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Hypothesis

Cytosolic triglycerides and oxidative stress in central obesity: the missing link between excessive atherosclerosis, endothelial dysfunction, and β-cell failure?

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Abstract

Central obesity is increasingly recognized as a risk factor for atherosclerosis and type 2 diabetes mellitus. Here we present a hypothesis that may explain the excess atherosclerosis, endothelial dysfunction and progressive β-cell failure. Central obesity is associated with increased cytosolic triglyceride stores in non-adipose tissues such as muscles, liver and pancreatic β-cells. A high cytosolic triglyceride content is accompanied by elevated concentrations of cytosolic long-chain acyl-CoA esters, the metabolically active form of fatty acids. These esters inhibit mitochondrial adenine nucleotide translocators, resulting in an intramitochondrial ADP deficiency. In vitro, such ADP deficiency is a potent stimulator of mitochondrial oxygen free radical production, and we assume that this mechanism is also active in vivo. The decline of organ function with normal ageing is thought to be due, at least partly, to a continuous low-grade mitochondrial oxygen free radical production. In tissues containing increased cytosolic triglyceride stores this process will be accelerated. Tissues with a high-energy demand or poor free radical scavenging capacity, such as pancreatic β-cells, are likely to be more susceptible to this process. This is how we explain their gradual dysfunctioning in central obesity. Likewise we propose that the enhanced production of oxygen free radicals in endothelial cells, or vascular smooth muscle cells, leads to the increased subendothelial oxidation of LDL and atherosclerosis, as well as to the endothelial dysfunction and microalbuminuria.

Keywords: Insulin resistance; Non-insulin-dependent diabetes mellitus; Adenine nucleotide translocase; Oxidative stress; Free radicals; Atherosclerosis; Oxidative phosphorylation; Obesity; Endothelial dysfunction

1. Introduction

Central obesity is associated with an enhanced cardiovascular morbidity and mortality and with type 2 diabetes mellitus [1]. The association of central obesity with numerous cardiovascular risk factors — also known as the insulin resistance syndrome (IRS) — can only partly explain the excess cardiovascular disease. Vigorous search for 'new' cardiovascular risk factors and pathophysiological phenomena that may explain the accelerated atherosclerotic process is ongoing. Even less understood is the link between central obesity and pancreatic β-cell failure that leads to hyperglycaemia. Although a toxic effect of intracellular fatty acids has been implicated, this 'lipotoxicity' hypothesis is still devoid of an underlying mechanism [2].

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Hyperglycaemia-induced oxidative stress has been suggested to promote microalbuminuria and endothelial dysfunction in subjects with diabetes mellitus. But this obviously does not explain the association of microalbuminuria and endothelial dysfunction with central obesity in normoglycaemic individuals [3,4].

In this paper we propose a pathophysiological mechanism that may explain these phenomena. Principally, we suggest that the 'normal' decline of organ function with ageing, which is ascribed to a continuous low-grade production of extremely toxic oxygen free radicals (OFR) by respiring mitochondria [5,6], is enhanced by excessive cytosolic triglyceride (cTG) stores in non-adipose tissues. We suggest that this can not only accelerate the atherosclerotic process, induce endothelial dysfunction and microalbuminuria, but may also lead to β-cell failure.

2. Cytosolic triglycerides and insulin resistance

Virtually all tissues contain cTG as a source of cytosolic long-chain acyl-CoA esters (cLCAC), the metabolically active form of fatty acids. Increased cTG with central adiposity is not limited to the adipose tissues, but also in non-adipose tissues, e.g. muscles, liver and pancreatic β-cells — a high cTG is demonstrable [7,8]. Due to a steady-state balance between cTG and cLCAC, concentrations of the latter are increased in central obesity [9]. Their pathophysiological role is now emerging. Within muscles, cLCAC impair glucose utilization as a consequence of substrate competition (Randle cycle), and induce insulin resistance [7]. In pancreatic β-cells, cLCAC have been shown to lower the setpoint for glucose-induced insulin secretion, which contributes to the hyperinsulinaemia that compensates for the impaired peripheral glucose utilization in the IRS [9]. Our hypothesis implies a role for cLCAC that goes beyond these phenomena, i.e. their involvement in alterations of oxidative phosphorylation and mitochondrial respiration.

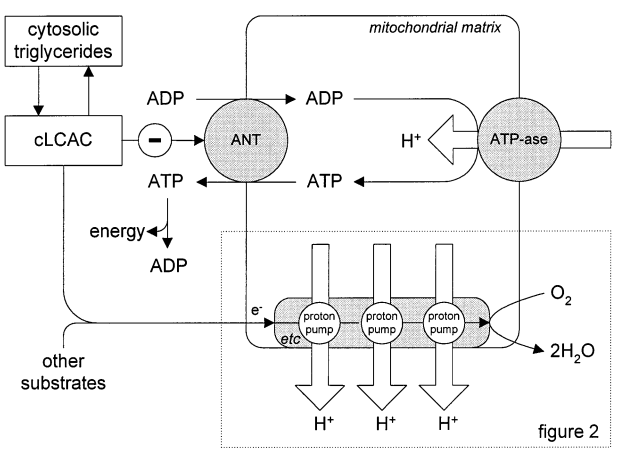

Fig. 1. Schematic representation of the relation between mitochondrial proton pumping and ATP synthesis. Proton pumps in the mitochondrial electron transfer chain (ETC) create a proton (H^+) gradient across the mitochondrial membrane. The major part of the proton gradient is consumed for the phosphorylation of ADP to ATP in the mitochondrial matrix by ATP-synthase. Inhibition of ANT by cLCAC will decrease the intramitochondrial ADP availability, which in turn will lead to a reduced consumption of the protonic gradient by ATP-synthase. A new steady state balance will be reached at a higher protonic gradient. Following entrance in the mitochondrial matrix, LCAC stimulate proton pumping by serving as an electron donor. In case of a decreased consumption of the protonic gradient this donation of electrons will further stimulate the accumulation of electrons along the ETC (see also Fig. 2).

3. Mitochondrial superoxide anion production

Most of the ATP consumed by energy requiring processes in cells is supplied by mitochondrial oxidative phosphorylation [10]. There, in a series of reactions, electrons originating from fuel molecules are transferred in a controlled way to oxygen. Upon acceptance of four electrons, oxygen is completely reduced to water [11]. Most of the energy released during the transfer of these electrons is captured by proton pumps that build-up a proton gradient across the mitochondrial membrane. The energy accumulated in this protonic gradient is the driving force for the enzyme ATP-synthase that phosphorylates ADP to ATP [10,12]. The ATP formed is exchanged for cytosolic ADP by the adenosine nucleotide translocator (ANT), thus providing a continuous supply of ADP necessary to sustain the oxidative phosphorylation process (Fig. 1) [10].

When the intramitochondrial ADP concentration drops, the process of phosphorylation slows down, which in turn lowers the protonic gradient consumption by ATP-synthase. This results in a diminished steady state activity of the proton pumps at a higher protonic gradient. This increased protonic gradient impairs the flow of electrons through the electron transfer chain (ETC) [10,11,13]. As a consequence electrons accumulate along the ETC [11,14]. This increases the likelihood of accidental transfer of a single electron from the ETC to oxygen (Fig. 2) [11,15]. By acceptance of a single electron, oxygen is converted to the superoxide anion, an OFR. Elevated local oxygen concentrations, resulting from the decreased flux of oxidative phosphorylation, may further aggravate OFR production [11]. Many in vitro studies have documented that ADP deficiency can induce superoxide anion and/or OFR production [11].

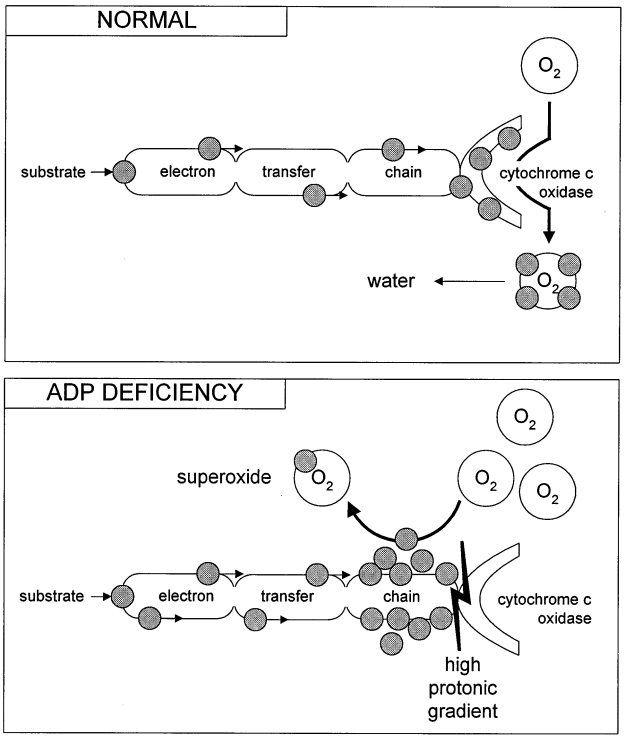

Fig. 2. Scheme of electron transfer chain changes induced by a high protonic gradient. Normally, electrons supplied by fuel molecules are transferred along the electron transfer chain (ETC) to molecular oxygen. The terminal component of the ETC, cytochrome c oxidase, binds oxygen untill it has accepted four electrons, which then is released as water. With ADP deficiency, a high protonic gradient impairs the flow of electrons along the ETC. The oxygen consumption decreases because less electrons become available at cytochrome c oxidase. At the same time electrons accumulate along proximal components of the ETC. This, and higher tissue oxygen concentrations, increase the transfer of single electrons to oxygen, and consequently increase superoxide anion production. A ● represents an electron.

4. Hypothesis

In the previous section we have shown that a drop in intramitochondrial ADP concentrations may lead to an increased OFR production. The essence of our hypothesis is that this situation can occur in vivo in the presence of physiologic concentrations of LCAC, through an inhibitory effect of LCAC on the ADP/ATP exchange by ANT [10,16]. Common LCAC, with chain lengths of the carbon moiety in the range of 12–18 carbon atoms, have been shown to exert this effect pronouncedly [17–21]. Below [17–20], and above these values [17,21], the inhibitory potency of LCAC decreases as a function of the number of carbon atoms. Differences in degree of unsaturation and stereoisomery of double bonds hardly result in any variation of inhibitory potency [17].

Our hypothesis is strongly corroborated by the observation that inhibition of ANT by atractyloside derivatives induces tissue damage through OFR production [22].

Induction of ADP deficiency may not be the only mechanism by which cLCAC stimulate superoxide anion production. As a substrate cLCAC supplies electrons (Fig. 1) [23], thereby contributing to a further accumulation of electrons along the ETC.

5. Potential consequences of the hypothesis

Superoxide anions are OFR. Subsequent chemical reactions lead to their conversion to extremely aggressive hydroxyl radicals, singlet oxygen, and strong non-radical oxidants such as hydrogen peroxide. After reacting with nitric oxide, superoxide anions form the extremely toxic peroxynitrite radical. All these highly reactive molecules can damage or destroy lipid membranes, DNA molecules, and proteins, resulting in cellular injury and malfunction, and cell death [6]. The extent to which tissues contain OFR scavenging capacity determines their susceptibility to cytotoxic damage.

Pancreatic β-cells have a much lower scavenging capacity than most other tissues, and they are unable to adapt their level of anti-oxidant enzyme expression in response to chronic oxidative stress [24]. OFR may in the first instance stimulate the growth of pancreatic β-cells [25], while the increased cLCAC concentrations lower the set point of pancreatic β-cells for glucose-induced insulin secretion [9,26]. In this way insulin resistance may in the first instance lead to hyperinsulinaemia. After maximal β-cell hyperplasia has been achieved, the ongoing increased OFR production will progressively destroy pancreatic β-cells and result in insulin deficiency. This time course of initial hyperplasia of pancreatic β-cells in response to insulin resistance, and destruction later on, is very characteristic for the development of type 2 diabetes mellitus [26–28].

The progressive accumulation of amyloid deposits formed from polymerized islet amyloid polypeptide (IAPP) during islet cell destruction in the later stages of the disease [29], may also be in line with our hypothesis. The causative factors of formation of the IAPP fibrils are unknown, but recent studies with mice expressing human IAPP suggest obesity as a permissive factor [30]. The increased cLCAC concentrations associated with obesity may provoke the OFR induced modifications necessary to form IAPP fibrils [31].

Increased OFR production by endothelial cells and vascular smooth muscle cells may promote subendothelial oxidation of LDL particles [32]. Together with the enhanced susceptibility to oxidation of the small dense LDL particles associated with central obesity [1,33], this may explain the excess atherosclerosis occurring in IRS. In vitro studies have indeed shown that endothe-

lial cells can accumulate cTG [34,35]. Such cells should also have elevated cLCAC concentrations and an increased OFR production. These studies also evidenced that endothelial cells can become dysfunctional in the absence of hyperglycaemia or subendothelial oxidation of LDL [34,35]. Thus, central obesity associated endothelial dysfunction [3,4], as amongst others reflected by microalbuminuria, may be attributed to an increased cTG.

6. Superimposed hyperglycaemia

Once failure of pancreatic β-cells ensues, hyperglycaemia may further stimulate mitochondrial superoxide production, especially in tissues not requiring insulin for glucose transport. In these tissues, mass effects of glucose and low cytosolic ATP/ADP ratios resulting from existing LCAC-mediated ANT inhibition, may stimulate glycolysis and mitochondrial efflux of citrate [9,36–40]. The former is accompanied by a net phosphorylation of cytosolic ADP to ATP, resulting in a further drop of mitochondrial ADP supply [36–38]. The latter results in an increased cytosolic malonyl-CoA synthesis [9,39,40]. The resulting elevated cytosolic level of malonyl-CoA will inhibit the rate at which LCAC enter the mitochondria. As a consequence cLCAC concentrations may be expected to rise, resulting in a greater inhibition of ANT [41].

In tissues that require insulin for glucose transport, the insulin deficiency that underlies hyperglycaemia is likely to at least oppose the effect of hyperglycaemia on glycolysis and malonyl-CoA synthesis [42]. Moreover, insulin deficiency may decrease the potency of malonyl-CoA to inhibit the rate at which LCAC enter the mitochondria in these tissues [43].

We therefore suggest that hyperglycaemia, once it ensues, exacerbates the postulated effects of LCAC on mitochondrial OFR production in tissues not requiring insulin for glucose transport.

7. Conclusions

It is currently not known how central obesity and insulin resistance can induce pancreatic β-cell failure and endothelial dysfunction. The occurrence of excessive atherosclerosis is also incompletely understood. An increased production of OFR through inhibition of ANT by cLCAC in tissues with increased amounts of cTG, can explain these phenomena. In pancreatic β-cells an increased OFR production may be expected to give rise to accelerated destruction on the long term, while an increased OFR production in endothelial cells may cause endothelial dysfunction and increase the rate of subendothelial LDL oxidation, together promoting the atherosclerotic process. Once hyperglycaemia ensues it may exacerbate the hypothesized pathophysiological process.

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