

## Measurement of salusin- $\beta$ without the addition of NP-40 or Tween-20 in coronary slow-flow phenomenon

To the Editor,

We read the study entitled "Relationship of serum salusin beta levels with coronary slow flow" by Akyüz et al. (1) with great interest. In their study, they reported that salusin- $\beta$  concentrations were associated with the coronary slow-flow phenomenon. We congratulate them for their contribution to the pathophysiology of the coronary slow-flow phenomenon. However, salusins (salusin- $\alpha$  and salusin- $\beta$ ) require specific biochemistry tubes for analysis, particularly when salusin- $\beta$  is analyzed in serum or plasma (2). If not, the reliability of the results is doubtful. Therefore, we wish to make the following contributions to this study conducted by Akyüz et al. (1).

Salusins were discovered by Shichiri et al. (3) in 2003, and they are present in biological fluids and tissues in two forms: salusin- $\alpha$  (comprising 28 amino acids) and salusin- $\beta$  (comprising 20 amino acids). Several studies have demonstrated that these peptides were associated with conditions such as hypertension, atherosclerotic cardiovascular disease, acute coronary syndrome, and vascular resistance (3, 4). Therefore, analyzing both salusin- $\alpha$  and salusin- $\beta$  together while performing research on salusin will be more useful in elucidating physiopathological events.

In their studies, the authors examined solely the levels of salusin- $\beta$  and did not follow an optimal way to collect samples during sample collection. For example, because salusin- $\beta$  studied by them adhered to the edges of propylene biochemistry tubes, its concentrations were measured to be extremely low if low doses of NP-40 or Tween-20 were not included (2), which has not been mentioned in the Material and Method section of the study by Akyüz et al. (1). Furthermore, the authors stated that they received 500 Kallikrein Inhibitor Units (KIU) in biochemistry tubes containing aprotinin to protect them from salusin- $\beta$  protease enzymes. However, commercially available biochemistry tubes containing aprotinin have EDTA. In this case, if a tube with EDTA is selected, plasma is obtained instead of serum (5). However, the authors stated that they added 500 KIU aprotinin into the plain biochemistry tube in the Material and Method section. However, in our country, aprotinin has been removed from commercial sale several years ago. In this case, if it was obtained from abroad, the brand and country from where it was obtained has not been indicated. Therefore, the situation regarding aprotinin remains ambiguous, and it would be useful to clarify this situation.

Upon combining previous laboratory findings and our own laboratory experience, we consider that it would be useful to carefully reinterpret this study performed by Akyüz et al. (1) be-

cause the study of salusin- $\beta$  without the addition of NP-40 or Tween-20 would not provide appropriate clarification.

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### Author's Reply

To the Editor,

NP-40 and Tween-20 are two detergents for cell lysis and protein extraction that have hydrophobic-hydrophilic interactions among molecules in biological specimens. They are used to lyse cells to release soluble proteins that are present in a cell and are used in both immunoassay and electrophoresis procedures (1, 2). We measured salusin- $\beta$  levels using ELISA in serum samples (3). It is well known that serum comes from the liquid portion of the blood by removing cells. Therefore, the use of these two detergents played no role in our study, similar to that observed in other protein analysis studies with serum samples.

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## Perivascular adipose tissue in cardiovascular diseases

To the Editor,

We congratulate Grigoras et al. (1) on their comprehensive and perceptive review titled "Perivascular adipose tissue in cardiovascular diseases-an update". However, some additional comments may be of interest.

Grigoras et al. (1) report that the perivascular adipose tissue (PVAT) differs in properties depending on its anatomical location. Other authors have also reported similar findings (2). These properties, together with the anatomical structural variations, may eventually facilitate the use of a specific treatment for a specific blood vessel, in addition to the usual general measures. In this context, we also need to consider that blood vessels, at different locations, may have different receptor distributions (3).

An abnormal PVAT is probably associated with abnormal peri-organ and intra-organ fat at other sites, and this may indirectly increase the risk of vascular events (4-6). Grigoras et al. (1) also mention the potential role of various drugs on PVAT. This will be an area of considerable interest for further research (1, 6).

Grigoras et al. (1) discuss a carotid model in the context of PVAT. Indeed, other authors have reported links between the PVAT and internal carotid arteries (ICA) stenosis (7). Furthermore, the pericarotid fat density has been associated with an increased risk of stroke and transient ischemic attack in patients with unilateral ICA stenosis  $\geq 50\%$ – $99\%$  (8).

The review by Grigoras et al. (1) made a considerable contribution to the field discussed above.

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