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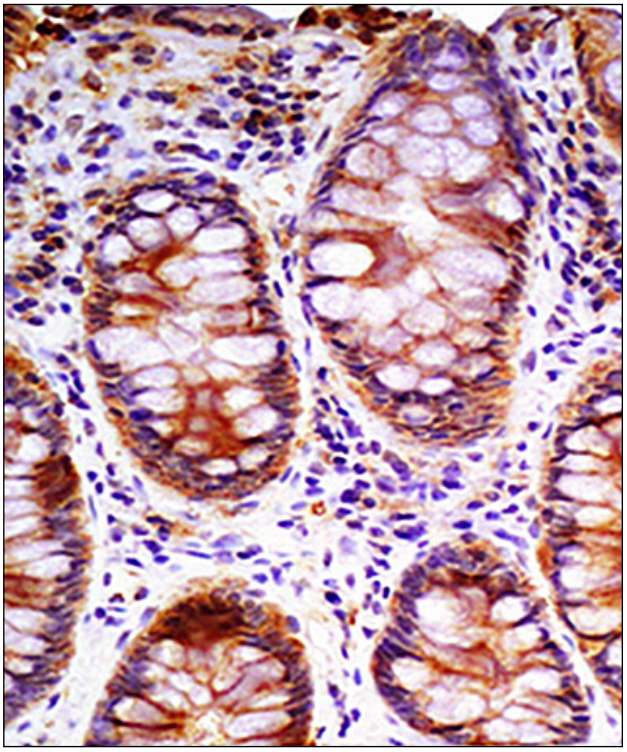

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Acidemia and Blood Free Fatty Acids: Analysis of Cardiovascular Risk Factors in a New Context

António Heitor Reis

Abstract: Following a hypothesis developed in an earlier paper, here it is discussed how deviations of blood pH from the normal range (namely states of acidemia) together with high blood levels of free fatty acids (FFA) may offer a rationale for many important risk factors for cardiovascular diseases (CVD) by shaping a context for formation of fatty acid micelles and vesicles with an acidic core, which fuse with the endothelia, disrupt vital cell processes, and thereby may initiate atherosclerotic plaque formation. Acidemia may arise primarily from dysregulation of the systemic buffers that control blood pH, chronic diseases of kidneys and lungs, inappropriate diet, or may be induced by some common drugs. The level of free fatty acids may be increased and maintained high by chronic stress, and adrenergic shocks. Elevated concentrations of blood FFA in a context of acidemia allow to understand important cardiovascular aspects: the increased risk of menopausal women, the protective effects of physical exercise, the changes in vascular behavior characteristic of metabolic acidosis/acidemia, the role of diet in the pH balance, on how some known medicines like metformin, steroids, NSAIDS, proton pump inhibitors, and calcium supplements may influence CVD risk, and an explanation is offered for the role of statins. [*Discovery Medicine* 23(126):183-188, March 2017]

Introduction

In an earlier paper (Reis, 2016) an interpretive framework was proposed that provides a rationale for many well-known features of cardiovascular diseases (CVD). In that paper it was discussed how acidemia together with high blood levels of free fatty acids (FFA) may shape a context for formation of fatty acid micelles and vesicles with an acidic core, which fuse with the endothelia, disrupt vital cell processes, and may initiate atherosclerotic plaque formation. The same process was proposed to play a role in acute events, namely heart attacks and thromboembolic strokes. It was also shown how this interpretative context is backed by a broad body of literature published in the last decades.

In fact, some striking features of CVD hardly find a rationale within the current views of CVD development. For instance, patients may develop angina and myocardial ischemia with clean coronaries (Abdelghani *et al.*, 2015; Baroldi and Silver, 2004). On the other hand, on the basis of observations made during autopsies, some cardiovascular pathologists have sustained that blockage of coronary arteries by thrombus is an effect secondary to the heart attack (Baroldi and Silver, 2004). Some others advocate that atherosclerotic plaques are caused by a single injurious agent that shall be very different from the traditionally alleged ones (Frink, 2002).

The possible role of the interplay between acidemia and FFA on CVD was reviewed and discussed (Reis, 2016). Because it is known that blood pH in normal conditions is tightly regulated (Kellum, 2000) (namely though the systemic buffers) little attention is usually paid to small deviations from the normal range (7.38-7.42). Yet, as stated by Pizzorno (2015): "a growing body of research has documented not only that acidosis is a real phenomenon, but that it is now known to contribute to a wide range of diseases." Moreover, metabolic acidosis is often asymptomatic, or may present as fatigue, anorexia, confusion, tachycardia, tachypnea, and dehydration (Pizzorno, 2015). Metabolic acidosis, and thus blood acidemia, can have many causes, though is more often

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due to kidney dysfunction (Kraut and Madias, 2016) or induced by uptake of medications and drugs (Liamis, *et al*., 2010), or even by diet (Adeva and Souto, 2011).

By itself, a state of acidemia might not cause CVD. However, acidemia may dramatically change the behavior of blood FFA: at slightly lower pH, FFA are fully protonated and form an oil phase, while at higher pH they are fully deprotonated and form FFA micelles and vesicles (Hentrich and Szostak, 2014; Markvoort *et al*., 2010; Morigaki and Walde, 2007; Chen and Szostak, 2004). Hyperventilation as a compensatory mechanism that develops to restore normal pH levels causes pH imbalances in the blood flowing from the lungs to the heart. It was proposed that if a state of acidemia occurs together with high blood concentration of FFA, micelles and fatty acid (FA) vesicles with an acidic core may develop, fuse with the endothelia, and initiate plaque formation. The process of micelle and FA vesicle formation, and fusion with the endothelia, is described in the biomedical literature, and occurs in special conditions of local blood pH imbalance (see Reis, 2016). The blood concentration of FFA may increase mainly as a consequence of elevated cortisol levels, or adrenergic shocks (Newsholme and Leech, 2010).

In what follows we analyze some common risk factors of CVD that may find a rationale within the proposed interpretive framework (Reis, 2016). We also offer explanations for the increase in the risk of CVD in people taking some well-known drugs. It should be noted that risk is here understood in the sense of the existence of any association (causal or not) between a particular health condition and CVD.

Blood pH and Free Fatty Acids

Blood pH is tightly regulated by several mechanisms in the body, which may be divided into 3 groups: (i) buffering activity; (ii) Excretion of acids and bases through sweat, stomach, urine, and feces; and (iii) removal of carbon dioxide by the lungs.

Buffers, which consist of a weak acid and a weak base at equilibrium with each other, work by taking the extra protons (H^+) and making the base component of the buffer to react with the protons to turn into the conjugate acid therefore neutralizing most of the extra protons. Important buffers are the bicarbonate buffer system that promotes the interconversion of blood bicarbonate and protons to carbon dioxide and water, and the phosphate and protein buffer systems that operate in the internal fluid of all cells (Das, 2005). Bones, which are the largest alkaline reserve in the body, also represent an important buffer. Bone buffering operates through the uptake of H^+ from the blood with release of bone Ca^{2+} to the blood, or by adsorption of HCO_3^- onto the crystal surfaces of bone minerals (Lemann *et al*., 2003; Krieger *et al*., 2004).

Circulating FFA mainly originate from lipolysis in the adipose tissue, and their concentration in plasma is usually in the range 100 mmol/L-1 mmol/L (Frayn, 2005). Hyperadrenergic states, namely those occurring as a response to catecholamine stimulus, increase plasma FFA levels markedly (Newsholme and Leech, 2010; Frayn, 2005).

In fact, some lines of evidence exist that point to the association of FFA, and specifically FA micelles and vesicles, with CVD (Srinivasan, 2012; Bobryshev *et al*., 2007, Pilz *et al*., 2007; 2006). Vesicle remnants were found in atherosclerotic plaques (Bobryshev *et al*., 2007, Pilz *et al*., 2007), and it is known that in case they cause damage to the endocardium, the myocardial function is compromised in respect to contractibility and electric activity (Brutsaert *et al*., 1988). Cardiopathologists who analyzed post-mortem lesions in the heart [e.g., contraction band necrosis (Baroldi and Silver, 2004)] have pointed out that they do not seem to be caused by usual injurious factors (Frink, 2002). Frink put forward the hypothesis that a special injurious agent should initiate the process of atherosclerotic plaque formation. In our hypothesis the role of Frink's "infectious agent" (and toxins) is played by the FFA vesicles with an acidic core.

In the following we show how the hypothesis of elevated FFA levels in the context of acidemia, leading to FA vesicle formation, offers explanations for major CVD risk factors.

Understanding CVD Risk Factors in the Light of the Proposed Hypothesis

In the proposed framework, boost of blood FFA concentration in a state of acidemia forms the context for increased risk of CVD events (Reis, 2016). Therefore, in such context CVD risk must increase with dysregulation of the main mechanisms that tightly control blood pH, i.e., the systemic buffers, kidney function, and respiratory function. In general, the proper functioning of these mechanisms is affected by many diseases, uptake of drugs, inappropriate diet, and age. Moreover, continuous stress increases and keeps high FFA levels in the blood, while adrenergic shocks may suddenly raise these levels (Newsholme and Leech, 2010).

Elevated blood levels of FFA

Some studies (e.g., Srinivasan, 2012; Pilz *et al*., 2006) have associated raised blood levels of FFA with CVD. Increase in plasma catecholamine levels, with the consequent increase in blood FFA concentration, has been associated with myocardial infarction and stroke (Hachinski *et al*., 1986). Moreover, events of myocardial infarction (MI) and sudden cardiac death (SCD) peak in the morning (Muller *et al*., 1987). Coincidently, both the blood levels of cortisol and FFA also peak in the early morning (Portaluppi and Lemmer, 2007; Muller *et al*., 1987). SCD and MI events have also been found to markedly increase after catastrophes, situations that are conducive to the occurrence of surges of plasma FFA [e.g., war (Meisel *et al*., 1991), earthquake (Leor *et al*., 1996), or sport events (Kirkup and Merrick, 2003)]. In effect, increased plasma levels of both norepinephrine and epinephrine were found in patients at the time of MI events (Slavíková, 2007; Remme *et al*., 1987; Schomig, 1988). To the knowledge of this author no published study addresses the simultaneous measurement of blood pH and the concentrations of FFA. Although strong association was found between elevated concentrations of FFA and CVD events, we speculate that a stronger association would emerge if only CVD events in a context of acidemia were considered.

Aging, and the decline of the systemic buffers, and renal and respiratory functions

It is known that the systemic pH buffers decline with age (Amodu and Abramowitz, 2013; Frassetto and Sebastian, 1996). Similar decline occurs with the renal function (Weinstein and Anderson, 2010; Frassetto *et al*., 1996), and the respiratory function (Sharma and Goodwin, 2006). Therefore, acidemia states are increasingly likely to occur with aging. Some authors (e.g., Pizzorno, 2015; Amodu and Abramowitz, 2013) pointed out that subclinical acidosis increases with age, and is very common in the elderly, whilst on the other hand, age is a well established factor for CVD (Mozaffarian *et al*., 2015). Within the proposed framework (Reis, 2016) we are able to associate aging with CVD due to the fact that decline of the mechanisms that control blood pH leads to increase of the frequency of acidemia states with aging, and therefore forms one of the conditions of the context for CVD development.

Statistics shows that the frequency of CVD in premenopausal women is lower than in males of the same age (Mozaffarian *et al*., 2015) but greatly increases in menopause, age at which it reaches the same pattern of CVD frequency in males. In fact, because during the women's fertile age the slightly alkaline environment in the uterus is vital for conception and fetus development, its pH must be tightly controlled (Dale *et al*., 1998). On the other hand, a study (animal model) (Marcus, 1965) showed that estrogens turn uterus more alkaline, whilst in premenopausal women they attenuate blood pressure, glucocorticoid, and catecholamine responses to psychological stress (Komesaroff, 1999; Rosano and Panina, 1999). The fact that estrogens prevent deviations from the normal blood pH, and attenuate the effect of catecholamines might explain the lower incidence of CVD among women during their fertile age. Furthermore, as referred above, because bones are the greatest alkaline reserve in the body, a sign that in menopausal women blood acidemia is likely to be frequent is the increased loss of bone mass (osteoporosis), namely via the resorption of calcium in the blood (Pizzorno, 2015; Krieger *et al*., 2004; Lemann *et al*., 2003).

The decline of the respiratory function with age (namely in smokers and other people affected by environmental pollution), which is key to regulate blood pH homeostasis, also contributes to CVD risk (Reis, 2016; Sharma and Goodwin, 2006).

On the other hand, the pH buffering capacity can be increased through physical exercise (Wasserman *et al*., 2012; Röcker *et al*., 1994) thereby explaining why moderate physical exercise is a factor protective against CVD.

As an additional outcome of the present framework, hyperventilation as part of the process compensatory to acidemia described (Reis, 2016) enables the understanding the changes in the vascular behavior that are characteristic of metabolic acidosis/acidemia, namely: (i) peripheral arteriolar vasodilatation; (ii) venoconstriction of peripheral veins; and (iii) vasoconstriction of pulmonary arteries (Brandis, 2017). Such sequence may be understood as follows: (i) in an acidemia state the blood coming from the heart is more alkaline (Reis, 2016), therefore most of calcium ions are bound to albumin, hence its concentration in the blood is low thereby allowing vasodilatation (Widmaier *et al*., 2010); (ii) as the blood bathes tissues in the state of acidosis its pH drops, hence calcium ions are released from albumin binding sites, and consequently are available for inducing vein constriction; and (iii) the lower pH blood with increased level of calcium ions is transported to the right atrium and ventricle, and then to the lungs, thereby explaining constriction of pulmonary arteries.

The vasoconstriction (peripheral veins and pulmonary arteries) characteristic of acidemia certainly plays a role

in hypertension, which is an established risk for CVD.

Cholesterol and statins

In the framework discussed here, the role of cholesterol as a primary injurious agent is not addressed, but its function is discussed as part of the repairing mechanism put into action after the endothelial injury. Interestingly, the model of arterial occlusion by thrombus with origin in plaque rupture as the primary cause of heart attack has been challenged by the observations made in autopsy showing that heart attacks and myocardial infarction occur even in the absence of thrombus occluding the coronary arteries (e.g., Abdelghani *et al*., 2015; Baroldi and Silver, 2004; Silver *et al*., 1980; Roberts, 1972), while acute coronary occlusions may occur without concomitant MI (Baroldi and Silver, 2004).

Some authors have pointed out that the beneficial action of statins with respect to CVD prevention and management -- though many questions have been raised about statin side effects (Hippisley-Cox and Coupland, 2010) -- is due to pleiotropic effects other than cholesterol lowering (e.g., Nordmann and Briel, 2012). In fact, in respect to CVD as it is analyzed in the proposed framework, statins produce mixed results: while they contribute to acidosis (Liamis *et al*., 2010; Neale *et al*., 2004; Goli *et al*., 2002) they also lower plasma FFA concentrations (Sahebkar *et al.*, 2016). As discussed above, peaks of FFA are key to CVD development, and thereby the lowering of plasma FFA levels may be the reason for the protective effect of statins.

Diet

Diet may contribute to the acid load in the body. Animal proteins and cereal grains increase the body acid load, while fruits and vegetables turn it more alkaline (Amodu and Abramowitz, 2013; Adeva and Souto, 2011). Sugar in the diet also generates acid in the gastrointestinal tract (Halperin and Kamel, 1996). In modern societies diet is generally high in meat, cereal derived products, and sugar, which promote acidity and, within the present framework, thereby the ground for CVD development. Some authors have pointed out the increase in acid load caused by shift to the modern diet, and namely the pathophysiologic effects of the post-agricultural inversion of the potassium-to-sodium and base-to-chloride ratios in the human diet (Sebastian *et al*., 2002; Frassetto *et al*., 2001). On the other hand, many studies have evinced the protective effect of the Mediterranean diet (alkalinity promoter) against CVD (Estruch *et al*., 2013).

Uptake of common drugs

Taking of some medications may affect the acid-base balance (Liamis *et al*., 2010) and according to the proposed framework may thus affect the risk of CVD. Beyond known drugs that have been associated with acidosis, such as metformin (the most prescribed antidiabetic drug in the world) (Liamis *et al*., 2010), steroids (Liamis *et al*., 2010; Hulter *et al*., 1980), and NSAIDS (Hunter *et al*., 2011; Liamis *et al*., 2010), other common drugs have been associated with the increase in CVD risk. For instance, proton pump inhibitors (PPI) have been associated with increased CVD risk (Shah *et al*., 2015). This fact is also understood within the present framework as PPI, by inhibiting secretion of protons into the stomach, block one of the ways by which the body gets rid of intercellular acid. Also calcium supplementation has been found to increase MI risk (Li *et al*., 2010). Actually, excessive calcium concentration in the blood may offset the buffering action of bone, because calcium ions release from bone (and thus uptake of protons in bone) is counteracted by the strong concentration of calcium ions in the blood.

Conclusions

The proposed interpretive framework in which states of acidemia together with high blood levels of free fatty acids (FFA) shape a context for cardiovascular diseases (CVD) offers a rationale for many well-known features of CVD.

The published literature shows that mild acidemia states are very common, mainly due to decline of systemic buffers with aging, chronic kidney disease, respiratory diseases, inappropriate diet, and drugs, at the same time that adrenergic shocks that increase the blood concentration of FFA are also common in modern daily life. Associations between acidemia and increased blood concentrations of free fatty acids, and cardiovascular risk factors are also easily found in the literature.

Important CVD risk factors are analyzed, which construe a rationale in the proposed framework. Specifically, aging, protective factors, and the role of some common drugs on CVD are addressed in relation with blood pH, and in particular an explanation is offered for the role of statins based on their ability to reduce the blood concentration of free fatty acids.

The present study does not extensively cover all identified CVD risk factors. Analysis must proceed in order to investigate if other CVD risk factors do fit in the proposed context.

Disclosure

The author reports no conflicts of interest.

References

Abdelghani R, Patel H, Patel C, Gadi S. Seeing is believing? Angina and myocardial ischemia with clean coronaries. *J Clin Exp Cardiol* 6:4, 2015.

Adeva MM, Souto G. Diet-induced metabolic acidosis. *Clin Nutr* 30(4):416-421, 2011.

Amodu A, Abramowitz MK. Dietary acid, age, and serum bicarbonate levels among adults in the United States. *Clin J Am Soc Nephrol* 8:2034-2042, 2013.

Baroldi G, Silver M. The etiopathogenesis of coronary heart disease: a heretical theory based on morphology. Eurekah, Landes Bioscience, 2004.

Bobryshev VY, Killingsworth MC, Huynh TG, Lord RSA, Grabs AJ, Valenzuela SM. Are calcifying matrix vesicles in atherosclerotic lesions of cellular origin? *Basic Res Cardiol* 102:133-143, 2007.

Brandis K. Acid-base physiology. In: *Metabolic Acidosis-Metabolic Effects*. From http://www.anaesthesiamcq.com/AcidBaseBook/ab5_4.php (accessed on Jan. 20, 2017).

Brutsaert DL, Meulemans AL, Sipido KR, Sys SU. Effects of damaging the endocardial surface on the mechanical performance of isolated cardiac muscle. *Circ Res* 62:358-366, 1988.

Chen IA, Szostak JW. A kinetic study of the growth of fatty acid vesicles. *Biophys J* 87:988-998, 2004.

Dale B, Menezo Y, Cohen J, DiMatteo L, Wilding M. Intracellular pH regulation in the human oocyte. *Hum Reprod* 13(4):964-970, 1998.

Das D. *Biochemistry, 12th Ed*. p206. Academic Pubs, 2005.

Estruch R, Ros E, Salas-Salvadó J, *et al*., PREDIMED Study Investigators. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 368:1279-1290, 2013.

Frassetto L, Sebastian A. Age and systemic acid-base equilibrium: analysis of published data. *J Gerontol Biol Sci* 51(1):B91-B99, 1996.

Frassetto LA, Morris RC Jr, Sebastian A. Effect of age on blood acid-base composition in adult humans: role of age-related renal functional decline. *Am J Physiol* 271:F1114-F1122, 1996.

Frassetto L, Morris RC Jr, Sellmeyer DE, Todd K, Sebastian A. Diet, evolution and aging-the pathophysiologic effects of the post-agricultural inversion of the potassium-to-sodium and base-to-chloride ratios in the human diet. *Eur J Nutr* 40:200-213, 2001.

Frayn KN. Plasma non-esterified fatty acids: why are we not measuring them routinely? *Ann Clin Biochem* 42:413-414, 2005.

Frink RJ. *Inflammatory atherosclerosis: characteristics of the injurious agent*. Heart Research Foundation, Sacramento, CA, 2002.

Goli AK, Goli SA, Byrd RP Jr, Roy TM. Simvastatin-induced lactic acidosis: a rare adverse reaction? *Clin Pharmacol Ther* 72:461-464, 2002.

Hachinski VC, Smith KE , Silver MD, Gibson CJ, Ciriello J. Acute myocardial and plasma catecholamine changes in experimental stroke. *Stroke* 17(3):387-390, 1986.

Halperin ML, Kamel KS. D-lactic acidosis: Turning sugar into acids in the gastrointestinal tract. *Kidney Int* 49:1-8, 1996.

Hentrich C, Szostak JW. Controlled growth of filamentous fatty acid vesicles under flow. *Langmuir* 30(49):14916-14925, 2014.

Hippisley-Cox J, Coupland C. Unintended effects of statins in men and women in England and Wales: population based cohort study using the QResearch database. *BMJ* 340:c2197, 2010.

Hulter HN, Licht JH, Bonner EL Jr, Glynn RD, Sebastian A. Effects of glucocorticoid steroids on renal and systemic acid-base metabolism. *Am J Physiol* 239(1):F30-F43, 1980.

Hunter LJ, Wood DM, Dargan PI. The patterns of toxicity and management of acute nonsteroidal anti-inflammatory drug (NSAID) overdose. *Open Access Emerg Med* 3:39-48, 2011.

Kellum JA. Determinants of blood pH in health and disease. *Critical Care* 4:6-14, 2000.

Kirkup W, Merrick DW. A matter of life and death: population mortality and football results. *J Epidemiol Community Health* 57:429-432, 2003.

Komesaroff PA, Esler MD, Krishnankutty S. Estrogen supplementation attenuates glucocorticoid and catecholamine responses to mental stress in perimenopausal women. *J Clin Endocrinol Metab* 84(2):606-610, 1999.

Kraut JA, Madias NE. Metabolic acidosis of CKD: an update. *Am J Kidney Dis* 67(2):307-317, 2016.

Krieger NS, Frick KK, Bushinsky DA. Mechanism of acid-induced bone resorption. *Curr Opin Nephrol Hypertens* 13(4):423-436, 2004.

Lemann J, Bushinsky DA, Hamm LL. Bone buffering of acid and base in humans. *Am J Physiol Renal Physiol* 285:811-832, 2003.

Leor J, Poole WK, Kloner RA. Sudden cardiac death triggered by an earthquake. *N Engl J Med* 334:413-419, 1996.

Li K, Kaaks R, Linseisen J, Rohrmann S. Association of dietary calcium intake and calcium supplementation with myocardial infarction and stroke risk and overall cardiovascular mortality in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition Study (EPIC-Heidelberg). *Heart* 98(12):920-925, 2012.

Liamis G, Milionis HJ, Elisaf M. Pharmacologically-induced metabolic acidosis: a review. *Drug Saf* 33(5):371-391, 2010.

Marcus SL. Effects of estrogen and progesterone on the pH of uterine fluid in the cat. *Am J Obstet Gynecol* 91(1):114-117, 1965.

Markvoort AJ, Pfleger N, Staffhorst R, Hilbers PAJ, Van Santen RA, Killian, De Kruijff B. Self-reproduction of fatty acid vesicles: a combined experimental and simulation study. *Biophys J* 99:1520-1528, 2010.

Meisel SR, Kutz I, Dayan K, *et al.* Effect of Iraqi missile war on incidence of acute myocardial infarction and sudden death in Israeli civilians. *Lancet* 338:660-661, 1991.

Morigaki K, Walde P, Fatty acid vesicles. *Curr Opin Colloid*

Interface Sci 12(2):75-80, 2007.

Mozaffarian D, Benjamin EJ, Go AS, *et al*. On behalf of the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics -- 2015 update: a report from the American Heart Association. *Circulation* 13:e29-e322, 2015.

Muller JE, Ludmer PL, Willich SN, *et al.* Circadian variation in the frequency of sudden cardiac death. *Circulation* 75:131-138, 1987.

Neale R, Reynolds TM, Saweirs W. Statin precipitated lactic acidosis? *J Clin Pathol* 57:989-990, 2004.

Newsholme E, Leech A. *Functional biochemistry in health and disease*. Wiley Blackwell Publishing, Hoboken, NJ, 2010.

Nordmann AJ, Briel M. Statins: pleiotropic, but less than previously thought. *Eur Heart J* 33:1551-1552, 2012.

Pilz S, Scharnagl H, Tiran B, Seelhorst U, *et al*. Free fatty acids are independently associated with all-cause and cardiovascular mortality in subjects with coronary artery disease. *J Clin Endocrinol Metab* 91(7):2542-2547, 2006.

Pilz S, Scharnagl H, Tiran B, Wellnitz B, et al. Elevated plasma free fatty acids predict sudden cardiac death: a 6.85-year follow-up of 3315 patients after coronary angiography. *Eur Heart J* 28:2763-2769, 2007.

Pizzorno J. Acidosis: an old idea validated by new research. *Integr Med* 14(1):8-12, 2015.

Portaluppi F, Lemmer B. Chronobiology and chronotherapy of ischemic heart disease. *Adv Drug Deliv Rev* 59:952-965, 2007.

Reis AH. On the etiology of cardiovascular diseases: A new framework for understanding literature results. *Med Hypotheses* 92:94-99, 2016.

Remme WJ, Kruijssen DA, Look MP, Bootsma M, de Leeuw PW. Systemic and cardiac activation and severity of myocardial ischemia in humans. *J Am Coll Cardiol* 23:82-91, 1994.

Roberts WC. Coronary arteries in fatal acute myocardial infarction. *Circulation* 45:215-230, 1972.

Röcker K, Striegel H, Freund T, Dickhuth HH. Relative functional buffering capacity in 400-meter runners, long-distance runners and untrained individuals. *Eur J Appl Physiol Occup Physiol* 68(5):430-434, 1994.

Rosano GM, Panina G. Oestrogens and the heart. *Therapie* 54(3):381-385, 1999.

Sahebkar A, Simental-Mendía LE, Pedone C, *et al*. Statin therapy and plasma free fatty acids: A systematic review and meta-analysis of controlled clinical trials. *Br J Clin Pharmacol* 81(5):807-818, 2016.

Schomig A. Adrenergic mechanisms in myocardial infarction: cardiac and systemic catecholamine release. *J Cardiovasc Pharmacol* 12:S1-S7, 1988.

Sebastian A, Frassetto LA, Sellmeyer DE, Merriam RL, Morris RC Jr. Estimation of the net acid load of the diet of ancestral preagricultural Homo sapiens and their hominid ancestors. *Am J Clin Nutr* 76(6):1308-1316, 2002.

Shah NH, LePendu P, Bauer-Mehren A, *et al.* Proton pump inhibitor usage and the risk of myocardial infarction in the general population. *PLoS One* 10(6):e0124653, 2015.

Sharma G, Goodwin J. Effect of aging on respiratory system physiology and immunology. *Clin Interv Aging* 1(3):253-260, 2006.

Silver MD, Baroldi G, Mariani F. The relationship between acute occlusive coronary thrombi and myocardial infarction studied in 100 consecutive patients. *Circulation* 61:219-227, 1980.

Slavíková J, Kuncová J, Topolcan O. Plasma catecholamines and ischemic heart disease. *Clin Cardiol* 30:326-330, 2007.

Srinivasan A, Ramaswamy V, Kuruvilla S, Sehgal PK, Balakrishnan K. Calcified atherosclerotic plaque - where exactly is the calcium and what does it contain? *Ind J Thorac Cardiovasc Surg* 28(1):6-14, 2012.

Wasserman K, Hansen JE, Sue DY, *et al. Principles of Exercise Testing and Interpretation, 5th Ed*. Chp. 2. Lippincott Williams & Wilkins, Philadelphia, PA, 2012.

Weinstein JR, Anderson S. The aging kidney: physiological changes. *Adv Chronic Kidney Dis* 17(4):302-307, 2010.

Widmaier EP, Raff E, Strang KT. *Muscle. Vander's Human Physiology: The Mechanisms of Body Function (12th ed.)*. pp250-291. McGraw-Hill, New York, NY, 2010.