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REVIEW

Research update for articles published in EJCI in 2011

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Carotid intima–media thickness is associated with plasma lipoprotein-associated phospholipase A2 mass in nondiabetic subjects but not in patients with type 2 diabetes [1] (Robin P.F. Dullaart)

Lipoprotein-associated phospholipase A(2) (Lp-PLA2), as measured in total plasma, has been identified as an independent marker of cardiovascular disease (CVD). Since the publication of our study in which we showed that intima–media thickness is related to plasma Lp-PLA2 mass in nondiabetic subjects but not in type 2 diabetic subjects, several reports have appeared demonstrating that the effect Lp-PLA2 mass on progression of (sub)clinical atherosclerosis is divergent compared with the effect of Lp-PLA2 activity [2–4]. Among other explanations, it has become clear that the distribution of Lp-PLA2 between apolipoprotein B-containing lipoproteins (apoB lipoproteins; its main carrier in plasma) and high-density lipoproteins (HDL) is pathophysiologically important in establishing CVD risk conferred by Lp-PLA2 [5]. In this context, it will be instrumental to know whether Lp-PLA2-specific activity is different between apoB lipoproteins and HDL and varies according to the degree of metabolic dysregulation attributed to obesity and diabetes mellitus [6].

Plasma n-3 and n-6 fatty acids and the incidence of atrial fibrillation following coronary artery bypass graft surgery [7] (Gudrun V. Skuladottir and Olafur S. Indridason)

Based on our results, suggesting a U-curve relationship between n-3 LC-PUFA in plasma phospholipids (PL) and risk of POAF in CABG patients, we speculated that n-3 LC-PUFA

supplements might prevent POAF in patients with low levels of these fatty acids in plasma phospholipids (PL), but might be harmful in those with high levels [7]. Subsequent interventional studies have neither demonstrated a beneficial nor an adverse effect of n-3 LC-PUFA supplementation on the incidence of POAF following open-heart surgery [8–11]. These studies have not reported the effect of n-3 LC-PUFA separately in those with high and low baseline fatty acid levels in plasma PL, and no other study has been published to date examining the association between fatty acid levels in plasma PL, and POAF following CABG. We have examined POAF incidence in relationship to levels of fatty acids in RBC membranes in CABG patients and observed a linear increase in incidence of POAF with higher levels of n-3 LC-PUFA and DHA in RBC membranes [12].

Depressive symptoms predict mortality in elderly subjects with chronic heart failure [13] (Gianluca Testa and Pasquale Abete)

Depression affects 13–77% of patients with chronic heart failure (CHF), and it is independently associated with hospitalization and mortality in elderly CHF patients [14]. Depression is an obstacle to CHF self-care, and it is associated with nonadherence to medications and diet recommendations [14]. Particularly in elders, depression is associated with impaired cognition, and it may interfere with the ability to learn, perceive symptoms, judge severity of symptoms and make decisions about symptoms [14]. Finally, depression often leads to social isolation and therefore to a poor social support, which is important in self-care [15]. Antidepressant drug in CHF patients with depression should be considered, especially selective serotonin reuptake [14]. The Sertraline Against Depression and Heart Disease in Chronic Heart Failure study confirms these data [16]. Finally, physical activity whose positive effect on the heart is still unknown [17], appears to be effective in reducing depression in elderly patients with CHF [18].

Angiotensin receptor blockers are associated with a lower remodelling score of stenotic aortic valves [19] (Nancy Côté and Patrick Mathieu)

We previously documented that after correction for covariates, angiotensin receptor blockers (ARBs) are significantly associated with a lower tissue remodelling score of calcific aortic valve disease (CAVD) [19]. More recently, we identified in 477 patients with CAVD that the fibrotic process is related to the disease severity [20]. Moreover, we found that the fibrotic process of the aortic valve correlated significantly with the expression level of interleukin-6 (IL-6). Furthermore, it was established that patients under a therapy with ARBs had a lower expression of IL-6 in stenotic aortic valves and had a decrease level of fibrosis in valvular tissues. Hence, these findings highlight that fibrosis, mineralization and inflammation have important relationships in the pathobiology of CAVD. Furthermore, it gives prominence to the concept that the renin–angiotensin system (RAS) is involved in the pathogenesis of CAVD [21]. Hence, it is possible that blocking the RAS downstream of cascade with ARBs may decrease the remodelling process [22]. Therefore, more evidence has accumulated indicating that ARBs lower the remodelling process of aortic valves, which emphasizes the need to perform clinical trials to evaluate whether this class of medication could be used as a therapeutic avenue in CAVD.

Metastatic pheochromocytoma: does the size and age matter? [23] (Karel Pacak)

In our previous study, we concluded that the size of pheochromocytoma and age at first presentation are independent factors associated with metastatic disease [23]. Recently, we have found that metastatic disease derived from pheochromocytoma or paraganglioma (an extra-adrenal tumour) in childhood or adolescence is almost always associated with the presence of succinate dehydrogenase subunit B/D (SDHB/D) gene mutations [24]. The percentage of patients presenting with metastatic disease resulting from pheochromocytoma significantly increases with age, especially after age 50, and the metastatic interval significantly decreases with increasing age regardless of whether patients present with a hereditary or nonhereditary tumour (unpublished observations). We also found that the size of any pheochromocytoma or paraganglioma over 6 cm is an independent risk factor to develop metastases [25]. Our recent data suggest that the age at diagnosis of SDHB-related paraganglioma or pheochromocytoma is an independent survival predictor in these patients (unpublished observations).

Sensitive troponin and N-terminal pro–brain natriuretic peptide in stable angina [26] (Gjin Ndrepepa)

In our observational study that included 869 patients with stable coronary artery disease (CAD) treated with percutaneous coronary intervention, we concluded that combined use of high-sensitivity cardiac troponin T (hs-TnT) and N-terminal pro–brain natriuretic peptide (NT-proBNP) performed better than either biomarker alone for prediction of long-term (4-year) all-cause or cardiac mortality. Since its publication, evidence has gathered that the combined use of hs-TnT and NT-proBNP improves risk prediction in patients with CAD

and other cardiac diseases [27–30]. Thus, among patients with low-to-moderate likelihood of acute coronary syndromes, combined use of conventional or hs-TnT and NT-proBNP enabled a better reclassification of patients [27]. In another recent study of patients admitted to coronary care unit with chest pain, an excellent risk prediction was achieved by combining Hs-TnT and NT-proBNP in an easily used algorithm [28]. Moreover, another recent study showed that combination of NT-proBNP and hsTnT was found to be the strongest predictor of outcome in patients with aortic stenosis [29]. Of note, in patients with stable CAD, any detectable hs-TnT level was significantly associated with all-cause mortality, which was particularly pronounced in patients with NT–pro-BNP > 400 ng/L [30].

Expression of melatonin in platelets of patients with aspirin-induced asthma [31] (Helen V. Evsyukova)

As platelets are one of the extrapineal sources of melatonin in the humans, the reduced melatonin synthesis in platelets of aspirin-induced asthma (AIA) patients determines the low diurnal melatonin production in these patients [32]. The absence of melatonin in platelets of patients with AIA may lead to the impairments of receptors. It was found that patients with aspirin-exacerbated respiratory disease are associated with signal-regulated palmitoylation of RGS7BP gene polymorphism [33]. The low melatonin content in platelets of patients with AIA determines the abnormal reaction of platelets to exogenous melatonin, which we had found in these patients [32]. Melatonin at a dose of 0·01 pg/mL plasma increased the intensity and the rate of the first phase of the ADP-induced platelet aggregation only in patients with AIA, which is due to the opening of the receptor-operated channels for calcium and/or Ca^{2+} mobilization from intracellular stores. It was discovered that melatonin is able to elevate intracellular free calcium in humans' platelets by inositol 1, 4, 5-trisphosphate–independent mechanism [34].

The association of serum potassium level with left ventricular mass in patients with primary aldosteronism [35] (Yen-Hung Lin, Yi-Lwun Ho, and TAIPAI study group)

In a recent study, Kurisu *et al.* showed serum potassium level is associated with left ventricular diastolic function and left ventricular mass index (LVMI) in patients with primary aldosteronism (PA) [36]. In this study, 85 patients with PA (21 with hypokalaemia and 64 normokalaemic) were enrolled. Not only LVMI, serum potassium level was significantly correlated with several diastolic function parameters, including left atrial volume index, E/septal e', E/lateral e'. It was different from our study [35]. In our study, patients with PA had a greater impairment of cardiac diastolic function then patients with essential hypertension. Among patients with PA, serum potassium level was significantly correlated with LVMI but not diastolic function, which also measured by E/septal e' and E/lateral e'.

Measurement of nasal nitric oxide by hand-held and stationary devices [37] (Francesca Santamaria and Mauro Maniscalco)

Hand-held devices successfully analyse nasal nitric oxide (nNO) in primary ciliary dyskinesia (PCD) and cystic fibrosis (CF) [37]. A study of PCD and CF compared success rates, discriminatory capacity and agreement between a portable unit and two stationary devices, applying tidal breathing and velum closure [38]. The portable tidal breathing showed high success rate and discriminative power, and equal agreement vs. the stationary device.

Hand-held units analyse nNO through nasal exhalation. To explore whether continuous aspiration discriminates PCD, we measured nNO with a hand-held device during oral breathing through mouthpiece in 23 patients with PCD (4·6–32·8 years) and 23 age-matched controls [39]. Median nNO was 12 (PCD) and 506 ppb (controls) ($P < 0·001$). Sensitivity and specificity were as follows:

Cut-off (ppb)	Sensitivity (%)	Specificity (%)
36	78	100
44	83	100
51	87	100
53	91	100
58	96	100
138	100	100

We concluded that hand-held nNO by continuous aspiration distinguishes PCD from controls.

Nasal NO for screening PCD is used by few centres. Nevertheless, it is easy to perform and reproducible, and the availability of either sampling techniques overcoming poor cooperation by young children or hand-held devices will increase its application in the paediatric age [40]. Despite a central role has been definitely assigned to nNO measurement to discriminate between patients with PCD and healthy subjects, usefulness in young children are sparsely reported [41]. Yet, infants and preschool children with recurrent upper respiratory tract infections require special attention because this clinical picture may mimic PCD. Unfortunately, as a rare

diagnosis like PCD is often not considered, appropriate therapeutic management at a specialized respiratory reference centre is frequently delayed and this may eventually results in more risk of developing upsetting respiratory complications. Therefore studies aimed at using nNO in preschool children should be encouraged.

The assessment of nNO requires a relatively small cooperation from the patient, and is rapid and easy to perform. As the prevalence of PCD in the world population is likely underestimated, a larger diffusion of nNO portable equipment as first-line tool in the diagnostic work-up for PCD would hopefully result in a larger number of new PCD diagnoses, particularly among children and adolescents. Moreover, the markedly lower cost and the very simple use of portable devices compared with stationary analysers would make them affordable at either the general practitioner office or at secondary paediatric centres.

Modelling of cardiovascular response to graded orthostatic stress: role of capillary filtration [42] (Nandu Goswami and Keith M. Sharp)

A challenge for subject-specific modelling is that additional data are needed, in particular for the capillary filtration mechanism identified as the prominent cause of postflight orthostatic intolerance (POI) in the original article [42].

No new studies have since been reported that model individual subjects, however, reviews have summarized the importance of modelling [43] and transdisciplinarity to understand POI [44]. Recent results consistent with the influence of capillary filtration include compression stockings that prevent POI in astronauts [45], reduced blood pressure and cerebral blood flow velocity in astronauts unable to finish a stand test [46] and fluid loading with exercise to restore orthostatic tolerance after bed rest [47]. Evidence for other factors include altered vasoconstriction after spaceflight in rats [48], compromised cerebral autoregulation in astronauts [46], and gender differences in baroreflex response [49]. New experiments including blood volume measurements are needed to validate the capillary filtration mechanism.

Volume regulating hormone responses to repeated head-up tilt and lower body negative pressure [50] (Andreas Roessler and Nandu Goswami)

A challenge for repeated head-up tilt and lower body negative pressure experiments is that additional data are needed, in particular for confirmation of the hormonal response patterns to repeated central hypovolaemia and after the stress applications in the original article [50]. In [50], we reported that both adrenocorticotropic hormone and aldosterone progressively increased with repeated stresses and were elevated above baseline values during each rest period.

No new studies have since been reported that studied specifically these two models of hypovolaemia. However, recent results consistent with patterns such as elevated levels of aldosterone and ACTH have been seen following 10 [Goswami *et al*., 2013, manuscript in preparation] and 20 min [51] of presyncope, induced by head-up tilt and graded lower body negative pressure. New experiments are needed to validate these hormonal responses using the two models of repeated central hypovolaemia and across gender and in ageing populations.

Hormonal and Plasma Volume Changes After Presyncope [51] (Helmut Hinghofer-Szalkay and Nandu Goswami)

In the original article, we reported that during the 20-min supine postsyncopal period, aldosterone and ACTH continue increasing [51]. Since then, we have also observed that in the 10-min supine postsyncopal period, aldosterone and ACTH continue to rise (Goswami *et al*., 2013, manuscript in preparation). It appears that the hormone-specific endocrine activation patterns in the recovery phase are also present following a shorter period of recovery.

Similar patterns of hormone-specific endocrine activation, the magnitude of which cannot be explained by the haemoconcentration/haemodilution, have also been seen during repeated orthostatic challenges [50]: 30 min of head-up tilt and lower body negative pressure elevated the levels of aldosterone and ACTH, and they stayed elevated during the 30-min recovery periods poststress. The existence of this close correlation of aldosterone to ACTH should be assessed during exposure to different stresses, across different age groups and gender.

Methylcobalamin effects on diabetic neuropathy and nerve protein kinase C (PKC) in rats [52] (Soroku Yagihashi and Hiroki Mizukami)

Currently, we are studying on the other cellular signals involving in the pathogenesis of diabetic neuropathy using *in vitro* Schwann cells and diabetic animal models as well. We found that molecules such as Akt, S6RP and PI3K are also crucial for the cellular dysfunction and related to PKC activity. For the activation of these signals, we also found that insulin receptors and also glucagon-like peptide 1 (GLP-1) receptors mediate the activation of cellular signals, which are impaired in diabetic conditions. However, detailed mechanisms of how these signals are impaired remain to be explored. We will pursue in the next few years in this issue (Unpublished data).

Estradiol-dependent perforin expression by human regulatory T-cells [53] (Silvia Sánchez-Ramón and Marta Tejera-Alhambra)

In our work [53], we showed that estradiol (E2) expanded frequencies and enhanced *in vitro* suppressive function of regulatory T lymphocytes (TReg) (CD4 + CD25 + FoxP3+), increasing in parallel their perforin expression in healthy individuals. Another authors confirmed E2 effects on TReg suppressive function by cytokine-mediated mechanisms and its relevance in bone remodellation [54]. We have recently shown that in multiple sclerosis (MS), high E2 doses enhanced TReg function and induced a TReg phenotype in activated CD4 + CD25-T lymphocytes, which could be probably explained by highly E2-receptor distribution in TReg [55]. We also observed than during MS pregnancy, a period during which MS usually ameliorates, a expansion of TReg occurs [55], as we previously described for healthy pregnant women [53]. In another work, we demonstrated that during MS relapses, perforin-expressing TReg are actively recruited to the cerebrospinal fluid (CSF), highlighting its role in TReg function [56]. Women presented increased frequencies of CSF TReg than men [56].

Cost analysis and cost-effectiveness of NT-proBNP-guided heart failure specialist care in addition to home-based nurse care [57] (Christopher Adlbrecht and Martin Hülsmann)

Postdischarge NT-proBNP-guided heart failure specialist care in addition to home-based nurse care (HNC) was cost-effective in patients recently hospitalized for decompensated systolic heart failure [57]. Based on the fact that results may in part driven by the chosen method of analysis, recently our data were recalculated on an intention-to-treat analysis including sophisticated analytical models (Markov model and Monte Carlo simulation) [58]. These data entirely supported our conclusion. NT-proBNP is also a potent tool for the identification of patients at highest risk of heart failure in primary care [59]. In how far this diagnostic tool is cost-effective in this context has to be elucidated. As there are different biomarkers available, the question rises in how far they are equipotent in this context. A recent meta-analysis revealed that the application of NT-proBNP-guided therapy reduced all-cause mortality and HF-related hospitalization, but BNP-guided therapy did not [60]. Taken together, evidence that has accumulated since publication of our article reinforces the conclusion made.

Assessment of serum sodium to urinary sodium divided by (serum potassium)^2 to urinary potassium as a screening tool for primary aldosteronism [61] (Olivier Steichen)

About 5% of all hypertensive patients have PA [62]. Most experts and guidelines therefore advocate hormonal screening in patients with a higher pretest probability of PA: young-onset, high-grade or resistant hypertension; hypertension associated with hypokalaemia or adrenal mass. However, many patients will go unnoticed under this policy and will be denied the specific interventions known to lessen the increased cardiovascular risk associated with PA. Willenberg *et al.* proposed the serum sodium/urinary sodium)/(serum potassium^2/urinary potassium (SUSPPUP) ratio to better recognize patients with high pretest probability of PA [63], but we showed that it does not perform better than plasma potassium to this end. We are not aware of any new study evaluating the SUSPPUP ratio, or any other tool, as an aid to select patients for hormonal screening. Japanese guidelines now recommend universal screening [64], but the clinical and economical evidence for this recommendation is not straightforward.

The relation between thyroid-stimulating hormone and measures of adiposity in patients with manifest vascular disease [65] (Jan Westerink and Frank L.J. Visseren)

In 2011, we showed that higher plasma level of thyroid-stimulating hormone (TSH) is associated with increased visceral adipose tissue (VAT) thickness in patients with manifest vascular disease [65]. Since then, Moon *et al.* found no relation between plasma TSH levels and any adipose tissue compartment in a healthy population [66]. However, the methodology was compromised by the small size, the limited adjustment for potential confounders and a potential for collinearity. In a separate small study, Muscogiuri *et al.*, found that plasma TSH levels were indeed associated with both body mass index (BMI) and VAT but only performed simple correlation [67]. This study also concurs with our findings that VAT is more closely associated with TSH than insulin resistance. Finally, we showed in the same population of patients with manifest vascular disease that higher plasma levels of TSH are associated with an increased risk of myocardial infarction, an association with is most prominent in patients without visceral adiposity [68].

Expression of osteoprotegerin in human fat tissue; implications for chronic kidney disease [69] (Anna Witasp and Peter Stenvinkel)

Vascular calcification is a common feature of the phenotype of chronic kidney disease (CKD). The soluble receptor osteoprotegerin (OPG) is increased in patients with CKD and is thought to prevent vascular calcification via inhibition of bone resorption and osteoclastogenesis. Our study from 2011 demonstrated expression of *OPG* in human subcutaneous adipose tissue and found that *OPG* mRNA levels were lower in patients with CKD compared with controls. These findings accord with a newly proposed crosstalk between bone-acting cytokines and adipose tissue. Subsequent studies have further shown that adipose tissue *OPG* expression is associated with increased bone-mineral density [70,71], and that inflammation modulates adipose tissue expression and secretion of OPG [70]. Today, the molecular link between bone, adipose tissue and CKD complications remain to be unravelled. Recent clinical studies show that OPG is associated with aortic stiffness [72,73], myocardial damage (3) and moderate coronary artery calcification [74] in nondialysis patients with CKD.

Intestinal epithelial cell proliferation, apoptosis and expression of tight junction proteins in patients with obstructive jaundice [75] (Stelios F. Assimakopoulos and Chrisoula D. Scopa)

To the best of our knowledge, there is no new clinical evidence regarding intestinal cellular alterations in patients with obstructive jaundice. However, recent experimental studies have reinforced the pivotal role of enterocytes' tight junctions (TJs) disruption in the pathophysiology of obstructive jaundice-associated intestinal hyperpermeability and bacterial translocation [76–78]. According to recent animal studies, these phenomena occur even from the first day of biliary obstruction, which indicates that are independent of the magnitude and duration of hyperbilirubinaemia [76]. This finding is in accordance with our results showing lack of interrelation between bilirubin levels and intestinal TJs' expression. Additionally, decreased enterocyte proliferation due to bile deprivation was also reinforced as an important mechanism associated with gut barrier dysfunction in experimental studies [78–80]. Restoration of intestinal TJs' integrity and promotion of enterocytes' proliferation by internal biliary drainage or administration of probiotics prevented intestinal barrier dysfunction in experimental obstructive jaundice [77,78].

Lymphatic vasculature is increased in heart valves, ischaemic and inflamed hearts, and in cholesterol-rich and calcified atherosclerotic lesions [81] (Ivana Kholová and Seppo Ylä-Herttuala)

The lymphatic system is crucial for the regulation of tissue fluid homeostasis, fat metabolism, and elicitation of immune responses. Recently, we have studied lymphatic vasculature in normal and diseased human hearts [81]. Enhancing the published data, we have shown lymphangiogenesis in a large series of aortic and mitral valves involved with endocarditis (unpublished data). Another study showed lymphangiogenesis in advanced aortic valve stenosis [82]. We have revealed lymphatic capillaries invading the intima in advanced atherosclerotic plaques [81]. Interestingly, lymphatic vessels were shown to be crucial during macrophage reverse cholesterol transport to bring lipidated apoA-1 or HDL-C to plasma in mouse experimental model [83]. Stimulation of lymphatic vascular growth may be useful in various clinical settings. In experimental pig model of lymphoedema, lymph node transfer and perinodal injection of VEGF-C were shown to improve lymphatic vessels regeneration and lymph node function [84,85].

Use of cardio–ankle vascular index in chronic dialysis patients [86] (Te-Chao Fang)

Our previous study showed 'After 1-year follow-up, de novo arterial stiffness in dialysis patients as determined by cardio-ankle vascular index (CAVI) was significantly associated with age and initial serum phosphorus' [86]. In the last 2 years, no new evidence exists. Two similar studies using arterial pulse wave velocity (PWV) instead of CAVI in dialysis patients were reported on aortic calcification [87,88]. One study enrolled 212 haemodialysis patients during a follow-up for 69 $\pm$ 45 months and showed that aortic arch calcification was positively associated with age, dialysis vintage, arterial stiffness, parathyroid hormone level and negatively correlated with body weight and BMI [87]. The other study enrolled 155 peritoneal dialysis patients and after 2 years showed that most patient had a progressive increase in carotid–femoral PWV. The magnitude of increase was related to systolic blood pressure, serum calcium level and the baseline normalized protein nitrogen appearance [88].

YKL-40 in type 2 diabetic patients with different levels of albuminuria [89] (Clemens Hoebaus and Gerit-Holger Schernthaner)

Following our conclusions, other authors investigated YKL-40 in subjects with diabetes and albuminuria [90–92]. They found

a significant association of plasma but not urinary YKL-40 with early stages of diabetic nephropathy. Some even suggested YKL-40 as an early marker of disease [92]. We and others have investigated in the meantime the association of macrovacular disease and YKL-40 in subjects with type 2 diabetes mellitus (T2DM) [93,94]. YKL-40 was identified as independent predictor of cardiovascular mortality in subjects with T2DM [93]. All the new evidence so far suggests that YKL-40 is most likely involved in the biology of accelerated atherosclerosis in subjects with T2DM and albuminuria, but cell culture, animal and associated experimental studies are still rare.

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Appendix Statements made in the Conclusions of the Abstract of original articles published by the European Journal of Clinical Investigation in 2011 and current status for each statement as judged by the authors of each original study.

		Current status for the statement				
References	**Statements made in 2011**	**Reinforced** ***n*** **= 23**	**Modified** ***n*** **= 3**	**Weakened** ***n*** **= 0**	**No new evidence** ***n*** **= 11**	**Other** ***n*** **= 1**
[1]	Plasma lipoprotein-associated phospholipase A(2) (Lp-PLA2) may relate to early stages of atherosclerosis development. In diabetes mellitus, in contrast, the association of intima media thickness (IMT) with plasma Lp-PLA2 mass is abolished, which could be partly ascribed to redistribution of Lp-PLA2 mass from apolipoprotein B-containing lipoproteins towards HDL. These findings raise questions about the usefulness of plasma Lp-PLA2 mass measurement as a marker of subclinical atherosclerosis in type 2 diabetes mellitus		X*			
[7]	This study suggests that n-3 LC-PUFA supplements might prevent POAF in CABG patients with low baseline levels of these fatty acids in plasma PL, but may be harmful in those with high levels. AA may play an important role in electrophysiological processes				X	
[13]	Depression symptoms predict long-term mortality in elderly subjects without and, even more, with chronic heart failure (CHF). Thus, depression can be considered a strong predictor of death in CHF elderly subjects	X				
[19]	Angiotensin receptor blockers (ARBs) were associated with lower aortic valve weight and less pronounced tissue remodelling. Further studies are needed to determine if ARBs could be used as a therapeutic avenue in AS	X				
[23]	In conclusion, regardless of a genetic background, the size of a primary pheochromocytoma and age of its first presentation are two independent risk factors associated with the development of metastatic disease	X				
[26]	Combined use of high-sensitivity cardiac troponin T and N-terminal pro–brain natriuretic peptide improves long-term risk prediction of mortality in patients with stable coronary heart disease	X				
[31]	It can be concluded that the reduced melatonin synthesis in platelets of patients with aspirin-induced asthma may determine a low daytime melatonin production and may lead to impairments in platelet receptors and ion channels. This results in disturbances in calcium homoeostasis, which may be a cause of platelet activation and pathological response to exogenous melatonin and acetylsalicylic acid	X†				
[35]	Serum potassium level is significantly associated with left ventricular mass index in PA patients. Compared with essential hypertensive patients, PA patients had a greater impairment of cardiac diastolic function	X				

Appendix *Continued*

References	Statements made in 2011	Current status for the statement				
		Reinforced *n* = 23	Modified *n* = 3	Weakened *n* = 0	No new evidence *n* = 11	Other *n* = 1
[37]	The hand-held device is as effective as the stationary analyzer for assessing nasal nitric oxide during silent and humming exhalation. Its wider use might result in an increased number of subjects suspected to have primary ciliary dyskinesia	X				
[42]	The feasibility of subject-specific simulations of cardiovascular response to orthostatic stress was demonstrated, providing stronger evidence that capillary filtration is a prominent mechanism in causing orthostatic intolerance. These results may have clinical and spaceflight applications				X	
[50]	We speculate that the observed differences in blood pressure and hormonal responses to LBNP and HUT are caused by divergent effects of blood pooling in the splanchnic region, despite similar reductions in splanchnic perfusion. Apparently with repeated central hypovolaemia, especially by the 3rd application of stress, plasma aldosterone levels rise (along with ACTH), conceivably increasing its volume-guarding effect				X	
[51]	We conclude that during the 20-min supine post-syncopal period, plasma volume, plasma renin activity (PRA) and adrenomedullin and vasopressin (AVP) return closer to baseline but aldosterone and ACTH continue increasing. The magnitude of observed concentration changes cannot be explained by haemoconcentration/haemodilution, rather it appears that the observed changes are indicative of hormone-specific endocrine activation patterns in the recovery phase				X	
[52]	This study suggested that correction of impaired neural signalling of protein kinase C (PKC) may be a major attribute to the beneficial effects of methycobalamin (MC) on diabetic nerve	X				
	This study suggested that correction of oxidative stress–induced damage may be a major attribute to the beneficial effects of MC on diabetic nerve	X				
[53]	Our data demonstrate the presence of functional regulatory T-cells (TReg) cytotoxic properties in biological systems	X				
	Our data support the concept that oestradiol (E2) enhances the number and function of TReg suggesting the potential interaction between E2 and immunoregulatory mechanisms	X				
[57]	NT-BNP-guided heart failure specialist care in addition to home-based nurse care (HNC) is cost effective and cheaper than standard care	X				
	HNC for patients with heart failure is cost neutral as compared to standard care	X				
[61]	The [(serum sodium/urinary sodium)/(serum potassium^2/urinary potassium)] (SUSPPUP) ratio was outperformed by serum potassium as a screening tool for primary aldosteronism (PA) in this large validation sample				X	

Appendix *Continued*

References	Statements made in 2011	Current status for the statement				
		Reinforced *n* = 23	Modified *n* = 3	Weakened *n* = 0	No new evidence *n* = 11	Other *n* = 1
	The value of SUSPPUP ratio as an adjunct to serum potassium for PA is questionable because of the low specificity of their combination				X	
[65]	In conclusion, higher thyroid-stimulating hormone (TSH) plasma levels in the normal range are associated with more visceral adipose tissue (VAT) in patients with manifest vascular disease above the age of 66 years	X				
	No association was found between plasma TSH levels and weight or body mass index		X‡			
	The relation between TSH levels and VAT may provide an explanation for the increased cardiovascular risk associated with elevated TSH plasma concentrations within the normal range				X	
[69]	Human subcutaneous adipose tissue (SAT) expresses osteoprotegerin (OPG)	X				
	Human SAT does not express alpha-2-HS-glycoprotein (AHSG)				X	
	OPG expression is reduced in patients with chronic kidney disease stage 5 (CKD-5) when compared to controls, despite increased circulating protein levels					X§
[75]	Decreased enterocyte proliferation might represent an important mechanism for intestinal barrier dysfunction and hyperpermeability in patients with extrahepatic cholestasis	X				
	Altered tight junction (TJ) protein expression might represent an important mechanism for intestinal barrier dysfunction and hyperpermeability in patients with extrahepatic cholestasis	X			X	
	The potential pharmacological modulation of these factors (decreased enterocyte proliferation, and altered TJ protein expression) may lead to better control of intestinal permeability in the jaundiced patient with improved clinical outcome	X				
[81]	The highest number of lymphatics was found in valves in infective endocarditis	X				
	Increases in lymphatics also accompanied major cardiac pathological changes, such as acute and chronic ischaemia, progressive atherosclerosis, myocarditis and hypertrophy	X				
	Thus, blocking of excess lymphangiogenesis might be useful in progressive atherosclerosis, whereas stimulation of lymphatic vascular growth and function might be useful in cardiac hypertrophy and heart failure	X				
[86]	After 1-year follow-up, de novo arterial stiffness in dialysis patients as determined by cardio-ankle vascular index (CAVI) was significantly associated with age.				X	

Appendix *Continued*

References	Statements made in 2011	Current status for the statement				
		Reinforced *n* = 23	Modified *n* = 3	Weakened *n* = 0	No new evidence *n* = 11	Other *n* = 1
	After 1-year follow-up, de novo arterial stiffness in dialysis patients as determined by CAVI was significantly associated with initial serum phosphorus.				X	
[89]	This is the first report of a significant elevation of YKL-40 in type 2 diabetic patients with albuminuria	X				
	In addition, we observed a significant association with macrovascular disease	X				
	Because we detected an association between YKL-40 with renal, micro- and macrovascular disease, this protein could play an important for the increased risk of type 2 diabetic patients with albuminuria for the development of cardiovascular disease		X¶			

*Plasma Lp-PLA2 activity but not Lp-PLA2 mass has been shown to predict progression of subclinical coronary atherosclerosis, as measured by coronary artery calcification in subjects without and with type 1 diabetes[2]; on the other hand, higher Lp-PLA2 activity was found to confer increased cardiovascular risk, contrasting lower risk attributable to higher Lp-PLA2 mass levels [3]

†There are no data on the activity of different G-proteins (Gi, G0, Gs) and calcium homoeostasis in platelets of aspirin-induced asthma patients

‡Authors' findings pertain to patients with manifest vascular disease. Others have found such a relation in healthier populations.

§Whereas several recent studies on CKD patients have further demonstrated that circulating OPG levels are inversely associated with decreased renal function (5), no new evidence exist on the *OPG* adipose tissue expression in CKD.

¶All the new evidence so far suggests that YKL-40 is most likely involved in the biology of accelerated atherosclerosis in subjects with T2DM and albuminuria, but cell culture, animal and associated experimental studies are still rare.

