

while platelet aggregation returned to baseline after 7 to 8 weeks (5). We also demonstrated a progressive reduction in platelet sensitivity to aspirin in long-term aspirin-treated patients.

The genomic response to a pharmacological “challenge” with aspirin has also been demonstrated. After aspirin administration, a set of platelet-enriched genes and proteins associated with platelet function and cardiovascular complications have been identified. Moreover, increased platelet expression of glycoprotein IIIa with aspirin treatment has been demonstrated.

In conclusion, we suggest a better definition of the impact of prior aspirin use, because the capability of aspirin in changing protein platelet expression may be useful to better understand high on-aspirin treatment platelet reactivity.

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#### REPLY: Aspirin Treatment and Outcomes After Percutaneous Coronary Intervention

Results of the ISAR-ASPI Registry

We thank Dr. Pulcinelli and colleagues for their interest in our publication (1). The high proportion of patients (>90%) presenting on aspirin therapy in our cohort likely would not enable a reliable analysis of the role of the presence or absence of this therapy on the platelet response to aspirin as measured in this study.

However, the fact that a significantly lower proportion of patients taking aspirin showed high on-aspirin platelet reactivity (91.2% in patients with vs. 93.8% in those without high on-aspirin platelet reactivity;  $p = 0.0006$ ) does not support the authors' hypothesis. In addition, as mentioned in the results section of our paper (1), prior aspirin therapy was also included in the multivariate model for the primary outcome, the composite of death or stent thrombosis at 1 year. From this model, prior aspirin therapy was associated with an adjusted hazard ratio of 0.66 (95% confidence interval: 0.45 to 0.96;  $p = 0.03$ ) for the occurrence of the primary outcome, suggesting a protective role against ischemic events.

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## Does Size Matter?

In Search of a Physiological Definition of Myocardial Atrophy

Mechanical unloading using left ventricular assist devices (LVADs) induces reverse remodeling of the failing myocardium to levels that are possibly superior to any other strategy. However, evidence of a



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significant and reliable curative efficacy is scarce. This paradox is explained by insufficient and/or possibly detrimental effects of mechanical unloading, despite a regression of pathological changes from the subcellular to the multicellular level. Because a reduction of cardiomyocyte size is a universal finding after LVAD treatment, we and others have called this effect “*atrophy*,” which indicates *a set of features that make myocyte mass and function inadequate to sustain the workload of the heart when it is reloaded*. In the paper by Diakos et al. (1) published in the *Journal*, the effect of LVAD treatment on myocardial atrophy was specifically investigated, and it was concluded that no atrophy occurred in these patients. In this paper, atrophy was predominantly defined as the evidence of subnormal cardiomyocyte size. A set of associated parameters were also considered, and we congratulate the investigators for the substantial set of data and the effort to further characterize this interesting population of patients. However, we have some concerns on the interpretation of the data presented.

This was not the first study to investigate the effect of unloading of the failing hearts on myocardial atrophy. We and others measured myocyte size after LVAD treatment in patients and found similar results (2). We also performed several studies using the rat heterotopic abdominal heart transplantation (HAHT) model in normal and failing hearts. HAHT induces a very substantial degree of unloading, and we showed that this could reverse the hypertrophic response of an established model of murine heart failure after 1 week of unloading and also induce substantial myocyte atrophy, to subnormal levels, of normal and failing myocytes after 4 weeks of unloading (3). This suggests that the degree of atrophy depends, not surprisingly, on the degree of unloading.

More than 10 years ago, we showed that, in patients treated with combined LVAD and pharmacological therapy, including clenbuterol, the maintenance of normal cell size was not a determinant of clinical recovery. Rather, functional improvements in excitation-contraction coupling were associated with clinical recovery, which suggested that even a myocyte of normal size can be dysfunctional (2). We also showed that reductions in cell size, within certain limits from normal, did not produce dysfunction and could even be associated with functional improvement in failing myocytes after unloading (3). We termed this “physiological” hypotrophy or atrophy, suggesting again that the absolute cell size is not deterministic of cell function, and that the consequences of the reduction in cell size depend upon the pathological substrate.

Another concern regarding this study was that most of the experiments showed only values before and after LVAD, and did not compare these values with the normal myocardium. Considering that the pre-LVAD samples represent a substantially diseased tissue, the lack of improvement in ultrastructural features, for example, suggests a lack of cellular recovery. Furthermore, with the evidence that hypertrophy and atrophy share a number of signalling pathways (4), the lack of difference between pre- and post-LVAD does not rule out atrophy. In addition, with respect to t-tubules, the investigators limited their analysis to the distance of ryanodine receptors from the sarcolemma—which was used as an index of atrophy for an unclear reason—with no reference to the parameters that were associated with cellular functions, including calcium handling (density, regularity, morphology, and local excitation-contraction coupling) (5). We showed that t-tubular disruption is a feature of both overloaded and unloaded dysfunctional myocardium, and the current findings would be consistent with the dysfunction of overload (pre-LVAD) and atrophy (post-LVAD) (5). The divergence of these data might be partly ascribed to the heterogeneity of the patient population, together with differences in unloading and/or pharmacological management.

We suggest that the current data should not be used as an indication of normalization of function and lack of atrophy, but rather as further evidence that current clinical strategies of mechanical unloading are insufficient to produce meaningful recovery of the myocardium. More studies are needed to optimize these strategies and explore associated therapies.

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### REPLY: Does Size Matter?

In Search of a Physiological Definition of Myocardial Atrophy



We would like to thank Dr. Terracciano and colleagues for their interest in our study (1). The aim of our study was to: 1) address the long-standing issue of whether myocardial atrophy and degeneration could be induced as a result of prolonged ventricular assist device (VAD) unloading; and 2) address this issue by studying the effects of newer generation, continuous-flow VADs. Previous studies that used first-generation pulsatile VADs reported effects on myocardial hypertrophy (2). These studies did not focus on investigating myocardial atrophy and degeneration.

One aspect of our research approach was to define and try to identify atrophy pathologically both at the cellular and subcellular levels using digital histopathology, confocal microscopy, and electron microscopic analysis (methodologies that were standardized in previous publications from our group). Our *ex vivo* structural data were also supported by the *in vivo* echocardiographic data, which showed no left ventricular (LV) mass reduction to levels lower than the normal range and no LV function decline indicative of atrophy and degeneration. Along the same lines, we did not observe metabolic changes (e.g., excessive glycogen accumulation) that would have been an indication of atrophic remodeling.

Regarding the t-tubule microstructural analysis, we agree with Dr. Terracciano and colleagues that our approach is a starting point in the quest to understand remodeling in VAD patients at the subcellular level. Based on our recent work, we think that the applied t-tubule-ryanodine receptor relationship is a powerful concept for characterizing the t-system and associated functional remodeling. Specifically, we introduced the t-tubule-RyR relationship as a

quantitative marker of subcellular structural remodeling of the failing heart and restoration after cardiac resynchronization therapy (3). In our recent study on local depletion of the t-system in ventricular myocytes, we found that this structural marker could also help explain deficiencies in excitation-contraction coupling (4). These studies motivated us to apply this marker in the investigations on the myocardial tissue from VAD patients. Unlike the disruption of the t-tubule system that was observed by Ibrahim et al. (5) in rat hearts that were fully unloaded (and denervated) after heterotopic transplantation, in the case of the partial unloading induced by VADs, we did not observe any t-tubule system changes that would indicate atrophic remodeling. As shown by Ibrahim et al. (5) in their rodent model, “the degree of atrophy depends on the degree of unloading.” The major differences in the degree of unloading between the heterotopic transplantation model and the VAD-induced unloading could explain the differential results. Also, the heterotopically transplanted rat hearts had a heart failure (HF) history of only 4 weeks versus the chronically ill human hearts of end-stage VAD patients, which could have also played a role in the differential response.

In an effort to investigate whether signaling pathways associated with atrophic remodeling were activated, we performed an extensive molecular analysis. We did not find any gene and protein expression changes that were indicative of active atrophic remodeling. We acknowledge that these pathways are complex and not only associated with atrophic remodeling. For this reason, we did not interpret the molecular data as isolated evidence, but we did interpret it in conjunction with the structural, t-system microstructural, ultrastructural, metabolic, and echocardiographic functional phenotypes.

We also analyzed tissue specimens from 18 normal donor human hearts, and we believe this is one of the strengths of our study, which has seldom been seen in similar investigations due to a lack of available normal heart tissue. In our study, the myocardial structural analysis that was central for this investigation was performed in this sizeable cohort of normal donor heart specimens and in the remaining assays in as many normal heart samples as feasible.

We strongly agree that our data must not be used as an indication of normalization of cardiac function. The aim of this study was to investigate whether VAD-induced unloading could be detrimental and lead to atrophic remodeling and degeneration. We included patients who were unloaded with continuous-flow VAD for up to 1 year, and the evidence presented