UDP-induced relaxation is enhanced in aorta from female obese Otsuka Long–Evans Tokushima Fatty rats

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Abstract Uridine 5'-diphosphate (UDP) plays an important role in controlling vascular tone; however, UDP-mediated response in metabolic syndromes, including obesity and type 2 diabetes in females, remains unclear. In this study, we investigated UDP-mediated response in the aorta of female obese Otsuka Long–Evans Tokushima Fatty (OLETF) rats and control Long–Evans Tokushima Otsuka (LETO) rats. In OLETF rat aortas precontracted by phenylephrine (PE) (vs. LETO), (1) UDP-induced relaxation was increased, whereas acetylcholine (ACh)-induced relaxation was decreased; (2) no UDP- or ACh-induced relaxations were observed in endothelial denudation, whereas UDP-induced small contraction was observed; and (3) N^G-nitro-L-arginine [L-NNA, a nitric oxide (NO) synthase inhibitor] eliminated UDP-induced relaxation and small contraction, whereas caused contrasting responses by ACh, including slight relaxations (LETO) and contractions (OLETF). Indomethacin, a cyclooxygenase inhibitor, eliminated the difference in UDP- and ACh-induced relaxations between the groups by increased UDP-induced relaxation in the LETO group and increased ACh-induced relaxation in the

OLETF group. MRS2578, a P2Y₆ receptor antagonist, eliminated the difference in UDP-induced relaxations between the groups by decreasing UDP-induced relaxation in the OLETF group. MRS2578 had no effect on UDP-induced contraction in endothelium-denuded aortas. Therefore, these findings demonstrate opposite trends of relaxations by UDP and ACh in OLETF and LETO rat aortas. These differences may be attributed to the imbalance between NO and vasoconstrictor prostanoids upon stimulations. Increased UDP-induced relaxation in OLETF rat aorta may be caused by the activation of endothelial MRS2578-sensitive P2Y₆ receptor.

Keywords Obese · Female · P2Y₆ · Relaxation · UDP

Abbreviations

ACh	Acetylcholine
COX	Cyclooxygenase
EDCF	Endothelium-derived contracting factor
EDRF	Endothelium-derived relaxing factor
KHS	Krebs–Henseleit Solution
LETO	Long-Evans Tokushima Otsuka
L-NNA	N ^G -nitro-L-arginine
NO	Nitric oxide
NOS	Nitric oxide synthase
OLETF	Otsuka Long-Evans Tokushima Fatty
PE	Phenylephrine
SNP	Sodium nitroprusside
UDP	Uridine 5'-diphosphate

Shota Kobayashi and Takayuki Matsumoto contributed equally to this work and should be considered as co-first authors.

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Introduction

Extracellular nucleotides (ATP, ADP, UTP, and UDP) act as intercellular signal messengers responding to various stimuli

in (patho)physiological conditions in an autocrine or a paracrine manner through activation of the P2 purinergic receptors family [1, 2]. Based on molecular structure and signaling pathways, so far, the P2 purinergic receptors are subdivided into ionotropic receptors P2X (P2X_{1–7}) and metabotropic G-protein-coupled receptors P2Y (P2Y_{1,2,4,6,11–14}) [1, 2]. Among purinoceptors, P2Y₆ receptor, a receptor for UDP, plays a pivotal role in various responses, including inflammatory and immune responses and cardiovascular physiology [1–6]. For example, UDP has been shown to cause contractile responses in mouse coronary, mesenteric, and renal arteries; rat cerebral, intrapulmonary, mesenteric, and femoral arteries and human cerebral arteries [7–13]. Moreover, Bar et al. [14] observed that UDP led to endothelium-dependent relaxation in mouse aorta, but this relaxation was abolished in P2Y₆ receptor-deficient mice, and UDP-induced contraction was observed in aorta under nitric oxide (NO) synthase (NOS) inhibition, but this contraction was abolished in P2Y₆ receptor-deficient mice. Therefore, UDP plays an important role in the regulation of vascular function. However, UDP-mediated vascular responses in pathophysiological states, especially, in females, remain unexplored.

Metabolic syndrome, which is characterized by obesity, dyslipidemia, high blood pressure, and hyperglycemia, has been shown to increase risk for cardiovascular disease and diabetes [15]. The Otsuka Long–Evans Tokushima Fatty (OLETF) rat is a model of obesity and is characterized by hyperphagia-induced obesity due to a spontaneous lack of cholecystokinin CCK₁ receptors, and this rat is an established model of type 2 diabetes and hyperphagia-induced obesity [16–19]. In male OLETF rats, we [20–24] and others [25] demonstrated vascular dysfunction in several arteries. Conversely, in female OLETF rats, although reproductive or metabolic dysfunction were reported [19, 26, 27], we recently observed that impaired endothelium-derived, hyperpolarization-mediated relaxation was impaired in superior mesenteric arteries for this animal model [28]; no study has focused on the relationship between vascular function and nucleotides in female OLETF rats.

Therefore, we hypothesize that UDP-mediated vascular responses would be altered in aorta isolated from female OLETF rats compared to their genetic control, Long–Evans Tokushima Otsuka (LETO) rats. We investigated the effect of UDP on vascular tone in thoracic aorta isolated from these rats at 12 months of age, which is consistent with the age of male rats exhibiting vascular dysfunction [22–24].

Materials and methods

All experimental procedures were approved by the Hoshi University Animal Care and Use Committee, and all studies were conducted in accordance with the *Guide for the Care and*

Use of Laboratory Animals, published by the US National Institutes of Health, and the *Guide for the Care and Use of Laboratory Animals* adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (accredited by the Ministry of Education, Culture, Sports, Science, and Technology, Japan).

Detailed methods, including the experimental animals, vascular functional studies, drugs and materials, and statistical analysis, are described in the Supplementary Data.

Results and discussion

At the time of the experiment, body weight and nonfasting blood glucose were greater in the OLETF group [595.1 ± 17.2 g ($n = 12$), $P < 0.05$ and 164.7 ± 7.6 mg/dL ($n = 12$), $P < 0.05$] than in the age-matched LETO rats [308.2 ± 5.6 g ($n = 12$) and 115.7 ± 5.6 mg/dL ($n = 12$)].

As shown in Fig. 1a, UDP elicited relaxation in endothelium-intact aorta of both OLETF and LETO rats. Unexpectedly, UDP-induced relaxation was greater in aorta from OLETF rats than from LETO rats, whereas ACh-induced relaxation was impaired in the OLETF group compared to the LETO group (Fig. 1e). SNP-induced relaxation was similar in both groups (Supplementary Fig. S1a). In endothelium-denuded aorta, no relaxation was observed and a higher UDP concentration caused contraction (Fig. 1b). This UDP-induced contraction was lower in the OLETF group than in the LETO group (Fig. 1b). ACh-induced relaxation was completely eliminated by endothelium denudation in both groups (Fig. 1f). These results suggest that UDP induces endothelium-dependent vasorelaxant responses, and there is a differing extent of relaxant responses between UDP and ACh when comparing OLETF and LETO rats.

Since NO is a major endothelium-derived relaxing factor in thoracic aorta and NO bioavailability and/or signaling are impaired in various pathophysiological states, including obesity, hypertension, and diabetes [20–22, 29], we investigated the effects of a NOS inhibitor on UDP- and ACh-induced relaxation (Fig. 1c, g, respectively). Under NOS inhibition with L-NNA (10^{-4} M), UDP-induced contraction was lower in the OLETF group than in the LETO group (Fig. 1c). Conversely, ACh induced a slight relaxation in the LETO group and a contraction in the OLETF group (Fig. 1g). These results suggest that UDP-induced relaxation is largely mediated by NO and the contractile component is unmasked by NOS inhibition, which was lower in OLETF rat aorta (vs. LETO).

To regulate vascular tone, endothelial cells release relaxant [such as endothelium-derived relaxing factors (EDRFs)] and contractile factors [such as endothelium-derived contracting factors (EDCFs)], and the balance between these factors is altered at various disease states [20–22, 29–33]. Moreover, purinoceptor ligands release EDCFs [30, 33]. Among these

Fig. 1 Relaxation responses of thoracic aorta of female Otsuka Long-Evans Tokushima Fatty (OLETF) rats and Long-Evans Tokushima Otsuka (LETO) rats to uridine 5'-diphosphate (UDP) (a–d) and acetylcholine (ACh) (e–h) in intact endothelium (a, e), denuded endothelium (b, f), and in the presence of a nitric oxide synthase

inhibitor (L-NNA; 10^{-4} M) (c, g) or cyclooxygenase inhibitor (indomethacin; 10^{-5} M) (d, h). Results are shown as means $\pm$ SEM; $n = 4$ –8. Number of determinations is shown within parentheses. * $P < 0.05$ vs. LETO

factors, vasoconstrictor prostanoids play an important role in regulating vascular tone by functioning as EDCFs [24, 25, 29, 31, 32]. In addition, in male OLETF rats, EDCF-mediated contraction has been reported to increase in superior mesenteric arteries [20–22]. Moreover, Kagota et al. have reported impaired ACh-induced endothelium-dependent relaxation in OLETF rat aorta (vs. LETO rats), and this impairment was restored by indomethacin [25]. Thus, we investigated the effect of a COX inhibitor indomethacin on UDP- (Fig. 1d and supplementary Table S1) and ACh- (Fig. 1h and supplementary Table S1) induced relaxations in rat aorta. Indomethacin (10^{-5} M) eliminated the difference in UDP- and ACh-induced relaxations between the groups by increasing UDP-induced relaxation in the LETO group and increasing ACh-induced relaxation in the OLETF group. These results suggest that the component of contraction by COX-derived prostanoids may be the key contributor in determining the difference in relaxant responses to each UDP and ACh between LETO and OLETF rat aortas.

Further, we constructed concentration–relaxation curves for another specific $P2Y_6$ agonist MRS2693 (supplementary Fig. S1b) and for UDP in the presence of $P2Y_6$ antagonist MRS2578 (Fig. 2a) to investigate whether UDP-mediated response would activate $P2Y_6$ receptors. Similar to UDP-induced response (Fig. 1a), MRS2693-induced relaxation was greater in OLETF rat aorta than in LETO rat aorta (Fig. S1b). Moreover, the difference in UDP-induced relaxation between the groups was eliminated in the presence of MRS2578 resulting from the decreased UDP-induced relaxation in the OLETF rat aorta (Fig. 2a and Table S1). These findings suggest that the difference in UDP-induced relaxation between the OLETF and

LETO groups is caused by the relaxant component of MRS2578-sensitive $P2Y_6$ receptor activation.

Although this is the first study to investigate aortic vascular function using female OLETF rats, impaired endothelium-dependent relaxation induced by ACh was observed in previous studies using female models of metabolic diseases [34, 35]. We very recently observed that the female OLETF rat at approximately 1 year of age manifests obesity, hypertension, hyperglycemia, hyperinsulinemia, and hyperlipidemia [28]. Endothelial dysfunction is observed in animals suffering from each of the above factors [31, 32]; thus, in female OLETF rats aged 12 months, endothelial dysfunction may result from the long-term suffering of aforementioned phenomena. A novel and important finding is that UDP-induced relaxation is increased from OLETF rat aorta (vs. LETO rats) even if ACh-induced relaxation is decreased in OLETF rat aorta (vs. LETO rat aorta). There are different interactions of endothelium-derived factors with UDP and ACh in aorta isolated from female OLETF and LETO rats. Mechanisms underlying these alterations based on our results are shown in Fig. 2c. NO (relaxant effect) and prostanoids (contractile effect) participate in the UDP-induced aortic response in LETO rats, but the prostanoid component is smaller in the response of OLETF rat aorta. Because UDP-induced relaxation was similar between both the groups under COX inhibition, the NO component upon UDP stimulation was also similar between both the groups. Moreover, with regard to the vasomotor properties of endothelium-derived NO (relaxation) and prostanoids (contraction), (1) UDP-induced relaxation in endothelium-intact PE-precontracted aorta (viz., relaxation > contraction) and (2) UDP-induced contraction was greater in endothelium-

Fig. 2 **a** Relaxation responses of thoracic aorta of female Otsuka Long–Evans Tokushima Fatty (OLETF) rats and Long–Evans Tokushima Otsuka (LETO) rats to uridine 5′-diphosphate (UDP) in the presence of the selective P2Y₆ antagonist MRS2578 (10^{-5} M). **b** Contractile responses of the endothelium-denuded aorta of OLETF and LETO rats to UDP in the absence (vehicle, dimethyl sulfoxide) and presence of MRS2578 (10^{-5} M). Results are shown as means $\pm$ SEM; $n = 6$ –8. Number of determinations is shown within parentheses. * $P < 0.05$, LETO vehicle vs. OLETF vehicle. # $P < 0.05$, LETO MRS2578 vs. OLETF MRS2578. **c** Summary of the present results is described in detail in the main text

denuded preparations (Fig. 2b) than in intact endothelium preparations (Fig. S1c). If prostanoids were predominant, the response would have decreased by endothelial denudation. Considering these results, NO is predominant rather than prostanoids upon UDP stimulation (Fig. 2c). In contrast, NO and prostanoids contributed to the ACh-induced aortic response in OLETF rats, whereas prostanoid contributed less to the response in LETO rat aorta. Varying contributions of endothelium-derived factors are seen in arteries of various disease models, including hypertension and diabetes [31, 32]. We speculate that the mode of generation and/or release of endothelium-derived factors (EDRFs and EDCFs) upon UDP or ACh stimulation in endothelial cells may be altered as a result of chronic diseases because both NO and COX-derived prostanoids could be generated by activation of the P2Y₆ and muscarinic receptors [1, 2, 20–22]. In the present study, a UDP-induced contractile response was observed under endothelium denudation and NOS inhibition, and it was decreased in OLETF rat aorta than in LETO rat aorta. Moreover, the UDP-induced contraction was greater with NOS inhibition [OLETF, $-15.92 \pm 3.69\%$ (% relaxation of PE-induced precontraction) ($n = 8$); LETO, $-28.5 \pm 3.10\%$ ($n = 8$)] rather than in endothelium denudation [OLETF, $-7.31 \pm 1.70\%$ ($n = 7$); LETO, $-14.13 \pm 2.64\%$ ($n = 8$)]. Therefore, we cannot rule out the

possibility that P2Y₆ activation on smooth muscle cells may generate prostanoids. However, further investigations are required; for example, time-course alterations in both aortic expressions of P2Y₆ receptors and NOS and COX activities in this model need clarification.

Notably, the present study has limitations. Some reports suggest that UDP induces vasorelaxation and vasoconstriction in various arteries [7–13]. Moreover, Bar et al. [14] have demonstrated, using P2Y₆ receptor knockout mice, that functional P2Y₆ receptors were present in endothelial and smooth muscle cells. We found that MRS2578 only affects the elimination of the difference between UDP-induced relaxation in PE-precontracted aortas in the OLETF and LETO groups; however, relaxation still existed in the presence of MRS2578 (Fig. 2a). Moreover, Fig. 2b shows that MRS2578 did not affect UDP-induced contraction in endothelium-denuded aortas in both OLETF and LETO groups. Because another specific P2Y₆ agonist MRS2693 also caused relaxation, which was increased in OLETF rat aorta than in LETO rat aorta (supplemental Fig. S1b), P2Y₆ receptor stimulation definitely caused vasorelaxation in rat aorta under PE precontraction. However, at present, we can only report receptors, namely, “MRS2578-sensitive” or “MRS2578-insensitive” P2Y₆ receptors (Fig. 2c). Moreover, we speculate that the activation

component of MRS2578-sensitive P2Y₆ receptor by UDP stimulation in endothelium may be attributable to the difference in UDP-induced relaxations between the OLETF and LETO groups. However, we cannot define which effect preferably increased NO or suppressed prostanoid productions upon MRS2578-sensitive P2Y₆ receptor stimulation by UDP. Furthermore, the possible mechanisms underlying the activation of other receptors, such as GPR17 [36], or heterodimers, such as angiotensin type 1/P2Y₆ receptors [37, 38], in our findings may be related to UDP-mediated responses. Because contrasting trends are observed in UDP-induced contraction in endothelium-denuded aorta between the groups, that is, between the basal and PE-precontracted aorta, the role of P2Y₆ (probably MRS2578-sensitive receptor) in contraction may also be different between OLETF and LETO rat aortas. Currently, the relationship between these putative P2Y₆ receptors and vascular functional properties in endothelium and smooth muscle cells remain unclear in female OLETF rat aortas. Thus, further investigations of these mechanisms are required.

Conclusions

In the present study, we observed that UDP-induced relaxation was increased in aorta from female OLETF rats compared to control LETO rats. These alterations may result from changes in the production of endothelium-derived factors on UDP stimulation. Although the precise pathophysiological significance of UDP signaling in the female obese state is not yet clear, we believe that our findings should stimulate further interest in the UDP-mediated vasomotor response as a potential therapeutic target in female metabolic syndrome-associated vascular complications.

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Compliance with ethical standards

Conflicts of interest Shota Kobayashi declares that he has no conflict of interest.

Takayuki Matsumoto declares that he has no conflict of interest.
Makoto Ando declares that he has no conflict of interest.
Maika Iguchi declares that he/she has no conflict of interest.
Shun Watanabe declares that he has no conflict of interest.
Kumiko Taguchi declares that she has no conflict of interest.
Tsuneo Kobayashi declares that he has no conflict of interest.

Ethical approval This study was approved by the Hoshi University Animal Care and Use Committee, and all studies were conducted in accordance with the Guide for the Care and Use of Laboratory

Animals, published by the US National Institutes of Health, and the Guide for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (accredited by the Ministry of Education, Culture, Sports, Science, and Technology, Japan).

References

1. Burnstock G, Ralevic V (2014) Purinergic signaling and blood vessels in health and disease. *Pharmacol Rev* 66:102–192. <https://doi.org/10.1124/pr.113.008029>
2. Erlinge D, Burnstock G (2008) P2 receptors in cardiovascular regulation and disease. *Prinergic Signal* 4:1–20. <https://doi.org/10.1007/s11302-007-9078-7>
3. Burnstock G (2017) Purinergic signaling in the cardiovascular system. *Circ Res* 120:207–228. <https://doi.org/10.1161/CIRCRESAHA.116.309726>
4. Zhang Z, Wang Z, Ren H, Yue M, Huang K, Gu H, Liu M, Du B, Qian M (2011) P2Y₆ agonist uridine 5'-diphosphate promotes host defense against bacterial infection via monocyte chemoattractant protein-1-mediated monocyte/macrophage recruitment. *J Immunol* 186:5376–5387. <https://doi.org/10.4049/jimmunol.1002946>
5. Stachon P, Peikert A, Michel NA, Hergeth S, Marchini T, Wolf D, Dufner B, Hoppe N, Ayata CK, Grimm M, Cicko S, Schulte L, Reinohl J, von zur Muhlen C, Bode C, Idzko M, Zirlik A (2014) P2Y₆ deficiency limits vascular inflammation and atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 34:2237–2245. <https://doi.org/10.1161/ATVBAHA.114.303585>
6. Riegel AK, Faigle M, Zug S, Rosenberger P, Robaye B, Boeynaems JM, Idzko M, Eltzschig HK (2011) Selective induction of endothelial P2Y₆ nucleotide receptor promotes vascular inflammation. *Blood* 117:2548–2555. <https://doi.org/10.1182/blood-2010-10-313957>
7. Vial C, Evans RJ (2002) P2X₁ receptor-deficient mice establish the native P2X receptor and a P2Y₆-like receptor in arteries. *Mol Pharmacol* 62:1438–1445
8. Malmisjo M, Hou M, Pendergast W, Erlinge D, Edvinsson L (2003) Potent P2Y₆ receptor mediated contractions in human cerebral arteries. *BMC Pharmacol* 3:4
9. Vonend O, Stegbauer J, Sojka J, Habel S, Quack I, Robaye B, Boeynaems JM, Rumb LC (2005) Noradrenaline and extracellular nucleotide cotransmission involves activation of vasoconstrictive P2X₁(,3)- and P2Y₆-like receptors in mouse perfused kidney. *Br J Pharmacol* 145:66–74
10. Kauffenstein G, Tamarelle S, Prunier F, Roy C, Ayer A, Toutain B, Billaud M, Isakson BE, Grimaud L, Loufrani L, Rousseau P, Abraham P, Procaccio V, Monyer H, de Wit C, Boeynaems JM, Robaye B, Kwak BR, Henrion D (2016) Central role of P2Y₆ UDP receptor in arteriolar myogenic tone. *Arterioscler Thromb Vasc Biol* 36:1598–1606. <https://doi.org/10.1161/ATVBAHA.116.307739>
11. Haanes KA, Spray S, Syberg S, Jorgensen NR, Robaye B, Boeynaems JM, Edvinsson L (2016) New insight on pyrimidine signalling within the arterial vasculature—different roles for P2Y₂ and P2Y₆ receptors in large and small coronary arteries of the mouse. *J Mol Coll Cardiol* 93:1–11. <https://doi.org/10.1016/j.jmcc.2016.01.025>
12. Mitchell C, Syed NI, Tengah A, Gurney AM, Kennedy C (2012) Identification of contractile P2Y₁, P2Y₆, and P2Y₁₂ receptors in rat intrapulmonary artery using selective ligands. *J Pharmacol Exp Ther* 343:755–762. <https://doi.org/10.1124/jpet.112.198051>
13. Matsumoto T, Tostes RC, Webb RC (2012) Alterations in vasoconstrictor responses to the endothelium-derived contracting factor

- uridine adenosine tetraphosphate are region specific in DOCA-salt hypertensive rats. *Pharmacol Res* 65:81–90. <https://doi.org/10.1016/j.phrs.2011.09.005>
14. Bar I, Guns PJ, Metallo J, Cammarata D, Wilkin F, Boeynaems JM, Bult H, Robaye B (2008) Knockout mice reveal a role for P2Y6 receptor in macrophage, endothelial cells, and vascular smooth muscle cells. *Mol Pharmacol* 74:777–784. <https://doi.org/10.1124/mol.108.046904>
 15. Ballantyne CM, Hoogeveen RC, McNeill AM, Heiss G, Schmidt MI, Duncan BB, Pankow JS (2008) Metabolic syndrome risk for cardiovascular disease and diabetes in the ARIC study. *Int J Obes* 32:S21–S24. <https://doi.org/10.1038/ijo.2008.31>
 16. Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M, Natori T (1992) Spontaneous long-term hyperglycemic rat with diabetic complications. Otsuka Long-Evans Tokushima Fatty (OLETF) strain. *Diabetes* 41:1422–1428
 17. Kawano K, Hirashima T, Mori S, Natori T (1994) OLETF (Otsuka Long-Evans Tokushima Fatty) rat: a new NIDDM rat strain. *Diabetes Res Clin Pract* 24:S317–S320
 18. Moran TH, Bi S (2006) Hyperphagia and obesity of OLETF rats lacking CCK1 receptors: developmental aspects. *Dev Psychobiol* 48:360–367
 19. Schroeder M, Zagoory-Sharon O, Shbiro L, Marco A, Hyun J, Moran TH, Bi S, Weller A (2009) Development of obesity in the Otsuka Long-Evans Tokushima Fatty rat. *Am J Physiol Regul Integr Comp Physiol* 297:R1749–R1760. <https://doi.org/10.1152/ajpregu.00461.2009>
 20. Matsumoto T, Kakami M, Noguchi E, Kobayashi T, Kamata K (2007) Imbalance between endothelium-derived relaxing and contracting factors in mesenteric arteries from aged OLETF rats, a model of type 2 diabetes. *Am J Physiol Heart Circ Physiol* 293: H1480–H1490
 21. Matsumoto T, Noguchi E, Ishida K, Kobayashi T, Yamada N, Kamata K (2008) Metformin normalizes endothelial function by suppressing vasoconstrictor prostanoids in mesenteric arteries from OLETF rats, a model of type 2 diabetes. *Am J Physiol Heart Circ Physiol* 295:H1165–H1176. <https://doi.org/10.1152/ajpheart.00486.2008>
 22. Matsumoto T, Nakayama N, Ishida K, Kobayashi T, Kamata K (2009) Eicosapentaenoic acid improves imbalance between vasodilator and vasoconstrictor actions of endothelium-derived factors in mesenteric arteries from rat at chronic stage of type 2 diabetes. *J Pharmacol Exp Ther* 329:324–334. <https://doi.org/10.1124/jpet.108.148718>
 23. Matsumoto T, Watanabe S, Kawamura R, Taguchi K, Kobayashi T (2014) Epigallocatechin gallate attenuates ET-1-induced contraction in carotid artery from type 2 diabetic OLETF rat at chronic stage of disease. *Life Sci* 118:200–205. <https://doi.org/10.1016/j.lfs.2013.11.016>
 24. Matsumoto T, Watanabe S, Ando M, Yamada K, Iguchi M, Taguchi K, Kobayashi T (2016) Diabetes and age-related differences in vascular function of renal artery: possible involvement of endoplasmic reticulum stress. *Rejuvenation Res* 19:41–52. <https://doi.org/10.1089/rej.2015.1662>
 25. Kagota S, Yamaguchi Y, Nakamura K, Kunitomo M (2000) Altered endothelium-dependent responsiveness in the aortas and renal arteries of Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model of non-insulin-dependent diabetes mellitus. *Gen Pharmacol* 34:201–209
 26. Amon L, Hazut N, Tabachnik T, Weller A, Koren L (2016) Maternal testosterone and reproductive outcome in a rat model of obesity. *Theriogenology* 86:1042–1047. <https://doi.org/10.1016/j.theriogenology.2016.03.033>
 27. Schroeder M, Kronfeld-Schor N, Weller A (2013) Selective leptin insensitivity and alterations in female-reproductive patterns linked to hyperleptinemia during infancy. *PLoS One* 8:e59937. <https://doi.org/10.1371/journal.pone.0059937>
 28. Matsumoto T, Kobayashi S, Ando M, Watanabe S, Iguchi M, Taguchi K, Kobayashi T (2017) Impaired endothelium-derived hyperpolarization-type relaxation in superior mesenteric arteries isolated from female Otsuka Long-Evans Tokushima Fatty rats. *Eur J Pharmacol* 807:151–158. <https://doi.org/10.1016/j.ejphar.2017.03.062>
 29. McCarthy CG, Wenceslau CF, Gouloupoulou S, Ogbi S, Matsumoto T, Webb RC (2016) Autoimmune therapeutic chloroquine lowers blood pressure and improves endothelial function in spontaneously hypertensive rats. *Pharmacol Res* 113:384–394. <https://doi.org/10.1016/j.phrs.2016.09.008>
 30. Alsaqati M, Chan SL, Ralevic V (2014) Investigation of the functional expression of purine and pyrimidine receptors in porcine isolated pancreatic arteries. *Purinergic Sig* 10:241–249. <https://doi.org/10.1007/s11302-013-9403-2>
 31. Matsumoto T, Gouloupoulou S, Taguchi K, Tostes RC, Kobayashi T (2015) Constrictor prostanoids and uridine adenosine tetraphosphate: vascular mediators and therapeutic targets in hypertension and diabetes. *Br J Pharmacol* 172:3980–4001. <https://doi.org/10.1111/bph.13205>
 32. Vanhoutte PM, Shimokawa H, Feletou M, Tang EH (2017) Endothelial dysfunction and vascular disease—a 30th anniversary update. *Acta Physiol* 219:22–96. <https://doi.org/10.1111/apha.12646>
 33. Ishida K, Matsumoto T, Taguchi K, Kamata K, Kobayashi T (2011) Mechanisms underlying altered extracellular nucleotide-induced contractions in mesenteric arteries from rats in later-stage type 2 diabetes: effect of ANG II type 1 receptor antagonism. *Am J Physiol Heart Circ Physiol* 301:H1850–H1861. <https://doi.org/10.1152/ajpheart.00502.2011>
 34. Naderali EK, Brown MJ, Pickavance LC, Wilding JP, Doyle PJ, Williams G (2001) Dietary obesity in the rat induces endothelial dysfunction without causing insulin resistance: a possible role for triacylglycerols. *Clin Sci* 101:499–506
 35. Pieper GM, Siebeneich W, Moore-Hilton G, Roza AM (1997) Reversal by L-arginine of a dysfunctional arginine/nitric oxide pathway in the endothelium of the genetic diabetic BB rat. *Diabetologia* 40:910–915
 36. Ciana P, Fumagalli M, Trincavelli ML, Verderio C, Rosa P, Lecca D, Ferrario S, Parravicini C, Capra V, Gelosa P, Guerrini U, Belcredito S, Cimino M, Sironi L, Tremoli E, Rovati GE, Martini C, Abbracchio MP (2006) The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor. *EMBO J* 25:4615–4627. <https://doi.org/10.1038/sj.emboj.7601341>
 37. Nishimura A, Sunggip C, Tozaki-Saitoh H, Shimauchi T, Numaga-Tomita T, Hirano K, Ide T, Boeynaems JM, Kurose H, Tsuda M, Robaye B, Inoue K, Nishida M (2016) Purinergic P2Y6 receptor heterodimerize with angiotensin AT1 receptors to promote angiotensin II-induced hypertension. *Sci Signal* 9:ra7. <https://doi.org/10.1126/scisignal.aac9187>
 38. Sunggip C, Nishimura A, Shimoda K, Numaga-Tomita T, Tsuda M, Nishida M (2017) Purinergic P2Y6 receptors: a new therapeutic target of age-dependent hypertension. *Pharmacol Res* 120:51–59. <https://doi.org/10.1016/j.phrs.2017.03.013>