# Reply to 'Salusins: newly identified bioactive peptides with hemodynamic and mitogenic activities'

**To the editor:**

In 2003, salusins were described as "newly identified bioactive peptides with hemodynamic and mitogenic activities," with an intravenous salusin-β dose of 1 nmol per kilogram body weight causing marked falls in blood pressure (~50%) and heart rate (~30%) in anesthetized rats, and with evidence that a reduction in cardiac output was responsible for the hypotension[1]. Subsequently, independent researchers confirmed the hypotensive effect of salusin-β in anesthetized rats, but found no evidence for a cardiac depressant effect, and concluded that the fall in blood pressure was due to peripheral vasodilatation[2]. However, a later study reported cardiac depressant effects of salusins, with evidence indicating that the hypotensive and bradycardic effects were vagally mediated, whereas the negative inotropism was a direct effect of the peptide on the myocardium[3].

We have established a system for monitoring regional hemodynamic actions of peptides in conscious, chronically instrumented rats, and this has been used to identify the cardiovascular effects of a range of newly identified peptides[4]. Thus, we were in an ideal position to address the apparent controversy regarding the underlying hemodynamic changes (cardiac versus vascular) responsible for the hypotensive effects of salusin-β.

We initially purchased salusin-β (human) from Bachem, and we administered intravenous doses ranging from 0.1 nmol/kg up to 10 nmol/kg to conscious, male Sprague Dawley rats, chronically instrumented with pulsed Doppler flow probes and intravascular catheters to monitor blood pressure, heart rate and renal, mesenteric and hindquarter vascular conductances. All surgical and experimental procedures were carried out with approval of the University of Nottingham Local Ethical Review Committee, under Home Office Project and Personal Licence authority. There were no cardiovascular effects detectable at any dose. The peptide sequence was analyzed in-house (using electrospray tandem mass spectrometry on a quadrupole time-of-flight device) and confirmed, although other peptide fragments were also present. As it was possible that these fragments may have interfered with the biological activity of the full peptide, a purified sample of salusin-β was synthesized in-house on an ABI 433A synthesizer using proprietary FastMoc reagents and chemistry protocols starting from H-Pro-2-ClTrt-resin, purchased from Merck Biosciences. However, when tested *in vivo*, we found this peptide also to be devoid of any activity. Finally, salusin-β (human) was purchased from Peptide Institute, but at doses of 1, 3 and 10 nmol/kg it was inactive *in vivo*.

Our experimental paradigm was not identical to that described in the original study[1], in which the salusin was made in-house and the rats were under pentobarbital anesthesia, but we cannot replicate this condition because the original material is no longer available and, at least in the United Kingdom, pentobarbital is no longer available as a long-term anesthetic for experimental use. However, the strain of rat used is the same in all publications (Sprague-Dawley), we used peptide obtained from three different sources, and the recording site (abdominal aorta) in the original study[1] was the same as in ours, albeit accessed from the femoral rather than the caudal artery. The presence of pentobarbital anesthesia would be expected to interfere with normal cardiovascular regulatory mechanisms (baroreceptor reflexes) such that animals may be more prone to a hypotensive effect of the peptide. But, although the initial experiments[1,2] were confined to anesthetized rats, Shichiri and colleagues[3] have since shown hypotensive and bradycardic effects in conscious rats, making it unlikely that the phenomenon is peculiar to the anesthetized state. Furthermore, our more detailed cardiovascular measurements enable any cardiovascular action to be determined in the absence of overt hypotension. In conclusion, we are unable to substantiate the published findings on the cardiovascular effects of human salusin-β *in vivo*.

COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

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**Shichiri *et al.* reply:**

Gardiner *et al.* state that in conscious, chronically instrumented rats they could not detect cardiovascular effects of salusin-β peptide, which they either purchased or synthesized and purified themselves. The absent hemodynamic response to salusin-β in their animals could be due to variability in the expression or function of a salusin-β receptor, which remains uncloned[1]. However, another possible explanation that deserves close attention relates to an unexpected physical property of this peptide. While struggling to establish an assay system sufficiently sensitive to measure immunoreactive salusin-α (ref. 2) and salusin-β in biological fluids, we recently observed that human salusin-β peptide readily binds to commonly used polypropylene and glass tubes, syringes and plates (unpublished data). The magnitude of this interaction between salusin-β and polypropylene or glass was such that any true binding of the peptide to its specific antibody could be masked. Furthermore, human plasma, serum and urine contain as yet unidentified substances that interfere with the specific binding of salusin-β to its antibody, thus making the accurate detection of this peptide even more difficult. When salusin-β dissolved in distilled water at a concentration of 10 μg/ml was left to sit for several hours in polypropylene tubes