

Cardiac compliance is acutely modulated through cGMP-PKG signalling

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INTRODUCTION

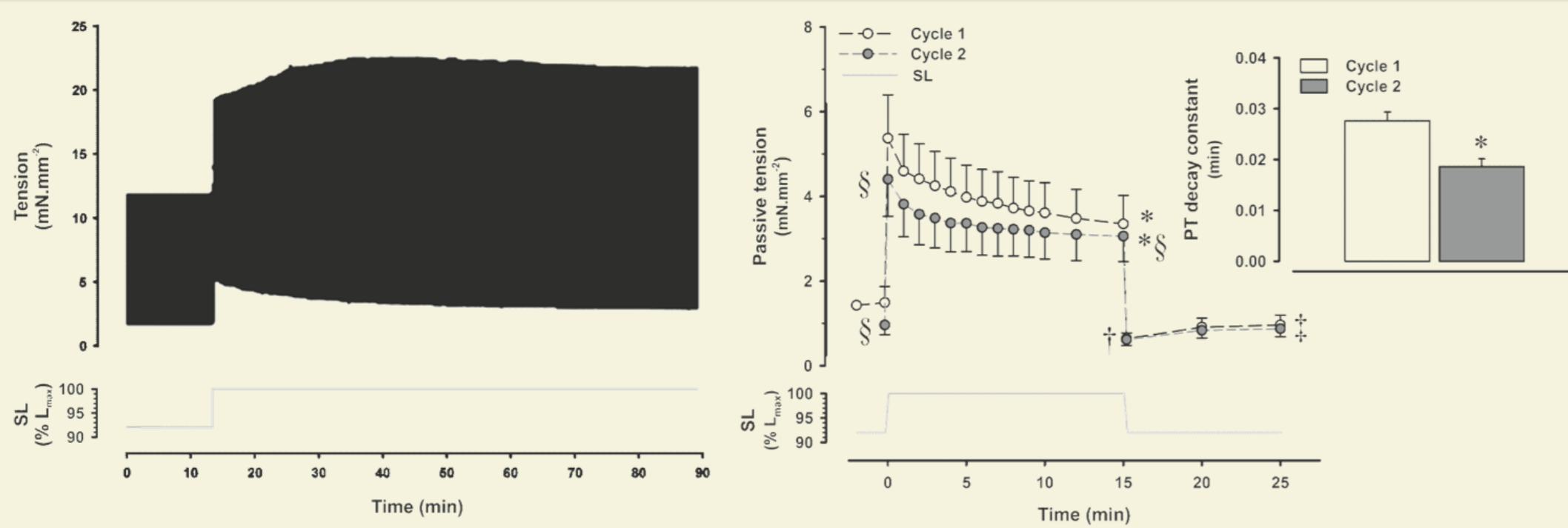
Increased diastolic stiffness due to **titin hypophosphorylation** and low **protein kinase G (PKG)** activity is a hallmark of **heart failure with preserved ejection fraction (HFpEF)**. Acute load sensitivity is a well-known factor of decompensation in HFpEF, which has been mainly ascribed to vascular and systolic stiffening, but other factors may be at play.

We aimed to investigate whether there is a diastolic response to acute **volume overload (VO)**, cardiac **stretch-induced compliance (SIC)**, and to ascertain the potential involvement of titin phosphorylation and cGMP-PKG-signalling in this response in several animal models and in the human heart.

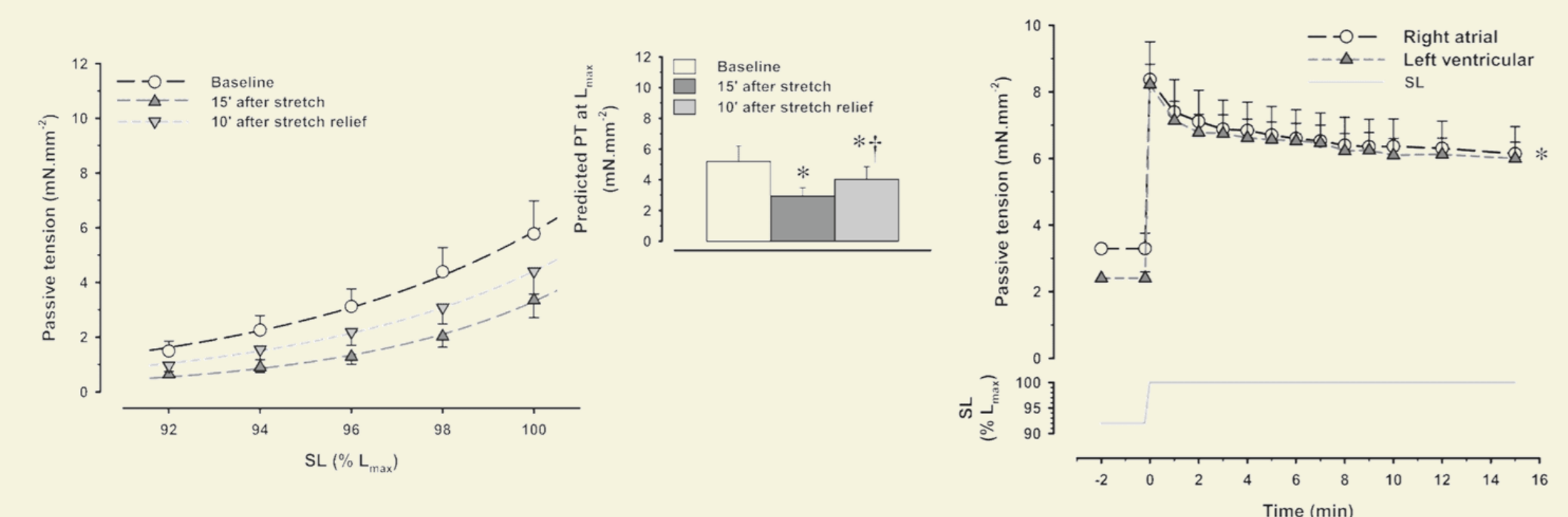
METHODS

- **Intact rat hearts**, strips dissected from the LV or right atrium of **cardiac surgery patients** and **rabbit papillary muscles** were acutely stretched for 15 minutes.
- **Skinned cardiomyocytes** from the LV of control and stretched hearts were incubated with either **PKG** or **Protein Phosphatase 1**.
- Rabbit muscles were incubated with a **PKG inhibitor**, a **NO synthase inhibitor**, a **NO scavenger** or a **natriuretic peptide (NP) receptor A antagonist**.
- After gel electrophoresis, **Titin phosphorylation** was measured with Pro-Q Diamond stain and indexed to total-protein signals using SYPRO Ruby.
- Myocardial **cGMP** levels and vasodilator-stimulated phosphoprotein (**VASP**) **phosphorylation** were quantified.
- Hemodynamic **pressure-volume response** to VO was assessed in sham and transverse aortic constriction (**TAC**) rats and was also assessed by echocardiography in **healthy volunteers** and invasively in **cardiac surgery patients** before and after leg elevation and Trendelenburg positioning, respectively.

RESULTS

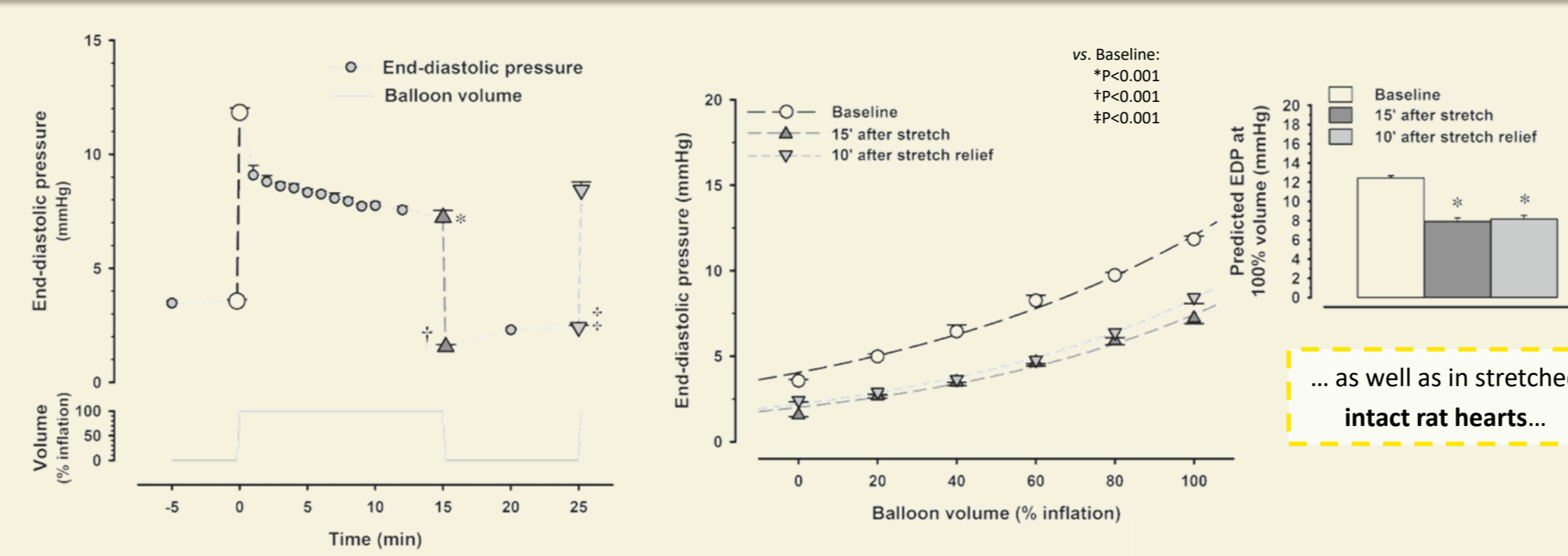


Acute stretch of **isolated rabbit papillary muscles** from a sarcomere length (SL) of 92% to 100% of L_{max} leads to a **decrease in passive tension (PT)** over 15 min (* $P < 0.001$), which drops below baseline values after stretch relief ($\dagger P < 0.01$). During the following 10 mins, it slightly rises, though remaining below baseline PT ($\ddagger P < 0.01$). In a second stretch cycle, PT rises significantly less and drops to lower values ($\S P < 0.001$ vs 1st cycle) at a faster rate.

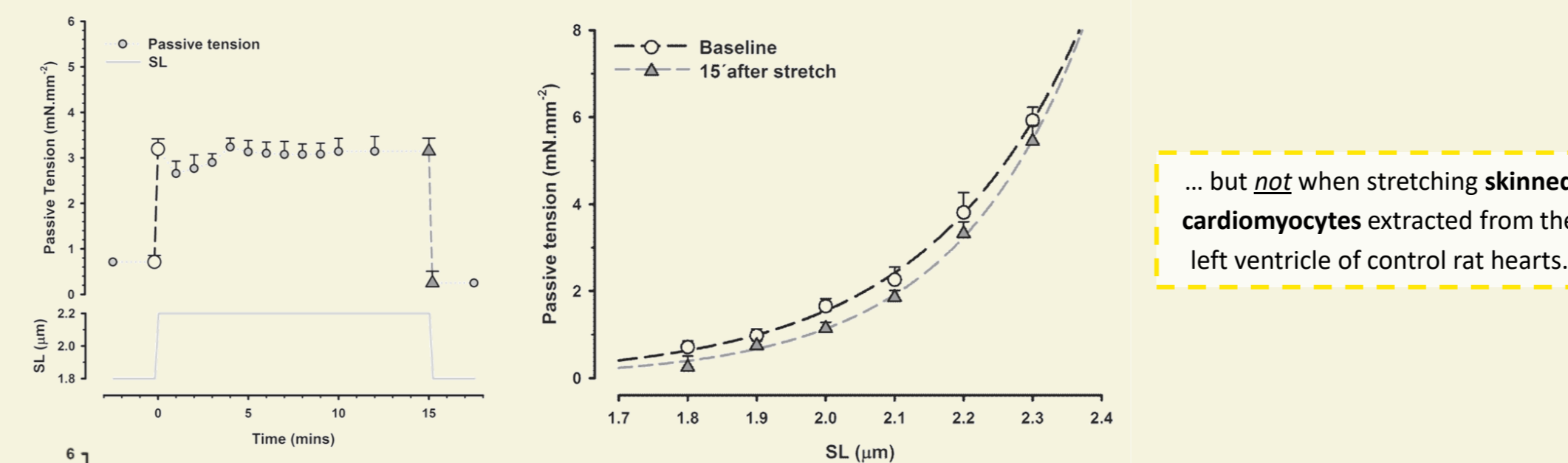


SL-PT relationships were downward shifted after 15 mins of stretch and did not fully return to baseline after 10 mins of stretch relief.

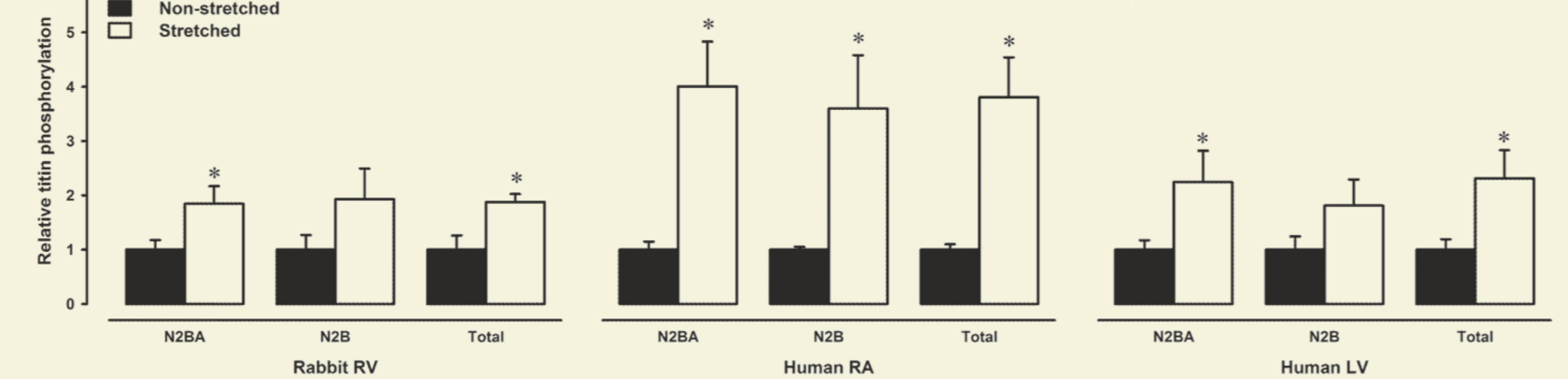
The same behaviour was observed in **muscle strips from cardiac surgery patients** (* $P < 0.001$ vs initial PT at L_{max})...



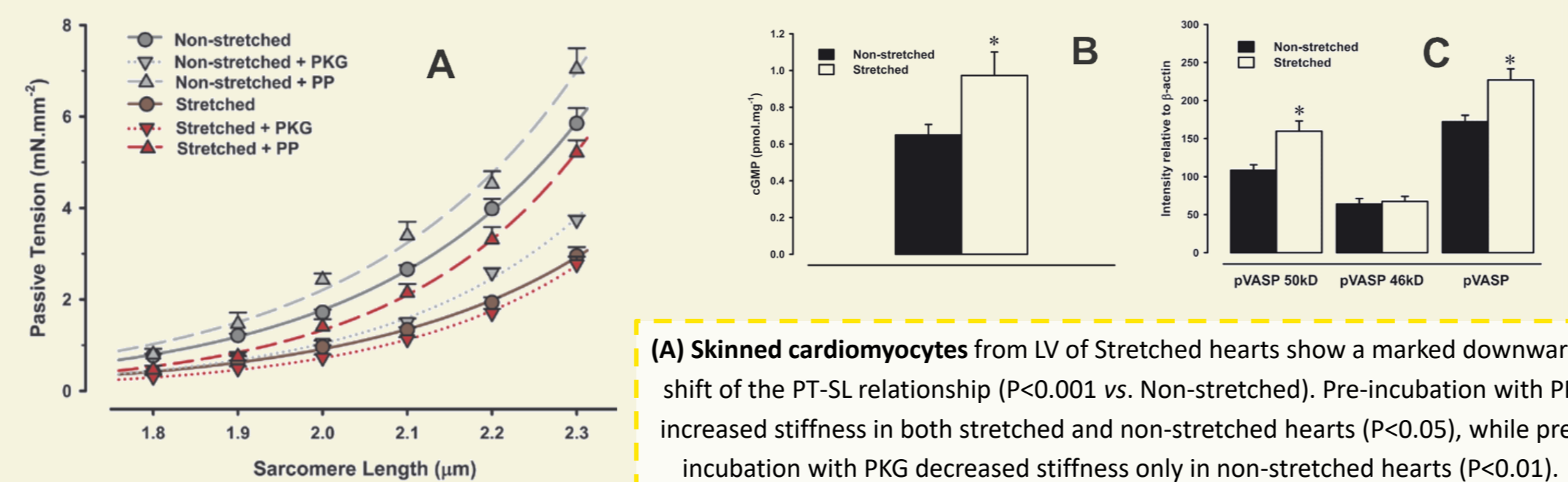
... as well as in stretched intact rat hearts...



... but **not** when stretching skinned cardiomyocytes extracted from the left ventricle of control rat hearts.



Titin phosphorylation increased in Stretched samples compared with their Non-stretched counterparts (* $P < 0.05$).



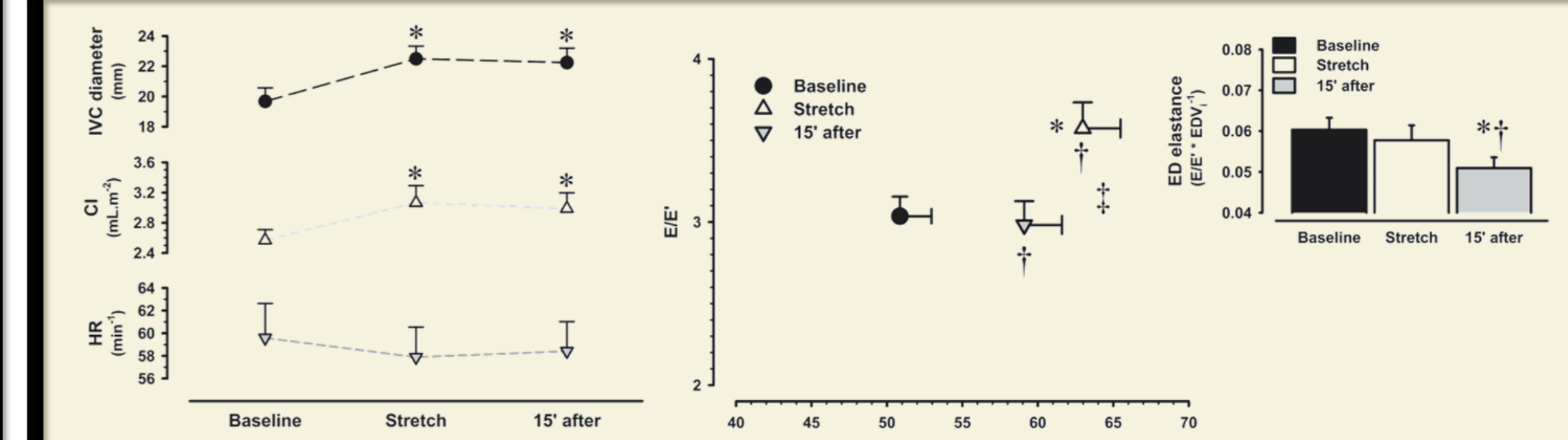
(A) **Skinned cardiomyocytes** from LV of Stretched hearts show a marked downward shift of the PT-SL relationship ($P < 0.001$ vs. Non-stretched). Pre-incubation with PP increased stiffness in both stretched and non-stretched hearts ($P < 0.05$), while pre-incubation with PKG decreased stiffness only in non-stretched hearts ($P < 0.01$).

(B) **cGMP concentration** and (C) **VASP phosphorylation** levels were higher in stretched hearts (* $P < 0.05$ vs non-stretched hearts).

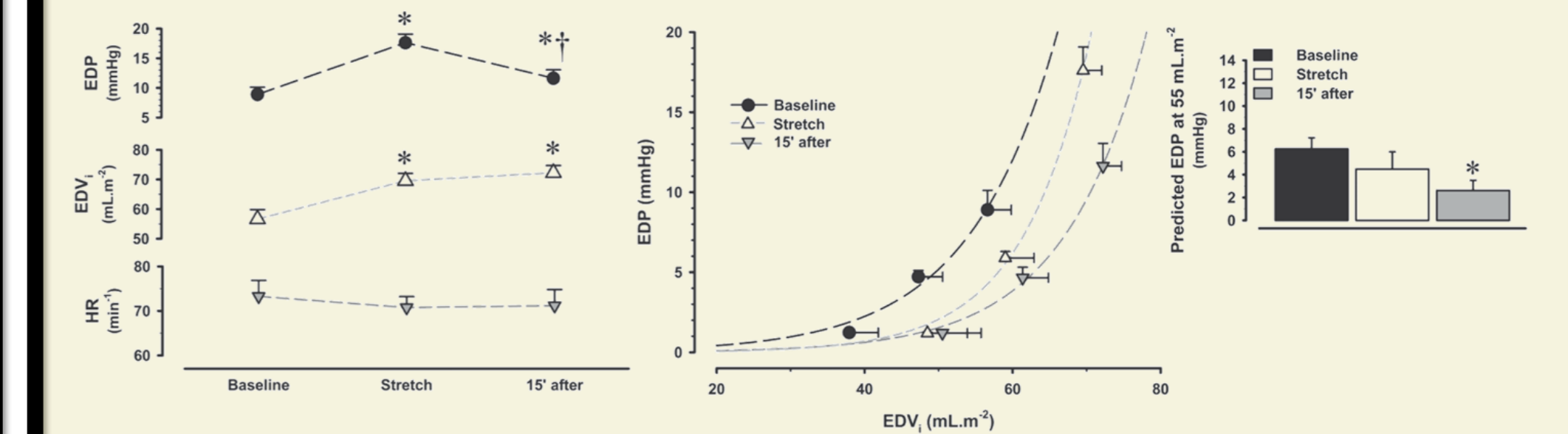
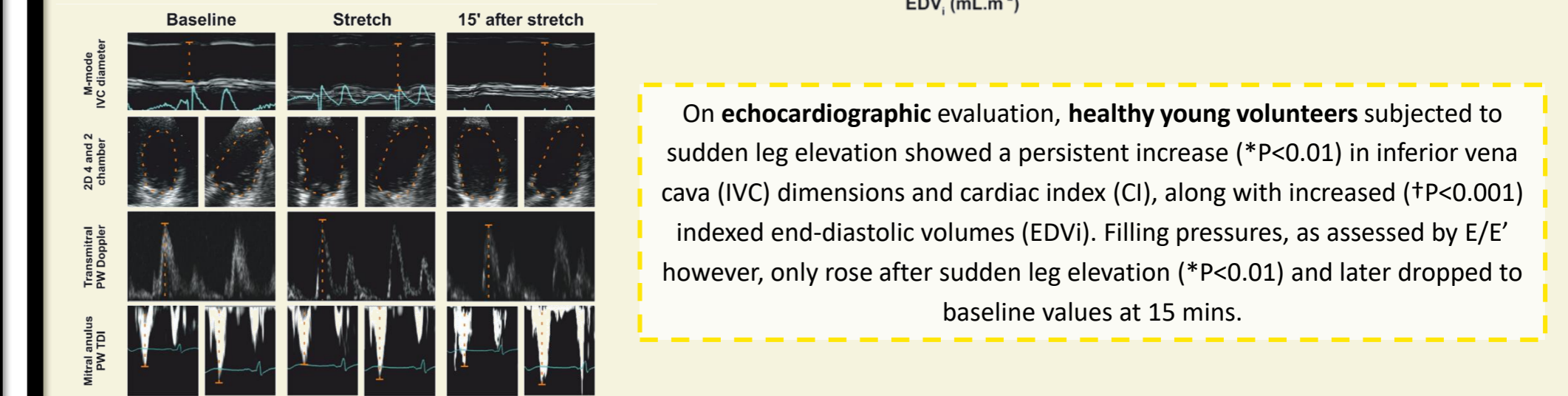


Rabbit muscle strips were stretched upon pre-incubation with vehicle (no drug), NP receptor-A antagonist (A-71915), NO synthase inhibitor (LNA), NO scavenger (HC), all of the above (A-71915 + LNA + HC) or with PKG inhibitor (PKGi). **Only joint inhibition or PKGi significantly attenuated PT fall** (* $P < 0.01$).

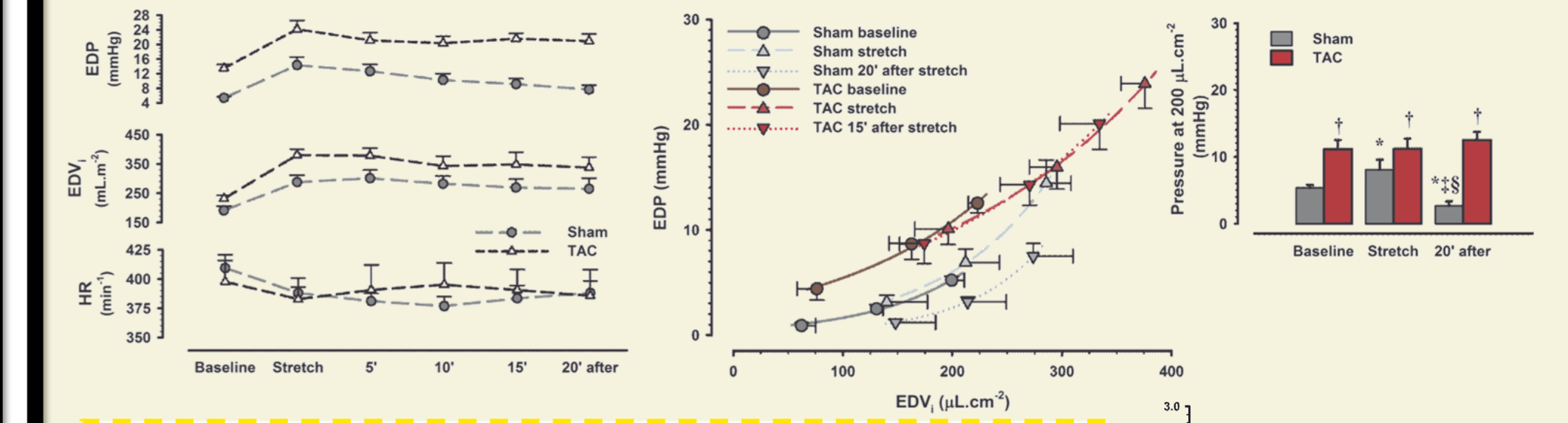
Accordingly, **PKGi blunted the increase in titin phosphorylation** observed in stretched muscles.



On **echocardiographic** evaluation, **healthy young volunteers** subjected to sudden leg elevation showed a persistent increase (* $P < 0.01$) in inferior vena cava (IVC) dimensions and cardiac index (CI), along with increased (* $P < 0.001$) indexed end-diastolic volumes (EDVi). Filling pressures, as assessed by E/E' however, only rose after sudden leg elevation (* $P < 0.01$) and later dropped to baseline values at 15 mins.



On **invasive pressure-volume** hemodynamic evaluation, **cardiac surgery patients** underwent Trendelenburg positioning and showed a sustained increase in EDVi (* $P < 0.01$ vs baseline). While an initial rise was observed, (* $P < 0.01$ vs baseline), EDP later dropped ($\dagger P < 0.001$ vs stretch) despite increased filling volumes



In **rats with LV hypertrophy** induced by TAC and their respective shams, **pressure-volume** evaluation of diastolic response to volume loading (stretch) was performed EDP fell only in Sham and not in TAC ($\dagger P = 0.024$ vs stretch).

TAC showed less **titin phosphorylation** (* $P < 0.05$) and no differences between Stretched and Non-stretched LV, whereas stretched Sham LV showed increased phosphorylation compared with their non-stretched counterparts ($\dagger P < 0.001$).

CONCLUSIONS

- **Myocardial compliance** increases in response to acute VO by a novel mechanism that is mediated by **titin phosphorylation** through **cGMP-PKG** signalling.
- The findings translated to human physiology and may be abolished in the **hypertrophic heart**, suggesting a potential role in the pathophysiology of HFpEF.