

A new hypothesis on vascular calcification: the exhausting buffer syndrome (EBS)

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Here we propose that the Western world lifestyle disrupts phosphate metabolism and homeostasis due to caloric or acidic hyperphagia. Psychic factors such as social defeat due to stressed social coexistence characterized by reduced activity and chronic hypoventilation (hypercapnia) also play a role. At least two mechanisms mediate the harmful vascular effects of phosphates with intracellular acidosis being a feature in both of them. First, insufficient lifestyle and adjacent diet together with the psychosomatic mechanism of social defeat (mainly through chronic hypercapnic acidosis) lead to insulin resistance characterized by the classical Cardiometabolic Syndrome. Secondly, overload with fixed acids caused by renal insufficiency or acidic diet (due to intracellular metabolic acidosis) leads to our here proposed Exhausting Buffer Syndrome (EBS) which tends to elevate serum inorganic phosphate levels. These two mechanisms overlap and are regulated through genetically determined processes that drive the disruption of phosphate metabolism and lead to vascular calcification. To have a lower intake of calories and less acidic foods combined with low-grade hypocapnia, might be one of several solutions.

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Keywords: Cardiometabolic Syndrome, Exhausting Buffer Syndrome, intracellular acidosis, social defeat

INTRODUCTION

An increasing number of publications claim that phosphates promote vascular calcification even at high-normal serum concentrations or if consumed them in slightly higher amounts than the Recommended Dietary Allowance (RDA) (Ellam and Chicho 2012). This hypothesis is disturbed by the fact that in a cross-sectional survey, the plasma phosphate levels were inversely correlated with the occurrence of Cardiometabolic Syndrome, which also worsens arteriosclerosis (Park et al. 2009). According to Håglin (2001), hypophosphatemia or phosphate deficiency can cause disturbed metabolism in the

Cardiometabolic Syndrome. These findings suggest that there should be at least two pathological metabolic pathways that result in vascular calcification and lead to divergent serum phosphate levels.

The authors have undertaken to answer the question if inorganic phosphate (Pi) and hypercapnia are significant factors in the aetiology of, among others, cardiovascular disorders. The hypothesis is based on the previous two theories of the first author. The first work published a simplified dynamic cytoplasm model stating that cytoplasmic growth is due to a cascade of events (Sikter 2019): 1) Pi uptake and 2) conversion of phosphate to divalent HPO_4^{2-} ; 3) HPO_4^{2-} seems to be the messenger of insulin, which

is incorporated into ATP in mitochondria (Bose et al. 2003). The generated ATP-energy is necessary for life phenomena supported by insulin; 4) Then other cytoplasm builder ions (Mg^{2+} , K^+ , Zn^{2+}) cumulate in the cytosol; 5) Eventually build-up the proteins. The circle of anabolism closes, then it starts over again on a higher energy level, it is a virtuous circle (Sikter 2019). The three parts (cytoplasm builder ions, ATP and the functioning proteins) form a union and help each other's building-up.

On the other hand, reduced amounts of ATP or lack of either cytoplasm builder ions results in cell protein deterioration and a stop in cytoplasmic constructions which trigger catabolism. One of the most aggressive stimuli of catabolism is acidosis. Catabolism takes the form of a vicious circle and evenly affects all cytoplasmic builder participants as well as ATP (Sikter 2019). The primary role of P_i in cytoplasm building-up is supported by the fact that hormones having an anabolic effect as insulin, IGF-I and vitamin D_3 , all reason a positive P_i balance in the organism (Tenenhouse 2005). It can be considered that anabolism starts with the transport of P_i into the cells as a second messenger.

NEW PATHOPHYSIOLOGICAL HYPOTHESIS

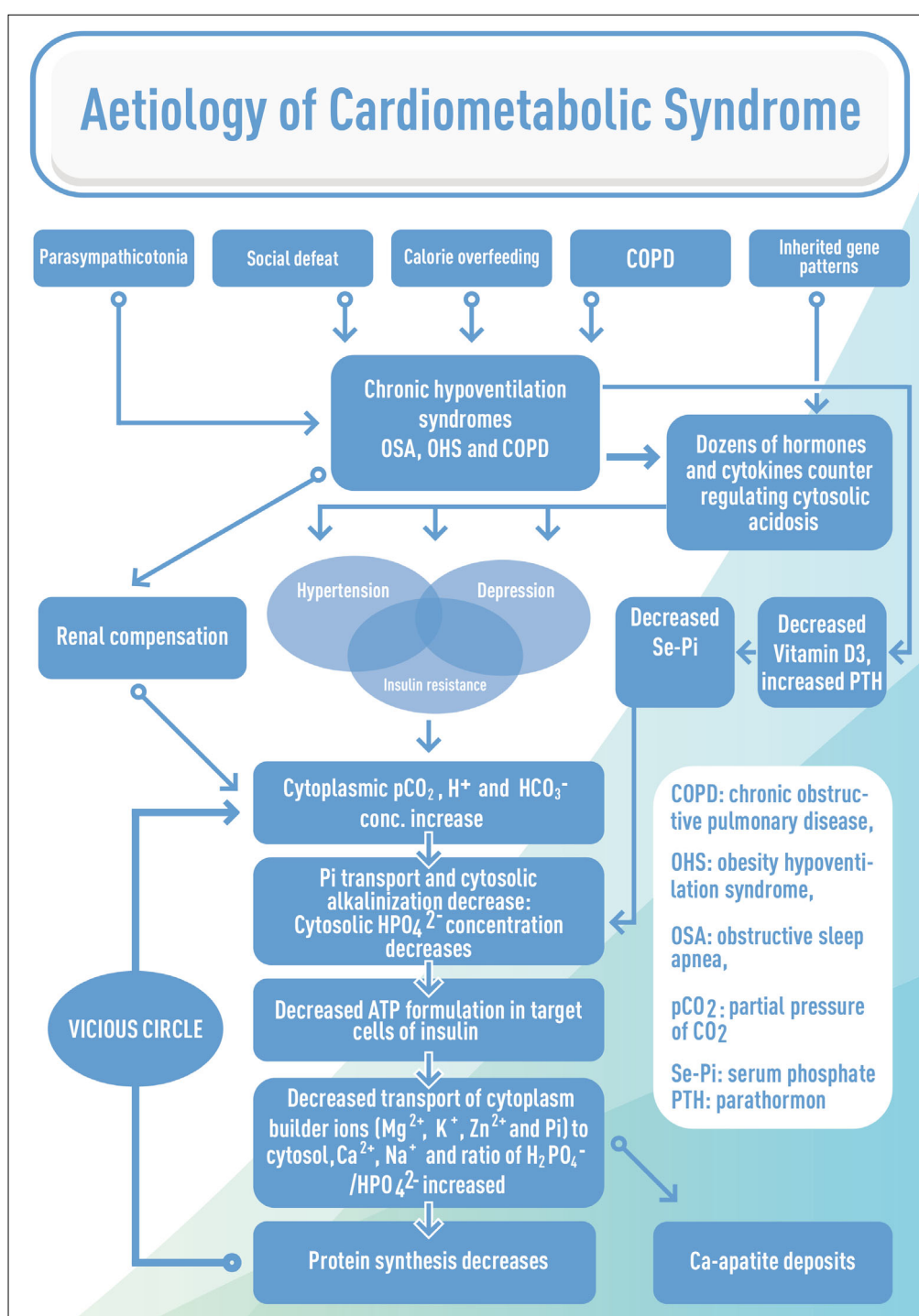
The second hypothesis is that chronic low-grade hypercapnia promote developing Cardiometabolic Syndrome (Sikter et al. 2017). Vascular calcification is a characteristic disorder in with an unhealthy Western world lifestyle and is rarely seen in wildlife. Social defeat is a source of chronic stress, and the defeated individual spiritually accepts the defeat, which causes significant alterations in behaviour and health (Brouillard et al. 2016). According to the authors' current hypothesis, this type of stress response is a parasympathetic phenomenon, and differ fundamentally from current stress models which explain stress with sympatheticotonia and adrenergic excess. Social defeat results in hypercapnia that may persist in the long run and slows down the organism's working, both mentally and physically, through alteration in metabolic pathways (Toyoda 2017). This type of stress forms is inevitable during social coexistence and may be one of the most important psychosomatic pathophysiological mechanisms.

In Western world populations, the proportion of chronically hypoventilating individuals increases significantly at the age 50+, reaching 50% by age 60 (Sikter et al. 2017, Castenada et al. 2018). The most

frequent hypoventilation syndrome is obstructive sleep apnoea (OSA) characterized by intermittent hypoxia and hypercapnia and elevated serum bicarbonate levels (Eskandari et al. 2017). The low-grade hypercapnia could cause Cardiometabolic Syndrome, hypertension and depression (Sikter et al. 2017). That is, chronic hypercapnia and intracellular acidosis alone are not sufficient to induce insulin resistance. There are dozens of unidentified hormones and cytokines, which counter-regulate acidic cytosol trying to restore normal pH levels leading to dysregulation and remodelling of metabolism (Figure 1). During chronic hypercapnia intracellular acidosis further persists in most cells/tissues, because the compensatory processes of respiratory acidosis are incomplete (Adler et al. 1965). Further differentiation of the clinical picture is determined by humoral responses elicited by cytosolic acidosis. Individuals have genetically determined hormonal-humoral patterns for defence, which is why the response to intracellular acidosis manifest in different clinical outcomes that overlap. In the case of insulin resistance, HPO_4^{2-} concentrations in insulin target cells do not increase sufficiently after the insulin effect, and that results in impaired mitochondrial ATP formation (Bose 2003, Petersen et al. 2005). It will ultimately lead to disruption of cytoplasmic homeostasis through decreases in Mg^{2+} , K^+ , Zn^{2+} and HPO_4^{2-} and a subsequent increase in the antagonist Ca^{2+} , Na^+ , and $H_2PO_4^- / HPO_4^{2-}$ ratio. Some studies attribute vascular calcification to ATP deficiency and others to Mg^{2+} lack. These mechanisms explain why intracellular vascular calcification would occur in Cardiometabolic Syndrome and related Diabetes Mellitus 2 (Tsutsumi and Sasase 2019, ter Braake et al. 2017). Metabolic acidosis can induce insulin resistance and cardiovascular risk (Suoto et al. 2011). While that intracellular acidosis result in insulin-post-receptor defect in skeletal muscle cells in Cardiometabolic Syndrome (Posa and Baba 2020).

The authors' opinion is that the absence of cytoplasm-builder ions (Mg^{2+} , K^+ , Zn^{2+} and HPO_4^{2-}) as well as the accumulation of their antagonist (Ca^{2+} , Na^+) and the increase in the $H_2PO_4^- / HPO_4^{2-}$ ratio in the cytosol, are jointly responsible for vascular calcification. In Cardiometabolic Syndrome, the chronic hypercapnia and the cavalcade of compensatory humoral effects often cause moderate hypophosphatemia due to the decreased 25-hydroxyvitamin D and increased PTH (Barceló et al. 2013).

Figure 1. According to the hypothesis, chronic hypercapnia is associated with a decreased sympathetic activity, which is a parasympathetic phenomenon (see the text). On the other hand, increased parasympathictonia usually means bradypnea. The social defeat also causes bradypnea and hypercapnia. Polyphagia leads to obesity and hypoventilation (see OHS). OSA, OHS and COPD are the three chronic hypoventilation disorders, create hypercapnia on average (Sikter et al. 2017). Renal compensation can partially decrease elevated intracellular acidosis. Dozens of hormones and cytokines also try to reduce elevated intracellular H^+ levels. Depending on which hormones act genetically most strongly in an individual case: insulin resistance, hypertension, depression, etc. will be the determining clinical picture (or a combination of these and others) (Sikter et al. 2017). Insulin resistance decreases the P_i transport to the target cells of insulin, and also HPO_4^{2-} concentration in the same cells. Then the ATP formation, ion transports, and finally, protein synthesis also decrease (a cascade of events). The vicious circle is a part of the first hypothesis (Sikter 2019). Ca-apatite deposits are the consequences of ATP deficiency and altered intracellular ion-pattern (see the text). Chronic hypercapnia tends to decrease Vitamin D_3 and elevates PTH levels which decrease the serum phosphate levels (Barceló et al. 2013).



THE TOXICITY OF P_i

The other pathway of vascular calcification develops through the insertion of high serum P_i levels. Deterioration of kidney function in chronic kidney diseases (CKD) is a textbook example, where chronic metabolic acidosis includes a decreased cytosolic pH and higher serum P_i levels, leading to disturbed P_i metabolism and vascular calcifications (Yamada and Giachelli 2017). Renal function declines with age, and the pathophysiology of this renal metabolic acidosis is similar to CKD (Frassetto and Sebastian 1996). The third common reason for chronic metabolic acidosis is the lifelong consumption of acidic foods (Adeva and Suoto 2011). On the one hand, the buffering capacity of the intracellular proteins is high, while the healthy kidneys excrete most of the fixed acid loads in the urine - although according to the hypothesis, not the whole - so it can take decades for intracellular pH to change significantly. A decrease in pH will result in a change in enzyme function and energetic insufficiency (May et al. 1987, Sikter 2019). This acidic overload can mimic reduced kidney function leading to similar conditions as in CKD.

There is an overlap between the two metabolic pathway. The result of all these processes is the Cardiometabolic Syndrome and a hyperphosphatemic pathways being the EBS as hypothesized by the authors of this current paper. The primary common cause (cytosolic acidosis) and the final common route of calcification (lack of ATP and cytoplasm builder ions) are similar in the two syndromes. The main differences are metabolic (versus respiratory) acidosis, the coexisting higher serum phosphate levels in the case of EBS, with insulin resistance being only optional. These two pathways meet at the intracellular level, as the cytosolic ion-constellations and ATP-deficiency are the same as shown in Figure 1. According to the hypothesis, cytosolic acidosis causes those changes, which start the whole process. That is why vascular calcification seems to be preventable, regardless of its hormonal-humoral background.

TESTING

Given metabolic syndromes are clinical states, it is not possible to develop an *in vivo* or *in vitro* animals model or controlled study that mimics the *in vivo* metabolic and biochemical status in the patients. In addition to this, there is no developed tool for measuring intracellular pH in a clinical set-up. Multiple publications have studied chronic social

defeat stress in rodents, and this kind of testing may be applicable to studying Cardiometabolic Syndrome (Chuang et al. 2010). It is unclear from the Brouillard and colleagues' study if the associated hypoventilation also manifest through a persistent increase in pCO₂ levels (Brouillard et al. 2016). It is therefore recommended to supplement the study with blood gas test before, after and three weeks following the experiment. Intracellular buffering power and capacity can theoretically testable *in vitro*, processing the method of Saleh et al. for humans (Saleh et al. 1991).

RECOMMENDATIONS

Intake of inadequately composed foods leads to intracellular acidification which results in vascular calcification. To avoid the acidification of the organism and risk of cardiometabolic diseases require physical activity and lower calorie intake, including fewer animal proteins and fat substituted with fruits, nuts, legumes and vegetables. Intracellular acidosis may be mitigated through a careful reduction in pCO₂ levels through increased respiratory volume and increase in exercise. Mechanisms that significantly affect serum P_i levels is often overlooked. Decreasing pCO₂ levels usually cause hypophosphatemia due to intracellular alkalosis, leading to increased glycolysis and metabolism (Knochel 1977, Relman 1972). Consequently, this mechanism restores the decreased intracellular transport of phosphates caused by intracellular acidosis because P_i is shifted from serum to cells. This hypothesis is impossible to test, however, our best guess - if the hypothesis is valid - is that controlled chronic hypocapnia would slow, stop, or reverse the cytosolic acidification mechanism and its consequences. However, so far, no method has yet been developed to increase respiratory volume reducing pCO₂ level in a controlled method except for non-physiological mechanical ventilation. Bicarbonate therapy fails to reduce acidosis-induced insulin resistance likely because of the paradoxically elevated intracellular acidosis due to compensatory rising pCO₂ levels (Harris and Dawson-Hughes 2010; Forni et al. 2020). A meta-analysis of seven trials was reviewed (Shang et al. 2021). The conclusion of the seven controlled studies (altogether enrolling 691 participants) using mechanical ventilation (CPAP) in OSA had the following positive result: "Continuous positive airway pressure treatment significantly improved glycaemic control and insulin resistance, as shown by the decreased HbA1c levels, fasting glucose levels and HOMA-IR values in patients with type 2 diabetes and OSA."

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REFERENCES

- Adeva MM, Souto G. (2011): Diet-induced metabolic acidosis. *Clin Nutr.* 30(4):416–421.
- Adler S, Roy A, Relman AS. (1965): Intracellular acid-base regulation. II. The interaction between CO₂ tension and extracellular bicarbonate in the determination of muscle cell pH. *J Clin Invest.* 44(1):21–30.
- Barceló A, Esquinas C, Javier Piérola J, et al. (2013): Vitamin D status and parathyroid hormone levels in patients with obstructive sleep apnea. *Respiration* 86(4):295–301.
- Bose S, French S, Evans FJ, Joubert F, Balaban RS. (2003): Metabolic network control of oxidative phosphorylation: multiple roles of inorganic phosphate. *J Biol Chem.* 278(40):39155–39165.
- Ter Braake AD, Shanahan CM, de Baaij JHF. (2017): Magnesium counteracts vascular calcification passive interference or active modulation? *Arterioscler Thromb Vasc Biol.* 37(8):1431–1445.
- Brouillard C, Carrive P, Camus F, Bénoliel JJ, Similowski J, Sévoz-Couche C. (2016): Long-lasting bradypnea induced by repeated social defeat. *Am J Physiol Regul Integr Comp Physiol.* 311(2):R352–R364.
- Castaneda A, Jauregui-Maldonado E, Ratnani I, Varon J, Surani S. (2018): Correlation between metabolic syndrome and sleep apnea. *World J Diabetes* 9(4):66–71.
- Chuang J-C, Cui H, Mason BL et al. (2010): Chronic social defeat stress disrupts regulation of lipid synthesis. *J Lipid Res.* 51(6):1344–1353. doi: 10.1194/jlr.M002196.
- Ellam TJ, Chico TJA. (2012): Phosphate: The new cholesterol? The role of the phosphate axis in non-uremic vascular disease. *Atherosclerosis* 220:310–318.
- Eskandari D, Zou D, Grote L, Schneider H, Penzel T, Hedner J. (2017): Independent associations between arterial bicarbonate, apnea severity and hypertension in obstructive sleep apnea. *Respir Res.* 18(1):130. doi: 10.1186/s12931-017-0607-9.
- Forni LG, Hodgson LE, Selby NM. (2020): The Janus faces of bicarbonate therapy in the ICU: not sure! *Intensive Care Med.* 46:519–521 <https://doi.org/10.1007/s00134-019-05842-4>
- Frassetto L, Sebastian A. (1996): Age and systemic acid-base equilibrium: analysis of published data. *J Gerontol A Biol Sci Med Sci.* 51(1): B91–B99.
- Häglin L. (2001): Hypophosphatemia: cause of the disturbed metabolism in the metabolic syndrome. *Medical Hypothesis* 56.6: 657–663.
- Harris SS, Dawson-Hughes B. (2010): No effect of bicarbonate treatment on insulin sensitivity and glucose control in non-diabetic older adults. *Endocrine.* 38(2):221–6. doi: 10.1007/s12020-010-9377-6.
- Knochel JP. (1977): The pathophysiology and clinical characteristics of severe hypophosphatemia. *Arch Intern Med.* 137(2): 203–220.
- May RC, Kelly RA, Mitch WE. (1987): Mechanisms for defects in muscle protein metabolism in rats with chronic uremia. Influence of metabolic acidosis. *J Clin Investig.* 79:1099–1103.
- Park W, Kim BS, Eun J, et al. (2009): Serum phosphate levels and the risk of cardiovascular disease and metabolic syndrome: A double-edge sword. *Diabetes Res Clin Pract.* 83(1):119–125.
- Petersen KF, Dufour S, Shulman GI. (2005): Decreased insulin-stimulated ATP synthesis and phosphate transport in muscle of insulin-resistant offspring of type 2 diabetic parents. *PLoS Med.* 2(9):e233. doi:10.1371/journal.pmed.0020233
- Posa DK, Baba SP. (2020): Intracellular pH regulation of skeletal muscle in the milieu of insulin signaling. *Nutrients* 12(10):2910; doi.org/10.3390/nu12102910
- Relman AS. (1972): Metabolic consequences of acid-base disorders. *Kidney Int.* 1(5):347–359.
- Saleh AM, Rombola G, Batlle DC. (1991): Intracellular H⁺ buffering power and its dependency on intracellular pH. *Kidney Int.* 39(2):282–288. doi: 10.1038/ki.1991.34.
- Shang W, Zhang Y, Wang G, Han D. (2021): Benefits of continuous positive airway pressure 1on glycaemic control and insulin resistance in patients with type 2 diabetes and obstructive sleep apnoea: A meta-analysis. *Diabetes Obes Metab.* 23(2):540–548. doi: 10.1111/dom.14247.
- Sikter A. (2019): Psychosomatic molecular mechanisms of metabolic syndrome and type 2 diabetes. Part 1. A theory for modeling the cytoplasm and diseases. *Acta Scientific Medical Sciences* 4(1) 124–140. doi: 10.31080/ASMS.2019.04.
- Sikter A, Rihmer Z, Guevara R. (2017): New aspects in the pathomechanism of diseases of civilization, particularly psychosomatic disorders. Part 2. Chronic hypocapnia and hypercapnia in the medical practice. *Neuropsychopharmacol Hung.* 19(3):159–169.
- Souto G, Donapetry C, Calviño J, Adeva MM. (2011): Metabolic acidosis-induced insulin resistance and cardiovascular risk. *Metab Syndr Relat Disord.* 9(4):247–253. doi: 10.1089/met.2010.0108
- Tenenhouse HS. (2005): Regulation of phosphorus homeostasis by the type IIa Na/phosphate cotransporter. *Annu Rev Nutr.* 25:197–214.
- Toyoda A. (2017): Social defeat models in animal science: What we have learned from rodent models. *Anim Sci J.* 88(7):944–952.
- Tsutsumi K, Sasase T. (2019): Cellular calcification induced by inorganic polyphosphate involves ATP depletion and opening of the mitochondrial permeability transition pore (mPTP). *FEBS Open Bio.* 9(9):1617–1622.
- Yamada S, Giachelli CM. (2017): Vascular calcification in CKD-MBD: Roles for phosphate, FGF23, and Klotho. *Bone.* 100:87–93.

Egy új hipotézis az érlemeszesedés mechanizmusáról: kimerülő puffer szindróma (KPSZ)

Az a véleményünk, hogy a nyugati világ életmódja – a kalorikus vagy savasító ételek túlfogyasztása – megzavarja a foszfát anyagcserét és a szervezet homeosztázisát. Pszichés tényezőket, mint például a stresszes társadalmi együttélés miatti „társadalmi vereséget” (social defeat) csökkent fizikai és mentális aktivitás, valamint krónikus hipoventiláció (hiperkapnia) jellemzi. Legalább két mechanizmus közvetíti a foszfátok káros érrendszeri hatásait, bár az intracelluláris acidózis mindkettőre jellemző. Először is, a nem megfelelő életmód és étkezés, valamint a társadalmi vereség pszichoszomatikus mechanizmusa (főleg krónikus hiperkapniás acidózis révén) inzulinrezisztenciához vezet, amelyet a klasszikus kardiometabolikus szindróma jellemez. Másodsor, a veseelégtelenség vagy a savasító étrend által okozott fix savakkal való túlterhelés az itt javasolt Kimerülő Puffer Szindrómához (KPSZ) vezet intracelluláris metabolikus acidózis okozása révén, melynél a szérum szerves foszfát szintjének emelkedése jelentkezik. Ez a két mechanizmus átfedésben van, melyeket genetikailag meghatározott folyamatok szabályoznak és a foszfát-anyagcsere megzavarását eredményezik, és végül az érrendszeri meszesedésbe torkoltnak. Javasolt az alacsonyabb kalória bevitele és kevésbé savasító ételek fogyasztása, mérsékelt fokú hipokapnia előidézése mellett számos más megoldás is lehetséges.

Kulcsszavak: kardiometabolikus szindróma, kimerülő puffer szindróma, intracelluláris acidózis, társadalmi vereség