Long-term Activation of Semicarbazide-Sensitive Amine Oxidase Lowers Circulating Levels of Uric Acid in Diabetic Conditions

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Summary

Uric acid is involved in nitrogenous waste in animals, together with ammonia and urea. Uric acid has also antioxidant properties and is a surrogate marker of metabolic syndrome. We observed that the elevated plasma uric acid of high-fat fed mice was normalized by benzylamine treatment. Indeed, benzylamine is the reference substrate of semicarbazide-sensitive amine oxidase (SSAO), an enzyme highly expressed in fat depots and vessels, which generates ammonia when catalysing oxidative deamination. Ammonia interferes with uric acid metabolism/solubility. Our aim was therefore to investigate whether the lowering action of benzylamine on uric acid was related to an improvement of diabetic complications, or was connected with SSAO-dependent ammonia production. First, we observed that benzylamine administration lowered plasma uric acid in diabetic db/db mice while it did not modify uric acid levels in normoglycemic and lean mice. In parallel, benzylamine improved the glycemic control in diabetic but not in normoglycemic mice, while plasma urea remained unaltered. Then, uric acid plasma levels were measured in mice invalidated for AOC3 gene, encoding for SSAO. These mice were unable to oxidize benzylamine but were not diabetic and exhibited unaltered plasma uric levels. Therefore, activated or abolished ammonia production by SSAO was without influence on uric acid in the context of normoglycemia. Our observations confirm that plasma uric acid increases with diabetes and can be normalized when glucose tolerance is improved. They also show that uric acid, a multifunctional metabolite at the crossroads of nitrogen waste and of antioxidant defences, can be influenced by SSAO, in a manner apparently related to changes in glucose homeostasis.

Introduction

Uric acid is one of the most important molecules of nitrogenous waste in animals, together with ammonia and urea. In man, uric acid is sadly known for causing gout attack (arthritis) when its circulating values exceed the normal range 3-7 mg/dL and crystallize between bone joints. For this reason the dietary income of uric acid, mainly due to foods that contain proteins and purines, has been recommended to be limited. However, uric acid has antioxidant properties (Becker *et al.*, 1991), it has also been considered as a surrogate marker of metabolic syndrome since its circulating levels have been repeatedly reported to increase with cardiovascular diseases, diabetes and obesity (Yoo *et al.*, 2005). These multiple aspects of uric acid complicates our understanding of its physiological role, together with the fact that kidney, which eliminates this metabolite is also actively reclaiming it. Therefore uric acid cannot be merely considered as a nitrogen waste. Similarly, it is not the sole trigger of human kidney pathologies. Once its renal filtration/reabsorption (clearance) is altered, uric acid, which is slightly soluble in water, precipitates in urine and generates kidney stones. However, only approx. 10 % of the kidney stones are constituted by uric acid - or more precisely urate - crystals, while calcium oxalate crystals are found in most of the nephropathies (Coe *et al.*, 2005). Accordingly, not all the kidney stones are accompanying metabolic diseases such as diabetes or obesity (Maalouf, 2011), while many of them coexist with inflammatory, irritating or infectious diseases (Soble *et al.*, 1999). Nevertheless, overproduction of uric acid in blood is probably more responsible than its decreased clearance in the appearance of troubles in its circulating levels during the onset of metabolic syndrome (Reaven, 1999).

We have recently observed that long-term oral treatment with benzylamine decreased plasma uric acid in mice fed a high-fat diet (HFD) (Iffiú-Soltész *et al.*, 2010). Indeed, benzylamine is the reference substrate of semicarbazide-sensitive amine oxidase- (SSAO), an enzyme highly expressed in fat depots (Bour *et al.*, 2007) and in vessels (Nunes *et al.*, 2011). This enzyme mainly located at the plasma membrane exhibits its catalytic site at the cell surface, i.e. in the interstitial milieu (for adipocytes or vascular smooth muscle cells) or in the blood (for endothelial cells). SSAO, also called vascular adhesion protein-1, is currently being renamed primary amine oxidase (PAO) because there are now many inhibitors that exhibit higher affinity and selectivity than semicarbazide itself (O'Rourke *et al.*, 2008), and therefore will be referred to as SSAO/PAO throughout this report. SSAO/PAO, such as many other amine oxidases, including the widely known monoamine oxidases (MAO-A and MAO-B), generates ammonia when catalysing the oxidative deamination of any soluble amine substrate of endogenous or exogenous origin (methylamine, tryptamine, benzylamine, polyamines…).

The administration of benzylamine supplies more substrate to SSAO/PAO and to MAO-B and increases ammonia formation in way that was suspected to modify the nitrogen waste and to influence the circulating levels of uric acid, by modifying its production or by reducing its solubility (Bowyer *et al.*, 1979). Alternatively, the SSAO/PAO activation, alongside its production of ammonia, generates other end-products of oxidative deamination: the aldehyde corresponding to the oxidized primary amine and hydrogen peroxide. Excess of the latter product is known to be involved in oxidative stress but hydrogen peroxide has also been demonstrated to mimic several insulin actions and is involved in the antidiabetic properties of benzylamine (Carpéné, 2009; Iffiú-Soltész *et al.*, 2010; Mercader *et al.*, 2010).

Our aim was therefore to investigate whether the lowering effect of benzylamine treatment on circulating uric acid was a direct consequence of amine oxidase-related ammonia production or was more related to an improvement of the glucose handling in the treated diabetic mice.

The following data bring evidence that activation of SSAO/PAO in normoglycemic mice does not reduce uric acid circulating levels and that the invalidation of SSAO/PAO does not influence its levels, either.

Methods

Animals and treatments

All mice were handled in accordance with the guidelines of the French National Institute of Medical Research (INSERM) for experimental animal care. They were housed under specific pathogen-free conditions, at 20 to 22°C, with a 12-hour light-dark cycle and free access to food and water. Male db-/- and their heterozygotes (db-/+) for the naturally occurring mutation of the *db* gene, encoding for leptin receptor, were offspring of genitors from Charles River Laboratories France. After weaning (4-week old), the males were separated in two groups, one receiving benzylamine in the drinking water at 0.5% (changed weekly) while the other group of littermates remained untreated. At the age of 6 weeks, the db-/- animals, phenotyped on their overweight were separated from their lean db-/+ littermates to facilitate the weekly follow-up of growth and food consumption. At the end of treatment (11 weeks of age), body mass and unfasted blood glucose were measured in all the groups as well as urea, uric acid and various metabolic circulating parameters.

AOC3-KO mice (knock out for SSAO/PAO) generously given by Pr S. Jalkanen and colleagues (Turku, Finland) on C57Bl6 background were crossed with FVB/N mice for two generations in our breeding center (Service Zootechnie, UMS 006, Anexplo, Toulouse, France). AOC3 gene invalidation was confirmed by RT PCR as previously reported (Stolen *et al.*, 2005). Homozygotes for the invalidated gene and homozygotes for the wild type gene were used as AOC3-KO and WT control, respectively with heterozygoteous littermates excluded from the study. Mice were sacrificed at 26-28 weeks of age after overnight fasting and subcutaneous white adipose (SCWAT) was frozen for subsequent determination of benzylamine oxidation.

Determination of circulating parameters and of tissue-bound amine oxidase activity

Assays of circulating uric acid were performed with a kit from Horiba ABX Medical (Montpellier, France) using an uricase-peroxidase method and a spectrophotometric detection at 550 nm of the hydrogen peroxide generated during the catalysis of uric acid into allantoin, based on the use of peroxidase and Amino-4-antipyrine / N-ethyl-N-(2-hydroxy-3-sulfopropyl) n-toluidine.

Urea was determined with a kit from Horiba ABX Medical (Montpellier, France) using an urease-glutamate dehydrogenase method. Urea is hydrolysed in presence of urease to produce ammonia, which combines with 2-oxoglutarate and NADH and is converted by glutamate dehydrogenase to glutamate and NAD. The decrease of NADH was spectrophotometrically detected at 340 nm. The volumes of samples and reactives were adapted to 96-well

microplates for use in a multi-analyser ABX Pentra 400. Blood glucose levels were determined using an Accu-Chek glucose monitor (Roche Diagnostic, Meylan, France). Amine oxidase activity was measured on homogenates of thawed SCWAT with 0.1 mM [^{14}C]-benzylamine in 200 mM phosphate buffer and in the presence of an antiprotease cocktail from Sigma as previously described (Visentin *et al.*, 2005). SSAO/ PAO activity was sensitive to semicarbazide 1 mM as previously documented (Prévot *et al.*, 2007) .

Chemicals

Benzylamine hydrochloride, semicarbazide, and other reagents were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). [^{14}C]-benzylamine (57 mCi/mmol) came from Amersham Biosciences (Buckinghamshire, UK).

Statistical analysis

Results are given as means ± S.E.M. Differences between BZA-treated or Knock out and respective control were determined by Student's *t* test.

Results

In vivo activation of SSAO/PAO and uric acid levels

In a previous experiment, oral administration of benzylamine at 510 mg/kg bw/day resulted in a decrease in plasma uric acid in mice fed a high-fat diet (HFD) (Iffiú-Soltész *et al.*, 2010). We observed that plasma uric acid fell from 365.9 ± 32.4 in HFD mice to 234.1 ± 13.6 µmol/l in the goup subjected to HFD + benzylamine (n = 12, p<0.001). Our first aim was to confirm whether such changes induced by continual administration of benzylamine could be reproduced in db-/- mice, a model of obesity and diabetes that is more drastic than the HFD-induced ones, and which offers the advantage to occur with the same standard chow given to lean control littermates.

Table 1 shows that administration of benzylamine in the drinking water, leading to a oral spontaneous ingestion of approximately 1000 mg/kg bw/day during 8 weeks, lowered the plasma uric acid in the obese and diabetic db-/- mice. Such reduction was concomitant with a reduction of the dramatically elevated blood glucose, a typical characteristic of this genetic model of diabetes associated to obesity. To distinguish whether the involved mechanism was dependent on the ammonia generation or on the mitigation of insulin resistance and/or improvement of glucose tolerance, the same treatment was performed in non-diabetic, non-obese mice. By contrast, this treatment did not modify the blood glucose or the plasma uric acid of the lean heterozygous control mice. Indeed, the db-/+ mice were normoglycemic and non-obese and they did not exhibit any detectable change in response to benzylamine treatment. Lastly, the db-/- mice did not exhibit any significant change in their circulating urea, and benzylamine was without any influence on this parameter in both genotypes. Therefore, benzylamine administration was able to modify the circulating levels of uric acid only when they were elevated and associated to a diabetic state or to metabolic syndrome. Moreover, the change of ammonia production that is suspected to occur once SSAO/PAO is activated by increasing its substrate availability was unable to alter the plasma urea in diabetic as in non-diabetic conditions, while it was associated with a clear decrease in uric acid only when there was a normalisation of the hyperglycemia. This latter beneficial issue was likely the consequence of the benzylamine oxidation by SSAO, since it has been reported in both in vitro and in vivo models that the insulin-like effects of SSAO/PAO substrates are mediated by one of the end products of amine oxidation: hydrogen peroxide.

Invalidation of SSAO/PAO and uric acid levels

Thus, it was decided to determine the uric acid levels in mice invalidated for the AOC3 gene, which encodes for SSAO/PAO. These mice were totally unable to oxidize benzylamine (Table 2) or any of the other SSAO substrates (not shown). Under standard conditions of

breeding, they appeared overall healthy and their plasma uric levels were unchanged compared to their respective controls (table 2). Therefore, the complete obliteration of the ammonia production mediated by the SSAO-dependent oxidation of endogenous or exogenous amines was not sufficient to alter uric acid production/elimination in such mice. Similarly, the complete lack of SSAO activity was without any consequence on the fasting glycemia (table 2).

To summarize our observations of the diverse mouse models studied, it can be suggested that SSAO/PAO activation by prolonged challenge to one of its substrates, or SSAO/PAO complete blockade, are not able on their own to modify uric acid levels when they are considered as being in the normal range. The decrease in uric acid seems to accompany the normalization of blood glucose that can be observed with SSAO substrate administration.

Discussion

The lowering effect of benzylamine on circulating uric acid in db-/- diabetic mice is in perfect agreement with the observed reduction in the levels of this metabolite in HFD-fed mice drinking benzylamine (Iffiú-Soltész *et al.*, 2010). In this former report, it was suggested that the oxidation of benzylamine was required to observe such effects. In fact, the acute effect of benzylamine on glucose tolerance, which leads to a reduction in the hyperglycemic excursion after a glucose load, has been reported to be abolished by previous treatment with semicarbazide, an inhibitor of SSAO/PAO (Iglesias-Osma *et al.*, 2004). Such a hypothesis of the involvement of SSAO/PAO activity in the antidiabetic action of prolonged benzylamine treatment totally fits with the fact that benzylamine is also active in db-/- mice regarding to the higher SSAO/PAO activity we found in the fat depots of db-/- when compared to their lean littermattes (Iffiú-Soltész *et al.*, 2011).Moreover, our data confirm that plasma uric acid is a surrogate marker of metabolic syndrome, which increases in type 2 diabetic conditions, and which can be almost normalized once the hyperglycemia is alleviated. It was the case here for the db-/- mice in untreated and benzylamine- treated conditions, respectively.

Indeed, it has already been proposed that a portion of the ingested benzylamine can be taken up through the intestinal barrier and can be oxidized in peripheral tissues (Iffiú-Soltész *et al.*, 2010). Such peripheral oxidation by SSAO/PAO and MAO-B facilitated glucose utilization, especially in fat cells (Iffiú-Soltész *et al.*, 2007; Mercader *et al.*, 2010) while SSAO substrates have been described also to increase glucose consumption inn cultured smooth muscle cells (El Hadri *et al.*, 2002) and by immune cells (Lin *et al.*, 2011). As a consequence, it appeared that the chronic benzylamine administration improves glucose disposal in diabetic mice by facilitating its use in peripheral tissues (Iffiú-Soltész *et al.*, 2007). However it remains unclear how this resulted in a decrease of uric acid once diabetes was partially or entirely prevented. Benzylamine oxidation was supposed to occur mainly in the WAT of the genetically obese/diabetic treated mice (owing to the elevated SSAO/PAO expression in the enlarged fat depots) (Iffiú-Soltész *et al.*, 2011), and by consequence, was improving one major sign of the metabolic syndrome: the elevated blood glucose. Then, others components of the metabolic syndrome of the db-/- model should have been normalized either, including the elevated levels of uric acid. The fact that benzylamine participated in lowering uricemia, without modifying urea levels may indicate that uric acid was more concerned as a metabolic syndrome marker than as a nitrogen catabolism end-product. While further studies on this model can pour more light on this issue, the verification we made in the non-diabetic, non-obese mice showed that benzylamine ingestion/oxidation on its own was unable to modify the

plasma uric acid levels. This definitively indicated that the changes induced by daily ingestion of benzylamine on uric acid turnover were more related to the observed mitigation of glucose tolerance and insulin sensitivity than to an alteration in the control of waste products of nitrogen metabolism.

To further test whether ammonia production by SSAO/PAO could influence uric acid metabolism/solubility, it was feasible to verify the consequences of the complete invalidation of this amine oxidase since the mice KO-AOC3 are viable (Stolen *et al.*, 2005) and devoid of capacity to oxidize benzylamine (Bour *et al.*, 2009).Our results show that the lack of SSAO/PAO is without noticeable influence on uric acid levels, at least in the context of normoglycemia. Therefore, the complete obliteration of the ammonia production mediated by the SSAO-dependent oxidation of endogenous or exogenous amines was not sufficient to alter uric acid production/elimination in the KO-AOC3 mice.

Taken together, our observations of long-term activation of SSAO/PAO by benzylamine, (a protoalkaloid found in several medicinal plants and present at low amount in edible vegetables) showed that the levels of uric acid can be modified only with the occurrence of an improvement of glucose intolerance and insulin resistance. It remains to be determined whether the role of SSAO/PAO in inflammatory process (O'Rourke *et al.*, 2008) (via its action on leukocyte adhesion (Jalkanen *et al.*, 2007)) is involved in such beneficial effect for reducing hyperuricemia. Nonetheless, when glycemic control is unaltered, any increase or blockade of SSAO/PAO activity has no detectable influence on uric acid production/elimination. Our supposed link of compensatory mechanism between ammonia production and nitrogen waste production was far from being evidenced, at least when SSAO/PAO is considered as instrumental to alter ammonia production in the organism. Similarly, our investigations did not allow to precise whether hyper uricemia is a causative process or a protective feedback in the metabolic syndrome.

Thus, uric acid, a multifunctional metabolite which is a the crossroads of nitrogen waste excretion and of antioxidant defences or glucose homeostasis, can be influenced by SSAO/PAO activity in an indirect manner that depends more on an overall improvement of glucose disposal in diabetic states than on nitrogen catabolism.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

References

BECKER BF, REINHOLZ N, LEIPERT B, RASCHKE P, PERMANETTER B, GERLACH E: Role of uric acid as an endogenous radical scavenger and antioxidant. *Chest* **100**: 176S-181S, 1991.

BOUR S, CASPAR-BAUGUIL S, IFFIÚ-SOLTÉSZ Z, NIBBELINK M, COUSIN B, MIILUNIEMI M, SALMI M, STOLEN C, JALKANEN S, CASTEILLA L, PÉNICAUD L, VALET P, CARPÉNÉ C: Semicarbazide-sensitive amine oxidase/vascular adhesion protein-1 deficiency reduces leukocyte infiltration into adipose tissue and favors fat deposition. *Am J Pathol,* **174**: 1075-1083, 2009.

BOUR S, DAVIAUD D, GRES S, LEFORT C, PRÉVOT D, ZORZANO A, WABITSCH M, SAULNIER-BLACHE J-S, VALET P, CARPÉNÉ C: Adipogenesis-related increase of semicarbazide-sensitive amine oxidase and monoamine oxidase in human adipocytes. *Biochimie* **89**: 916-925, 2007.

BOWYER RC, MCKULLOCH RK, BROCKIS JG, RYAN GD: Factors affecting the solubility of ammonium acid urate. *Clinica Chimica Acta* **95**:17-22, 1979.

CARPÉNÉ C: Amine oxidases in adipose tissue-related disorders. In: *Copper Amine Oxidases: Structures, Catalytic Mechanisms and Role in Pathophysiology.* G. FLORIS, B. MONDOVI (eds), CRC Press, Boca Raton, 2009, pp177-194,.

COE FL, EVAN A, WORCESTER E: Kidney stone disease. *J Clin Invest* **115**: 2598–2608, 2005.

EL HADRI K, MOLDES M, MERCIER N, ANDREANI M, PAIRAULT J, FÈVE B: Semicarbazide-sensitive amine oxidase in vascular smooth muscle cells: differentiation-dependent expression and role in glucose uptake. *Arterioscler Thromb Vasc Biol* **22**:89-94, 2002.

IFFIÚ-SOLTÉSZ Z, MERCADER J, DAVIAUD D, BOUCHER J, CARPÉNÉ C: Increased primary amine oxidase expression and activity in white adipose tissue of obese and diabetic db-/- mice. *J Neural Transm* in press:PMID: 21298297, 2011.

IFFIÚ-SOLTÉSZ Z, PRÉVOT D, GRÈS S, BOUR S, SZÖKŐ É, KNAUF C, BURCELIN R, FERNÁNDEZ-QUINTELA A, LOMBA A, MILAGRO F, CARPÉNÉ C: Influence of benzylamine acute and chronic administration of benzylamine on glucose tolerance in diabetic and obese mice fed on very high-fat diet *J Physiol Biochem* **63**:3 05-316, 2007.

IFFIÚ-SOLTÉSZ Z, WANECQ E, LOMBA A, PORTILLO MP, PELLATI F, SZÖKO E, BOUR S, WOODLEY J, MILAGRO FI, MARTINEZ JA, P. V, CARPÉNÉ C: Chronic benzylamine administration in the drinking water improves glucose tolerance, reduces body weight gain and circulating cholesterol in high-fat diet-fed mice. *Pharmacol Res* **61**: 355-363, 2010.

IGLESIAS-OSMA MC, GARCIA-BARRADO MJ, VISENTIN V, PASTOR-MANSILLA MF, BOUR S, PREVOT D, VALET P, MORATINOS J, CARPÉNÉ C: Benzylamine exhibits insulin-like effects on glucose disposal, glucose transport, and fat cell lipolysis in rabbits and diabetic mice. *J Pharmacol Exp Ther* **309**: 1020-1028, 2004.

JALKANEN S, KARIKOSKI M, MERCIER N, KOSKINEN K, HENTTINEN T, ELIMA K, SALMIVIRTA K, SALMI M: The oxidase activity of vascular adhesion protein-1 (VAP-1) induces endothelial E- and P-selectins and leukocyte binding. *Blood* **110**: 1864-1870, 2007.

LIN Z, LI H, LUO H, ZHANG Y, LUO W: Benzylamine and methylamine, substrates of semicarbazide-sensitive amine oxidase, attenuate inflammatory response induced by lipopolysaccharide. *Int Immunopharmacol* in press:PMID: 21414430 2011.

MAALOUF NM: Metabolic syndrome and the genesis of uric acid stones. *J Renal Nutr* **21**: 128–131, 2011.

MERCADER J, IFFIÚ-SOLTESZ Z, BRENACHOT X, FÖLDI A, DUNKEL P, BALOGH B, ATTANÉ C, VALET P, MÁTYUS P, CARPÉNÉ C: SSAO substrates exhibiting

insulin-like effects in adipocytes as a promising treatment option for metabolic disorders. *Future Med Chem* **2**: 1735-1749, 2010.

NUNES SF, FIGUEIREDO IV, PEREIRA JS, TEIXEIRA DE LEMOS E, REIS F, TEIXEIRA F, CARAMONA MM: Monoamine oxidase and semicarbazide-sensitive amine oxidase kinetic analysis in mesenteric arteries of patients with type 2 diabetes. *Physiol Res* **60**: in press, 2011.

O'ROURKE AM, WANG EY, MILLER A, PODAR K, SCHEYHING K, HUANG L, KESSLER C, GAO H, TON-NU H-T, MACDONALD MT, JONES DS, LINNIK MD: Anti-inflammatory effects of LJP 1586 [Z-3-Fluoro-2-(4-methoxybenzyl)allylamine Hydrochloride], an amine-based inhibitor of semicarbazide-sensitive amine oxidase activity. *J Pharmacol Exp Ther* **324**: 867-875, 2008.

PRÉVOT D, SOLTESZ Z, ABELLO V, WANECQ E, VALET P, UNZETA M, CARPÉNÉ C: 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, 2007.

REAVEN G: Syndrome X: 10 years after. *Drugs* **58** (suppl 1): 19-20, 1999.

SOBLE JJ, HAMILTON BD, STREEM SB: Ammonium acid urate calculi: a reevaluation of risk factors. *J Urol* **161**: 869-873, 1999.

STOLEN CM, MARTTILA-ICHIHARA F, KOSKINEN K, YEGUTKIN GG, TURJA R, BONO P, SKURNIK M, HANNINEN A, JALKANEN S, SALMI M: Absence of the endothelial oxidase AOC3 leads to abnormal leukocyte traffic in vivo. *Immunity* **22**: 105-115, 2005.

VISENTIN V, BOUR S, BOUCHER J, PREVOT D, VALET P, ORDENER C, PARINI A, CARPÉNÉ C: 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, 2005.

YOO TW, SUNG KC, SHIN H, KIM BJ, KIM BS, KANG JH, LEE MH, PARK JR, KIM H, RHEE EJ, LEE WY, KIM SW, RYU SH, KEUM DG: Relationship between serum uric acid concentration and insulin resistance and metabolic syndrome. *Circ J* **69**: 928-933, 2005.

Table 1.   Influence of prolonged administration of the SSAO/PAO substrate benzylamine on circulating uric acid and urea levels in genetically obese and diabetic mice and in their lean control.

group:	db-/-	BzA db-/-	db-/+	BzA db-/+
group size	(12)	(7)	(11)	(10)
body mass, g	34.1 ± 0.9	36.8 ± 1.5	24.3 ± 0.7 †	23.1 ± 1.0
glycemia, mg/dL	416.1 ± 33.8	265.7 ± 42.0**	86.1 ± 8.5 †	78.6 ± 6.8
uric acid, µM	458.4 ± 30.3	299.6 ± 21.4***	288.4 ± 20.0 †	272.6 ± 21.2
urea, mM	7.6 ± 1.2	6.0 ± 0.5	5.5 ± 0.7	5.4 ± 0.4

Obese and diabetic db-/- males and their lean controls (db-/+) were treated by benzylamine supplementation in drinking water in order to administrate 1 millimole/kg body weight/day as detailed in Methods (Bza-treated). Results are means ± SEM of the number of cases indicated in parentheses. Different from corresponding control males drinking only water at: **: $p < 0.01$; ***: $p < 0.001$. Different from untreated obese at †: $p < 0.001$.

Table 2. Influence of genetic invalidation of AOC3 gene on circulating glucose and uric acid levels in mouse, and of $[^{14}C]$-benzylamine oxidation in adipose tissue.

group:	WT	AOC3-KO
group size	(13)	(13)
body mass, g	31.7 ± 1.8	33.1 ± 1.9
blood glucose, mg/dL	102.7 ± 5.2	100.1 ± 7.1
plasma uric acid, μM	234.2 ± 20.0	253.3 ± 20.0
Bza oxidation in SCWAT, nmol/mg protein/min	2.173 ± 0.335	0.010 ± 0.001 ***

Eleven-week old mice of both genders with genetic invalidation of the AOC3 gene (AOC3 KO) and their wild type (WT) control were euthanized in fasting conditions. Results are means ± SEM of 13 mice, except for benzylamine (Bza) oxidation performed on 10 subcutaneous white adipose tissue preparations in each genotype. Different from corresponding control at: ***: $p < 0.001$.