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REPORT

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Phospholipase Cγ2 is required for basal but not oestrogen deficiency–induced bone resorption [1] (Dávid Győri and Attila Mócsai)

Phospholipase Cγ2 (PLCγ2) is a critical regulator of osteoclast differentiation and basal bone resorption, which was demonstrated by Kertész *et al.* in agreement with previous reports [1]. The role of the PLCγ isoenzyme in bone resorption and in adhesion receptor signalling of osteoclasts was further emphasized in a recent review [2] reinforcing the statements made by Kertész *et al.*, [1]. Moreover, Dr. Faccio's group provided genetic evidence that targeting PLCγ2 can suppress inflammatory osteolysis [3]. This is in contrast with our observation that PLCγ2 does not play a major role in ovariectomy-induced bone loss, indicating that inflammatory osteolysis and oestrogen deficiency–triggered bone resorption may utilize different signalling pathways. It is also interesting to note the similarity between the impaired osteoclastogenesis seen in the genetic deficiency of PLCγ2 and the phosphoinositide 3-kinase isoform PI3Kβ [4], another molecule that seems to play an important role in osteoclast adhesion receptor signalling.

Predominant role of obesity/insulin resistance in oxidative stress development [5] (Massimo D'Archivio and Paulina Ormazabal)

The evidence provided in the last years reinforces our statement that obesity and insulin resistance play a relevant role in oxidative stress development. The adipose tissue of obese insulin-resistant subjects shows increased oxidative stress compared with insulin-sensitive patients [6]. This increase appears to be directly correlated with BMI [7] and exacerbated by abdominal adiposity [8].

Our statement that to treat obesity and insulin resistance might be a useful strategy in counteracting systemic oxidative stress has been confirmed only for obesity. Indeed, a recent paper showed that hypocaloric diet and regular moderate exercise are effective in reducing oxidative stress along with weight loss in obese patients [9]. In addition, it has been shown that consumption of green leafy vegetables, together with a fat-rich meal, improves antioxidant defences in obese subjects [10]: vegetable consumption has been encouraged as a component of nutritional education for obese and insulin-resistant patients.

Visceral fat positively correlates with cholesterol synthesis in dyslipidaemic patients [11] (Maria Anastasia Ricci and Graziana Lupattelli)

Our previous paper focused on the role of visceral fat on cholesterol metabolism, observing a positive correlation with a cholesterol synthesis marker (lathosterol) and a negative one with absorption ones (sitosterol and campesterol) [11]. Later studies indirectly confirmed this result: in primary hyperlipaemias, anthropometric and metabolic indices of visceral adiposity (BMI, waist circumference, HOMA-IR, triglycerides) all showed a positive correlation with lathosterol, the surrogate index of cholesterol synthesis [12,13]; BMI was the only independent predictor of lathosterol values, and HOMA-IR, index of insulin resistance strictly associated with visceral fat amount, was a negative predictor of sitosterol levels [12]. All these data confirmed our first observation.

In this setting, we had also suggested the use of ultrasonographic detection of abdominal adiposity finalized to a tailored therapeutic approach [11]. Later on, no further evidences emerged regarding the usefulness of ultrasonography in this context and more studies are desirable.

Use of clopidogrel and calcium channel blockers and risk of major adverse cardiovascular events [14] (Morten Schmidt)

Following our study [14], evidence from three studies has accumulated. First, a post hoc analysis of the CREDO trial found no evidence of decreased clopidogrel efficacy with use of calcium channel blockers (CCBs) when examining the combined effect on death, myocardial infarction and stroke at 1 year [HR 0·74 (95% CI: 0·45–1·21) for CCB users and 0·87 (0·62–1·23) for CCB nonusers] [15].

Second, a cohort study of 1258 patients undergoing percutaneous coronary intervention found that a clinically relevant interaction between amlodipine and clopidogrel on the 12-month composite outcome of cardiac death, myocardial infarction, stent thrombosis and ischaemic stroke was only observed in CYP3A5 non-expressing individuals, who lacked an alternate CYP3A-metabolizing pathway after inhibition of CYP3A4 by amlodipine [16].

Finally, a randomized, controlled, open label trial found no substantial difference in 30-day on-treatment platelet reactivity comparing amlodipine with non-CCB standard therapy in 98 clopidogrel-treated patients with ischaemic heart disease [17].

TGF-β1 content in atherosclerotic plaques, TGF-β1 serum concentrations and incident coronary events [18] (Christian Herder and Barbara Thorand)

Our results [18] indicated that *high* TGF-β1 content in human atherosclerotic plaques and *high* serum levels of TGF-β1 are not associated with *reduced* risk of coronary events. No new data became available regarding the predictive value of TGF-β1 in human plaques for cardiovascular disease (CVD), but there is novel evidence which extends our statement on the relevance of TGF-β1 in the circulation. Serum TGF-β1 was also not associated with risk of death from cardiac disease in the Japan

Collaborative Cohort (JACC) Study [19]. In the Cardiovascular Health Study (CHS), plasma TGF-β1 was not associated with any CVD outcomes [20], but showed a *positive* association with all-cause mortality [21]. In the CHS subgroup with high CRP, *high* plasma TGF-β1 was associated with *increased* CVD risk [20]. However, it is unclear whether this association is attributable to profibrotic properties of TGF-β1 or reflects a compensatory, anti-inflammatory response to CVD risk factors.

Inflammatory activation and carbohydrate-antigen-125 levels in subjects with atrial fibrillation [22] (Natale Daniele Brunetti and Francesco Santoro)

Several evidences have linked inflammation to the initiation and perpetuation of atrial fibrillation (AF), but it is still not clear whether inflammation is a consequence or a cause of AF [23]. After AF catheter ablation, baseline neutrophil/lymphocyte ratio predicted recurrence at long-term follow-up [24], while C-reactive protein and fibrinogen only predicted early AF recurrence [25].

Carbohydrate-antigen(CA)-125 levels, which may reflect the presence of heart failure and structural heart disease, should however be considered for risk stratification in subjects with AF. Recent evidence shows that increased CA-125 levels are related to atrial remodelling [26] and mitral regurgitation [27], which are strictly linked to initiation and perpetuation of AF. We are currently focusing on possible prognostic value of CA-125 among patients affected by Takotsubo cardiomyopathy.

In conclusion, structural heart disease should always be considered in patients with AF, and CA-125 may represent an easy tool for the identification of clinically significant heart remodelling.

Six-year prognosis of diabetic patients with coronary artery disease [28] (Carlo Lombardi and Alessandra Dei Cas)

We studied the 6 years outcomes of patients with diabetes mellitus (DM) and coronary artery disease (CAD). Patients had a relatively good prognosis. Comorbidities, heart failure (HF) and renal impairment, were the main determinants of survival [28].

Observational data on long-term survival in subjects with DM undergoing coronary angiography are scarse. In the Bypass Angioplasty Revascularization Investigation 2 Diabetes, 5-year survival was slightly higher (86·7%) compared with our study (75·5%) [29]. Another study has shown a 17·94% mortality during a 28 months follow-up of octogenarians admitted for acute coronary syndrome [30].

Consistent with our data, short- and long-term survival in subjects with DM has been shown to be mainly affected by age, serum glucose levels [31], impaired renal function [32] and HF, suggesting that traditional cardiovascular risk factors are still the main determinants of outcomes in these patients.

Interaction between Glycemic Control and Serum Insulin-like Growth Factor 1 on the Risk of Retinopathy in Type 2 Diabetes [33] (Tzu-En Wu and Harn-Shen Chen)

We conducted a study to investigate the risk of diabetic retinopathy in patients with type 2 diabetes [33]. Our data suggested serum IGF-1 was a contributing factor in severe diabetic retinopathy, and this effect may be masked by poor glycaemic control. We further reported that the association with severe retinopathy was the strongest in the UACR, followed by HbA1c, insulin therapy, diabetes duration, smoking, eGFR and then serum IGF-1 level [34]. Bazzaz *et al.* examined two polymorphisms of the IGF-1 gene in type 1 diabetes and health controls. They found that the main regulator of IGF-1 on microangiopathy resides elsewhere rather than the IGF-1 gene itself [35]. We have examined eye fundi in 43 acromegalic patients with diabetes [unpublished data]. There were 4 patients with proliferative diabetic retinopathy, but no nonproliferative diabetic retinopathy was found in our small group of patients. We reinforced our statement that the serum IGF-1 could be a contributing factor in diabetic retinopathy, although the effect may be masked by other risk factors.

Plasma myeloperoxidase level and peripheral arterial disease [36] (Helmuth Haslacher and Thomas Perkmann)

Our study, which shed light on the relationship between cardiovascular events in peripheral arterial disease (PAD) and myeloperoxidase (MPO) levels, has been recently confirmed by Ye *et al.* [37]. The authors investigated the prediction of a decreased ankle-brachial index (ABI), which is a surrogate of PAD and bears an increased risk of cardiovascular events, by known risk markers. In this study, elevated myeloperoxidase levels predicted lower ABI in adjusted multivariable linear regression models ($P < 0{\cdot}01$) [37].

Besides cardiovascular diseases, we could expand our hypothesis of oxidative stress represented by MPO to other general systemic conditions. In detail, mental stress enhanced the secretion of MPO by 20% in orchestra musicians during a performance situation [38,39]. At the same time, elderly marathon runners with high basal MPO levels indeed presented with lower executive cognitive functions [40].

These findings strengthen the conclusion that peripheral MPO levels might represent a reliable marker of systemic oxidative stress.

Effect of weight loss on C3 and C4 components of complement in obese patients [41] (Segundo A Gómez-Abril and Milagros Rocha)

To our knowledge, there is no new published evidence that a reduction in body weight following a VLCD produces reductions in complement C3. However, we do have unpublished data which indicate that weight loss after bariatric surgery is associated with a significant reduction in complement C3 that is maintained for up to 5 years postsurgery, thus reducing the presence of metabolic syndrome (MetS) by 77·2%.

In addition to this, growing evidence points to complement C3 as an emerging risk factor for metabolic, atherosclerotic/atherothrombotic and microangiopathic processes [42]. Phillips *et al.* showed that individuals with C3 concentrations in the top 50th percentile were at a higher risk of MetS, impaired insulin sensibility, hyperinsulinaemia and abdominal obesity than individuals in the bottom 50th percentile [43]. Interestingly, MetS was further augmented in high dietary fat consumers and smokers. Indeed, in this latter population, an independent association between cardiovascular and coronary heart disease and plasma complement C3 has been described [44,45], suggesting that a smoking habit affects the activation of C3 axis.

Impact of electrocardiographic interpretability on outcome in patients referred for stress testing [46] (Alberto Bouzas-Mosquera and Jesús Peteiro)

The association of baseline electrocardiographic abnormalities (BEA) (including those precluding a proper interpretation of exercise electrocardiography) with outcome has been confirmed in several studies. In postmenopausal women with coronary heart disease (CHD) or with risk factors for CHD, [47] BEA were associated with an annual rate of coronary death of 1·28% as compared with 0·53% among those with normal electrocardiograms. In elderly patients, BEA were also associated with an increased risk of cardiovascular death (HR 3·12, 95% CI 1·02–9·57) and all-cause mortality (HR 2·45, 95% CI 1·23–4·85) [48]. Likewise, in patients with chronic kidney disease included in the Cardiovascular Health Study [49], the presence of BEA was associated with a higher risk of cardiovascular events (HR 2·15, 95% CI 1·56–2·98) and death (HR 2·27, 95% CI 1·56–3·30). Finally, in the Health ABC study [50], BEA were also associated with an increased risk of CHD events (HR for major BEA 1·51, 95% CI 1·20–1·90).

Use of atorvastatin to inhibit hypoxia-induced myocardin expression [51] (Kou-Gi Shyu)

Hypoxia in neonatal cardiomyocytes increases myocardin expression and reactive oxygen species (ROS) to cause cardiomyocyte hypertrophy, which can be prevented by atorvastatin, a 3-hydroxy-3-methylglutaryl coenzyme reductase inhibitor (statin) by suppressing ROS and myocardin expression [51]. Li *et al.* demonstrated that atorvastatin could inhibit myocardin gene expression *in vivo* and *in vitro* [52]. Myocardin can cause cardiac hypertrophy. Jiang *et al.* recently demonstrated that inhibition of myocardin expression by interferon regulatory factor 9 could protect against cardiac hypertrophy [53]. These findings reinforce our statement that cardiomyocyte hypertrophy can be prevented by suppression of myocardin expression. However, Yang *et al.* reported that simvastatin, another statin, increased myocardin expression in embryomic stem cells [54]. Stretch can cause smooth muscle hypertrophy, and we found that cyclic stretch increased myocardin expression to cause rat aortic smooth muscle cell hypertrophy [55]. Statin may suppress myocardin expression in cardiovascular cells and increase myocardin expression in stem cells.

Cardiopulmonary assessment in primary ciliary dyskinesia [56] (Giuliana Valerio and Francesca Santamaria)

In the last decades, aerobic fitness (AF) has deserved great attention as sensitive marker of children and adolescents health status [57]. As AF is influenced by patients' physical activity (PA), as well as by cardiopulmonary, neuromuscular and musculoskeletal function, this issue received much attention in primary ciliary dyskinesia (PCD), a genetic disorder that may progress to chronic respiratory insufficiency.

Following our finding that VO2 peak (an index of AF) is significantly reduced in PCD children with impaired lung function [56], Madsen *et al.* confirmed these data in a larger sample of PCD children and adults [58]. Both studies also showed that VO2 peak is influenced by limitation in self-reported vigorous PA.

PCD youths with low PA and AF might develop additional risk factors for cardiovascular disease, including abdominal adiposity, elevated blood pressure and dyslipidaemia [59]. Assessment of cardiovascular risk in this population should prompt adequate preventive strategies concerning lifestyle.

Inorganic phosphate and FGF-23 predict outcome in stable systolic heart failure [60] (Richard Pacher and Max Plischke)

Since publishing our manuscript [60], information has been extended to c-terminal FGF23 [61]. Experimentally, some direct mediator effects of FGF23 on the heart were found as it increases cardiomyocyte cell size, the expression of gene markers of cardiac hypertrophy and total protein content of cardiac muscle. Furthermore, FGF23 was described as a novel regulator cardiac contractility [62]. Data confirm our results in

respect to phosphate as in this cohort there is also no association with total mortality, but with hospitalization [63]. Although Ess *et al.* confirmed that phosphate was correlated with Nt-proBNP, but found an independent association with death or heart transplantation in CHF [64].

Most important FGF-23 was proven in 3627 patients to be a possible marker for personalized medicine in heart disease. Benefit from ACE-inhibitors can be predicted by FGF-23 in a setting of stable IHD. This opens a complete new target for FGF23 [65].

Immunohistochemical expression of somatostatin receptor subtypes 2 and 5 in colorectal cancer [66] (Pavlos Msaouel and Michael Koutsilieris)

Since the publication of the above article, further data have accumulated indicating the association of somatostatin receptor (Sst) subtypes with lower tumour aggressiveness in non-neuroendocrine solid malignancies. Shi *et al.* reported that Sst promoter methylation is associated with decreased Sst protein expression in gastric cancer samples compared with normal gastric mucosa tissues [67]. Furthermore, Guo *et al.* showed that Sst3 expression is lower in poorly differentiated gallbladder cancer compared to moderately and highly differentiated gallbladder cancer [68]. In the same study, it was shown that Sst4 expression is higher in gallbladder cancer with lymph node metastasis compared with tumours without lymph node metastasis [68]. *In vitro* cell line experiments demonstrated that activation of Sst1 and Sst2/Sst5 heterodimer can inhibit proliferation of prostate cancer cell lines PC-3, DU-145 and LNCaP [69]. However, the clinical applications of these findings remain to be determined.

Decreased plasma sRAGE levels in COPD: influence of oxygen therapy [70] (Erica PA Rutten and Niki L Reynaert)

The statement that plasma sRAGE levels are decreased in patients with COPD compared to control subjects is confirmed by another manuscript published by our research group [71] and others [72]. Decreased levels of sRAGE in the patients using oxygen therapy in addition to an association of sRAGE with FEV1 and FEV1/FVC in the patients have been described. Although esRAGE was also decreased in patients with COPD when compared to control subjects, there was no association between esRAGE and lung function parameters [71]. A recent study again confirmed the results on plasma sRAGE and demonstrated that lower sRAGE was associated with greater progression of airflow limitation in 4 years in smokers with COPD [73]. These findings indicate that total sRAGE but not esRAGE may serve as marker of COPD disease state and severity and that sRAGE might have a protective role in the lung.

New findings by our group show an association of sRAGE with lung function and DLCO in patients with COPD [71]. Results from the ECLIPSE and TESRA cohort demonstrated an association between plasma sRAGE and emphysema severity by HRCT scan and DLCO in patients with COPD [72]. Regarding these new data, sRAGE could serve as a marker of disease severity. Concerning CML levels, only few data became available in patients COPD. In our previously mentioned study, plasma sRAGE was positively associated with CML in the patients [71]. Another study demonstrated both increased CML and sRAGE levels in patients with chronic heart failure but not in patients with COPD when compared to a control group [74]. Conclusions about CML levels in COPD cannot be drawn based on the currently available results.

Novel dual inhibitors of vascular endothelial growth factor and VEGFR2 receptor [75] (David L Vesely)

Four cardiac hormones (i.e. vessel dilator, long-acting natriuretic peptide (LANP), kaliuretic peptide (KP) and atrial natriuretic peptide (ANP) maximally decrease the VEGFR2 receptor in human pancreatic adenocarcinoma cells by 48%, 49%, 74% and 83%. Vessel dilator, LANP, KP and ANP decrease the VEGFR2 receptor by 77%, 89%, 88% and 67% in human small-cell lung cancer cells and by 48%, 92%, 64% and 71% in human prostate cancer cells, confirmed with Western blots. VEGF itself in pancreatic carcinoma cells decreases 42%, 58%, 36% and 40% secondary to vessel dilator, LANP, KP and ANP. VEGF likewise decreases in small-cell lung and prostate cancer cells. Conclusion: Four cardiac hormones are the first dual inhibitors of VEGF and the VEGFR2/KDR/Flk-1 receptor. There no evidence to refute this conclusion. Rather, VEGF stimulates cancer cells to grow by stimulating ras, MEK 1/2 and ERK 1/2 kinases and the 4 cardiac hormones decrease these kinases and cross-talk between VEGF and these kinases [76].

Postprandial remnant lipoprotein metabolism in autosomal recessive hypercholesterolaemia [77] (Hayato Tada and Masakazu Yamagishi)

Zhao *et al.* and several others have reported that uptake of certain fractions of lipoproteins, especially, VLDL remnant does not require LDL receptor adaptor protein 1 (LDLRAP1) [78]. Those basic data support our findings that clearance of remnant lipoproteins is preserved in autosomal recessive hypercholesterolaemia (ARH), which is caused by the loss of function mutations in LDLRAP1 gene. Also we have confirmed that catabolism of remnant lipoprotein fractions was

preserved in human ARH patient using stable isotope kinetic study [79]. We believe that that evidence could provide new insights into the novel pharmacological target for LDLRAP1 protein.

Low-frequency oscillations in cephalic vessels assessed by near-infrared spectroscopy [80] (Henrik Winther Schytz and Messoud Ashina)

We reported that near-infrared spectroscopy (NIRS) can be used to assess changes in low frequency oscillations (LFO) of cortical vessels between hemispheres and over time in healthy volunteers [80]. Since this report, Cooper *et al.* [81] demonstrated the importance motion correction techniques to minimize the impact of motion artefact in LFO NIRS analysis. In two NIRS studies, we investigated possible changes in LFO in obstructive sleep apnoea (OSA) and stroke patients. Patients with OSA showed a decrease in LFO amplitudes after continuous positive airway pressure therapy [82], which may reflect a decrease in sympathetic activity affecting cortical vessels. We also showed that LFO interhemispheric amplitude ratio was significantly altered in 16 stroke patients with hypoperfusion symptoms using LFO NIRS analysis [83]. Collectively, these studies demonstrated that NIRS might be used to assess alterations in LFO of cortical vessels in neurological diseases affecting cerebral haemodynamics.

Enhanced immunological response by dendritic cells in male hypogonadism [84] (Juan J Corrales and Alberto Orfao)

Following our previous observation about an enhanced immunological response of circulating CD16+ monocytes/dendritic cells (DCs) to antigen stimulation in male hypogonadism [84], we have recently analysed the effects of testosterone replacement therapy on the immunocellular response of these subjects [85]. Testosterone treatment induced overexpression of LAMP-2 on both classical (CD16-) and nonclassical (CD16+) monocytes/DCs, a very high correlation being observed between LAMP-2 expression on these cells and both the LH and FSH serum levels [85]. Of note, overexpression of LAMP-2 was detected both *ex vivo* and after immunogenic stimulation *in vitro* [85]. As LAMP-2 is a functional marker expressed during immune activation [86], our results support the occurrence of testosterone-induced immunological activation of CD16 monocytes/DCs, and therefore, a neuroendocrine modulation of the immune response. In this regard, overexpression of LAMP-2 may be potentially associated with lysosomal-mediated cell deletion and/or killing [87], and consequently, it may be involved in immunotolerance.

Recent findings expand on the results of our first publication in a different clinical context (e.g. testosterone replacement therapy in hypogonadal men), where we observed a testosterone-associated overexpression of LAMP2, both in nonclassical CD16+ monocytes/dendritic cells (DCs) and classical CD16− monocytes, as well as CD33+ myeloid DCs and CD33− plasmacytoid DCs. Therefore, testosterone replacement therapy is associated with activation of monocytes and DCs, under the above-described conditions.

As overexpression of LAMP-2, a biochemical marker of immune cell activation, in monocytes and DCs, was strongly correlated with the serum levels of both gonadotropins in hypogonadal men treated with testosterone, these results suggest that LH and FSH modulate the immune response in hypogonadal patients receiving testosterone treatment.

The pleiotropic effects of an increased release of the lysosomal contents of DCs and monocytes, as reflected by the increased LAMP-2 expression, suggest that LAMP-2 may also be associated with a regulatory effect on immune tolerance, and other immune functions such as antigen processing and presentation by MHC class II molecules in dendritic cells and their interaction with CD4+ T cells. In this regard, it has been shown that LAMP-2-deficient human B cells display an altered antigen presentation in MHC class II molecules [88].

Adipocyte chemerin release is induced by insulin without being translated to higher levels *in vivo* [89] (Christa Buechler and Andreas Schmid)

Chemerin is secreted from adipose tissue and the liver and is increased in obesity [90]. Recently, our group has shown that systemic chemerin is not affected by oral glucose uptake in nondiabetic individuals [89]. This finding has been confirmed by a study from Luque-Ramirez *et al.* published about 1 year later [91]. Of note, during a 4-h liquid meal test in women with normal glucose tolerance during third trimester of pregnancy and three to 4 months postpartum serum chemerin level does not change [92]. Fatty acids which are increased in obesity stimulate adipocyte chemerin release [93]. However, systemic chemerin is even modestly diminished in normal weight but not over-weight healthy probands after an oral fat challenge (unpublished own data). In over-weight males with the metabolic syndrome an oral fat load is accompanied by modestly reduced serum chemerin [94]. Therefore, postprandial hyperinsulinaemia and hyperlipidaemia do not contribute to higher serum chemerin levels.

Soluble CD163 is associated with body mass index and blood pressure in hypertensive obese Saudi patients [95] (Nasser M. Al-Daghri and Shaun Sabico)

In 2012, our group observed that soluble CD163 (sCD163), a marker of monocyte/macrophage activation, is associated with

body mass index (BMI) and blood pressure in a cohort of hypertensive obese patients [95]. A more recent evidence confirms our finding on sCD163's association with BMI, supporting sCD163 as an emerging biomarker for low-grade inflammatory conditions such as obesity [96]. The link between sCD163 and obesity is apparently modulated by diet-induced weight loss independent of physical activity. However, using a euglycaemic-hyperinsulinemic clamp technique, Kracmerova and colleagues observed that the association between sCD163 with CD163 mRNA expression in both adipose (AT) and subcutaneous tissue (ST) is only true for steady-state conditions and not during a weight-reducing hypocaloric diet, raising the possibility of a different mechanism at work on why sCD163 is altered on diet-induced weight loss [97]. No new evidence has been found to support the link between sCD163 and hypertension.

Plasma Lp-PLA2 mass and apoB-lipoproteins that carry Lp-PLA2 decrease after sodium [98] (Robin PF Dullaart and Michiel N Kerstens)

High dietary sodium lowers apolipoprotein B (apoB)-lipoproteins in humans, a finding that was reinforced in a hypercholesterolaemic mouse model, in which a high-sodium diet–ameliorated atherosclerosis development in addition [99]. The decrease in plasma lipoprotein-associated phospholipase A_2 (Lp-PLA2) after a sodium challenge [98] coincided with expected decreases in apoB-lipoproteins and in the total cholesterol/high-density lipoprotein (HDL) cholesterol ratio [100]. Lp-PLA2 that resides on apoB-lipoproteins is considered to be pro-atherogenic, whereas the small proportion of Lp-PLA2 that is associated with HDL may protect against atherosclerosis development [101]. It is, therefore, relevant to more precisely delineate the Lp-PLA2 load of atherogenic apoB-lipoproteins. We measured apoB-Lp-PLA2, which also decreased after sodium loading [98]. More recently, apoB-Lp-PLA2 was found to be elevated in hypercholesterolaemia and its change after statin treatment correlated with changes in oxidized low-density lipoproteins [102]. ApoB-Lp-PLA2 may represent a pathophysiologically relevant metric of the atherogenic potential of apoB-lipoproteins.

Prevalence of abnormal urinary albumin excretion in a population-based study in Spain: results from the HERMEX Study [103] (Nicolás Roberto Robles and Guadalupe Garcia Pino)

A post hoc analysis of our data found that statins therapy is independently associated with microalbuminuria [104]. This diagnosis could result in incorrect labelling of subjects as higher cardiovascular risk patients. This is not the first report of positive relationships between statins use and microalbuminuria [105].

Little is known on how microalbuminuria is interrelated to brain target organ disease. Delgado *et al*., [106] found an independent association between microalbuminuria and silent brain injury. Surprisingly, in most patients, these lesions did not coexist with heart or kidney involvement. This confirms the value of microalbuminuria as cardiovascular risk marker in Spanish population.

It has been reported data from the Fifth Korea National Health and Nutrition Examination Survey data from 2011. The prevalence of microalbuminuria was found to be 5·2%, and conventional risk factors for cardiovascular diseases are closely related to the presence of microalbuminuria in Korea [107].

Phosphorylated p38 and JNK MAPK proteins in hepatocellular carcinoma [108] (Yao-Tsung Yeh)

Although there is no direct evidence, accumulating articles have reinforced the oncogenic roles of p-p38 in hepatocellular carcinoma (HCC). Liang *et al*. demonstrated that forced expression of myeloid differentiation factor 88 (MyD88) was significantly associated with poor prognosis and survival; notably, MyD88 enhanced the activation of NF-κB and p38 to promote HCC cell proliferation and invasion *in vitro* [109]. TGF-β1/H2O2/HOCl-induced β3 integrin expression is also mediated by triggering the enhanced expression of p-p38 to promote metastatic phenotype [110]. Moreover, activation of p38 is involved in osteopontin (OPN) activation which is relevant to the metastasis of HCV-associated HCC [111], and in hypoxia-inducible factor 1α (HIF-1α)-promoting malignant phenotypes of HCC [112]. Similarly, no direct evidence reveals the antogonistic activity of p-JNK to p-p38. Nevertheless, activation of JNK/Beclin 1 pathway is a key modulator in histone deacetylase 6 (HDAC6)-mediated activation of caspase-independent autophagic cell death in liver cancer [113].

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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 2012 and current status for each statement as judged by the authors of each original study

		Current status for the statement				
References	**Statement made in 2012**	**Reinforced** ***N*** **= 27**	**Modified** ***N*** **= 0**	**Weakened** ***N*** **= 2**	**No new evidence** ***N*** **= 10**	**Other** ***N*** **= 1**
1	Our results indicate that phospholipase Cγ2 participates in bone resorption under basal conditions, likely because of its role in adhesion receptor signalling during osteoclast development	X				
	In contrast, phospholipase Cγ2 does not appear to play a major role in ovariectomy-induced bone loss				X	
5	Obesity and insulin resistance, more than type 2 diabetes, play the most relevant role in oxidative stress development	X				
	The correction of obesity might be a useful strategy in counteracting systemic oxidative stress	X				
	The correction of insulin resistance might be a useful strategy in counteracting systemic oxidative stress				X	
11	In hyperlipidaemic patients, the amount of visceral fat correlates with cholesterol synthesis	X				
	The use of ultrasonographic detection of abdominal adiposity allows a better characterization of cholesterol pathway, potentially useful for a tailored therapeutic approach				X	
14	Concomitant use of calcium channel blockers as a class did not modify the protective effect of clopidogrel and was not associated with increased cardiovascular risk among patients using clopidogrel after coronary stent implantation	X				
18	Our results indicate that high Transforming Growth Factor-β1 (TGF-β1) content in human atherosclerotic plaques and high serum levels of TGF-β1 are not associated with reduced risk of coronary events	X				

Appendix *Continued*

References	Statement made in 2012	Current status for the statement				
		Reinforced *N* = 27	Modified *N* = 0	Weakened *N* = 2	No new evidence *N* = 10	Other *N* = 1
22	Atrial fibrillation (AF) is characterized by an inflammatory activation. Impaired functional class in AF subjects might be associated with increased carbohydrate-antigen-125 levels and higher inflammatory markers	X				
28	Our study shows a relatively good prognosis of patients with type 2 diabetes mellitus. Comorbidities, namely heart failure and renal impairment, are main determinants of survival	X				
33	Our data suggested serum insulin-like growth factor-1 was a contributing factor in severe diabetic retinopathy and this effect may be masked by poor glycaemic control	X				
36	Hence, we suggest consideration of plasma myeloperoxidase (MPO) for the risk stratification of major adverse cardiac events (MACE) in patients with peripheral arterial disease (PAD)	X				
	In contrast, MPO-463G>A is not an independent risk factor for MACE in patients suffering from PAD				X	
41	Our findings show that in severely obese patients following a very low-calorie diet for 6 weeks produces reductions in factor C3, a biomarker of cardiovascular disease and a significant improvement in some features of metabolic syndrome	X				
	In this way, the above-mentioned diet may represent an effective strategy for treating obesity and related cardiovascular risk factors				X	
46	Uninterpretable electrocardiograms portend a worse prognosis in patients referred for stress testing	X				
51	Hypoxia in neonatal cardiomyocytes increases myocardin expression and reactive oxygen species (ROS) to cause cardiomyocyte hypertrophy, which can be prevented by atorvastatin by suppressing ROS and myocardin expression	X				
56	Assessment of resting pulmonary function and cardiopulmonary functional capacity could contribute to the evaluation of pulmonary impairment in primary ciliary dyskinesia (PCD) Given the benefit of physical exercise on airway clearance and on general health and quality of life, patients with PCD should be encouraged to adopt an active lifestyle	X				
60	The results of this study demonstrate the independent prognostic value of inorganic phosphate and fibroblast growth factor FGF-23 in heart failure even in the context of established risk markers	X*				
66	The present data confirm the relationship of above article, further data have accumulated indicating the association of somatostatin receptor Sst2 and Sst5 expression levels with reduced tumour aggressiveness	X				

Appendix *Continued*

References	Statement made in 2012	Current status for the statement				
		Reinforced *N* = 27	Modified *N* = 0	Weakened *N* = 2	No new evidence *N* = 10	Other *N* = 1
70	Plasma soluble Receptor for Advanced Glycation End Product (sRAGE) levels are lower in patients with chronic obstructive pulmonary disease (COPD) compared with healthy control subjects, and even lower levels in patients receiving long-term oxygen therapy	X				
	Because sRAGE correlated with lung function only in the whole group, sRAGE can be considered a marker of COPD, but not of disease severity. A lack of clear association between sRAGE, carboxymethyl lysine and systemic inflammation is furthermore evident			X		
75	Four cardiac hormones are the first dual inhibitors of vascular endothelial growth factor (VEGF) receptor and the vascular endothelial growth factor 2 receptor/kinase insert domain receptor/Foetal Liver Kinase-1 receptor (VEGFR2/KDR/Flk-1 receptor)				X	
77	These data demonstrate that unlike in familial hypercholesterolaemia (FH), remnant-like particles (RLP) clearance is preserved in autosomal recessive hypercholesterolaemia (ARH)	X				
	The preservation of postprandial RLP clearance may contribute to the mild phenotype of ARH compared with FH	X				
80	Our results show that low frequency oscillations phase shift arterial blood pressure – oxygenated haemoglobin (oxyHb) may be used as a robust measurement of differences in autoregulation between hemispheres and over time	X				
	In addition, we found a strong relation between oxyHb and velocity of the middle cerebral artery during paced breathing				X	
	Gain showed too large variation for clinical use, as the standard deviation was up to 100-fold of mean values				X	
84	These findings show an enhanced immunological response of circulating (activated) CD16+ dendritic cells to antigen stimulation, which was inversely related to testosterone and gonadotropin serum levels	X				
	Such findings suggest a modulation by the hypothalamic–hypophyseal–gonadal axis of the immune response and may have clinical implications for hypogonadic men, as regards susceptibility to autoimmune diseases and increased responses to antigenic stimuli	X				
89	Postprandial hyperinsulinaemia does not contribute to higher chemerin levels in nondiabetic individuals	X				
95	Taken together, these data demonstrate that the monocyte/macrophage activation-related soluble CD163 (sCD163) is positively associated with body mass index	X				

Appendix *Continued*

References	Statement made in 2012	Current status for the statement				
		Reinforced *N* = 27	Modified *N* = 0	Weakened *N* = 2	No new evidence *N* = 10	Other *N* = 1
	Taken together, these data demonstrate that the monocyte/macrophage activation-related sCD163 is positively associated with an increased arterial blood pressure				X	
98	Plasma lipoprotein-associated phospholipase A_2 (Lp-PLA2) mass levels decrease in response to a short-term oral sodium challenge				X	
	Plasma apoB-Lp-PLA2 decrease in response to a short-term oral sodium challenge					X[†]
103	A low frequency of abnormal urinary albumin excretion rate was detected in a randomly selected sample of Spanish general population				X	
	This finding agreed with the low rates of cardiovascular mortality and morbidity observed in Spain in spite of a high prevalence of classic cardiovascular risk factors	X				
108	Increasing phosphorylated-p38 (p-p38) levels in hepatocellular carcinoma (HCC) tissues were associated with tumour size and the formation of satellite tumours. High p-p38 expression could serve as a predictor for a poor survival for the patients with HCC	X				
	Simultaneous expression of phosphorylated Jun NH_2-terminal kinases (p-JNK) in HCC tissues might antagonize the promoting effect of p-p38 in human liver cancer	X				

*Extent information about the potential of FGF-23 as a marker for a personalized strategy in cardiac disease.

[†]The method used to assay apoB-Lp-PLA2, as described in this study, allows to document the load of apoB-lipoproteins with Lp-PLA2; it has been recently demonstrated that this estimate of the number of apoB-particles that contain Lp-PLA2 is elevated in hypercholesterolaemia and associates with changes in oxidized LDL in response to statin treatment; it is conceivable the apoB-Lp-PLA2 level represents a pathophysiologically relevant metric of the atherogenic potential of apoB-lipoproteins.