Receptors & Clinical Investigation 2014; 1: e344. doi: 10.14800/rci.344; © 2014 by Daniel L. Sparks, *et al.*
http://www.smartscitech.com/index.php/rci

REVIEW

Circulating nucleotide levels in health and disease

Daniel L. Sparks, Heather Doelle, Cynthia Chatterjee

Atherosclerosis, Genetics and Cell Biology Group, University of Ottawa Heart Institute, Ottawa, Ontario, Canada

Correspondence: Daniel L. Sparks
E-mail: dsparks@ottawaheart.ca
Received: September 15, 2014
Published online: November 15, 2014

The concentration of adenosine nucleotides (ATP&ADP) in the circulation may impact health and disease by controlling cellular metabolic pathways in tissues throughout the body. Fasting plasma nucleotide levels are normally relatively low in healthy subjects, but can increase by orders of magnitude in disease. High circulating nucleotide levels promote chronic purinergic signaling, which may disrupt the normal cellular metabolism in the body and initiate immune and inflammatory consequences. Strategies to control nucleotide levels in the bloodstream may therefore have therapeutic importance to halt the progression of metabolic disorders.

Keywords: ADP; ATP; nucleotide release; plasma nucleotides; purinergic signaling; postprandial; autophagy; apoptosis; metabolic disorders; inflammatory disease

To cite this article: Daniel L. Sparks, *et al*. Circulating nucleotide levels in health and disease. Receptor Clin Invest 2014; 1: e344. doi: 10.14800/rci.344.

Extracellular nucleotides and disease

Adenosine nucleotides in the circulation are potent autocrine/paracrine regulators of cellular metabolism and control both the immune and inflammatory response [1-3]. Nucleotide concentration is carefully controlled to regulate purinergic signaling by coordinating the activation of specific P2X and P2Y receptors (**Figure 1**). Acute and short-lived nucleotide signaling is required for normal cellular function, while chronic purinergic signaling may perturb various metabolic pathways [4-6]. We have shown that extracellular nucleotides regulate lipoprotein secretion from the liver, by controlling intracellular proteolytic pathways in the hepatocyte [7] (**Figure 1**). Other studies have shown that extracellular nucleotides may regulate glucose metabolism by controlling insulin signaling through the insulin receptor [8, 9]. Chronically elevated blood nucleotide levels and sustained purinergic signaling may therefore play a path physiological role in the development of metabolic disorders [6, 10, 11]. Nucleotide-induced signaling events

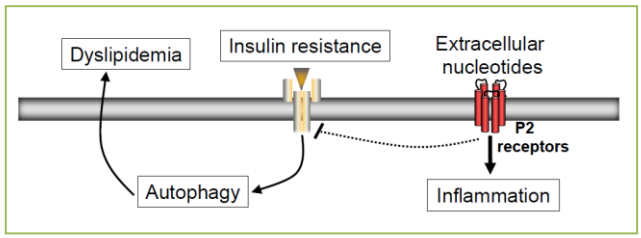

Figure 1. Elevated blood nucleotide levels promote chronic purinergic signaling. Activation of P2 receptors (P2X & P2Y) initiates inflammation through NF-κB and promotes autophagy and dyslipidemia through inhibition of insulin receptor and Akt pathways. The levels of extracellular nucleotides in the circulation may thereby perturb cellular metabolism and promote inflammatory disease.

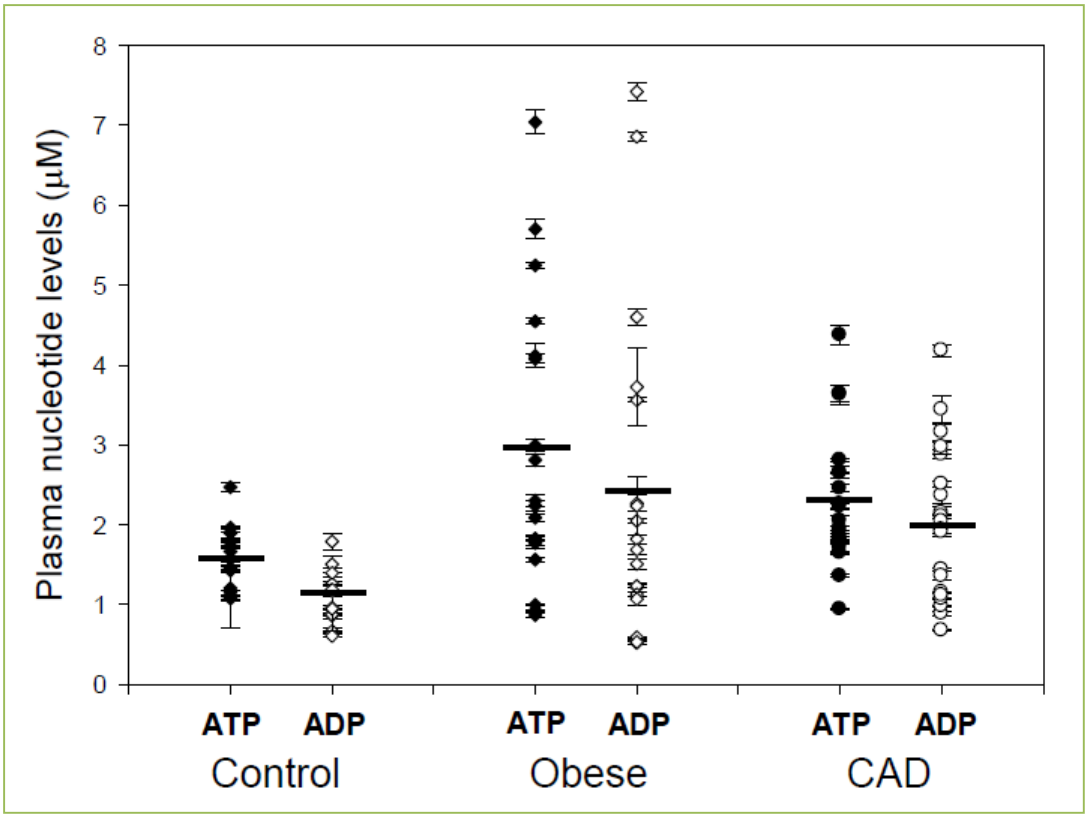

Figure 2. Fasting plasma nucleotide levels are elevated in obese and CAD patients. Plasma samples were isolated from fasted venous blood drawn from 16 control subjects, 18 morbidly obese subjects and 20 coronary artery disease (CAD) patients. Plasma nucleotide levels were measured with a bioluminescent assay and mean values for obese and CAD patients were almost double normal levels. Some obese and CAD patients had fasted nucleotide levels that were 4 to 7-fold elevated over controls.

also stimulate the release of pro-inflammatory cytokines and the activation of inflammatory pathways [12-14] (**Figure 1**). High circulating nucleotides may therefore impact the development of inflammatory disease by promoting an "injury response" in vascular tissues [11-13]. Consistent with this view, knockout of P2 receptors in mice significantly reduces inflammatory disease in the heart, liver, kidney and other tissues [15-20]. Factors that control the levels of extracellular nucleotides in the bloodstream may therefore have important regulatory effects on cellular metabolic pathways [5, 6, 21].

Extracellular nucleotides in the circulation

Nucleotides are released from various tissues in the body and then accumulate in the bloodstream and extracellular fluids. Studies three decades ago quantified human plasma nucleotide levels and showed that both ATP&ADP concentrations in EDTA plasma were near equimolar and in the low μM range [22, 23]. The measurement of adenosine nucleotide concentration in aqueous solutions has been accomplished by both HPLC and luminometry [23, 24]. Commercial bioluminescent assay kits are currently readily available and our characterizations show the analytical technique to be accurate and scalable for routine clinical investigations. These assays utilize luciferase to convert ATP and D-luciferin to oxyluciferin and light and then luminescence is quantified in a luminometer. Serum samples interfere with the luminescence assay reagents and therefore cannot be evaluated. Plasma samples exhibit minimal interference. We observe low μM levels of ADP and ATP in fasted EDTA plasma from healthy control subjects and from patients with various metabolic disorders (**Figure 2**). These estimations are in complete agreement with previous measurements made with luminescent assays [24, 25] and by HPLC [22, 23]. Our studies show that plasma nucleotide levels are unaffected by short term storage of the blood or plasma samples at room temperature or long term storage at -20ºC. Earlier work had suggested that plasma nucleotides are quite labile [26, 27] and our work shows that the stability of nucleotide measurements is directly proportional to the level of hemolysis. The consequence of hemolytic events on circulating nucleotides has long been a concern [26] and we have found that hemolysis decreases the measurement stability and

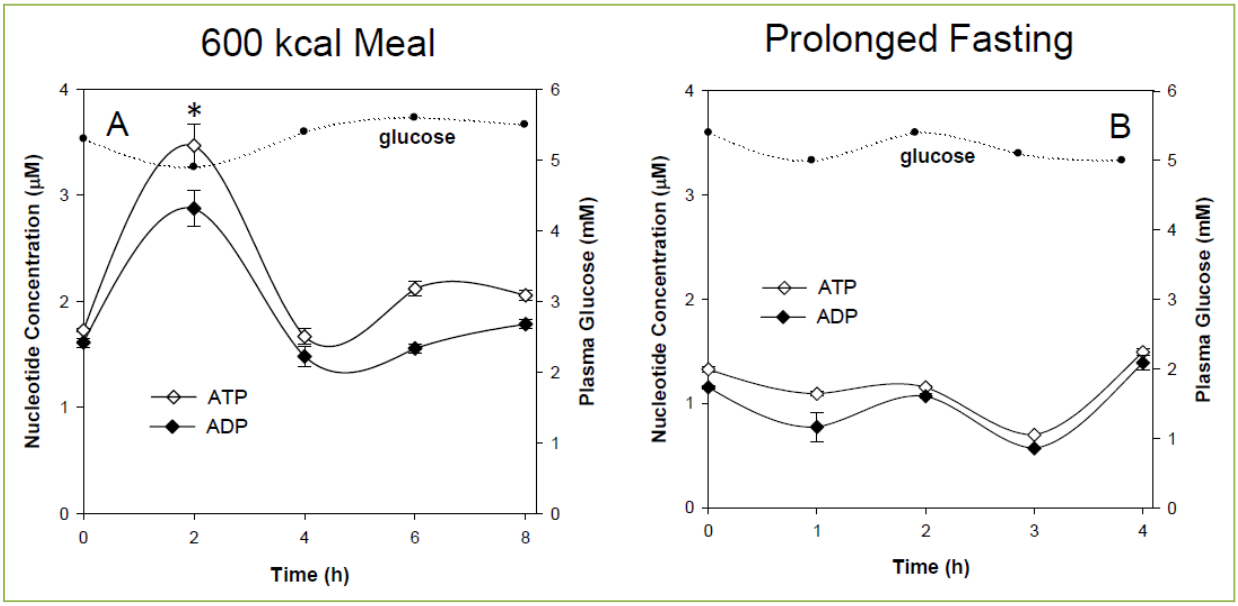

Figure 3. Plasma nucleotide levels exhibit a postprandial response. Plasma samples were isolated from venous blood drawn from a control subject after a 12 hour fast and (**A**) a 600 kcal meal or (**B**) prolonged fasting. Plasma nucleotide levels were measured with a bioluminescent assay and mean values are illustrated relative to plasma glucose levels. (**A**) Plasma nucleotide levels increase quickly and double in magnitude at 2 hours after a 600 kcal meal and then return to normal levels by 4 hours. (**B**) Prolonged fasting has little effect on plasma nucleotide levels.

increases ATP/ADP ratios by selectively increasing the level of ATP. ATP: ADP ratios therefore appear to be a marker for red cell hemolysis. A normal plasma sample ATP: ADP ratio is close to 1, while a hemolysed sample has an ATP: ADP ratio of >2. This would be expected considering the predominant intracellular nucleotide in red blood cells is ATP.

Stress and disease have been reported to increase circulating nucleotide levels [24, 28, 29] and we therefore set out to characterize what would be considered "normal" versus "abnormal" levels of ATP&ADP in the bloodstream. Nucleotide levels in fasted plasma samples from healthy control subjects (n=16) varied between 0.5 to 1.5 μM, with a mean of 1.0 μM for ATP and 0.9 μM for ADP (**Figure 2**). In healthy subjects, ATP concentrations were 20-30% greater than ADP concentrations and ATP: ADP ratios averaged 1.5. We also measured plasma nucleotide levels in morbidly obese subjects and in patients with coronary artery disease (CAD). Nucleotide levels were highly variable in the obese subjects and ranged from 0.5 to 7 μM. While mean nucleotide levels averaged ~2-fold greater than that measured in control subjects, several of the obese subjects had plasma nucleotide levels that were 4 to 7-fold elevated (**Figure 2**). High nucleotide levels observed in some morbidly obese subjects appeared to associate with early stage insulin resistance, since obese subjects with average nucleotide levels > 2.5μM (n=8) had elevated circulating insulin levels, 122+38 pmol/L. Nucleotide levels were also measured in fasting plasma samples from patients with angiographically confirmed CAD. Average nucleotide levels in CAD patients were elevated and approximately double that observed in control plasma samples (**Figure 2**), while ATP: ADP ratios were reduced to ~1.1 as a consequence of higher ADP levels.

Studies have shown that stress and injury may increase circulating nucleotide levels both acutely and chronically [5, 24, 28-30]. Early studies suggested that simple exercise can significantly increase plasma ATP levels [29], while both endotoxin and anaphylactic shock were shown to be associated with low plasma ATP and high ADP levels [24]. We have now shown that circulating nucleotide levels are also directly affected by a postprandial response. Plasma nucleotide levels were measured in peripheral venous plasma samples drawn from human subjects after a 12 hour fast and during a postprandial response to a meal. Circulating nucleotide levels increased significantly during a postprandial response (**Figure 3A**), relative to that observed after prolonged fasting (**Figure 3B**). ATP&ADP levels increased ~2-fold in the bloodstream of control subjects at 2 hours after a 600 kcal (60g carbohydrate, 30g fat, 15g protein) mixed nutrient meal (**Figure 3**). This increase in blood nucleotide level lasted for several hours and then returned to normal levels by 4 hours. In contrast, a 1,400 kcal (153g carbohydrate, 74g fat, 25g protein) meal promoted a 4 to 6-fold increase in plasma nucleotide levels by 4 hours (not shown).

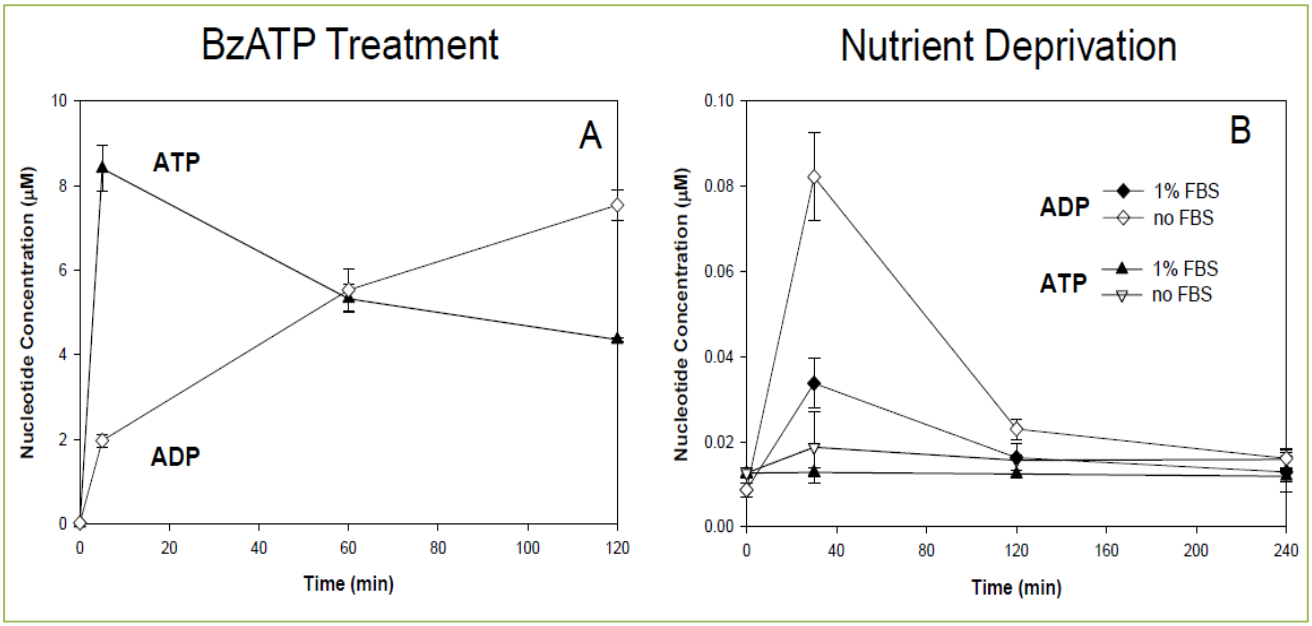

Figure 4. Liver cells can release both ATP and ADP into the media. Extracellular nucleotide concentration in the media of healthy liver cells averages ~20 nM, but increases after treatment with BzATP or after nutrient depletion. (**A**) The P2X agonist, BzATP, rapidly stimulates the release of both ATP and ADP from liver cells. ATP release is 4-fold greater than that for ADP and then ADP levels rise as ATP is degraded. (**B**) ATP levels are unaffected by a reduction in media serum (FBS), while ADP levels in the media increase 8-fold at 30 min. when serum levels are reduced. Newly released ADP is metabolized and cleared from the media by 4 hours.

Preliminary studies were also undertaken to determine the dietary components that contribute to the postprandial nucleotide response. An oral glucose dose (300 kcal/75g) had minimal effect on plasma nucleotide levels, while pure clarified butter fat (315 kcal/35g) increased blood nucleotide levels to a similar extent as the 600 kcal meal. This suggests that circulating nucleotide levels are sensitive to dietary fat. A specific dietary regime may therefore have therapeutic value to normalize elevated circulating nucleotide levels.

Cellular control of extracellular nucleotide levels

Extracellular nucleotide levels in the circulation are a product of both cellular release and extracellular degradation. Nucleotide release has been long known to be a phenomenon of all types of cells [31-33] and a consequence of various forms of cellular stress and injury [34]. ATP release has been shown to be regulated by intracellular [Ca^{2+}] and by P2X receptor activation [35]. ATP release may involve specific P2X receptors [36, 37], since activation of P2X receptors with the receptor agonist, BzATP (2'3'-O-(4-benzoyl-benzoyl)-adenosine 5'-triphosphate) increases intracellular [Ca^{2+}] and stimulates ATP release from different tissues [37-39]. **Figure 4A** illustrates the effect of BzATP treatment of hepatic cells on both ATP and ADP release. Media ATP levels peak at 5 min after the treatment and then fall over a 2 hour incubation, as the extracellular nucleotides are metabolized or degraded. In hepatic cells, BzATP stimulates a massive 300-fold pulse of ATP release at 5 min and a 4-fold lesser response with ADP [39]. ADP levels then increase in parallel to the clearance of ATP from the media. While this compound has been widely touted as a P2X7 receptor agonist, in hepatocarcinoma cells, gene silencing of both P2X4 and P2X7 was shown to have no effect on BzATP-induced nucleotide release [39], which may suggest that BzATP can also act through other P2 receptors [40].

Studies have primarily characterized the pathways that control ATP release [34, 35, 41, 42], however our work suggests that a cellular stress response preferentially stimulates the release of ADP from cultured cells [39, 43]. **Figure 4B** shows that a reduction in media serum can promote a pulse of ADP release from liver cells, which increases extracellular ADP levels 8-fold at 5-30 min. and then is cleared by 4 hours. This was a somewhat novel finding, as earlier work had suggested that most cells primarily release ATP [34] and only the circulating platelets were predisposed to ADP release. We have shown that ADP release is stimulated by P2X receptors, an increase in intracellular Ca^{2+} levels and by nutrient deprivation [39]. ADP release was shown to be stimulated by a reduction in media serum and/or glucose levels and by the calcium ionophore, ionomycin. Since intracellular ADP levels are normally very low, this work appeared to suggest that ADP is released through a Ca^{2+} regulated exocytic pathway, perhaps involving the vesicular nucleotide transporter

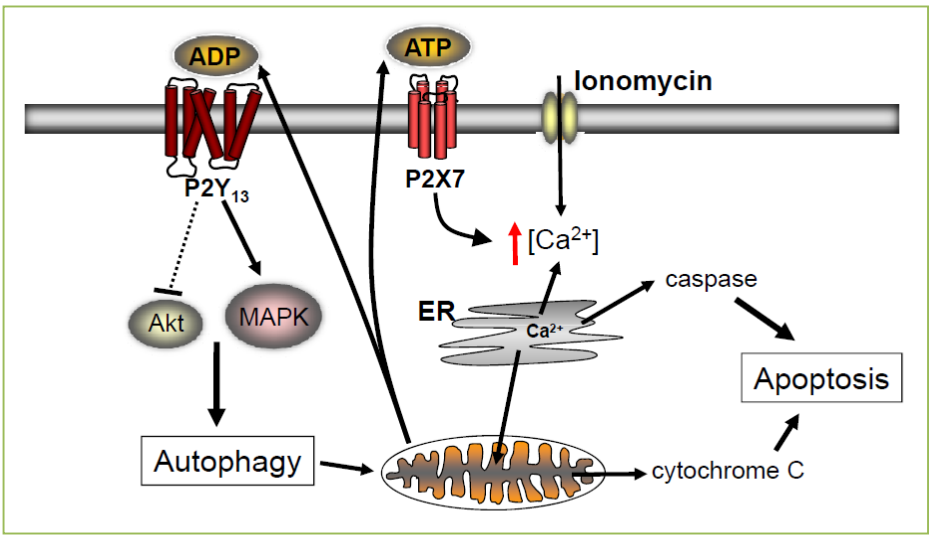

Figure 5. Extracellular nucleotides coordinate cellular autophagy and apoptosis. Nucleotide release from various tissues is stimulated by P2X receptor activation and increased intracellular [Ca^{2+}]. Extracellular ATP stimulates P2X receptors, increases intracellular Ca^{2+} and activates apoptotic pathways. Extracellular ADP stimulates P2Y receptors and activates cellular autophagy. Extracellular levels of ATP&ADP may therefore indirectly impact cellular survival pathways.

(VNUT) or by membrane ATPases, such as F_1-ATPase and ABC transporters [34]. Other work has suggested that nucleotide release may also be regulated by membrane channels, i.e. connexin hemichannels and pannexin, as described for ATP [44].

The pulse of ADP release into the media is short lived and media ADP levels were shown to return to near normal levels by 2 hours (**Figure 4B**), suggesting that nucleotide degradation may be important to the regulation of extracellular nucleotide levels. Nucleotide degradation has been previously described to affect circulating nucleotide levels. Nucleotides are degraded by specific ecto-nucleotidases, i.e. NTPDase1 and CD73 [45, 46] and knockout of the NTPDase1 (CD39) in mice was shown to cause an elevation in blood nucleotides and a phenotype that has all the hallmarks of metabolic syndrome, including inflammation, hypertriglyceridemia and insulin resistance [47]. Single nucleotide polymorphisms in human NTPDase1 gene have also been shown to be associated with type 2 diabetes and diabetic nephropathy [48]. This suggests that nucleotidases may also affect extracellular nucleotide levels.

Cellular stress and [Ca^{2+}] therefore control the release of cellular ATP&ADP and regulate their accumulation in the extracellular compartment. Intracellular Ca^{2+} levels also control cellular apoptotic pathways and it therefore appears that nucleotide release may be a universal apoptotic cell response (**Figure 5**). Studies have shown that extracellular ATP may be a chemotatic (find-me) signal released by apoptotic cells and utilized to promote the phagocytic clearance of the dying cell [49, 50]. Our research has shown that cellular stress and [Ca^{2+}] can also stimulate ADP release [39], but shows that ADP uniquely acts through P2Y receptors to activate a different survival pathway, cellular autophagy [7, 43]. Autophagy can block the induction of apoptosis, and vice versa, and these pathways are therefore thought to work antagonistically to regulate the turnover of cellular components and coordinate survival and death [51]. This may suggest that specific extracellular nucleotides play unique roles in regulating cellular survival pathways (**Figure 5**). ADP release and cellular autophagy are stimulated by a survival-starvation response [43], while ATP release is linked to a caspase-dependent activation of cellular apoptosis and the consequent clearance of dying cells [49].

Summary

Chronically elevated levels of adenosine nucleotides in the circulation and sustained purinergic signaling may play a pathological role in the development of metabolic disorders [6, 11, 53] and inflammatory disease [11-13]. Circulating nucleotide levels appear to be elevated in some morbidly obese subjects and patients with cardiovascular disease. Nucleotide levels are affected by a postprandial response to a meal and are sensitive to dietary composition. Since dietary intervention is a well-established therapy for both metabolic and inflammatory disease [53, 54], it therefore follows that diet modification may partly act to modulate extracellular nucleotide metabolism and suppress chronic purinergic signaling. Further characterization of how dietary nutrients and

caloric intake impacts circulating nucleotide levels may therefore contribute to the development of a simple and effective therapeutic approach to the treatment of metabolic and cardiovascular disease.

Conflicting interests

The authors have declared that no competing interests exist.

Acknowledgements

We would like to thank Dr. Ruth McPherson and Dr. Alexandre Stewart for their assistance with patient studies.

References

1. von Albertini M, Palmetshofer A, Kaczmarek E, Koziak K, Stroka D, Grey ST, *et al*. Extracellular ATP and ADP activate transcription factor NF-kappa B and induce endothelial cell apoptosis. Biochem Biophys Res Commun 1998; 248:822-9.
2. Di Virgilio F, Solini A. P2 receptors: new potential players in atherosclerosis. Br J Pharmacol 2002; 135:831-42.
3. Di Virgilio F, Boeynaems JM, Robson SC. Extracellular nucleotides as negative modulators of immunity. Curr Opin Pharmacol 2009; 9:507-13.
4. Di Virgilio F, Solini A. P2 receptors: new potential players in atherosclerosis. Br J Pharmacol 2002; 135:831-42.
5. Erlinge D, Burnstock G. P2 receptors in cardiovascular regulation and disease. Purinergic Signal 2008; 4:1-20.
6. Sparks DL, Chatterjee C. Purinergic Signaling, Dyslipidemia and Inflammatory Disease. Cell Physiol Biochem 2012; 30:1333-9.
7. Chatterjee C, Sparks DL. Extracellular nucleotides inhibit insulin receptor signaling, stimulate autophagy and control lipoprotein secretion. PLoS ONE 2012; 7:e36916.
8. Tan C, Salehi A, Svensson S, Olde B, Erlinge D. ADP receptor P2Y(13) induce apoptosis in pancreatic beta-cells. Cell Mol Life Sci 2010; 67:445-53.
9. Amisten S, Meidute-Abaraviciene S, Tan C, Olde B, Lundquist I, Salehi A, *et al*. ADP mediates inhibition of insulin secretion by activation of P2Y13 receptors in mice. Diabetologia 2010; 53:1927-34.
10. Di Virgilio F, Solini A. P2 receptors: new potential players in atherosclerosis. Br J Pharmacol 2002; 135:831-42.
11. Sellers MB, Tricoci P, Harrington RA. A new generation of antiplatelet agents. Curr Opin Cardiol 2009; 24:307-12.
12. Khakh BS, North RA. P2X receptors as cell-surface ATP sensors in health and disease. Nature 2006; 442:527-32.
13. Trautmann A. Extracellular ATP in the immune system: more than just a "danger signal". Sci Signal 2009; 2:e6.
14. Di Virgilio F, Boeynaems JM, Robson SC. Extracellular nucleotides as negative modulators of immunity. Curr Opin Pharmacol 2009; 9:507-13.
15. Hohenstein B, Renk S, Lang K, Daniel C, Freund M, Leon C, *et al*. P2Y1 gene deficiency protects from renal disease progression and capillary rarefaction during passive crescentic glomerulonephritis. J Am Soc Nephrol 2007; 18:494-505.
16. Hechler B, Freund M, Ravanat C, Magnenat S, Cazenave JP, Gachet C. Reduced atherosclerotic lesions in P2Y1/apolipoprotein E double-knockout mice: the contribution of non-hematopoietic-derived P2Y1 receptors. Circulation 2008; 118:754-63.
17. Ji X, Naito Y, Weng H, Endo K, Ma X, Iwai N. P2X7 deficiency attenuates hypertension and renal injury in deoxycorticosterone acetate-salt hypertension. Am J Physiol Renal Physiol 2012; 303:F1207-F1215.
18. Ayata CK, Ganal SC, Hockenjos B, Willim K, Vieira RP, Grimm M, *et al*. Purinergic P2Y(2) receptors promote neutrophil infiltration and hepatocyte death in mice with acute liver injury. Gastroenterology 2012; 143:1620-9.
19. Sun S, Xia S, Ji Y, Kersten S, Qi L. The ATP-P2X7 signaling axis is dispensable for obesity-associated inflammasome activation in adipose tissue. Diabetes 2012; 61:1471-8.
20. Hoque R, Sohail MA, Salhanick S, Malik AF, Ghani A, Robson SC, *et al*. P2X7 receptor-mediated purinergic signaling promotes liver injury in acetaminophen hepatotoxicity in mice. Am J Physiol Gastrointest Liver Physiol 2012; 302:G1171-G1179.
21. Di Virgilio F, Solini A. P2 receptors: new potential players in atherosclerosis. Br J Pharmacol 2002; 135:831-42.
22. Harkness RA, Simmonds RJ, Coade SB. Purine transport and metabolism in man: the effect of exercise on concentrations of purine bases, nucleosides and nucleotides in plasma, urine, leucocytes and erythrocytes. Clin Sci (Lond) 1983; 64:333-40.
23. Harkness RA, Coade SB, Webster AD. ATP, ADP and AMP in plasma from peripheral venous blood. Clin Chim Acta 1984; 143:91-8.
24. Jabs CM, Ferrell WJ, Robb HJ. Plasma changes in endotoxin and anaphylactic shock (ATP, ADP and Creatine phosphorus). Ann Clin Lab Sci 1979; 9:121-32.
25. Helenius M, Jalkanen S, Yegutkin G. Enzyme-coupled assays for simultaneous detection of nanomolar ATP, ADP, AMP, adenosine, inosine and pyrophosphate concentrations in extracellular fluids. Biochim Biophys Acta 2012; 1823:1967-75.
26. Coade SB, Pearson JD. Metabolism of adenine nucleotides in human blood. Circ Res 1989; 65:531-7.
27. Yegutkin GG, Wieringa B, Robson SC, Jalkanen S. Metabolism of circulating ADP in the bloodstream is mediated via integrated actions of soluble adenylate kinase-1 and NTPDase1/CD39 activities. FASEB J 2012; 26: 3875-83.
28. Forrester T, Lind AR. Identification of adenosine triphosphate in human plasma and the concentration in the venous effluent of forearm muscles before, during and after sustained contractions. J Physiol 1969; 204:347-64.
29. Forrester T. An estimate of adenosine triphosphate release into the venous effluent from exercising human forearm muscle. J Physiol 1972; 224:611-28.
30. Di Virgilio F, Solini A. P2 receptors: new potential players in atherosclerosis. Br J Pharmacol 2002; 135:831-42.

31. Bodin P, Bailey D, Burnstock G. Increased flow-induced ATP release from isolated vascular endothelial cells but not smooth muscle cells. Br J Pharmacol 1991; 103:1203-5.
32. Bodin P, Burnstock G. ATP-stimulated release of ATP by human endothelial cells. J Cardiovasc Pharmacol 1996; 27:872-5.
33. Bodin P, Burnstock G. Purinergic signalling: ATP release. Neurochem Res 2001; 26:959-69.
34. Lohman AW, Billaud M, Isakson BE. Mechanisms of ATP release and signalling in the blood vessel wall. Cardiovasc Res 2012; 95:269-80.
35. Lazarowski ER. Vesicular and conductive mechanisms of nucleotide release. Purinergic Signal 2012; 8:359-73.
36. Manohar M, Hirsh MI, Chen Y, Woehrle T, Karande AA, Junger WG. ATP release and autocrine signaling through P2X4 receptors regulate gammadelta T cell activation. J Leukoc Biol 2012; 92:787-94.
37. Gutierrez-Martin Y, Bustillo D, Gomez-Villafuertes R, Sanchez-Nogueiro J, Torregrosa-Hetland C, Binz T, *et al*. P2X7 receptors trigger ATP exocytosis and modify secretory vesicle dynamics in neuroblastoma cells. J Biol Chem 2011; 286:11370-81.
38. Brandao-Burch A, Key ML, Patel JJ, Arnett TR, Orriss IR. The P2X7 Receptor is an Important Regulator of Extracellular ATP Levels. Front Endocrinol (Lausanne) 2012; 3:41.
39. Chatterjee C, Sparks DL. P2X receptors regulate adenosine diphosphate release from hepatic cells. Purinergic Signal 2014 July 25 (Epub).
40. Feranchak AP, Roman RM, Schwiebert EM, Fitz JG. Phosphatidylinositol 3-kinase contributes to cell volume regulation through effects on ATP release. J Biol Chem 1998; 273:14906-11.
41. Fitz JG. Regulation of cellular ATP release. Trans Am Clin Climatol Assoc 2007;118:199-208.
42. Li A, Leung CT, Peterson-Yantorno K, Stamer WD, Mitchell CH, Civan MM. Mechanisms of ATP release by human trabecular meshwork cells, the enabling step in purinergic regulation of aqueous humor outflow. J Cell Physiol 2012; 227:172-82.
43. Chatterjee C, Sparks DL. Hepatic lipase release is inhibited by a purinergic induction of autophagy. Cell Physiol Biochem 2014; 33:883-94.
44. Iglesias R, Locovei S, Roque A, Alberto AP, Dahl G, Spray DC, *et al*. P2X7 receptor-Pannexin1 complex: pharmacology and signaling. Am J Physiol Cell Physiol 2008; 295:C752-C760.
45. Kukulski F, Levesque SA, Sevigny J. Impact of ectoenzymes on p2 and p1 receptor signaling. Adv Pharmacol 2011; 61:263-99.
46. Deaglio S, Robson SC. Ectonucleotidases as regulators of purinergic signaling in thrombosis, inflammation, and immunity. Adv Pharmacol 2011; 61:301-32.
47. Enjyoji K, Kotani K, Thukral C, Blumel B, Sun X, Wu Y, *et al*. Deletion of cd39/entpd1 results in hepatic insulin resistance. Diabetes 2008; 57:2311-20.
48. Friedman DJ, Talbert ME, Bowden DW, Freedman BI, Mukanya Y, Enjyoji K, *et al*. Functional ENTPD1 polymorphisms in African Americans with diabetes and end-stage renal disease. Diabetes 2009; 58:999-1006.
49. Elliott MR, Chekeni FB, Trampont PC, Lazarowski ER, Kadl A, Walk SF, *et al*. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature 2009; 461:282-6.
50. Chekeni FB, Elliott MR, Sandilos JK, Walk SF, Kinchen JM, Lazarowski ER, *et al*.. Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. Nature 2010; 467:863-7.
51. Marino G, Niso-Santano M, Baehrecke EH, Kroemer G. Self-consumption: the interplay of autophagy and apoptosis. Nat Rev Mol Cell Biol 2014; 15:81-94.
52. Di Virgilio F, Solini A. P2 receptors: new potential players in atherosclerosis. Br J Pharmacol 2002; 135:831-42.
53. Keane D, Kelly S, Healy NP, McArdle MA, Holohan K, Roche HM. Diet and metabolic syndrome: an overview. Curr Vasc Pharmacol 2014;11:842-57.
54. Andersen CJ, Fernandez ML. Dietary strategies to reduce metabolic syndrome. Rev Endocr Metab Disord 2013; 14:241-54.