



Hypothesis

The potential role of adenosine in the pathophysiology of the insulin resistance syndrome

Stephan J.L. Bakker^{a,*}, Rijk O.B. Gans^a, Jan C. ter Maaten^a, Tom Teerlink^b,
Hans V. Westerhoff^c, Robert J. Heine^b

^a Department of Internal Medicine, University Hospital Groningen, P.O. Box 30001, 9700 RB Groningen, The Netherlands

^b Research Institute for Endocrinology, Reproduction and Metabolism, University Hospital Vrije Universiteit, Amsterdam, The Netherlands

^c Department of Molecular Cell Physiology, Vrije Universiteit, Amsterdam, The Netherlands

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Abstract

An increased intracellular availability of the co-enzyme A esters of long-chain fatty acids is thought to underlie many aspects of the insulin resistance syndrome. However, the cause of clustering of a hyperdynamic circulation, sympathetic activation, hypertension, hyperuricaemia, and a raised haematocrit in the insulin resistance syndrome remains to be elucidated. We propose a mechanism that expands the etiological role of long-chain fatty acids. By inhibiting adenine nucleotide translocators, elevated intracellular concentrations of the co-enzyme A esters of long-chain fatty acids impair mitochondrial oxidative phosphorylation. This is expected to result in a chronic systemic increase in extracellular adenosine concentrations. As adenosine stimulates the sympathetic nervous system, induces systemic vasodilatation, stimulates erythropoiesis, and induces renal vasoconstriction with renal sodium retention, increased extracellular ADO concentrations may be the common denominator explaining the above-mentioned and still unexplained phenomena associated with the insulin resistance syndrome. Along the same lines, hyperuricaemia can be explained by the fact that adenosine is broken down to urate and because of increased renal urate retention. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Abdominal obesity comprises an increased risk for future development of coronary heart disease (CHD) and type 2 diabetes mellitus [1–8]. Insulin resistance, with compensatory hyperinsulinaemia, as well as other accompanying cardiovascular risk factors — their clustering nowadays known as the ‘insulin resistance syndrome’ (IRS) — are thought to underlie this increased risk [9–11]. Many components of IRS, including resistance to insulin-mediated glucose uptake, glucose intol-

erance, hyperinsulinaemia, hypertriglyceridaemia, low HDL cholesterol, and small dense LDL, have been related pathophysiologically to an increased mobilisation of free fatty acids (FFA) from intra-abdominal and peripheral fat depots [9–11]. The existence of an inverse relationship between FFA and glucose metabolism (Randle cycle) has been known since 1963 [12,13]. Only recently the causative role of elevated intramuscular co-enzyme A derivatives of long-chain fatty acids (LCFA-CoA) — the intracellular equivalent of circulatory FFA — has emerged from studies in rodents, and been brought into relationship with insulin resistance [14–18]. In parallel, evidence has accumulated from in vitro studies that an elevated intracellular LCFA-CoA concentration in rodent pancreatic β -cells alters their

* Corresponding author. Tel.: +31-503612943; fax: +31-503619069.

E-mail address: s.j.l.bakker@int.azg.nl (S.J.L. Bakker).

set point for insulin production in such a way that insulin resistance can be offset by hyperinsulinaemia [19–23]. Elevated tissue LCFA-CoA concentrations are also implicated in the elevated levels of circulatory triglycerides that accompany IRS. In the liver LCFA-CoA stimulate hepatic production of triglyceride-rich lipoproteins, and in peripheral tissues of rodents they delay clearance of these lipoproteins through a down-regulation of lipoprotein lipase activity [24,25]. In humans, it has been established that elevated levels of circulatory triglycerides, in turn, can explain the association of IRS with low HDL cholesterol concentrations and small dense LDL through stimulation of neutral lipid exchange by cholesteryl ester transfer protein (CETP) [26].

2. Pancreatic β -cell hypertrophy and subsequent dysfunction

The central role in the pathophysiology of IRS attributed to elevated LCFA-CoA concentrations in tissues is already of great biological plausibility [14–18]. However, this LCFA-CoA hypothesis did not include an explanation for the initial hypertrophy and following gradual decline in function of pancreatic β -cells after induction of an insulin-resistant state [27–29], until we recently expanded this hypothesis with the suggestion that elevated LCFA-CoA concentrations in tissues stimulate mitochondrial superoxide anion radical production [30]. Superoxide is an important growth factor but it also leads to the production of other, extremely toxic, oxygen free radicals (OFR). If sustained, the initial growth promoting effect may be negated by a hastened decline in function due to increased apoptosis and necrosis [31].

This expansion of the already existing LCFA-CoA hypothesis also allows for unification with the Barker hypothesis which relates impaired fetal growth with subsequent catch-up growth to cardiovascular disease and type 2 diabetes mellitus in adults [32,33]. In humans, lipolysis of FFA from adipose tissues is inhibited by insulin at much lower serum insulin concentrations than required for the stimulation of glucose uptake [34,35]. FFA become available during episodes of fasting to replace glucose as a source of energy for muscles and to provide the liver with reducing equivalents to stimulate endogenous glucose production to keep pace with insulin-independent glucose consumption by several tissues including the brain [36]. In addition, stored triglycerides in adipose tissues have a mass effect on lipolysis [37,38]. Infants who suffered from impaired growth in utero are born with a low pancreatic β -cell mass and a low fat mass [33,39,40]. Thus, any increase in fat mass relative to pancreatic β -cell mass will stimu-

late lipolysis through its mass effect. We hypothesise that increased intracellular LCFA-CoA concentrations will allow for adjustment of the low pancreatic β -cell mass to the higher fat mass by induction of compensatory hypertrophy through stimulation of mitochondrial superoxide production and lead to a compensatory increase in serum insulin concentrations [30]. This may be interpreted as a physiologic response to prevent an uncontrolled effect of stored triglycerides in adipose tissues on lipolysis, resulting in a new steady state balance between pancreatic β -cell mass and fat mass. As explained earlier, we hypothesise this response to occur at the expense of an increased gradual loss of function of pancreatic β -cells with time, and an earlier occurrence of glucose intolerance and type 2 diabetes mellitus [30]. This may explain why subjects with a low weight at birth, and a high body mass index thereafter, exhibit the highest risk of cardiovascular disease and type 2 diabetes mellitus [33,41]. The same sequence of events, albeit to a lesser extent, can be expected in subjects with a normal birth weight developing obesity.

Interestingly, the increase in LCFA-CoA in non-adipose tissues, and the accompanying toxic effect of an increased OFR production may not only explain an accelerated gradual decline in pancreatic β -cell function. In endothelial cells, increased OFR production may explain endothelial dysfunction and the stimulation of subendothelial LDL-oxidation that is supposed to underlie accelerated atherosclerosis [30]. In the liver, the toxic effects of increased OFR production may result in non-alcoholic steatohepatitis, another associate of IRS [42,43].

3. Mechanism underlying increased mitochondrial superoxide production

We have based our hypothesis of induction of increased mitochondrial superoxide production by increases in tissue LCFA-CoA concentrations on a combination of established metabolic phenomena. Firstly, many *in vitro* studies have consistently documented an increase in mitochondrial superoxide production in response to mitochondrial ADP deficiency [44]. Secondly, in animal tissues, LCFA-CoA have been shown to result in a concentration-dependent inhibition of the mitochondrial adenine nucleotide translocator (ANT) in the physiological range, *in vitro* and *in vivo* [45–50]. Because ANT serves to translocate ADP from the cytosol to the mitochondrial matrix for oxidative phosphorylation to ATP, it may be postulated that ANT inhibition by LCFA-CoA leads to mitochondrial ADP deficiency, and thus to increased mitochondrial superoxide production [30]. We are currently testing this hypothesis *in vitro*.

4. Cytosolic ADP excess, ATP deficiency and adenosine (ADO)

ANT not only serves to supply the mitochondrial matrix with cytosolic ADP, but also serves to supply the cytosol with the ATP produced from ADP in the mitochondrial matrix [45,47]. Both processes occur continuously in parallel because of the translocation of one molecule of ATP from the mitochondrial matrix to the cytosol in exchange for translocation of one molecule of ADP in the opposite direction. Thus, inhibition of ANT by elevated intracellular LCFA-CoA concentrations will not only result in mitochondrial ADP deficiency but also in a cytosolic ADP excess, and a parallel decrease in cytosolic ATP concentrations [30,47]. Consequently, the cytosolic adenylate kinase equilibrium, which buffers changes in cytosolic ATP concentrations, will shift towards production of one molecule of ATP and one molecule of AMP from two molecules of ADP (Fig. 1) [51–53]. This rise in the cytosolic AMP concentration will subsequently lead to an increased dephosphorylation of AMP to ADO, resulting in increased cytosolic ADO concentrations [54,55]. These elevated cytosolic ADO concentrations will impair facilitated diffusion of ADO from the extracellular space because of a lower concentration gradient [54]. The combination of continuous extracellular production of adenosine, presumably by membrane-bound ecto-5'-nucleotidase [54,56], and the lower gradient for facilitated diffusion towards the cytosol, will result in elevated extracellular ADO concentrations [54,57]. This will be accompanied by more outspoken ADO as well as elevated urate levels due to increased extracellular ADO breakdown. Studies in isolated animal hearts, and studies in animals and humans *in vivo* have shown this sequence of events to occur in response to any cause of relative or absolute impairment of mitochondrial oxidative phosphorylation of ADP in all respiring tissues [51–57]. Release of ADO by tissues with a deficit in ATP is viewed teleologically as a response aimed at restoring local ATP synthesis through an increased delivery of metabolic fuels and oxygen [58–60]. In conscious animals and humans, ADO induces acute local vasodilatation in non-renal tissues, and systemically ADO induces activation of the sympathetic nervous system in combination with respiratory stimulation [61–65]. Increased systemic ADO concentrations have been shown to result in renal sodium, urate and water retention in humans and in rodents [66–68]. ADO is also known to stimulate erythropoiesis [69–71]. These effects persist as long as ADO concentrations are elevated. Thus, prolonged systemic and renal effects of ADO may result in extracellular volume expansion and increase in red blood cell mass, as can be seen in the setting of high altitude adjustment [57,71,72]. Taken together, the acute effects with local

vasodilatation, sympathetic activation, and respiratory stimulation, and the chronic effects with an increase in circulatory volume and red blood cell mass, concord with the view that the effects of ADO promote metabolic fuel and oxygen delivery.

Extrapolation of these acute effects of ADO in conscious subjects of an initially unchanged mean arterial pressure and induction of renal sodium retention to the chronic effects of ADO results in the prediction of high volume hypertension.

5. ADO effects and the pathophysiology of the insulin resistance syndrome

In IRS, the chronic increase in intracellular LCFA-CoA concentrations in all non-adipose tissues is expected to be accompanied by a chronic systemic, rather than an acute local, increase in extracellular ADO concentrations. Thus, vasodilatation will be sustained and generalised. This may contribute to the well documented, but currently still unexplained, association of IRS with a hyperdynamic circulation [73–75]. In addition, the other aforementioned ADO effects fit nicely with the known phenomena associated with IRS (Fig. 2). For instance, the known stimulation of the sympathetic nervous system by elevated extracellular ADO concentrations could well explain the association of IRS with sympathetic activity and heart rate [76–78]. In conscious subjects, increased extracellular ADO concentrations leave the mean arterial blood pressure initially unchanged. The peripheral vasodilatation is counterbalanced by an increase in pulse rate [61–65]. However, in the long term it may be expected that an increased systemic extracellular ADO concentration will produce hypertension through extracellular volume expansion and increased red cell mass [66,67,69–71]. These phenomena may well be involved in the currently unexplained pathophysiology of the IRS associated hypertension [79–81]. Moreover, IRS has been shown to be associated with a high extracellular volume and a high haematocrit [73,74,82,83]. Interestingly, in humans, urate is the final breakdown product of ADO [84,85]. Thus, with chronically increased extracellular ADO concentrations, a parallel increase in urate production may be expected. It is therefore consistent with our hypothesis that serum urate levels have been recognised to be associated with IRS in humans [86–88]. The fact that it has been suggested that increased renal urate retention adds to an increased endogenous production in the establishment of hyperuricaemia in IRS states is strongly corroborative [87,88], because renal sodium retention induced by ADO is necessarily accompanied by renal urate retention [66,67].

6. Metabolic feedback regulation by ADO

In vitro and in vivo studies, also in humans, have demonstrated that increased extracellular ADO concen-

trations result in an amplification of insulin-dependent and insulin-independent glucose disposal in several tissues, including skeletal muscles [89,90], the heart [91–93], and adipose tissue [94,95]. Recruitment of

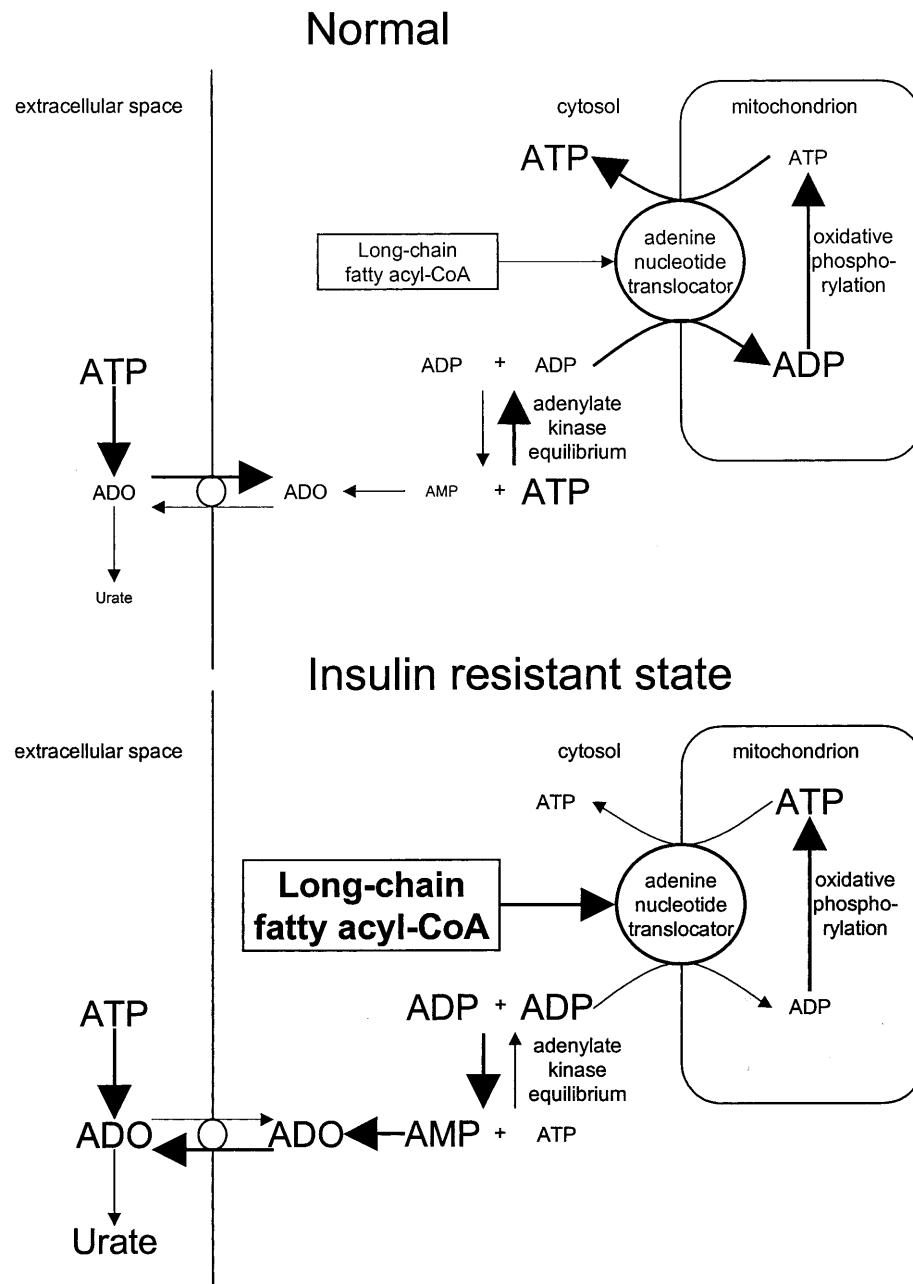


Fig. 1. Schematic representation of the relationship between inhibition of mitochondrial ANT by elevated intracellular long-chain fatty acyl-CoA ester (LCFA-CoA) concentrations and increased extracellular adenosine concentrations. Normally, with low intracellular LCFA-CoA concentrations, there is little inhibition of ANT, and there is rapid exchange of cytosolic ADP for mitochondrial ATP. Cytosolic ATP concentrations will be high, and cytosolic ADP concentrations low. As a consequence, the adenylate kinase equilibrium will be diverted towards formation of ADP from ATP and AMP, net resulting in low cytosolic AMP concentrations. Cytosolic ADO concentrations will also be low because the rate of formation of ADO from AMP is dependent on AMP concentrations. As a consequence, the flux of facilitated diffusion of ADO formed in the extracellular space will be directed towards the low concentrations in the cytosol, resulting in low extracellular ADO concentrations. With high intracellular LCFA-CoA concentrations in insulin resistant states there is inhibition of ANT. Consequently, cytosolic ATP concentrations will be low, and cytosolic ADP concentrations high. The adenylate kinase equilibrium will be diverted towards formation of ATP and AMP from ADP, resulting in high cytosolic AMP concentrations. Cytosolic ADO concentrations will be high, and the flux of facilitated diffusion of ADO will be directed towards the extracellular space in comparison to states in which cytosolic LCFA-CoA concentrations are low. Therefore, extracellular ADO concentrations will be high, resulting in a high rate of break-down to urate, and relatively high urate concentrations.

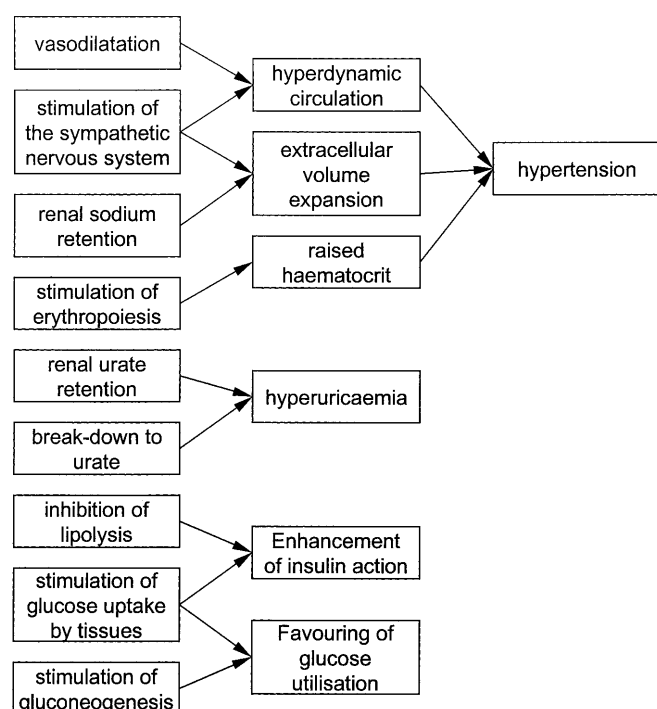


Fig. 2. ADO effects in relation to currently unexplained associations within the insulin resistance syndrome. The left panel shows documented effects of increased systemic ADO concentrations. The middle and the right panels show potential long-term consequences of increased systemic ADO concentrations.

metabolically active tissue through recruitment of collateral vessels and capillaries seems to be important [96]. Moreover, ADO has been shown to inhibit lipolysis from adipose tissue [94,97,98], and to stimulate hepatic gluconeogenesis [94,99], thus favouring the utilisation of glucose rather than fat as a source of energy. It has therefore been suggested that release of ADO from adipose tissues undergoing lipolysis serves as feedback regulation to adjust the rate of lipolysis to the rate of adipose tissue perfusion, preventing local toxicity of an uncontrolled increase in FFA concentrations [95].

Apparently, elevated extracellular ADO concentrations support maintenance of glucose and FFA homeostasis in mildly insulin deficient and/or insulin resistant states. Moreover, ADO seems to serve as a fine-tune regulation of energy metabolism by insulin in these states, and during exercise and fasting [94,95,99–101].

7. Conclusions

It remains to be demonstrated whether the relationship of intracellular LCFA-CoA with insulin resistance and pancreatic β -cell function is as important in hu-

mans as it appears to be in animal experiments. However, assuming this relationship to be present, it is possible to explain, at least partly, currently unexplained associations of IRS with increased sympathetic activity, elevated pulse rate, expansion of the extracellular volume, peripheral vasodilatation, elevated blood pressure, elevated levels of uric acid, and elevated haematocrit through increased interstitial and circulatory ADO concentrations, arising from inhibition of ANT by elevated tissue levels of LCFA-CoA.

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