mM [^{3}H]-glucose and 0.1 µM insulin, or with 0.1 mM vanadate alone (no add) or together with the indicated concentrations of insulin (ins), tyramine (tyr) or hydrogen peroxide (H_2O_2). Lipogenesis is expressed relative to glucose incorporation into cellular lipids without added agent set at 1 (basal). (A) young (white columns) control and TYR-treated rats (black columns). (B) Older (shaded columns) control and their age-matched TYR-treated rats (black columns), as described in Methods. Mean ± SEM of four rats per group.

In addition, the effect of tyramine plus vanadate was abolished by 1 mM phenelzine or impaired by 1 mM pargyline and semicarbazide (MAO- and SSAO-inhibitor, respectively) in young and mature rats (not shown). Altogether, our observations clearly indicated that the *in vitro* insulin-like effects of high doses of tyramine in adipocytes were not altered after repeated *in vivo* administration, ruling out any putative down-regulation of such responsiveness that was amine oxidase-dependent. Our *ex vivo* explorations also suggested that the direct lipogenic and antilipolytic effects of tyramine tended to contribute to the enhanced fat deposition observed in WAT of tyramine-treated rats. However, our investigations could not demonstrate that the *in vitro* insulin-like actions of tyramine on adipocytes were predominantly contributing to its *in vivo* facilitation of lipid accumulation. One concern is that acute insulin-like effects were observed at 90 min with millimolar doses of tyramine, while only 17 µmol/kg body weight were daily administered in rats for 10 days. Indeed, *in vivo* tyramine treatment corresponded to a less than twofold increase over the spontaneous tyramine dietary intake of rats, estimated to average 26 µmol/kg body weight/day, as the standard rodent chow they consumed contains around 45 mg tyramine/kg pellets [17].

How the effects of a treatment that brought additional amounts of tyramine reachable by nutritional supplementation can be mechanistically explained by *in vitro* effects obtained at pharmacological millimolar doses that are only reached in the richest tyramine-containing food items, such as cheeses or dry sausages (exceeding 300 mg/kg [9,30])? Obviously, the millimolar doses tested in our *in vitro* explorations could be qualified as supra-nutritional and were probably not occurring in any anatomical location of the treated rats, save from the point of i.p. injection. Nevertheless, the dose we used for *in vivo* repeated administration (3 mg/kg/day) was far from being toxic, since on a toxicological point of view, the no-observed-adverse-effect level (NOAEL) of tyramine is ≥180 mg/kg body weight/day (2000 ppm) [31]. Indeed, rather than comparing the *in vitro/in vivo* ratio of doses of tyramine that surround the adipocytes during acute and prolonged challenges it is of major importance to quantify - if feasible - the ratio of the trace amounts that are found in the milieu under baseline or fasting conditions *vs* under safe tyramine supplementation. In humans, only scarce information can be obtained from the literature, with mean baseline fasting levels of blood tyramine varying between 0.00049 and 0.0058 µmol/L *(*i.e. lower than 1 ng/mL) according to the individual status or to the laboratory performing the determinations [32,33]. Pharmacokinetic studies performed in humans indicate a plasma Cmax of 0.22 µmol/L after oral administration of 200 mg tyramine [33]. Thus, there is no doubt that plasma levels of tyramine, a member of the trace amine family, can be significantly increased upon ingestion. However it has been evidenced that doses of tyramine more than twice as high are required to increase in a similar way in plasma when it is ingested with a meal than when administered orally to fasting subjects in an experimental situation [33]. Similarly, it has been univocally reported that the amount of tyramine needed to produce a pressor response is increased when it is administered orally compared to intravenously: negligible changes are observed after a 200-mg oral ingestion (in the absence of any other drug treatment), while a detectable pressor response is obtained after only 4 mg of intravenously administered tyramine [34]. One can suppose that it is the same issue for the insulin-like effects reported here for tyramine. A clear difference lies in the fact that the *in vivo* pressor response to tyramine is exaggerated by MAO blockade while, here, its *in vitro* antilipolytic and lipogenic effects are inhibited by MAO blockade and potentiated by vanadate.

In fact, the ingestion of tyramine-rich food has no cardiovascular effect in most of the individuals due to the purported highly effective enzymatic barriers that inactivate tyramine in the intestines and liver before it can reach the systemic circulation. This expected high first-pass elimination of tyramine by gut wall and liver is managed by peripheral MAO and is responsible for the very high clearance of the dietary amine [33]. In this view, and after considering that MAO and other amine oxidases are involved in the abovementioned insulin-like effect of tyramine in adipocytes, we compared the richness of these enzymatic activities in different anatomical locations.

3.3. Comparison of tyramine oxidation by adipose and intestinal tissues

To assume whether dietary amine could act in adipose tissues beyond the intestinal barrier, as accepted in the case of adrenergic neurons involved in the hypertensive reactions referred to as tyramine pressor response, we compared tyramine oxidation in rat gut and WAT. Tyramine was oxidized mainly by MAO in jejunum and ileum homogenates (Figure 2A). An equivalent MAO-dependent oxidation was found in WAT, when oxidase activity was (biochemically) expressed as nmoles of substrate oxidized/mg protein/min. Noteworthy, an additional SSAO-dependent

oxidation of tyramine occurred in WAT only. Comparison of the amine oxidase richness in terms of nmoles of tyramine oxidized per gram of wet tissue gave a different pattern since gut is much richer in proteins than WAT. However, this latter data expression still supported a (physiologically) higher SSAO richness in WAT than in intestine and an overall non-negligible capacity of the adipose tissue to oxidize tyramine (Figure 2B).
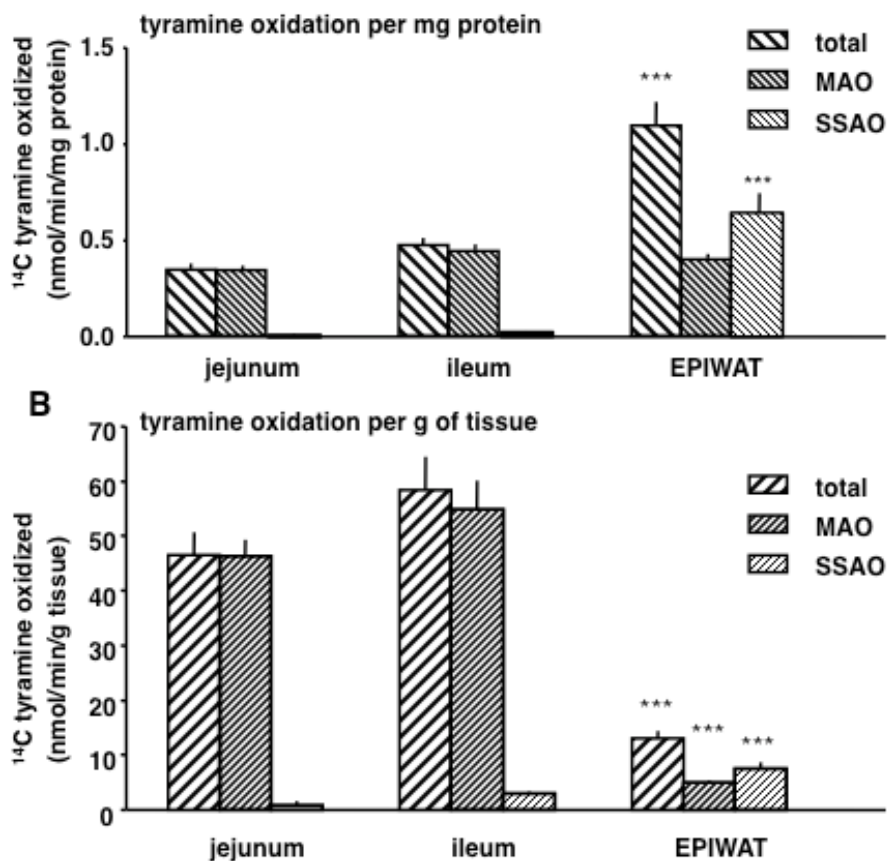
Figure 2. Tyramine oxidation in rat intestinal and adipose tissues. Oxidation of 0.5 mM tyramine was measured without any addition (total) in the presence of the indicated rat tissue homogenate. MAO corresponds to the oxidation that was inhibited by 0.5 mM pargyline preincubation while SSAO activity corresponds to oxidation inhibited by 1 mM semicarbazide preincubation. Expression of tyramine oxidation on a per mg protein basis (A) or on a per g of tissue basis (B), with 132 ± 4 and 121 ± 4 mg protein/g for jejunum and ileum, or 12 ± 1 mg protein/g for adipose tissue. Mean $\pm$ SEM. of 5 (jejunum), 4 (ileum) or 7 (EPIWAT) preparations. Different from corresponding oxidation in intestinal preparations at: ***$p < 0.001$.

Although pharmacokinetics indicates a low bioavailability of tyramine once ingested, it appears possible that not all the ingested tyramine is oxidized at the intestinal level and that a small percentage can reach adipocytes. Even in the absence of drug treatment, the larger mass of fat depots relative to intestinal tract may play in favor of a possible contribution of adipose amine oxidases in the metabolism of tyramine, especially in obese individuals. Moreover, oleamide, a slip agent that can be found in plasticware behaves as a strong inhibitor of monoamine oxidases [35] and, as a food

contaminant, might partially inhibit MAO in gut during digestion, therefore improving the crossing of the intestinal barrier by the ingested amine.

When intentionally administered in humans, tyramine not only triggers its widely known hypertensive effect (improved by MAO blockade), since it also activates sympathetic nerves such as those ending in endocrine pancreas (decreasing insulin secretion) [28], gut (increasing ghrelin release) [36] or eyes (provoking mydriasis). Again, in humans, tyramine has been reported to elicit local release of endogenous norepinephrine in adipose tissue and to stimulates lipolysis when administered via subcutaneous microdialysis probes but not when directly tested in isolated adipocytes [37]. By contrast, we have observed in rodent models tyramine metabolic effects that are inhibited by MAO and/or SSAO blockade: glucose transport and antilipolysis in adipocytes [16-18]. We also reported that tyramine activates glucose uptake in skeletal and cardiac muscles [38].

We further confirmed here that tyramine exerts rapid *in vitro* insulin-like effects in fat cells when present at 100 μM to 1 mM, such as lipolysis inhibition and lipogenesis activation. Taken together, these observations agree with the fact that tyramine favors fat deposition when repeatedly injected to rats. These somewhat "insulin-like" actions of tyramine were not desensitized after prolonged administration, ruling out the involvement of down-regulatable receptors in such effects. Additionally, the fact that submillimolar doses of tyramine were necessary to reproduce insulin actions in adipocytes and the synergism with vanadate indicated the involvement of a non-receptor-mediated mechanism, as already evidenced for glucose uptake stimulation [15]. Similar interpretations were proposed by Bairras and coworkers who reported that tyramine potentiates the poor antilipolytic effect of insulin in adipocytes from old rats [39]. However, to our knowledge, a complete study of such antilipolytic actions still lacks for human adipocytes since only a lack of direct lipolytic effect of tyramine has been reported [37] and no putative antilipolytic effects have been investigated.

4. Conclusion

In conclusion, our results underscore direct and rapid tyramine effects in rat fat cells. These effects are "insulin-like": inhibition of lipolysis and stimulation of glucose incorporation into lipids. They are dependent on amine oxidation and might participate to diet-induced fattening, especially when considering that tyramine is a biogenic amine present in foods. Thus, it cannot be ruled out that, alongside its well-recognized pressor effect, this dietary amine exerts *in vivo* some effects related with adipocyte main functions. We thus propose that, rather than being considered only hazardous for cardiovascular complications, this molecule naturally occurring in foods may have an interest for diabetes and related metabolic diseases especially when glucotoxicity needs to be reduced. Indeed, as it is the case for its derivative, N-methyltyramine [40], the administration of tyramine is devoid of any slimming effect, a somewhat astonishing observation for an agent endowed with catecholaminergic properties, except if one takes into account the activation of glucose utilization and the antilipolytic effects we report here on adipocytes. These direct influences on fat cells tend to promote fattening rather than lipid mobilization, which appears to result from indirect adrenergic activation and might be subjected to desensitization. Whether high oral doses or long-term supplementation of tyramine or its presence in functional foods for type 2 diabetes [41] will act *in vivo* as an insulin sensitizer or will exhibit antihyperglycemic action deserves to be further studied in insulin-resistant models or patients, for completing preliminary observations performed on type 1-diabetic rats [17-19] and our present findings.

Acknowledgments

Conflict of Interest

There is no conflict of interest to declare for any author of this work.

References

1. Maguire JJ, Parker WA, Foord SM, et al. (2009) International Union of Pharmacology. LXXII. Recommendations for trace amine receptor nomenclature. *Pharmacol Rev* 61: 1-8.
2. Fankhauser C, Charieras T, Caille D, et al. (1994) Interaction of MAO inhibitors and dietary tyramine: a new experimental model in the conscious rat. *J Pharmacol Toxicol Methods* 32: 219-224.
3. Cantarini MV, Painter CJ, Gilmore EM, et al. (2004) Effect of oral linezolid on the pressor response to intravenous tyramine. *Br J Clin Pharmacol* 58: 470-475.
4. DeMarcaida JA, Schwid SR, White WB, et al. (2006) Effects of tyramine administration in Parkinson's disease patients treated with selective MAO-B inhibitor rasagiline. *Mov Disord* 21: 1716-1721.
5. Finberg JP, Gillman K (2011) Selective inhibitors of monoamine oxidase type B and the "cheese effect". *Int Rev Neurobiol* 100: 169-190.
6. Marquet A, Kupas K, Johne A, et al. (2012) The effect of safinamide, a novel drug for Parkinson's disease, on pressor response to oral tyramine: a randomized, double-blind, clinical trial. *Clin Pharmacol Ther* 92: 450-457.
7. Gillman PK (2016) Monoamine Oxidase Inhibitors: a review concerning dietary tyramine and drug interactions. *PsychoTropical Commentaries* 1: 1-90.
8. Tiller JW, Dowling JT, Tung LH, et al. (1985) Tyramine-induced cardiac arrhythmias. *N Engl J Med* 313: 266-267.
9. Komprda T, Sladkova P, Dohnal V (2009) Biogenic amine content in dry fermented sausages as influenced by a producer, spice mix, starter culture, sausage diameter and time of ripening. *Meat Sci* 83: 534-542.
10. Ubaldo JC, Carvalho AF, Fonseca LM, et al. (2015) Bioactive amines in Mozzarella cheese from milk with varying somatic cell counts. *Food Chem* 178: 229-235.
11. Rumore MM, Roth M, Orfanos A (2010) Dietary tyramine restriction for hospitalized patients on linezolid: an update. *Nutr Clin Pract* 25: 265-269.
12. Jansen SC, Van Dusseldorp M, Bottema KC, et al. (2003) Intolerance to dietary biogenic amines: a review. *Ann Allergy Asthma Immunol* 91: 233-240.
13. Shen SH, Wertz DL, Klinman JP (2012) Implication for functions of the ectopic adipocyte copper amine oxidase (AOC3) from purified enzyme and cell-based kinetic studies. *PLoS One* 7: e29270.

14. Visentin V, Prevot D, Marti L, et al. (2003) Inhibition of rat fat cell lipolysis by monoamine oxidase and semicarbazide-sensitive amine oxidase substrates. *Eur J Pharmacol* 466: 235-243.

15. Marti L, Morin N, Enrique-Tarancon G, et al. (1998) Tyramine and vanadate synergistically stimulate glucose transport in rat adipocytes by amine oxidase-dependent generation of hydrogen peroxide. *J Pharmacol Exp Ther* 285: 342-349.

16. Carpéné C, Daviaud D, Boucher J, et al. (2006) Short- and long-term insulin-like effects of monoamine oxidases and semicarbazide-sensitive amine oxidase substrates in cultured adipocytes. *Metabolism* 55: 1397-1405.

17. Visentin V, Marq P, Bour S, et al. (2003) Effect of prolonged treatment with tyramine on glucose tolerance in streptozotocin-induced diabetic rats. *J Physiol Biochem* 59: 225-232.

18. Visentin V, Bour S, Boucher J, et al. (2005) Glucose handling in streptozotocin-induced diabetic rats is improved by tyramine but not by the amine oxidase inhibitor semicarbazide. *Eur J Pharmacol* 522: 139-146.

19. Lino CdS, Sales TdP, Gomes PB, et al. (2007) Anti-diabetic activity of a fraction from Cissus verticillata and tyramine, its main bioactive constituent, in alloxan-induced diabetic rats. *Am J Pharmacol Toxicol* 2: 178-188.

20. Carpéné C, Schaak S, Guilbeau-Frugier C, et al. (2016) High intake of dietary tyramine does not deteriorate glucose handling and does not cause adverse cardiovascular effects in mice. *J Physiol Biochem* 72: 539-553.

21. Iffiu-Soltesz Z, Prévot D, Carpéné C (2009) Influence of prolonged fasting on monoamine oxidase and semicarbazide-sensitive amine oxidase activities in rat white adipose tissue. *J Physiol Biochem* 65: 11-23.

22. Carpéné C, Galitzky J, Saulnier-Blache JS (2016) Short-term and rapid effects of lysophosphatidic acid on human adipose cell lipolytic and glucose uptake activities. *AIMS Molec Sci* 3: 222-237.

23. Atgié C, Sauvant P, Ambid L, et al. (2009) Possible mechanisms of weight loss of Siberian hamsters *(Phodopus sungorus sungorus)* exposed to short photoperiod. *J Physiol Biochem* 65: 377-386.

24. Fowler CJ, Tipton KF (1981) Concentration dependence of the oxidation of tyramine by the two forms of rat liver mitochondrial monoamine oxidase. *Biochem Pharmacol* 30: 3329-3332.

25. Carpéné C, Les F, Hasnaoui M, et al. (2016) Anatomical distribution of primary amine oxidase activity in four adipose depots and plasma of severely obese women with or without a dysmetabolic profile. *J Physiol Biochem* 73: 475-486.

26. Prévot D, Soltesz Z, Abello V, et al. (2007) Prolonged treatment with aminoguanidine strongly inhibits adipocyte semicarbazide-sensitive amine oxidase and slightly reduces fat deposition in obese Zucker rats. *Pharmacol Res* 56: 70-79.

27. Yraola F, Garcia-Vicente S, Marti L, et al. (2007) Understanding the mechanism of action of the novel SSAO substrate $(C_7NH_{10})6(V_{10}O_{28}).2H_2O$, a prodrug of peroxovanadate insulin mimetics. *Chem Biol Drug Des* 69: 423-428.

28. Gilliam LK, Palmer JP, Taborsky GJ, Jr. (2007) Tyramine-mediated activation of sympathetic nerves inhibits insulin secretion in humans. *J Clin Endocrinol Metab* 92: 4035-4038.

29. Carpéné C, Iffiú-Soltész Z (2011) Monoaminergic systems and anti-obesity drug discovery: chronic administration of sympathicomimetic amines, re-uptake inhibitors, or amine oxidase

inhibitors? *In: Anti-Obesity Drug Discovery and Development (Atta-ur-Rahman & Iqbal Choudhary M Editors) Bentham Sciences Publishers* 1: 114-130.

30. Suzzi G (2003) Biogenic amines in dry fermented sausages: a review. *Int J Food Microbiol* 88: 41-54.

31. Til HP, Falke HE, Prinsen MK, et al. (1997) Acute and subacute toxicity of tyramine, spermidine, spermine, putrescine and cadaverine in rats. *Food Chem Toxicol* 35: 337-348.

32. Yonekura T, Kamata S, Wasa M, et al. (1988) Simultaneous determination of plasma phenethylamine, phenylethanolamine, tyramine and octopamine by high-performance liquid chromatography using derivatization with fluorescamine. *J Chromatogr* 427: 320-325.

33. VanDenBerg CM, Blob LF, Kemper EM, et al. (2003) Tyramine pharmacokinetics and reduced bioavailability with food. *J Clin Pharmacol* 43: 604-609.

34. Korn A, Da Prada M, Raffesberg W, et al. (1988) Tyramine pressor effect in man: studies with moclobemide, a novel, reversible monoamine oxidase inhibitor. *J Neural Transm Suppl* 26: 57-71.

35. McDonald GR, Hudson AL, Dunn SM, et al. (2008) Bioactive contaminants leach from disposable laboratory plasticware. *Science* 322: 917.

36. Mundinger TO, Cummings DE, Taborsky GJ (2006) Direct stimulation of ghrelin secretion by sympathetic nerves. *Endocrinology* 147: 2893-2901.

37. Adams F, Boschmann M, Schaller K, et al. (2006) Tyramine in the assessment of regional adrenergic function. *Biochem Pharmacol* 72: 1724-1729.

38. Morin N, Visentin V, Calise D, et al. (2002) Tyramine stimulates glucose uptake in insulin-sensitive tissues in vitro and in vivo via its oxidation by amine oxidases. *J Pharmacol Exp Ther* 303: 1238-1247.

39. Bairras C, Ferrand C, Atgié C (2003) Effect of tyramine, a dietary amine, on glycerol and lactate release by isolated adipocytes from old rats. *J Physiol Biochem* 59: 161-167.

40. Stohs SJ, Hartman MJ (2015) A review of the receptor binding and pharmacological effects of N-methyltyramine. *Phytother Res* 29: 14-16.

41. Hamasaki H (2016) Functional foods for type 2 diabetes. *AIMS Med Sci* 3: 278-297.