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Adiponectin and p53 mRNA in epicardial and subcutaneous fat from heart failure patients [1] (Rosa María Agra and Sonia Eiras)

Our findings showed that p53 mRNA expression levels are upregulated in epicardial adipose tissue (EAT) from patients with heart failure (HF). This upregulation was also found in myocardium [2]. Our group have described its upregulation in EAT by sympathetic system. However, in myocardium, Lu TM et al [2] have described the low Sirt1 expression as upregulator of the proapoptotic molecules through p53 [2] and Miller EJ et al showed that apoptosis and systolic dysfunction through p53 can be induced by partial liver kinase B1 deficiency [3]. Thus, in mice with myocardial infarction the early administration of olmesartan prevents cardiac rupture through the inhibition of p53 apoptosis activity [4] and also in vitro knockout of miR-155 in cardiac fibroblasts improves cardiac remodeling by down-regulation of p53. Even, in endothelial cells, its suppression can also increase angiogenesis through Hif-1 regulation [5-6]. These results suggest that p53 is a mediator and a therapeutic target in HF.

Dynamics of serum-induced endothelial cell apoptosis in patients with myocardial infarction [7] (Cesar Rios-Navarro and Vicente Bodi)

Recently, several in vivo studies have reinforced our previous conclusion. Firstly, the integrity and functionality of the endothelial cells (EC) after a myocardial infarction (MI) were evaluated in a rodent model of MI by determining nuclear damage, cell-to-cell connections and endothelium permeability. In consonance with our results, EC injury post-MI began during the ischemic conditions, reaching its maximum after reperfusion [8]. Secondly, EC dysfunction plays a pivotal role in microvascular obstruction (MVO), which is a strong long-term predictor of mortality post-MI. Our group studied MVO sequentially in a controlled swine model of reperfused MI. It was demonstrated that this process started immediately after reperfusion and peaked at the acute phase (Figure 1) [9], coinciding with the time frame in which the EC viability was mostly compromised. Lastly, it has been proposed that EC apoptosis may be triggered by NLRP3 inflammasome through the up-regulation of this pathway in MI derived-EC [10].

Increased soluble CD36 is linked to advanced steatosis in nonalcoholic fatty liver disease [11] (Carmelo García-Monzón and Águeda González-Rodríguez)

Since we reported that a positive correlation exists between serum levels of soluble CD36 (sCD36) and the histological grade of steatosis in non-alcoholic fatty liver disease (NAFLD) [11], no further evidence has been provided elsewhere modifying this statement. While in the last 2 years the key role of hepatocyte-specific CD36 expression in the development of fatty liver, by modulating the rate of free fatty acids uptake in hepatocytes [12-13] as well as the significance of sCD36 as prognostic biomarker for diabetic nephropathy [14] have been reinforced, no new

evidence exists so far on the value of sCD36 as non-invasive biomarker of advanced steatosis in NAFLD. We propose that measurement of circulating sCD36, alone or in combination with other risk factors we have recently reported [15], is useful to identify those NAFLD patients with higher degrees of steatosis. This hypothesis, however, must be validated in large cohorts of biopsy-proven NAFLD patients.

Incretins, amylin and other gut-brain axis hormones in children with coeliac disease [16] (Maria Papastamataki and Ioannis Papassotiriou)

Our publication revealed a different secretion pattern of gut-brain axis hormones in children with coeliac disease and especially in those with coeliac disease and type-1 diabetes mellitus, potentially acting as a model. Nowadays, new efforts are focused in elucidating the implication of neuropeptides in the microbiota-gut-brain-axis and to address the information carriers from the gut to the brain and vice-versa. To this end, the vagal and spinal afferent neurons, the immune mediators such as cytokines, the gut hormones and the gut-microbiota-derived signaling molecules, in the one way; and the sympathetic efferent neurons, the parasympathetic efferent neurons, the neuroendocrine factors involving the adrenal medulla and neuroendocrine factors involving the adrenal cortex, in the other way, are investigated step by step in these pathways in order to provide novel potential targets to treat metabolic disorders in several studies [17-19]. The role and cross-talk of Brain-Derived-Neurotrophic-Factor with gut-brain-axis hormones in patients with coeliac disease has currently been investigated by our group [20-21].

Irisin as a predictor of glucose metabolism in children: sexually dimorphic effects [22] (Nasser M. Al-Daghri and Shaun Sabico)

In 2014, our group observed that circulating irisin in children influence glucose levels in a sexual dimorphic manner with preponderance in girls [22]. Recent evidence confirms the sexual dimorphic differences in irisin levels in lean adults, not in obese subjects, but only after acute, high intensity endurance exercise [23]. Animal studies also demonstrated the effects of female sex hormones in irisin levels among ovariectomized female rats, but not in orchiectomized male rats [23]. Furthermore, the association of irisin levels in glycemic metabolism is reinforced in several recent studies, with elevated irisin levels being an independent predictor of diabetes mellitus and Homeostatic Model Assessment – Insulin Resistance (HOMA-IR) in adults [24-25]. The association of irisin however to insulin resistance is positive in adults as opposed to inverse association in children in our 2014 study. Circulating irisin's significant positive association between insulin resistance and pubertal stage were nevertheless demonstrated among obese children [26].

Recovery of upper limb muscle function in chronic fatigue syndrome with and without fibromyalgia [27] (Kelly Ickmans and Jo Nijs)

Recent work [28-29] strengthens the notion that patients fulfilling the diagnostic criteria for Chronic Fatigue Syndrome (CFS) and Fibromyalgia (FM) represent a more homogenous subgroup within the heterogeneous CFS population [27]. We found that the CFS+FM but not the CFS-only group presented with 1) significantly lower pressure pain thresholds; 2) enhanced temporal summation of pain; and 3) associations between descending nociceptive inhibition and cognitive performance [28]. Further, it was found that people with CFS+FM showed

significantly decreased cognitive performance compared to those with CFS alone [29]. Finally, fulfilment of the different diagnostic criteria for CFS did not generate a different clinical picture, whereas a diagnosis of CFS+FM selected symptomatically worse and more disabled patients [30]. Delayed muscle recovery following exercise, as shown in our 2014 study [27], might be due to impaired activation of AMP kinase, impaired stimulation of glucose uptake and diminished release of interleukin-6 as shown in skeletal muscle cultures of people with CFS [31].

Changes in adiponectin level and fat distribution in patients with type 2 diabetes [32] (Tomasz Miazgowski and Aleksandra Taszarek)

In addition to our initial results, further studies confirmed a relatively weak direct effect of metformin given alone on adiponectin level in patients with type 2 diabetes (T2DM). However, metformin given in combination with exanatide (GLP-1 agonist) [33] or exenatide and biphasic insulin aspart [34] significantly increased serum concentration of this adipokine. This effect is greatly influenced by improvements in insulin sensitivity and magnitude of weight reduction. On the other hand, although adiponectin is known to promote insulin sensitivity, new studies showed the paradoxical association between high serum adiponectin levels and increased cardiovascular mortality rate, suggesting an unexpected deleterious role of adiponectin on atherosclerotic processes in T2DM [35]. Similarly, while we could not demonstrate the effect of metformin on visceral fat (VF), recent studies suggest than other anti-diabetic agents, such as ipraglifrozin alone [36] or miglitol combined with sitagliptin [37], may be more potent in the reduction of VF.

Parvovirus B19 at the culprit coronary stenosis predicts outcome after stenting [38]

(Giampaolo Niccoli and Anna Severino)

Since our paper has been published, interesting studies have explored the possible mechanisms involved in the tropism of Parvovirus (PV) B19 for different cell types [39], including endothelial cells [40-41], erythroid progenitor cells [42] and T-cells [43]. Altered function of these cell types might be responsible for the occurrence of major adverse cardiac events (MACE) after coronary stenting in different ways, thus supporting the main observation of our paper. In particular, Anbarlou and collegues have shown that miRNAs may be involved in regulation of PVB19 replication in different cell types, allowing structural protein expression in non-permissive cells [39]. PVB19 uptake into endothelial cells seems to be enhanced by an antibody-mediated mechanism that involves a receptor for complement factor C1q [40]. Moreover, it has been hypothesized that B19V is a perpetrator of impaired endothelial regeneration and that B19V infection might result in dysfunctional endogenous vascular repair [41]. Finally, PVB19-specific CD4+ T-cells have been identified with high cytolytic potential [43] These cytolytic T-cells could have a role in the pathogenesis of some autoimmune disorders reported to be associated with PVB19, and also in target-lesion complication after coronary stent implantation.

Simvastatin and bezafibrate increase cholesterol efflux in men with type 2 diabetes [44]

(Robin P.F. Dullaart and Uwe J.F. Tietge)

The focus of high density lipoprotein (HDL) research is currently being shifted from HDL cholesterol quantification towards determination of HDL's functional properties and HDL subfraction measurement. Indeed, the documentation that the capacity of HDL to stimulate

efflux of cholesterol out of macrophages in vitro (cholesterol efflux capacity; CEC) predicts incident atherosclerotic cardiovascular events even independent from HDL cholesterol [45], a close predictor of CEC [46], has boosted interest in HDL functionality measurement. Intriguingly, CEC may predict renal graft failure as well [47]. While our study has demonstrated that simvastastin, bezafibrate and their combination increase CEC in diabetic men [44], variable results of lipid modifying treatment on CEC have been reported since using various statins in other patient groups [48]. No additional information has been published regarding the effect of statin and fibrate administration on other metrics of HDL functionality, including its anti-oxidative, anti- inflammatory and other potentially important atheroprotective properties.

Endothelial function and carotid intima-media thickness in giant-cell arteritis [49] (Franz Hafner and Thomas Gary)

Recent reports indicate that contrast-enhanced ultrasonography might improve the visualization of the carotid wall and its vascularization as a marker of disease activity [50].

In addition to previously published data, we investigated endothelial function and carotid intima-media thickness (C-IMT) in an age- and gender matched control group without vasculitis. Forty-five (64.4% female) subjects underwent measurement of flow-mediated dilatation (FMD) and C-IMT at baseline and 6 months thereafter. Mean FMD did not vary between baseline (2.6 ± 3.1%) and 6 month control [2.7 ± 3.1% (p=0.858)]. In contrast to the reported significant decrease of carotid IMT in giant cell arteritis under steroid treatment [49], C-IMT increased in control subjects between baseline (0.68 ± 0.10mm) and 6 month control [0.71 ± 0.09mm (p=0.004)]. These findings reinforce our statement that, in contrast to endothelial function, steroid therapy significantly improves carotid intima-media thickness in giant-cell arteritis, which reflects the specifity of giant-cell arteritis for cerebrovascular arteries [49].

Retinol-binding protein 4 levels and susceptibility to ischaemic events in men [51] (Judit Cubedo and Lina Badimona)

We reported decreased retinol-binding protein 4 (RBP4) systemic levels in men in the early-phase of acute-myocardial-infarction (AMI) and in familial-hypercholesterolemia (FH)-patients before the presentation of an ischemic event, and a potential vasculoprotective role of RBP4 through NO and PGI2 [51]. In line with these findings, decreased RBP4 levels were found in patients with high total-cholesterol levels [52], highlighting that the reported results are not exclusive to FH-patients. On the opposite side of cardiovascular risk, increased RBP4 levels were reported in patients with activated anti-atherosclerotic defences (Bartter's/Gitelman's-syndrome) together with an increased NO-mediated vasodilation, again supporting our results [53]. Interestingly, an association between RBP4 levels and coronary lesion complexity in women with stable coronary-artery-disease and acute-coronary-syndromes [54] was reported, underscoring the previously reported sex influence on RBP4 levels. All these evidence highlight the need for further studies to advance our mechanistic understanding of RBP4 function and to design clinical studies to determine the role of this intriguing adipokine in cardiovascular disease progression and clinical event presentation.

Residual thrombin generation potential is inversely linked to the occurrence of atherothrombotic events in patients with peripheral arterial disease [55] (Thomas Gremmel)

No new evidence on the association of thrombin generation potential with the occurrence of atherothrombotic events has accumulated in patients with peripheral arterial disease (PAD). However, in another study, we investigated the predictive value of protease-activated receptor

(PAR)-1 mediated platelet activation for ischemic complications in patients undergoing infrainguinal angioplasty with stent implantation for PAD [56]. We found that high PAR-1 mediated platelet surface expressions of P-selectin and activated glycoprotein IIb/IIIa were strong predictors for target vessel restenosis/reocclusion and atherothrombotic events in these patients. These findings are in line with our initial hypothesis that a low remaining thrombin generation potential in vitro may be the consequence of continuously ongoing thrombin generation in vivo with consecutive subclinical platelet activation. Pre-activated platelets may respond stronger to additional stimuli and thereby predispose patients for future cardiovascular events.

Inferior vena cava parameters predict re-admission in ischaemic heart failure [57]

(Federico Carbone and Fabrizio Montecucco)

In the two years following the publication of the study "Inferior vena cava parameters predict re-admission in ischaemic heart failure" [57], we were unable to find any new evidence about the early re-admission rate of patients with heart failure (HF) that was higher in ischaemic heart disease (IHD) group as compared to non-ischemic patients.

With regard to the statement that change in inferior vena cava (IVC) minimum diameter from admission to discharge was the best predictor of re-admission in patients with IHD, some studies partially reinforced it. The respiratory collapse of the IVC was consolidated as an approach to monitor the therapeutic response in patients admitted for HF [58]. Other studies confirmed the predictive role of IVC measurements (either as maximum diameter [59-60] and inspiratory collapse [61]) as predictor of mortality in patients with HF. Although further studies are required, the IVC parameters may be useful to stratify high-risk patients.

Short-term Type-1 diabetes differentially modulates 14-3-3 proteins in rat brain and liver [62] (Antonio Gnoni and Federica Taurino)

Recently, it has been reported [63] that in the cerebral cortices of diabetic animals with middle cerebral artery occlusion (MCAO) injury, the 14-3-3 proteins (14-3-3s) levels were decreased thus supporting our data [62].

It has been demonstrated [64] that in the brain cortex and hippocampus of insulin-resistant rat, the 14-3-3s mRNA and protein levels were altered. These results mainly corroborate our findings [62]; however, some discrepancy was reported (e.g. 14-3-3θ protein level). There are several possible explanations for this result: i) in [62] the protein levels were measured in both brain cytosol and nuclei while in [64] it was employed a tissue homogenate, ii) in [62] we used a type-1 diabetes mellitus (T1DM) animal-model that is insulin-sensitive.

Recent evidence [65] indicates that alterations in the 14-3-3s phosphorylation may contribute to the Parkinson's disease (PD). Interestingly, it was found that 14-3-3θ protein levels were altered in the brain cortex and substantia nigra of human PD brains, thus creating an intriguiging connnection with our results in T1DM brains [62].

Decreased cholesterol efflux capacity in patients with low cholesteryl ester transfer protein plasma levels [66] (Andreas Ritscha and Winfried März)

Our suggested concept that low plasma cholesteryl ester transfer protein (CETP) concentrations might lead to impaired HDL function within the reverse cholesterol transport is in good agreement with several subsequently published clinical studies. Firstly, following torcetrapib and dalcetrapib, the third CETP inhibitor evacetrapib failed to prevent cardiovascular events

despite an apparent substantial increase in high-density lipoprotein cholesterol (HDL-C) [67]. Secondly, we were able to show that parameters reflecting high-density lipoprotein (HDL) functionality are able to predict cardiovascular outcome [68]. Thirdly, cholesterol efflux capacity has been shown to be inversely associated with the incidence of cardiovascular events [69] and hence can serve as an independent measure for predicting all-cause and cardiovascular mortality [70]. We have extended these investigations to patients at high risk for cardiovascular events and found that low cholesterol efflux capacity constituted an independent risk factor for cardiovascular death [71]. Winding up the vast majority of these data is reinforcing our initial findings. Current evidence warrants that future research focuses on HDL function rather than on the HDL cholesterol plasma concentration.

New vasoactive peptides in cirrhosis: organ extraction and relation to the vasodilatory state [72] (Søren Møller and Nina Kimer)

Recent research has elaborated and developed statements related to copeptin and atrial natriuretic peptide. Copeptin is released by inflammation or hemodynamic stressful conditions [73] and is elevated in patients with heart failure and related to pro-ANP and reduced renal and systolic function [74]. Galectin 3 is a new marker of inflammation that is increased in heart failure and associated with pro-ANP, copeptin, and renal dysfunction [74]. The pathophysiological importance of galectin 3 in cirrhosis is yet unknown. Kerbert et al. confirmed increased copeptin in cirrhosis, and reported relations to circulatory dysfunction, severity of cirrhosis and to transplant-free survival [75]. Experimental and clinical results from the same group showed that copeptin correlated with MELD and MELD-Na scores indicating a potential benefit of combining copeptin with specific prognostic scores [76].

Data from the last two years reinforces the involvement of pro-ANP and copeptin in the pathophysiology of cirrhosis. New aspects include independent prognostic value of copeptin and development of new biomarkers of complications of cirrhosis, such as galectin 3.

Impaired resolution of inflammation in human chronic heart failure [77] (Marta Reina-Couto and Teresa Sousa)

We previously described that higher severity of heart failure (HF) is associated with reduced levels of lipoxins (LXs) [77]. Meanwhile, increasing evidence has accumulated regarding the cardiovascular and metabolic protective effects of specialized proresolving mediators that might delay the onset and progression of HF [78-79]. Post-hoc analysis of our data in HF patients revealed that plasma 15-epi-LXA4 (ng/mL) inversely correlates with triglycerides (TG, mmol/L)) and with the TG-to-high-density lipoprotein cholesterol ratio (TG/HDL-c), which is in line with recent reports that LXs promote reverse cholesterol transport [78]. Additionally, we observed inverse correlations between the urinary excretion of LXs (U-LXA4 and U-15-epi-LXA4, μg/day) and age (years), and positive correlations of U-15-epi-LXA4 (μg/day) vs weight (kg) and vs estimated glomerular filtration rate (eGFR, mL/min). These results emphasize the interest in the therapeutic use of drugs that stimulate 15-epi-LXs production, like aspirin, statins and ticagrelor [80-81].

Delayed vasodilation is associated with cardiovascular risk [82] (Cesare Tripolino and Concetta Irace)

In a recent issue we have explored the physical properties of carotid artery (i.e. elasticity) in subjects with different kinetic of brachial artery flow mediated dilation (FMD) [83]. Increased arterial stiffness is an independent predictor of morbidity and mortality and thus a useful cardiovascular risk biomarker [84]. Our results demonstrated that subjects with delayed

vasodilation had increased carotid stiffness (4.7±1.6 vs 7.4±3.9) and reduced distensibility (12.8±7.4 vs 16.3±5.6) compared with subjects having an early vasodilatory response.

To further define the cardiovascular risk associated with delayed FMD, we have explored its relationship with the low flow mediated constriction [85]. In condition of low flow (such as during cuff inflation), brachial artery constrains and this phenomenon is associated with cardiovascular risk factors and coronary artery disease [86]. In our population, subjects with delayed FMD had a more pronounced low flow mediated constriction than individuals with early response (0.67±5.2 vs 1.22±4.7).

Urinary proteomics in obstructive sleep apnoea and obesity [87] (Ian W Seetho and John PH Wilding)

We postulated that there may be a role for urinary proteomic profiling with capillary electrophoresis-mass spectrometry (CE-MS) in characterising urinary profiles in severely obese populations with obstructive sleep apnoea (OSA) [87]. Following on from this study, we studied the urinary peptide profiles in severely obese subjects with OSA on continuous positive airway pressure (CPAP) treatment and controls without OSA. A urinary proteome comprising 15 peptides was identified using CE-MS that showed differential expression between the two groups. Although correction for multiple testing did not reach significance, sequences were determined for 8 peptides interestingly demonstrating origins from collagens, fibrinogen beta chain and T-cadherin that may be associated with perturbations in vascular function and a potential mechanistic link with cardiovascular disease that is observed in OSA [88]. The evidence that has accumulated thus far reinforces our hypothesis that urinary proteomics may have a potential role in studying OSA, including investigating mechanisms underlying OSA complications and its treatment.

Subclassification of left ventricular hypertrophy based on dilation stratifies coronary artery disease patients with distinct risk [89] (Baotao Huang and Mao Chen)

The clinical relevance of the new classification of left ventricular hypertrophy has been tested in other cohorts of general population and patients with hypertension. A recent published review by Garg et al. summarized the accumulating evidence elaborately [90]. In general, longitudinal studies showed that no matter eccentric or concentric hypertrophy, subjects with dilated left ventricle are at higher risk of adverse prognosis; furthermore, all but one studies corroborated the benign phenotype of eccentric nondilated left ventricular hypertrophy [91, 92]. Nevertheless, the predictive value of the new classification of left ventricular (LV) geometry beyond or independent of left ventricular mass was questioned [93]. In additional, the relationship between time-varying changes of left ventricular geometry and clinical outcomes has not been evaluated. Other studies, using two- or three- dimensional echocardiographic technology, observed structural and functional characteristics of left ventricle, left atria, and right ventricle across different LV geometry in hypertensive subjects.

Advanced oxidation protein products and ischaemia-modified albumin in obstructive sleep apnea [94] (Serkan Ozben and Tomris Ozben)

In our previous study, we detected high systemic oxidative stress as reflected by increased advanced oxidation protein products, total oxidative status and reduced total antioxidative capacity with no marked change in ischaemia-modified albumin (IMA) levels in obstructive sleep apnea syndrome (OSAS) [94]. Further studies performed after our study reported significantly increased IMA levels and oxidative stress which decreased after continuous positive airway pressure [95-98]. While oxidative stress results of these studies are in

accordance with our data, IMA results are in contrast to ours and weaken our conclusion. This discrepancy on IMA might be due to differences in study design, variability in sampling and methods and different demographical characters of the patients. Conflicting results exist between IMA levels and BMI and obesity. In order to accept IMA as a potential biomarker, multivariate analysis and validation studies need to be performed in a large number of OSAS patients.

Renal denervation in multiple renal arteries [99] (Michiel Voskuil and Willemien Verloop)

After publication of our manuscript in 2014, more insight has been gathered regarding the technical challenges in renal denervation. After publication of the neutral outcome of the randomised HTN-3 trial [100], more attention has come to this subject. Particularly, assessing a reliable read-out of the procedure seems crucial. New techniques using assessment of sympathetic renal nerve activity recently showed a residual source of sympathetic tone in accessory arteries. This may result in persistent hypertension in these patients and thereby explain the large response variability after renal denervation in general [101]. These publications led to an increased call for strict selection of patients and guidance of the procedure [102]. Also, different designs of future studies using a more aggressive ablation technique hopefully move this interesting field of research forward [103].

Recurrent thyroid cancers have more peritumoural lymphatic vasculature than nonrecurrent thyroid cancers [104] (Tommi Hakala and Ivana Kholová)

The lymphatic system plays an essential role in the tissue fluid homeostasis, immune surveillance and fat metabolism. Despite its crucial functions, it is still somewhat forgotten part of the circulatory system [105]. We have studied lymphatic vasculature in thyroid cancer that is the most common endocrine malignancy. A high density of peritumoural lymphatic vessels was found in recurrent papillary carcinomas being a potential marker for more aggressive and recurrence-prone tumours [104]. Tumour microenvironment including immune cells is crucial for tumour development. Furthermore, angiogenesis and lymphangiogenesis have a pivotal role in tumour growth and spread. Tumour and immune cells produce pro- and anti-angiogenic factors in the tumour microenvironment [106]. Lymphangiogenesis has an emerging role in various inflammatory processes. Lymphatic vasculature is markedly remodelled by inflammation and may itself impact inflammation [107, 108]. The cross-roles of tumour development, inflammation and lymphangiogenesis await further studies.

Gender, age and risk of ST segment elevation myocardial infarction [109] (Ville Kytö and Jussi Sipilä)

There is accumulating evidence on gender differences of epidemiology, pathophysiology and outcomes of coronary artery disease [110]. In addition to ST segment elevation myocardial infarction (STEMI), men also have increased risk of two other acute coronary subtypes, namely non-ST-elevation myocardial infarction (NSTEMI) and unstable angina. In the same background population in which men had a 3.0 fold risk for STEMI [110], their risk of both NSTEMI [109] and UAP [111] was 2.4 times that of women. Adjusted to age and co-morbidity, this translates to

13% higher risk of myocardial infarction presenting with ST-elevations in men compared to women [112]. Incidence of myocardial infarction increases with age and data on differences in MI presentation in different age groups is mounting. Occurrence of NSTEMI appears to increase more rapidly with aging (61 % per 5-year increase in age) [111] than that of STEMI (41 % per 5-year increase in age) [113].

Treatment with Evasin-3 abrogates neutrophil-mediated inflammation in mouse acute pancreatitis [114] (Federico Carbone and Fabrizio Montecucco)

In the two years following the publication of the study "Treatment with Evasin-3 abrogates neutrophil-mediated inflammation in mouse acute pancreatitis" [114], two studies confirmed our results about the chemokine up-regulation in mouse models of acute pancreatitis [115, 116]. Specifically, Merza and co-workers observed a 20-fold increase of CXCL2, whereas data from Yu and colleagues showed a rise of serum CXCL1 and CXCL2 in the pancreatic tissue by 3-fold and 8-fold, respectively.

With regard to the CXC chemokine inhibition with Evasin-3 as potential therapeutic strategy interferring with damages in acute pancreatitis and related pulmonary complications, we were unable to find any new evidence. However, in two studies pharmacological inhibition of chemokine expression was demonstrated to effectively reduce leukocyte infiltration in pancreatic and pulmonary tissues during an acute pancreatitis [115, 116]. Although further studies are required, CXC chemokine inhibition may be a promising approach in the treatmenet of acute pancreatitis [117].

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References

[1] Agra RM, Teijeira-Fernández E, Pascual-Figal D, Sánchez-Más J, Fernández-Trasancos A, González-Juanatey JR, et al. Adiponectin and p53 mRNA in epicardial and subcutaneous fat from heart failure patients. *Eur J Clin Invest* 2014;**44**:29-37.

[2] Lu TM, Tsai JY, Chen YC, Huang CY, Hsu HL, Weng CF, et al. Downregulation of Sirt1 as aging change in advanced heart failure. *J Biomed Sci* 2014;**21**:57.

[3] Miller EJ, Calamaras T, Elezaby A, Sverdlov A, Qin F, Luptak I, et al. Partial Liver Kinase B1 (LKB1) Deficiency Promotes Diastolic Dysfunction, De Novo Systolic Dysfunction, Apoptosis,

and Mitochondrial Dysfunction With Dietary Metabolic Challenge. *J Am Heart Assoc* 2015;**5**. pii: e002277.

[4] Chen B, Lu D, Fu Y, Zhang J, Huang X, Cao S, et al. Olmesartan prevents cardiac rupture in mice with myocardial infarction by modulating growth differentiation factor 15 and p53. *Br J Pharmacol* 2014;**171**:3741-53.

[5] Sano M, Minamino T, Toko H, Miyauchi H, Orimo M, Qin Y, et al. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. *Nature* 2007;**446**:444-8.

[6] Oka T, Morita H, Komuro I. Novel molecular mechanisms and regeneration therapy for heart failure. *J Mol Cell Cardiol* 2016;**92**:46-51.

[7] Forteza MJ, Novella S, Trapero I, Hermenegildo C, Ruiz-Sauri A, Chaustre F, et al. Dynamics of serum-induced endothelial cell apoptosis in patients with myocardial infarction. *Eur J Clin Invest* 2014;**44**:46-53.

[8] Hollander MR, de Waard GA, Konijnenberg LS, Meijer-van Putten RM, van den Brom CE, Paauw N, et al. Dissecting the effects of ischemia and reperfusion on the coronary microcirculation in a rat model of acute myocardial infarction. *PLoS One* 2016;**11**:e0157233.

[9] Hervas A, de Dios E, Forteza MJ, Minana G, Nunez J, Ruiz-Sauri A, et al. Intracoronary infusion of thioflavin-S to study microvascular obstruction in a model of myocardial infarction. *Rev Esp Cardiol* 2015;**68**:928-34.

[10] Liu Y, Lian K, Zhang L, Wang R, Yi F, Gao C, et al. TXNIP mediates NLRP3 inflammasome activation in cardiac microvascular endothelial cells as a novel mechanism in myocardial ischemia/reperfusion injury. *Basic Res Cardiol* 2014;**109**:415.

[11] García-Monzón C, Lo Iacono O, Crespo J, Romero-Gómez M, García-Samaniego J, Fernández-Bermejo M, et al. Increased soluble CD36 is linked to advanced steatosis in nonalcoholic fatty liver disease. *Eur J Clin Invest* 2014;**44**:65-73.

[12] Wang C, Yan Y, Hu L, Zhao L, Yang P, Moorhead JF, et al. Rapamycin-mediated CD36 translational suppression contributes to alleviation of hepatic steatosis. *Biochem Biophys Res Commun* 2014;**447**:57-63.

[13] Wilson CG, Tran JL, Erion DM, Vera NB, Febbraio M, Weiss EJ. Hepatocyte-Specific Disruption of CD36 attenuates fatty liver and improves insulin sensitivity in HFD-fed mice. *Endocrinology* 2016;**157**:570-85.

[14] Shiju TM, Mohan V, Balasubramanyam M, Viswanathan P. Soluble CD36 in plasma and urine: a plausible prognostic marker for diabetic nephropathy. *J Diabetes Complications* 2015;**29**:400-6.

[15] García-Monzón C, Vargas-Castrillón J, Porrero JL, Alonso MT, Bonachía O, Castillo MJ, et al. Prevalence and risk factors for biopsy-proven non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in a prospective cohort of adult patients with gallstones. *Liver Int* 2015;**35**:1983-91.

[16] Papastamataki M, Papassotiriou I, Bartzeliotou A, Vazeou A, Roma E, Chrousos GP, et al C. Incretins, amylin and other gut-brain axis hormones in children with coeliac disease. *Eur J Clin Invest* 2014;**44**:74-82.

[17] Fournel A, Drougard A, Duparc T, Marlin A, Brierley SM, Castro J, et al. Apelin targets gut contraction to control glucose metabolism via the brain. *Gut* 2015; Nov 12. pii: gutjnl-2015-310230. doi: 10.1136/gutjnl-2015-310230.

[18] Emge JR, Huynh K, Miller EN, Kaur M, Reardon C, Barrett KE, et al. Modulation of the microbiota-gut-brain axis by probiotics in a murine model of inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol* 2016; **310**:G989-98.

[19] Khamsi R. Metabolism in mind: New insights into the 'gut-brain axis' spur commercial efforts to target it. *Nat Med* 2016;**22**:697-700.

[20] Hadjivassiliou M, Rao DG, Grìnewald RA, Aeschlimann DP, Sarrigiannis PG, Hoggard N, et al. Neurological Dysfunction in Coeliac Disease and Non-Coeliac Gluten Sensitivity. *Am J Gastroenterol* 2016;**111**:561-7.

[21] Russo F, Chimienti G, Clemente C, Ferreri C, Orlando A, Riezzo G. A possible role for ghrelin, leptin, brain-derived neurotrophic factor and docosahexaenoic acid in reducing the quality of life of coeliac disease patients following a gluten-free diet. *Eur J Nutr* 2015; Dec 21. [Epub ahead of print].

[22] Al-Daghri NM, Alkharfy KM, Rahman S, Amer OE, Vinodson B, Sabico S, et al. Irisin as a predictor of glucose metabolism in children: sexually dimorphic effects. *Eur J Clin Invest* 2014;**44**: 119-24.

[23] Zugel M, Qiu S, Laszlo R, Bosnyak E, Weigt C, Muller D, et al. The role of sex, adiposity and gonadectomy in the regulation of irisin secretion. *Endocrine* 2016; [Epub ahead of print].

[24] Huh JH, Ahn SV, Choi JH, Koh SB, Chung CH. High serum irisin level as an independent predictor of diabetes mellitus: a longitudinal population-based study. *Medicine (Baltimore)* 2016;**95**: e3742.

[25] Fukushima Y, Kurose S, Shinno H, Thu HCT, Tamanoi A, Tsutsumi H, et al. Relationships between serum irisin levels and metabolic parameters in Japanese patients with obesity. Obesity Science & Practice 2016;**2**:203-9.

[26] Reinehr T, Elfers C, Lass N, Roth CL. Irisin and its relation to insulin resistance and puberty in obese children: a longitudinal analysis. *J Clin Endocrinol Metab* 2015; **100**: 2123-30.

[27] Ickmans K, Meeus M, De Kooning M, Lambrecht L, Nijs J. Recovery of upper limb muscle function in chronic fatigue syndrome with and without fibromyalgia. *Eur J Clin Invest* 2014;**44**:153-9.

[28] Ickmans K, Meeus M, De Kooning M, Lambrecht L, Pattyn N and Nijs J. Associations Between Cognitive Performance and Pain in Chronic Fatigue Syndrome: Comorbidity with Fibromyalgia Does Matter. *Pain Physician* 2015;**18**:E841-52.

[29] Ickmans K, Meeus M, De Kooning M, Lambrecht L, Pattyn N, Nijs J. Can recovery of peripheral muscle function predict cognitive task performance in chronic fatigue syndrome with and without fibromyalgia? *Phys Ther* 2014;**94**:511-22.

[30] Meeus M, Ickmans K, Struyf F, Kos D, Lambrecht L, Willekens B, et al. What is in a name? Comparing diagnostic criteria for chronic fatigue syndrome with or without fibromyalgia. *Clin Rheumatol* 2016;**35**:191-203.

[31] Brown AE, Jones DE, Walker M and Newton JL. Abnormalities of AMPK activation and glucose uptake in cultured skeletal muscle cells from individuals with chronic fatigue syndrome. *PloS One* 2015;**10**:e0122982.

[32] Miazgowski T, Dziwura-Ogonowska J, Safranow K, Iskierska K, Widecka K. Changes in adiponectin level and fat distribution in patients with type 2 diabetes. *Eur J Clin Invest* 2014;**44**:192-9.

[33] Quan H, Zhang H, Wei W, Fang T. Gender-related different effects of a combined therapy of Exenatide and Metformin on overweight or obesity patients with type 2 diabetes mellitus. *J Diabetes Complications* 2016;**30**:686-92.

[34] Su K, Lv C, Ji Z, Wang Y, Wang H, Bai Y, et al. Phase III Study on Efficacy and Safety of Triple Combination (Exenatide/Metformin/Biphasic Insulin Aspart) Therapy for Type 2 Diabetes Mellitus. *Am J Ther* 2016; published online on Feb 03,2016; doi: 10.1097/MJT.0000000000000431

[35] Ortega Moreno L, Copetti M, Fontana A, De Bonis C, Salvemini L, Trischitta V, et al. Evidence of a causal relationship between high serum adiponectin levels and increased cardiovascular mortality rate in patients with type 2 diabetes. *Cardiovasc Diabetol* 2016;**5**:17.

[36] Yamamoto C, Miyoshi H, Ono K, Sugawara H, Kameda R, Ichiyama M, et al. Ipragliflozin effectively reduced visceral fat in Japanese patients with type 2 diabetes under adequate diet therapy. *Endocr J* 2016;63:589-96.

[37] Mikada A, Narita T, Yokoyama H, Yamashita R, Horikawa Y, Tsukiyama K et al. Effects of miglitol, sitagliptin, and initial combination therapy with both on plasma incretin responses to a mixed meal and visceral fat in over-weight Japanese patients with type 2 diabetes; "the MASTER randomized, controlled trial". *Diabetes Res Clin Pract* 2014;106:538-47.

[38] Niccoli G, Severino A, Pieroni M, Cosentino N, Ventrone MA, Conte M, et al.

Parvovirus B19 at the culprit coronary stenosis predicts outcome after stenting. *Eur J Clin Invest* 2014;**44**:209-18.

[39] Anbarlou A, AkhavanRahnama M, Atashi A, Soleimani M, Arefian E, Gallinella G. Possible involvement of miRNAs in tropism of Parvovirus B19. *Mol Biol Rep* 2016;**43**:175-81.

[40] von Kietzell K, Pozzuto T, Heilbronn R, Grössl T, Fechner H, Weger S. Antibody-mediated enhancement of parvovirus B19 uptake into endothelial cells mediated by a receptor for complement factor C1q. *J Virol* 2014;**88**:8102-15.

[41] Schmidt-Lucke C, Zobel T, Schrepfer S, Kuhl U, Wang D, Klingel K, et al. Impaired endothelial regeneration through human parvovirus B19-infected circulating angiogenic cells in patients with cardiomyopathy. *J Infect Dis* 2015;**212**:1070-81.

[42] Bua G, Manaresi E, Bonvicini F, Gallinella G. Parvovirus B19 replication and expression in differentiating erythroid progenitor cells. *PLoS One* 2016;**11**:e0148547.

[43] Kumar A, Perdomo MF, Kantele A, Hedman L, Hedman K, Franssila R. Granzyme B mediated function of Parvovirus B19-specific CD4(+) T cells. *Clin Transl Immunology* 2015;**4**:e39.

[44] Triolo M, Annema W, de Boer JF, Tietge UJ, Dullaart RPF. Simvastatin and bezafibrate increase cholesterol efflux in men with type 2 diabetes. *Eur J Clin Invest* 2014;**44**:240-8.

[45] Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, et al. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med* 2014;**371**:2383-93.

[46] Annema W, Dikkers A, de Boer JF, van Greevenbroek MM, van der Kallen CJ, Schalkwijk CG, et al. Impaired HDL cholesterol efflux in metabolic syndrome is unrelated to glucose tolerance status: the CODAM study. *Sci Rep* 2016;**6**:27367.

[47] Annema W, Dikkers A, de Boer JF, Dullaart RP, Sanders JS, Bakker SJ, et al. HDL Cholesterol Efflux Predicts Graft Failure in Renal Transplant Recipients. *J Am Soc Nephrol* 2016;**27**:595-603.

[48] Brownell N, Rohatgi A. Modulating cholesterol efflux capacity to improve cardiovascular disease. *Curr Opin Lipidol* 2016;**27**:398-407.

[49] Hafner F, Haas E, Belaj K, Froehlich H, Gary T, Eller P, et al. *Eur J Clin Invest* 2014;**44:**249-56.

[50] Schinkel AF, van den Oord SC, van der Steen AF, van Laar JA, Sijbrands EJ. *Eur Heart J Cardiovasc Imaging* 2014;**15**:541-6.

[51] Cubedo J, Padro T, Cinca J, Mata P, Alonso R, Badimon L. Retinol-binding protein 4 levels and susceptibility to ischaemic events in men. *Eur J Clin Invest* 2014;**44**:266-75.

[52] Jugnam-Ang W, Pannengpetch S, Isarankura-Na-Ayudhya P, Thippakorn C, Isarankura-Na-Ayudhya C, Lawung R, et al. Retinol-binding protein 4 and its potential roles in hypercholesterolemia revealed by proteomics. *EXCLI Journal* 2015;**14**:999-1013.

[53] Calo LA, Maiolino G, Pagnin E, Vertolli U, Davis PA. Increased RBP4 in a human model of activated anti-atherosclerotic and antiremodelling defences. *Eur J Clin Invest* 2014;**44**:567-72.

[54] Liu Y, Wang D, Chen H, Xia M. Circulating retinol binding protein 4 is associated with coronary lesion severity of patients with coronary artery disease. *Atherosclerosis* 2015;**238**:45-51.

[55] Gremmel T, Koppensteiner R, Ay C, Panzer S. Residual thrombin generation potential is inversely linked to the occurrence of atherothrombotic events in patients with peripheral arterial disease. *Eur J Clin Invest* 2014;**44**:319-24.

[56] Gremmel T, Steiner S, Seidinger D, Koppensteiner R, Panzer S, Kopp CW. In vivo and protease-activated receptor-1-mediated platelet activation but not response to antiplatelet therapy predict two-year outcomes after peripheral angioplasty with stent implantation. *Thromb Haemost* 2014;**111**:474-82.

[57] Carbone F, Bovio M, Rosa GM, Ferrando F, Scarrone A, Murialdo G, et al. Inferior vena cava parameters predict re-admission in ischaemic heart failure. *Eur J Clin Invest* 2014;**44**:341-9.

[58] Yavasi O, Unluer EE, Kayayurt K, Ekinci S, Saglam C, Surum N, et al. Monitoring the response to treatment of acute heart failure patients by ultrasonographic inferior vena cava collapsibility index. *Am J Emerg Med* 2014;**32**:403-7.

[59] Cubo-Romano P, Torres-Macho J, Soni NJ, Reyes LF, Rodriguez-Almodovar A, Fernandez-Alonso JM, et al. Admission inferior vena cava measurements are associated with mortality after hospitalization for acute decompensated heart failure. *J Hosp Med* 2016 Jun 6. doi: 10.1002/jhm.2620.

[60] Torres D, Cuttitta F, Paterna S, Garofano A, Conti G, Pinto A, et al. Bed-side inferior vena cava diameter and mean arterial pressure predict long-term mortality in hospitalized patients with heart failure: 36 months of follow-up. *Eur J Intern Med* 2016;**28**:80-4.

[61] Asahi T, Nakata M, Higa N, Manita M, Tabata K, Shimabukuro M. Respiratory Collapse of the Inferior Vena Cava Reflects Volume Shift and Subsequent Fluid Refill in Acute Heart Failure Syndrome. *Circ J* 2016;**80**:1171-7.

[62] Taurino F, Stanca E, Vonghia L, Siculella L, Sardanelli AM, Papa S, et al. Short-term type-1 diabetes differentially modulates 14-3-3 proteins in rat brain and liver. *Eur J Clin Invest* 2014;**44**:350-8.

[63] Jeon SJ, Sung JH, Koh PO. Hyperglycemia decreases expression of 14-3-3 proteins in an animal model of stroke. *Neurosci Lett* 2016;**626**:13-8.

[64] Bock H, Zimmer AR, Zimmer ER, de Souza DO, Portela LV, Saraiva-Pereira ML. Changes in Brain 14-3-3 Proteins in Response to Insulin Resistance Induced by a High Palatable Diet. *Mol Neurobiol* 2015;**52**:710-8.

[65] Slone SR, Lavalley N, McFerrin M, Wang B, Yacoubian TA. Increased 14-3-3 phosphorylation observed in Parkinson's disease reduces neuroprotective potential of 14-3-3 proteins. *Neurobiol Dis* 2015;**79**:1-13.

[66] Scharnagl H, Heuschneider C, Sailer S, Kleber ME, März W, Ritsch A. Decreased cholesterol efflux capacity in patients with low cholesteryl ester transfer protein plasma levels. *Eur J Clin Invest* 2014;**44**:395-401.

[67] Nicholls SJ, Lincoff AM, Barter PJ, Brewer HB, Fox KA, Gibson CM, et al. Assessment of the clinical effects of cholesteryl ester transfer protein inhibition with evacetrapib in patients at high-risk for vascular outcomes: Rationale and design of the ACCELERATE trial. *Am Heart J* 2015;**170**:1061-9.

[68] Zewinger S, Drechsler C, Kleber ME, Dressel A, Riffel J, Triem S, et al. Serum amyloid A: high-density lipoproteins interaction and cardiovascular risk. *Eur Heart J* 2015;**36**:3007-16.

[69] Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, et al. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med* 2014;**371**:2383-93.

[70] Liu C, Zhang Y, Ding D, Li X, Yang Y, Li Q, et al. Cholesterol efflux capacity is an independent predictor of all-cause and cardiovascular mortality in patients with coronary artery disease: A prospective cohort study. *Atherosclerosis* 2016;**249**:116-24.

[71] Ritsch A, Scharnagl H, Marz W. HDL cholesterol efflux capacity and cardiovascular events. *N Engl J Med* 2015;**372**:1870-1.

[72] Kimer N, Goetze JP, Bendtsen F, Møller S. New vasoactive peptides in cirrhosis: organ extraction and relation to the vasodilatory state. *Eur J Clin Invest* 2014;**44**:441-52.

[73] Di M, V, Weil D, Cervoni JP, Thevenot T. New prognostic markers in liver cirrhosis. *World J Hepatol* 2015; **7**:1244-50.

[74] Stoltze GF, Bosselmann H, Kistorp C, Iversen K, Kumler T, Gustafsson F, et al. Galectin 3: association to neurohumoral activity, echocardiographic parameters and renal function in outpatients with heart failure. *BMC Cardiovasc Disord* 2016; **16**:117.

[75] Kerbert AJ, Weil D, Verspaget HW, Moreno JP, van HB, Cervoni JP, et al. Copeptin is an independent prognostic factor for transplant-free survival in cirrhosis. *Liver Int* 2016; 36:**530**-537.

[76] Kerbert AJ, Verbeke L, Chiang FW, Laleman W, van der Reijden JJ, van DW, et al. Copeptin as an Indicator of Hemodynamic Derangement and Prognosis in Liver Cirrhosis. *PLoS One* 2015; **10**:e013826.

[77] Reina-Couto M, Carvalho J, Valente MJ, Vale L, Afonso J, Carvalho F, et al. Impaired resolution of inflammation in human chronic heart failure. *Eur J Clin Invest* 2014;**44**:527-38.

[78] Demetz E, Schroll A, Auer K, Heim C, Patsch JR, Eller P, et al. The arachidonic acid metabolome serves as a conserved regulator of cholesterol metabolism. *Cell Metab* 2014;**20**:787-98.

[79] Kain V, Ingle KA, Colas RA, Dalli J, Prabhu SD, Serhan CN, et al. Resolvin D1 activates the inflammation resolving response at splenic and ventricular site following myocardial infarction leading to improved ventricular function. *J Mol Cell Cardiol* 2015;**84**:24-35.

[80] Vital SA, Becker F, Holloway PM, Russell J, Perretti M, Granger DN, et al. Formyl-Peptide Receptor 2/3/Lipoxin A4 Receptor Regulates Neutrophil-Platelet Aggregation and Attenuates Cerebral Inflammation: Impact for Therapy in Cardiovascular Disease. *Circulation* 2016;**133**:2169-79.

[81] Ye Y, Birnbaum GD, Perez-Polo JR, Nanhwan MK, Nylander S, Birnbaum Y. Ticagrelor protects the heart against reperfusion injury and improves remodeling after myocardial infarction. *Arterioscler Thromb Vasc Biol* 2015;**35**:1805-14.

[82] Irace C, Padilla J, Carallo C, Scavelli F, Gnasso A. Delayed vasodilation is associated with cardiovascular risk. *Eur J Clin Invest* 2014;**44**:549-56.

[83] Tripolino C, Gnasso A, Carallo C, Scavelli FB, Irace C. Difference in carotid artery elasticity in subjects with different brachial artery kinetic of vasodilatation. *J Hum Hypertens* 2016;**30**:493-497.

[84] Cohn JN. Arterial stiffness, vascular disease, and risk of cardiovascular events. *Circulation* 2006;**113**:601–603.

[85] Irace C, Tripolino C, Scavelli FB, Carallo C, Gnasso A. Brachial low-flow-mediated constriction is associated with delayed brachial flow-mediated dilation. *J Atheroscler Thromb* 2016;**23**:355-363.

[86] Spiro JR, Digby JE, Ghimire G, Mason M, Mitchell AG, Ilsley C, et al. Brachial artery low-flow-mediated constriction is increased early after coronary intervention and reduces during recovery after acute coronary syndrome: characterization of a recently described index of vascular function. *Eur Heart J* 2011;**32**:856-866.

[87] Seetho IW, Siwy J, Albalat A, Mullen W, Mischak H, Parker RJ, et al. Urinary proteomics in obstructive sleep apnoea and obesity. *Eur J Clin Invest*. 2014;**44**:1104-15.

[88] Seetho IW, Ramirez-Torres A, Albalat A, Mullen W, Mischak H, Parker RJ, et al. Urinary proteomic profiling in severe obesity and obstructive sleep apnoea with CPAP treatment. *Sleep Sci*. 2015;**8**:58-67.

[89] Huang BT, Peng Y, Liu W, Zhang C, Huang FY, Wang PJ, et al. *Eur J Clin Invest*. 2014;**44**: 893-901.

[90] Garg S, Drazner MH. Refining the classification of left ventricular hypertrophy to provide new insights into the progression from hypertension to heart failure. *Curr Opin Cardiol.* 2016;**31**:387-93.

[91] Cuspidi C, Facchetti R, Bombelli M, Sala C, Tadic M, Grassi G, et al. Risk of mortality in relation to an updated classification of left ventricular geometric abnormalities in a general population: the Pamela study. *J Hypertens*. 2015;**33**:2133-40.

[92] Garg S, de Lemos JA, Ayers C, Khouri MG, Pandey A, Berry JD, et al. Association of a 4-Tiered Classification of LV Hypertrophy With Adverse CV Outcomes in the General Population. *JACC Cardiovasc Imaging*. 2015;**8**:1034-41.

[93] de Simone G, Izzo R, Aurigemma GP, De Marco M, Rozza F, Trimarco V, Stabile E, De Luca N, Trimarco B. Cardiovascular risk in relation to a new classification of hypertensive left ventricular geometric abnormalities. *J Hypertens*. 2015;**33**:745-54.

[94] Ozben S, Huseyinoglu N, Hanikoglu F, Guvenc TS, Yildirim BZ, Cort A, et al. Advanced oxidation protein products and ischaemia-modified albumin in obstructive sleep apnea. *Eur J Clin Invest* 2014;**44**:1045-52.

[95] Dogan D, Ocal N, Aydogan M, Tasci C, Arslan Y, Tapan S, et al. Assessment of the role of serum ischemia-modified albumin in obstructive sleep apnea in comparison with interleukin-6. *Postgrad Med* 2016; **128**:603-8.

[96] Uygur F, Tanriverdi H, Can M, Ornek T, Erboy F, Altinsoy B, et al. The Impact of Obstructive Sleep Apnoea and Nasal Continuous Positive Airway Pressure on Circulating Ischaemia-Modified Albumin Concentrations. *Mediators Inflamm* 2016;**2016**:8907314.

[97] Eisele HJ, Markart P, Schulz R. Obstructive Sleep Apnea, Oxidative Stress, and Cardiovascular Disease: Evidence from Human Studies. *Oxid Med Cell Longev* 2015;**2015**:608438.

[98] Passali D, Corallo G, Yaremchuk S, Longini M, Proietti F, Passali GC, et al. Oxidative stress in patients with obstructive sleep apnoea syndrome. *Acta Otorhinolaryngol Ital* 2015;35:420-5.

[99] Verloop WL, Vink EE, Spiering W, Blankestijn PJ, Doevendans PA, Bots ML, et al. Renal denervation in multiple renal arteries. *Eur J Clin Invest*. 2014;**44**:728-35.

[100] Bhatt DL, Kandzari DE, O'Neill WW, D'Agostino R, Flack JM, Katzen BT, et al; SYMPLICITY HTN-3 Investigators. A controlled trial of renal denervation for resistant hypertension. *N Engl J Med*. 2014;**370**:1393-401.

[101] De Jong MR, Hoogerwaard AF, Gal P, Adiyaman A, Smit JJ, Delnoy PP, et al. Persistent Increase in Blood Pressure After Renal Nerve Stimulation in Accessory Renal Arteries After Sympathetic Renal Denervation. *Hypertension*. 2016;**67**:1211-7.

[102] Nair GK, Massé S, Asta J, Sevaptisidis E, Azam MA, Lai PF, et al. The need for and the challenges of measuring renal sympathetic nerve activity. *Heart Rhythm*. 2016;**13**:1166-71.

[103] Kandzari DE, Kario K, Mahfoud F, Cohen SA, Pilcher G, et al. The SPYRAL HTN Global Clinical Trial Program: Rationale and design for studies of renal denervation in the absence (SPYRAL HTN OFF-MED) and presence (SPYRAL HTN ON-MED) of antihypertensive medications. *Am Heart J*. 2016;**171**:82-91.

[104] Hakala T, Sand J, Kellokumpu-Lehtinen PL, Huhtala H, Leinonen R, Kholová I. Recurrent thyroid cancers have more peritumoural lymphatic vasculature than nonrecurrent thyroid cancers. *Eur J Clin Invest*. 2014;**44**:825-32.

[105] Adamczyk LA, Gordon K, Kholová I, Meijer-Jorna LB, Telinius N, Gallagher PJ, et al. Lymph vessels: the forgotten second circulation in health and disease. *Virchows Archiv* 2016; **469**: 3.

[106] Galdiero MR, Varricchi G, Marone G. The immune network in thyroid cancer. *OncoImmunology* 2016; **5**: e1168556.

[107] Kim H, Kataru RP, Koh GY. Inflammation-associated lymphangiogenesis: a double-edged sword? *J Clin Invest* 2014; **124**: 936.

[108] Niinimäki E, Mennander AA, Paavonen T, Kholová I. Lymphangiogenesis is increased in heart valve endocarditis. *Int J Cardiol* 2016; **219**: 317.

[109] Kytö V, Sipilä J, Rautava P. Gender, age and risk of ST segment elevation myocardial infarction. *Eur J Clin Invest* 2014; **44**: 902–9.

[110] EUGenMed Cardiovascular Clinical Study Group, Regitz-Zagrosek V, Oertelt-Prigione S, Prescott E, Franconi F, Gerdts E, Foryst-Ludwig A, et al. Gender in cardiovascular diseases: impact on clinical manifestations, management, and outcomes. *Eur Heart J*. 2016; **37**: 24-34.

[111] Kytö V, Sipilä J, Rautava P. Association of age and gender with risk for non-ST-elevation myocardial infarction. *Eur J Prev Cardiol*. 2015; **22**: 1003-8.

[112] Kytö V, Sipilä J, Rautava P. Gender-specific and age-specific differences in unstable angina pectoris admissions: a population-based registry study in Finland. *BMJ Open* 2015; **5**:e009025.

[113] Kytö V, Sipilä J, Rautava P. Likelihood and Predictors of ST-elevation in Patients Hospitalized for Myocardial Infarction. *PLoS One*. 2014;**25**:e108440.

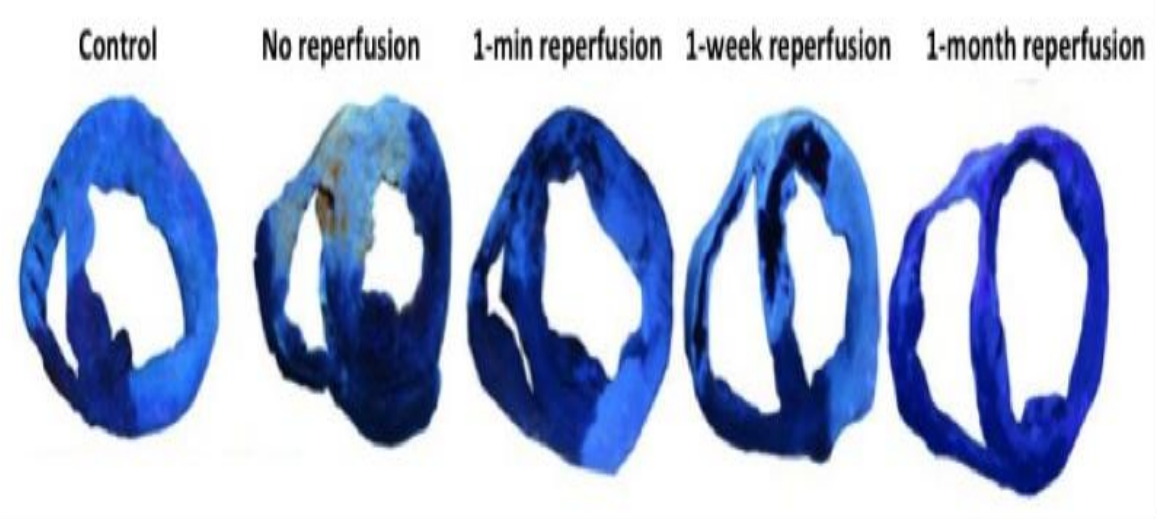
[114] Montecucco F, Mach F, Lenglet S, Vonlaufen A, Gomes Quindere AL, Pelli G et al. Treatment with Evasin-3 abrogates neutrophil-mediated inflammation in mouse acute pancreatitis. *Eur J Clin Invest* 2014;**44**:940-50.

[115] Merza M, Rahman M, Zhang S, Hwaiz R, Regner S, Schmidtchen A et al. Human thrombin-derived host defense peptides inhibit neutrophil recruitment and tissue injury in severe acute pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2014;**307**:G914-21.

[116] Yu C, Merza M, Luo L and Thorlacius H. Inhibition of Ras signalling reduces neutrophil infiltration and tissue damage in severe acute pancreatitis. *Eur J Pharmacol* 2015;**746**:245-51.

[117] Bonvin P, Power CA and Proudfoot AE. Evasins: Therapeutic Potential of a New Family of Chemokine-Binding Proteins from Ticks. *Front Immunol* 2016;**7**:208.

Figure 1 The dynamics of microvascular obstruction (MVO) in a controlled swine model of reperfused anterior myocardial infarction. Light blue represents the thioflavin-S myocardial uptake after infusion through the anterior descending coronary artery. Dark blue in the core of the perfused area indicates severe microvascular damage into the infarcted area, demonstrating the presence of MVO. This phenomenon was undetected during the ischemia period, while it appeared immediately after reperfusion, peaked after 1-week, and significantly decreased 1 month after reperfusion [9].



Appendix Statements made in the Conclusions of the Abstract of original articles published by the European Journal of Clinical Investigation in 2014 and current status for each statement as judged by the authors of each original study.

		Current status for the statement				
References	**Statements made in 2014**	**Reinforced n=22**	**Modified n=2**	**Weakened n=0**	**No new evidence n=5**	**Other n=1**
[1]	p53 mRNA expression levels, inversely correlated with adiponectin, increase in epicardial adipose tissue of heart failure patients and can be regulated by sympathetic activation pathway.	X				

[7]	Dynamic changes in endothelial cells viability occur in the setting of ST-segment elevation myocardial infarction patients reperfused with primary coronary intervention, these changes peak late after reperfusion, they are mainly the result of an increase of apoptosis and are associated with the presence of extensive myocardial oedema.	X				
[11]	Increased serum soluble CD36 is an independent factor associated with advanced steatosis in non-alcoholic fatty liver disease.				X	
[16]	Our study revealed a different secretion pattern of gut-brain axis hormones in children with coeliac disease compared with healthy controls.	X				
	The alterations in the axis were more pronounced in children with both coeliac disease and type 1 diabetes mellitus.				X	
[22]	Serum irisin levels were higher in girls than in boys and correlated negatively with Homeostatic Model		X[*]			

	Assessment – Insulin Resistance (HOMA-IR).					
[27]	This study reveals, for the first time, delayed recovery of upper limb muscle function in chronic fatigue syndrome + fibromyalgia, but not in chronic fatigue syndrome -only patients. The results underline that chronic fatigue syndrome is a heterogeneous disorder suggesting that reducing the heterogeneity of the disorder in future research is important to make progress towards a better understanding and uncovering of mechanisms regarding the nature of divers impairments in these patients.	X				
[32]	In postmenopausal women with newly diagnosed type 2 diabetes mellitus, lifestyle modifications alone or combined with metformin produced comparable changes in adiponectin levels. Weight reduction in patients treated with metformin was associated with significant decrease in %total body fat but not in regional fat depots.	X				

[38]	Parvovirus B19 DNA detected on the balloon used for dilatation of coronary stenosis before stent implantation is associated with major adverse cardiac events rate at follow-up, mainly due to clinically driven target lesion revascularization.	X				
[44]	Simvastatin and bezafibrate increase cholesterol efflux, parallel to high density lipoprotein cholesterol and Apolipoprotein A1 responses.		X[**]			
	The antioxidative and anti-inflammatory properties of high-density lipoprotein are not to an important extent affected by these therapeutic interventions.				X	
[49]	Steroid therapy has no influence on endothelial function but does significantly improve carotid intima-media thickness in giant-cell arteritis. This divergence of endothelial function and intima-media thickness reflects the specifity of giant-cell arteritis for cerebrovascular arteries thereby	X				

	sparing the brachial arteries.					
[51]	We show decreased serum retinol-binding protein 4 levels in men in the acute phase of acute-myocardial-infarction, being this decrease already detected in men with familial-hypercholesterolemia previous to the presentation of an ischaemic event.	X				
	The decrease in retinol-binding protein 4 levels could confer an increased susceptibility to the precipitation of an ischaemic event that could be mediated by the decrease in its vasculoprotective properties through nitric oxide and prostacyclin (PGI2).	X				
[55]	Residual thrombin generation potential is inversely correlated with protease-activated receptor-1 mediated platelet activation and linked to the occurrence of atherothrombotic events in patients with peripheral arterial disease.				X	
[57]	This pilot study showed a higher early re-admission rate in patients				X	

	with heart failure due to ischaemic heart disease.					
	In addition, the change in inferior vena cava minimum diameter from admission to discharge was the best predictor of re-admission in patients with ischaemic heart diseas.	X				
[62]	Our results indicate that during the early phase of streptozotocin-induced type 1 diabetes mellitus, the 14-3-3 proteins are affected in an isoform- and tissue-specific way.	X				
[66]	Our findings indicate that low plasma concentrations of cholesteryl ester transfer protein might indeed lead to impaired high-density lipoprotein function within the reverse cholesterol transport pointing towards an atheroprotective role of cholesteryl ester transfer protein at least in patients with high risk of coronary artery disease.	X				
[72]	Pro-atrial natriuretic peptide (proANP) is elevated in cirrhosis. Copeptin, proadrenomedullin and	X				

	proANP are related to portal pressure and seem associated with systemic haemodynamics. These propeptides may participate in development and perpetuation of vasodilatation and hyperdynamic circulation in cirrhosis.					
[77]	Higher severity of chronic heart failure is associated with reduced levels of lipoxins. Plasma lipoxin A4 (LXA4) appears to be a valuable marker for risk stratification in chronic heart failure.	X				
	Furthermore, the acetylsalicylic acid-related increase in urinary 15-epi-LXA4 suggests enhanced renal synthesis of this eicosanoid and may represent a disregarded benefit of acetylsalicylic acid.	X				
[82]	These results suggest that the magnitude of the flow mediated dilation and its latency are both important for identifying patients at risk of cardiovascular disease. Subjects with a delayed though significant vasodilation associated with a blunted early response exhibit the highest cardiovascular	X				

	risk.					
[87]	In this study, we report for the first time, urinary proteomic profile analyses using capillary electrophoresis-mass spectrometry (CE-MS) in obstructive sleep apnoea (OSA) and non-OSA obese groups. The differences in urinary proteomic profiles prior to adjustment for multiple testing, with increased metabolic syndrome in obese OSA subjects, suggest that there may be a role for CE-MS in characterising urinary profiles in severely obese populations with OSA.	X				
[89]	In patients with coronary artery disease (CAD), dilated left ventricular hypertrophy (LVH) and nondilated LVH provide distinct prognostic information. Eccentric nondilated LVH does not predict adverse outcomes.	X				
[94]	We conclude that high systemic oxidative stress in obstructive sleep apnea is reflected by increased advanced oxidation protein products without causing an					X

	increase in ischaemia-modified albumin.					
[99]	Based on our results and the high prevalence of multiple arteries, it seems reasonable not to exclude patients with multiple renal arteries from renal denervation (RDN). Current analysis suggests that blood pressure (BP) reduction may be less pronounced in patients with multiple renal arteries of whom not all arteries were treated	X				
[104]	Recurrent thyroid cancers expressed less intratumoural microvessels than thyroid adenomas. A high density of peritumoural lymphatic vessels was found in recurrent papillary cancers. High blood vessel density may be a marker for less aggressive tumours, while high peritumoural lymphatic vasculature is a marker for more aggressive and recurrence-prone tumours.	X				
[109]	Men have a tripled overall risk of ST segment elevation myocardial infarction (STEMI) compared with women with highest relative risk in	X				

	younger adults. Incidence rate of STEMI increases by estimated 41% per 5-year increase in age.					
[114]	Chemokine production and leucocyte infiltration are timely regulated in lung and pancreas during pancreatitis. CXC chemokine inhibition with Evasin-3 improved neutrophil inflammation and injury, potentially interfering with damages in acute pancreatitis and related pulmonary complications.	X				

[*] The correlation of serum irisin with insulin resistance is still controversial, at least in children, but the sexual dimorphism in irisin levels are reinforced with recent evidence.

[**] Variable results have been published in part dependent on type of cell system, procedure to isolate HDL, specific statin and patient category.