A Role for TRPV1 in Influencing the Onset of Cardiovascular Disease in Obesity

Nichola J. Marshall,* Lihuan Liang,* Jennifer Bodkin, Cecile Dessapt-Baradez, Manasi Nandi, Sophie Collot-Teixeira, Sarah-Jane Smillie, Kamal Lalgi, Elizabeth S. Fernandes, Luigi Gnudi, Susan D. Brain

Abstract—Obesity induced by Western diets is associated with type 2 diabetes mellitus and cardiovascular diseases, although underlying mechanisms are unclear. We investigated a murine model of diet-induced obesity to determine the effect of transient potential receptor vanilloid 1 (TRPV1) deletion on hypertension and metabolic syndrome. Wild-type and TRPV1 knockout mice were fed normal or high-fat diet from 3 to 15 weeks. High-fat diet-fed mice from both genotypes became obese, with similar increases in body and adipose tissue weights. High-fat diet-fed TRPV1 knockout mice showed significantly improved handling of glucose compared with high-fat diet-fed wild-type mice. Hypertension, vascular hypertrophy, and altered nociception were observed in high-fat diet-fed wild-type but not high-fat diet-fed TRPV1 knockout mice. Wild-type, but not high-fat diet-fed TRPV1 knockout, mice demonstrated remodeling in terms of aortic vascular hypertrophy and increased heart and kidney weight, although resistance vessel responses were similar in each. Moreover, the wild-type mice had significantly increased plasma levels of leptin, interleukin 10 and interleukin 1β, whereas samples from TRPV1 knockout mice did not show significant increases. Our results do not support the concept that TRPV1 plays a major role in influencing weight gain. However, we identified a role of TRPV1 in the deleterious effects observed with high-fat feeding in terms of inducing hypertension, impairing thermal nociception sensitivity, and reducing glucose tolerance. The observation of raised levels of adipokines in wild-type but not TRPV1 knockout mice is in keeping with TRPV1 involvement in stimulating the proinflammatory network that is central to obesity-induced hypertension and sensory neuronal dysfunction. (Hypertension. 2013;61:246-252.) ● Online Data Supplement

Key Words: obesity ■ glucose intolerance ■ TRPV1 protein, mouse ■ mice ■ hypertension

Transient receptor potential type 1 (TRPV1) is a nonselective ion channel, primarily localized to C- and Aδ-fiber sensory nerves. These fibres have a dual sensory-effenter role, involving nociception and release of vasodilator neuropeptides, such as calcitonin gene-related peptide. A range of endogenous activating mechanisms are known. Capsaicin is used as an experimental tool, as an agonist, and also as its continued application desensitizes and defunctionalizes sensory nerves. TRPV1 knockout (KO) mice possess a phenotype of reduced responsiveness to thermal thresholds, a lack of vasodilatation to capsaicin, and decreased joint inflammation. TRPV1 deletion is associated with proinflammatory consequences in cardiovascular alterations, such as the ones observed in sepsis, myocardial ischemia, and DOCA-salt–induced hypertension. We have demonstrated both anti- and proinflammatory effects of TRPV1 deletion in support of the concept that TRPV1 can act as a molecular integrator and can play pivotal roles in disease progression. TRPV1 channel activation and high calcium influx were originally shown to prevent adipogenesis, but not more recently. In vivo studies involving a capsaicin-containing diet, that on a chronic basis desensitizes sensory nerves in addition to the TRPV1 channel, are associated with a lean phenotype. Moreover, wild-type (WT) mice fed a 11% high-fat diet (HFD) for 3 to 25 weeks gained more weight than TRPV1KO mice, but only during the 15- to 25-week period, suggesting a link with age-onset obesity only. In contrast, capsaicin-pretreatment of Zucker rats (a model of the metabolic syndrome and type 2 diabetes mellitus) led to a greater increase in weight after 12 weeks of capsaicin feeding, with the capsaicin-fed rats protected against the normal deterioration of glucose handling. Also, HFD-TRPV1KO mice exhibited improved glucose tolerance compared with HFD-WT mice. Therefore, a complex picture has emerged of the role of TRPV1, with interest in the concept that TRPV1 may play a primary role in body weight regulation and metabolism.

Received July 18, 2012; first decision July 30, 2012; revision accepted September 25, 2012.
From the British Heart Foundation Centre of Cardiovascular Excellence and Centre of Integrative Biomedicine, King’s College London, London, United Kingdom.
The online-only Data Supplement is available with this article at http://hyper.ahajournals.org lookup/suppl/doi:10.1161/HYPERTENSIONAHA.112.201434/-/DC1.
*Dr Marshall and Dr Liang are joint first authors.
Correspondence to Susan D. Brain, Cardiovascular Division, King’s College London, Franklin-Wilkins Building, Waterloo Campus, London SE1 9NH, United Kingdom. E-mail sue.brain@kcl.ac.uk
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Hypertension is available at http://hyper.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.112.201434
Based on evidence that TRPV1 may influence obesity and glucose handling in a harmful manner, we have investigated the influence of TRPV1 in a model involving WT and TRPV1KO mice. Our results show that HFD-induced hypertension is not observed when TRPV1 is deleted and that HFD-WT mice become less responsive to the characteristic TRPV1-dependent thermal nociception.

Materials and Methods

An expanded Methods section is available in the online-only Data Supplement.

Mouse Models

C57BL6/129SVJ TRPV1KO mice and their genetically unaltered WT counterparts were bred and characterized. Mice were divided into sex-balanced groups and fed an HFD (35% fat from lard) or normal diet (ND; see the online-only Data Supplement for details), for 12 weeks from 3 weeks of age, according to the Animals (Scientific Procedures) Act, 1986, United Kingdom.

Blood Pressure and Additional Analysis

Blood pressure was assessed in restrained conscious mice using the tail-cuff CODA plethysmography technique and by a preimplanted telemetry device (PA-C10, DSI) in unrestrained conscious mice. At 15 weeks of age, after blood pressure measurements were complete, blood glucose was measured before (baseline) and after glucose administration (2mg/kg IP). Nociception assays are detailed in the online-only Data Supplement. At termination, external markers of obesity and development were taken and samples were collected for analysis.

Statistical Analysis

Results are shown as mean±SEM and evaluated by 2-way ANOVA and Bonferroni posttest, unless stated. Significance was accepted at P<0.05.

Results

Effect of HFD on Body Weight

Mice on the HFD gained significantly more weight than ND mice. Weight gain was similar in HFD-WT and HFD-TRPV1KO mice (Figure 1A). However, assessment of adipocyte size revealed that HFD-TRPV1KO mesenteric adipose tissue contained a significantly higher percentage of small adipocytes (1–20 µm) than that from HFD-WT mice (Figure 1B). Weight gain was paralleled by a significant increase in abdominal circumference (Table S1) and fat mass (Figure S1). Typically, mice ate less mass, despite increased caloric intake, over the first 28 days of feeding in the HFD group, irrespective of genotype (Figure 1C). Circulating levels of leptin increased in both HFD-WT and HFD-TRPV1KO mice (Figure S1). Adiponectin levels were significantly increased in HFD-WT but not HFD-TRPV1KO mice (Figure S1).

Effect on Blood Pressure and Vascular Hypertrophy

Blood pressure was significantly increased in HFD-WT mice only by week 15, measured by telemetry or tail cuff. Results for measurement by telemetry in conscious unrestrained mice are shown (Figure 2A through 2E). The use of tail cuff, involving restraint (associated with stress), reveals similar results but higher trends for all of the measurements (Figure S2). The vascular wall width, collagen deposition, and total aortic wall width analyzed as parameters of vascular hypertrophy were each significantly increased in HFD-WT mice in comparison with ND-fed WT counterparts and HFD-TRPV1KO mice (Figure 3A, 3B and Figure S3). Images of representative aortic sections are shown (Figure 3C). Despite the vessel wall increase in HFD-WT, no increased vascular cell adhesion protein 1 protein expression was observed (Figure S4). Myograph studies revealed that neither constrictor (phenylephrine) nor dilator (endothelial-independent vasodilator calcitonin gene-related peptide and the endothelial-dependent carbachol) responses differed in ND compared with HFD or in WT or TRPV1KO mice (Figure S5).

Effect on Glucose Tolerance

Baseline glucose levels were similar in WT and TRPV1KO mice but showed a nonsignificant raised trend in HFD mice and transient potential receptor vanilloid 1 (TRPV1) knockout (KO) mice. A, Body weight (n=12–17). B, Percentage of adipocytes based on cell size (in micrometers squared); n=4–5. C, Total food intake (n=2–3) over 28 days from 3 to 7 weeks. **P<0.01; ***P<0.001 vs respective normal diet control.

Figure 1. Effect of high-fat diet (HFD; 35%) on wild-type (WT) and transient potential receptor vanilloid 1 (TRPV1) knockout (KO) mice. A, Body weight (n=12–17). B, Percentage of adipocytes based on cell size (in micrometers squared); n=4–5. C, Total food intake (n=2–3) over 28 days from 3 to 7 weeks. **P<0.01; ***P<0.001 vs respective normal diet control.
Glucose administration in HFD-WT and HFD-TRPV1KO mice caused substantial increase in plasma glucose levels compared with ND mice. However, by comparison, after the administration of the glucose load, the HFD-TRPV1KO mice were able to reverse the high plasma levels quicker than the HFD-WT group (Figure 4B), with a significant difference when areas under the response curve (0–105 minutes) were compared for WT-HFD and WT-TRPV1KO mice (Figure 4C). In addition, the HFD-WT and HFD-TRPV1KO mice exhibited increased heart size and heart rate (Figure S6) when...
compared with ND mice. By comparison, kidney weight in HFD-WT mice was significantly increased compared with TRPV1KO mice (Figure 4D), although not when calculated as a kidney:body weight ratio (Figure S7), where no structural damage was observed (Figure S7).

Figure 4. Effect of high-fat diet (HFD; 35%) on (A) baseline blood glucose and (B) blood glucose levels of wild-type (WT) and transient potential receptor vanilloid 1 (TRPV1) knockout (KO) mice, after a 2-mg/kg glucose bolus (n=5–13). Results are analyzed by comparison of mean area under curve. C, Area under the curve between baseline and 105 minutes after glucose bolus treatment. Analyzed by unpaired t test, *P<0.05, **P<0.01, and ***P<0.001 vs respective normal diet control, and #P<0.05 and ##P<0.01 vs respective HFD-WT.

Figure 5. The effect of a high-fat diet (HFD) on circulating inflammatory cytokine levels. A, Interleukin (IL)-1β, (B) IL-6, (C) IL-10, and (D) IL-12 plasma levels in wild-type (WT) and transient potential receptor vanilloid 1 (TRPV1) knockout (KO) mice fed either a normal diet or HFD for 12 weeks. *P<0.05, **P<0.01, and ***P<0.001 vs respective normal diet control, and #P<0.05, ##P<0.01 vs HFD-fed TRPV1KO.
Effect on Nociceptive Thresholds

The characteristic phenotype of TRPV1KO mice is that they are insensitive to thermal nociceptive stimuli. Little is known about the impact of HFD on TRPV1-dependent heat sensitivity. Here, HFD-WT mice showed increased latency to heat when compared with ND-WT mice, indicating a loss in heat sensitivity in the skin of the HFD-WT mice (Figure S8). Both HFD-WT and HFD-TRPV1KO mice exhibited similar and reduced sensitivity to mechanical hyperalgesia when compared with their ND controls (Figure S8).

Effect on Plasma Protein Levels

Because obesity is associated with a low-grade inflammation, and inflammation is also a key event in cardiovascular disease, we determined levels of circulating cytokines in a subset of the mice. Plasma levels of interleukin (IL) 10 and IL-1β were significantly raised in HFD-WT but not HFD-TRPV1KO mice compared with normally fed mice. In addition, IL-10 and IL-1β levels were significantly higher in the HFD-WT when compared with the HFD-TRPV1KO group. There was evidence of raised levels (nonsignificant) of IL-6 and IL-12 in HFD-WT mice only (Figure 5). Tumor necrosis factor-α and interferon-γ levels were not raised beyond the baseline levels (Figure S8). The possibility of ongoing vascular inflammation was examined through measurement of circulating endothelin 1, but no significant increase in its levels were observed (Figure S9).

Discussion

This study provides novel evidence of a linked series of events whereby TRPV1 plays a primary and pivotal role in the development of the metabolic syndrome. The HFD-WT mice show an enhanced metabolic syndrome compared with the HFD-TRPV1KO mice, in terms of hypertension, glucose handling, and low-grade inflammation, despite similar weight gain. The results show, for the first time to our knowledge, that TRPV1 deletion is associated with protection against obesity-induced hypertension and inhibition of low-grade inflammation. In addition, the HFD-WT mice show an altered nociceptive phenotype with loss of sensitivity to heat touch that is at least partly dependent on TRPV1. The results are in keeping with the concept that a series of complex networks operate in obesity-driven dysfunction that leads to hypertension and that TRPV1 is an influential component in this network.

The HFD model was based on previous studies where obesity-induced hypertension was defined in C57Bl/6 mice. The results confirm that WT and TRPV1KO mice gain similar weight when fed an HFD from 3 to 15 weeks. Studies of TRPV1 in weight loss present mixed results and have often involved chronic administration of capsaicin, which causes the destruction and defunctionalization of the sensory nerves, in addition to the TRPV1 channel. Interestingly, a novel capsaicin analogue has been shown to be well tolerated but to have no effect on body weight in humans, despite significantly reducing abdominal fat in a manner that was linked with 2 common genetic variants. Our results, when taken with those already in the literature, indicate that TRPV1 is unlikely to play a major role in influencing weight gain. Of note, we show that HFD-WT mice possess a greater percentage of larger adipocytes in keeping with the concept that functional TRPV1 is implicated in normal preadipocyte-to-adipocyte differentiation. We confirm that lack of active TRPV1 is able to attenuate loss of glucose handling, as shown previously in HFD-TRPV1KO and TRPV1 antagonist-treated mice. Blood pressure was measured by tail cuff or telemetry in 3 separate experiments. All of the experiments showed that HFD-TRPV1KO mice were protected from HFD-induced hypertension. TRPV1 activation releases vasodilator neuropeptides, leading to peripheral vasodilatation. We originally hypothesized that TRPV1 deletion would trigger a loss of the vasodilator effect and hypertension, but this is not the case with TRPV1 presence associated with increased blood pressure in this HFD model. The obesity-induced high blood pressure in HFD-WT but not HFD-TRPV1KO mice was mirrored by an effect on vascular hypertrophy in HFD-WT mice, indicating that the onset of vascular remodeling and these 2 phenomenon may well be directly related. Further studies using antihypertensives would verify this. However, vascular inflammation, as assessed by vascular cell adhesion protein 1 immunohistochemistry, was not increased in either HFD-WT or HFD-TRPV1KO aortas. Vascular cell adhesion protein 1 is often upregulated in the aorta of the hypertensive mouse, as shown by this laboratory in the angiotensin II hypertension model. Myograph studies of mesenteric resistance vessels were carried out. Our results revealed that constrictor and dilator responses to phenylephrine, calcitonin gene-related peptide, and the endothelial-dependent carbachol remained intact, suggesting little vascular dysfunction at the mesenteric resistance vessel level at least. It has been shown recently that dietary capsaicin resists loss of endothelial-dependent relaxation and hypertension in HFD-fed mice, but whether this is associated with activating or desensitizing doses of capsaicin has been debated.

We observed an increased kidney weight in HFD-WT but not HFD-TRPV1KO mice, but not when calculated as a kidney:body weight ratio (Figure S14). This was not associated with structural damage, as shown by histology. Thus, no obvious link between kidney damage and hypertension was observed. Moreover, a similar increase in heart rate and weight gain was observed in HFD mice, irrespective of genotype, indicating that the heart, in our model, is influenced by the HFD in a similar manner, irrespective of genotype. TRPV1 deletion enhances damage after myocardial infarction, mainly because of a lack of neuropeptide release. Obesity and the onset of the metabolic syndrome are associated with an altered phenotype in terms of inflammatory markers and the presence of a low-grade inflammation, although mechanisms by which this translates into a systemic condition are unclear. Here, we found raised levels of leptin in HFD-WT and HFD-TRPV1KO mice, reaching significance in the HFD-WT mice, indicating that adipose tissue is involved at a primary level. Furthermore, levels of adiponectin and IL-10 were raised, both suggested to be anti-inflammatory in hypertension, although their levels have been shown to be high in patients with chronic heart conditions.
failure. The lack of detection of increased levels of IL-10 in HFD-TRPV1KO mice suggests that TRPV1 does not play a central role in influencing a shift from the T-helper 1 to a T-helper 2 cytokine profile. We found evidence for a range of circulating cytokines that included IL-1β, IL-6, and IL-12 in HFD-WT but not HFD-TRPV1KO mice. Of these levels, IL-1β reached significance, and we assume that the high levels of IL-10 acted to decrease the expression and release of proinflammatory cytokines, in keeping with its established activity. We did not detect increased circulating levels of tumor necrosis factor-α, which is considered to function as an autocrine/paracrine factor in adipose tissue, rather than a circulating factor, and these results are in keeping with that concept.

The inflammatory cytokine profile fit with the dysfunction of glucose handling and the hypertensive profile, rather than directly with adiposity. Indeed, it is tempting to link circulating levels of the cytokines with the observed increase in blood pressure, but the understanding of the exact role of the cytokines is at an early stage. Endogenous IL-10 has been suggested to limit the onset of angiotensin II–mediated damage and may be acting here to suppress inflammatory events at the vascular wall. We examined the possibility that vascular inflammation may be increased in HFD-WT but not HFD-TRPV1KO mice by measuring endothelin 1 levels. Endothelin 1 levels are suggested to become functionally significant at an early stage in the spontaneously hypertensive rats and to be linked with insulin resistance. The vasodilator potential of TRPV1 activation is suppressed in pigs with metabolic syndrome. A recent study has revealed a mechanism by which the vasoconstrictor peptide endothelin is released to mediate pressor responses in WT but not TRPV1KO mice after TRPV1 activation by capsaicin. However, increased circulating levels of endothelin could not be detected in this study. Both IL-1β and IL-6 have also been linked to hypertension and, in the case of IL-6, T-cell activation and insulin resistance. These collected findings suggest a need to learn more about the interplay between the different facets of the metabolic syndrome and the factors that lead to the onset of hypertension.

Finally, TRPV1KO mice typically demonstrate a slowed response in terms of paw withdrawal threshold to heat. A reduced response to thermal thresholds has been demonstrated in Zucker rats. Here, concomitant analysis of 2 distinct TRPV1-dependent pathways in the same mouse indicates that TRPV1-dependent sensitivity to heat is altered during the same time frame as the vascular changes occur, before the establishment of defined type II diabetes mellitus. We also demonstrate a loss of mechanical sensitivity that is TRPV1 independent. Thus, of note, changes in nociception sensitivity that are presently associated with diabetes mellitus occur at an early time point in this model that is more relevant to the metabolic syndrome.

In conclusion, we confirm TRPV1 deletion has no effect on the onset of weight gain in mice, even in the presence of an HFD, over 8 to 15 weeks. On the other hand, we provide evidence that TRPV1KO mice were protected from obesity-induced hypertension, low-grade inflammation, and glucose tolerance.

Perspectives

The results demonstrate for the first time a key influence of TRPV1 in the onset of obesity-induced hypertension, low-grade inflammation, and a related reduction in thermal sensation. These findings suggest that TRPV1 may play a critical role in the onset of symptoms of the metabolic syndrome. These changes are observed before any effect that TRPV1 may have in influencing weight gain. These results complement very recent findings that a TRPV1 antagonist protects against the onset of glucose tolerance in a murine model of type II diabetes mellitus. However, it is important to note that these results contrast with studies that have concentrated previously on cardiovascular disease (primarily ischemic conditions) where lack of TRPV1 is associated with a worsened phenotype. These studies provide further evidence of a critical regulatory role of TRPV1 in metabolic and cardiovascular regulation. They indicate a possible novel therapeutic approach whereby TRPV1 antagonists may protect against onset of the metabolic syndrome.

Acknowledgments

We acknowledge the support of Chi Teng Vong and Khadija Alawi.

Sources of Funding

This work was supported by the British Heart Foundation (to Dr Marshall); a Capacity Building Award in Integrative Mammalian Biology funded by the British Biotechnology Science Research Council, British Pharmacological Society, Higher Education Funding Council, Knowledge Transfer Network, Medical Research Council, and Scottish Funding Council (to Dr Liang and Brain); Arthritis Research United Kingdom (to Dr Fernandez; grant No. 19296); British Biotechnology Science Research Council (to Dr Smillie); and Diabetes United Kingdom (to Dr Dessapt-Baradez).

Disclosures

None.

References

These findings contrast those where TRPV1 deletion has been associated with a worsened phenotype, primarily ischemic diseases.

What Is Relevant?

• The studies provide further evidence of a critical regulatory role of TRPV1 in cardiovascular regulation.

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Hypertension. 2013;61:246-252; originally published online November 12, 2012; doi: 10.1161/HYPERTENSIONAHA.112.201434

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/61/1/246

Data Supplement (unedited) at:
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A role for TRPV1 in influencing the onset of cardiovascular disease in obesity.

Nichola J Marshall (BSc, PhD)*, Lihuan Liang (BSc, MSc)*, Jennifer Bodkin (BSc, MRes), Cecile Dessapt-Baradez (BSc, PhD), Sophie Collot-Teixeira (BSc, MSc, PhD), Sarah-Jane Smillie (BSc), Kamal Lalgi (BSc), Elizabeth S. Fernandes (BSc, MSc, PhD), Luigi Gnudi (MD, PhD, FRCP, FASN), Susan D Brain (BSc, PhD)

*Joint first authors

BHF Centre of Cardiovascular Excellence and Centre of Integrative Biomedicine, King’s College London, LONDON SE1 9NH, UK

Short Title: TRPV1 and obesity-induced hypertension

Word count:

Correspondence

Prof. SD Brain, Cardiovascular Division, King’s College London, Franklin-Wilkins Building, Waterloo Campus, LONDON SE1 9NH, UK

Tel: +44 207 848 4453 Fax: +44 207 848 3743 Email: sue.brain@kcl.ac.uk

Detailed Materials and Methods
Mouse Models
Adult age matched male and female C57BL6/129SVJ mice (with greater than 7 generations of backcrosses), either the genetically unaltered WT or TRPV1 receptor gene KO genotype lacking the TRPV1 receptor, were bred in house. The mice were characterised and genotyped as previously detailed.1-3 Both male and female were used for body weight (Fig 1) and tail cuff blood pressure (Fig 2) as stated below. Only males were used for telemetry (Fig 2) and myograph (S7). Otherwise only females were included in the rest of the study.

Obesity-Induced Hypertension
Male (n=3-6) and female (n=8-13) TRPV1KO mice and their WT counterparts were either fed a HFD4 (35% fat from lard, mainly triglycerides, with 58.4% Kcal from fat, sodium level: 0.15% and potassium level: 0.5%, Harlan Teklad, USA, custom diet reference TD 03584) or a normal diet (4% fat, mainly from fatty acids but also from plant or grain sources;6.8% Kcal from fat, sodium level: 0.25% and potassium level: 0.67%, Rodent Diet RM 1E, SDS, Essex, England) from 3 or 12 weeks of age as appropriate, ad libitum. Paired mice were selected from weaned WT and TRPV1KO colonies by body weight. Mice were housed communally in groups of 2 to 3 to avoid the stresses associated with single housing, whilst also reducing competition between mice for food. Mice were weighed weekly and had access to food and water ad libitum. Food intake was assessed daily by weighing food, in a subgroup of mice and the calorific intake of mice on the HFD was increased by approximately 30%.

Blood Pressure Analysis
Tail Cuff Plethysmography
Male (n=4) and female (n=13-17) TRPV1KO and WT mice were used for tail cuff experiments. Blood pressure was measured by tail cuff plethysmography, using the CODA 6 (Kent Scientific, UK) non-invasive blood pressure acquisition system for mice.5, 6 after two weeks training. Blood pressure was measured in the final 3-4 days of the study or the last 4 weeks of the study in a separate experiment.

Telemetry
Blood pressure and heart rate were measured using a telemetry device (PA-C10, DSI, NL), with the catheter tip placed in the left carotid artery and advanced towards the aortic arch. The transmitter body was placed subcutaneously in the right flank. Male WT normal diet (n=3), WT HFD (n=6), TRPV1KO normal diet (n=4), TRPV1KO HFD (n=6) mice were used. Telemetry surgery was carried when mice were of sufficient size for surgery (aged 12 - 13 weeks) and when HDF-mice were not too obese, in order to reduce risk of surgical problems. Implantation surgery was conducted using aseptic techniques under isoflurane anaesthesia (2.5%, Abbott Laboratories, UK, in 3L/minute O2). The catheter was secured using surgical braided silk (5.0, waxed, Pearsalls sutures) and the outer wound was closed with absorbable sutures (4.0, Ethicon, Johnson and Johnson). Intra-muscular buprenorphine (50μg/kg, Vetersgecis, Alstoe animal health) was given prior to surgery for pain relief. Mice were allowed to recover for two weeks in a quiet room before collecting data. Data was acquired for the last 3 days at the age of week 15 and then calculated by the DSI software (DSI Dataquest A.R.T.) and analysed in Microsoft Excel and GraphPad Prism 5.
TRPV1 and obesity-induced hypertension

**Thermal nociception**

The analysis of response to noxious thermal stimulus was performed in accordance with the original method described by Hargreaves *et al.*, using the Ugo Basile Model 7370 Plantar Test. Mice were placed in transparent boxes and allowed to acclimatise to their new surroundings for 30 minutes or until exploratory behaviour had ceased. An integral automatic timer was used to record the paw withdrawal latency. A cut off point of 22 seconds was used to prevent tissue damage. Measurements were taken in triplicate for each paw, and the mean of both paws was used to calculate average paw withdrawal latency.

**Mechanical nociception**

The sensitivity of mice to mechanical nociceptive stimulus was assessed as described by Keeble *et al.* using a dynamic Plantar Aesthesiometer (Ugo Basile model 37400). Mice were placed in perspex boxes on a wire mesh floor and allowed to acclimatise to their surroundings for 30 minutes, or until exploratory behaviour had ceased. After this time, a touch stimulator unit equipped with a von Frey filament (0.5mm diameter) was situated underneath the mice. The filament was directed to the plantar surface of the hind paw and raised at an increasing force of 10g/second, until the mouse withdrew the paw. A cut-off force of 50g was pre-set to prevent injury. The force at which the mouse withdrew its paw was automatically determined as paw withdrawal threshold (g). The mean of at least 3 measurements from each hind paw were used to calculate average paw withdrawal threshold.

**Glucose tolerance testing**

Mice were fasted for 6 hours during the light phase of the light-dark cycle. A small blood sample was obtained from the tails of conscious mice. This was then used to measure blood glucose using an Acu-Chek Compact Plus meter (Roche Diagnostics, Burgess Hill, UK). Baseline blood glucose was measured, then an i.p. glucose bolus of 2mg/kg was administered. Blood glucose was then sampled at 15 minute intervals thereafter.

**Collection of samples and measurements taken**

Under terminal anaesthesia (isofluorane, 2%), a blood sample was collected (0.7-1.5ml) by cardiac puncture, using a 25G needle and 1ml syringe washed through with heparin (500 I.U/ml heparin sodium) to prevent coagulation. The resulting blood sample was then centrifuged at 380g at 4°C to obtain plasma. Plasma was then aliquoted and stored at -80°C for further analysis.

The relationship between hip / waist size and obesity in mice was assessed through measure of the narrowest point just under the rib cage (waist) and widest (hip) diameter of the mouse body. In addition to this, it was felt important to determine whether an increase in body mass might correlated to an increased lean mass because of extra skeletomuscular development from the increased nutrients available from this HFD. Therefore, body length (tip of nose to base of tail), front paw (tip of longest toe to heel) and back paw length (tip of longest toe to apex of the knee joint) was measured also. A final body weight was taken. The animal was then killed by cervical dislocation. Tissues (heart, kidney, aorta and adipose tissue) were taken and stored for further analysis.

**Measurement of inflammatory mediators in plasma samples**


The levels of different inflammatory mediators were quantified in plasma samples obtained from WT and TRPV1KO mice fed with either normal or HFD. IL-1β, IL-6, IL-10, IL-12, TNFα and IFNγ, and the chemokine KC (CXCL1) levels were measured by using the multi-spot Mouse Pro-inflammatory ELISA assay kit (Mouse pro-inflammatory 7-plex kit, MS6000, Meso Scale Discovery, Maryland, USA) in accordance with manufacturer’s guidelines and results are are expressed as pg cytokine / ml. Plasma levels of leptin and adiponectin were quantified by by using a multiplex assay (Searchlight; Aushon Biosystems, MA, USA). Endothelin-1 (ET-1) was measured using an ET-1 ELISA kit purchased from Phoenix Pharmaceuticals (CA, USA) according manufacturer's instructions.

**Preparation of Aortic and Kidney sections for histology**

Approximately 5mm long sections of descending thoracic aorta and longitudinally-cut sections of kidney were preserved in 10% formaldehyde. Samples were processed overnight using a Tissue-Tek VIP Vacuum Infiltration processor (Sakura Tissue Tek VIP 1000, USA) and then embedded into paraffin blocks using a Sakura Tissue Tek III Embedding Centre. 4 μm thick transverse sections were cut from the paraffin blocks using a Reichert-Jung 2030 Biocut microtome and dried overnight onto positively charged slides, before being stained with Masson's trichrome stain.

**Aortic histology**

Aortic sections were analysed for markers of vascular hypertrophy as described by Liang et al. Masson's Trichrome-stained aortic sections were used to determine the width of aortic wall comprised of either intimal and medial layer or the collagen containing adventitial layer, at 40x magnification. Widths were determined by taking 8 measurements per section at approximately 45° angles. As 3-4 sections were measured per mouse, width measurements are therefore the mean of 24-32 measurements per mouse.

**Renal morphology**

To assess glomerular cross-section, the edge of the glomerulus was identified and carefully marked out. The area inside this boundary was then quantified. The number of nuclei (stained dark purple) within the glomerulus was counted. This gave an indication of cell proliferation within the glomerulus. Collagen deposition (stained blue by the Masson's trichrome stain) around the edge of the glomerulus was then measured by a blinded operator by grading the amount of blue stained material on a scale of 1 to 5 (where 1 is little to no blue-stained collagen, and 5 is an extensive layer of blue-stained collagen). The mesangial matrix area (stained pink by Masson's trichrome stain) was also graded in the same manner.

**Quantification of histological parameters**

Aorta and kidney sections were imaged using a colour video camera (Olympus U-CMAD3 colour view camera soft imaging system, Southend-on-Sea, UK) connected to a calibrated microscope (Olympus BX51, Southend-on-Sea, UK). Images were assessed using specialised morphometry software (Cell P, version 2.6, Olympus soft imaging solutions GmbH, ©1986-2007). All measurements were calibrated to 1μm. Representative 3-4 sections were selected for morphometric analysis. All samples were read by blinded operators.

**VCAM-1 immunohistochemistry in aortic sections**
For immunohistochemical analysis, the thoracic aorta was dissected and immersed in OCT compound, snap frozen in liquid nitrogen then stored at -80°C. Serial aortic cross sections (10µm) were cut using a cryostat. VCAM-1 (1:100 dilution; sc-8304, Santa Cruz Biotechnology, USA) expression was assessed by immunohistochemistry using (Envision HRP kit, Dako, UK). The mean of 4 measurements was taken and the mean ± sem from 2-4 sections/mouse from 3-4 mice in each case was determined (ProgReg C5, JENOPTIK, UK).

Adipocyte Analysis
10 µm sections of paraffinized mesenteric adipose tissues were stained with haemotoxylin and eosin (H&E). Six pictures from separate parts of each section were taken (200X magnification) and all counts performed by three different individuals, who were blinded to treatments. The area of 100 cells was measured on each section using the ProgRes programme (JENOPTIK, UK). The percentage of the different cell sizes was calculated.

Wire Myography
First-order mesenteric artery branches (approx. 20-40µm relaxed diameter) were isolated in ice cold Krebs (118mM NaCl, 24mM NaHCO₃, 1mM MgSO₄, 4mM KCl, 0.5mM NaH₂PO₄, 5.5mM glucose, and 2.5mM CaCl₂, all salts from Sigma, UK). They were carefully cleaned of fat, mounted and normalized to normal peripheral artery tension (13.3kPa) on a wire myograph (DMT 610M or 620) using 0.025 mm tungsten wire. Vessels were maintained throughout the experiment in 37°C Krebs solution gassed with air/5% CO₂. Endothelial function was examined using carbachol (10µM, Sigma, UK), where > 60% relaxation over 2 min was counted as endothelium intact. For relaxation studies, tissues were pre-constricted with phenylephrine (10nM, Sigma, UK, 5 minutes) then given cumulative concentration of human αCGRP (Phoenix, USA), or carbachol with the % of relaxation at 3 minutes calculated. In some experiments additional pharmacological mediators were used. Time and vehicle controls were collected for all vessels to ensure comparative functioning. Results from multiple vessels per mouse are averaged to produce each N number.

Statistical Analysis
Results are shown as mean ± standard error of the mean (s.e.m.), and evaluated by ANOVA + Bonferroni’s post test, using Graph Pad Prism San Diego California, USA www.graphpad.com. A significant difference from the null hypothesis was accepted when p<0.05.

References:


Supplemental Figures

<table>
<thead>
<tr>
<th>Measurement</th>
<th>WT normal diet, n=18</th>
<th>WT high (35%) fat diet, n=22</th>
<th>TRPV1KO normal diet, n=18</th>
<th>TRPV1KO high (35%) fat diet, n=21</th>
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<tbody>
<tr>
<td>Waist (mm)</td>
<td>67.8 ± 1.8</td>
<td>77.9 ± 1.8 ***</td>
<td>62.6 ± 1.1</td>
<td>74.3 ± 1.6 ***</td>
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<td>Abdomen (mm)</td>
<td>78.1 ± 1.7</td>
<td>93.8 ± 1.5 ***</td>
<td>75.2 ± 1.7</td>
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<td>Body length (mm)</td>
<td>97.9 ± 1.1</td>
<td>102.6 ± 1.0 **</td>
<td>98.6 ± 1.0</td>
<td>102.2 ± 0.8 **</td>
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<td>Front paw length (mm)</td>
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<td>Back paw length (mm)</td>
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<td>17.6 ± 0.2</td>
<td>17.9 ± 0.6</td>
<td>17.8 ± 0.2</td>
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</table>

Table S1. Developmental markers of WT and TRPV1KO mice following the feeding of either normal or HFD-fed from 3-15 weeks of age (n=13-17). Mean ± s.e.m. analysed by ANOVA + Bonferroni’s t-test, where ***=p<0.001, compared with respective normal diet control.
Figure S1. The effect of a HFD on the adipose tissue pad weights of WT and TRPV1KO mice fed either normal diet (shaded bars) or HFD (open bars) from 3-15 weeks of age, n=13-17. Where (A) abdominal, (B) dorsal and (C) mesenteric adipose tissue pads. Plasma levels of leptin (D) and adiponectin (E). Mean ± s.e.m. analysed by ANOVA + Bonferroni’s t-test, where ***=p<0.001, compared with respective normal diet control.
Figure S2: Effect of HFD (35%) on blood pressure measured by tail cuff. Mice were fed either HFD or normal diet for 12 weeks.
Figure S3: Effect of HFD (35%) on total aortic wall width of WT and TRPV1 KO mice fed either normal diet (shaded bars) or HFD (open bars) from 3-15 weeks of age, measured from Masson’s Trichrome stained aortic sections, n=10-14. ***=p<0.001 compared with respective normal diet control.
Figure S4. Effect of high (35%) fat diet on expression of VCAM-1 on aortic wall. A) Representative immunohistochemical staining of VCAM-1 in the thoracic aorta wall at 200x magnification. (B) Percentage of VCAM-1 expression in relation to total aortic area, n=3-4.
Figure S5. The effect phenylephrine, CGRP and carbachol on ND-WT and ND-TRPV1KO mouse and also HFD-WT and HFD-TRPV1KO mouse mesenteric arteries, as determined by myograph. (A) Effect of phenylephrine (0.1-10µM) and (B) CGRP (0.33-33nM) on endothelium intact and denuded mesenteric arteries. n = 5-7. (C) Effect of carbachol (1nM-10 µM) on endothelium intact mesenteric arteries. n=3-6. Dilator responses were examined in the presence of phenylephrine (10µM).
Figure S6: Effect of HFD (35%) on (A) total heart weight and (B) heart rate of WT and TRPV1KO mice fed either normal diet (shaded bars) or HFD (open bars) from 3-15 weeks of age. Lihuan to change A and B around.
TRPV1 and obesity-induced hypertension

Figure S7. Representative images (A-D) of kidney sections stained with Masson’s trichrome with WT and TRPV1KO mice fed either normal diet or HFD from 3-15 weeks of age. The effect of a HFD on the weight and morphology of kidneys (E) from WT and TRPV1KO mice fed either a normal or HFD from 3-15 weeks of age, n=6-9. Mean ± s.e.m. analysed by ANOVA + Bonferroni’s t-test, where *=p<0.05 compared with respective normal diet control. To investigate this increase in kidney weight observed in TRPV1 HFD kidney, morphometric analysis was undertaken using Masson's trichrome stain. From these sections, glomerular volume, glomerular nuclei number, mesangial matrix and collagen deposition was measured but no differences were observed.

<table>
<thead>
<tr>
<th></th>
<th>WT normal diet, n=6</th>
<th>WT high (35%) fat diet, n=8</th>
<th>TRPV1KO normal diet, n=9</th>
<th>TRPV1KO high (35%) fat diet, n=9</th>
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</thead>
<tbody>
<tr>
<td>Combined Kidney to Body Weight ratio (%)</td>
<td>1.15 ± 0.13</td>
<td>0.96 ± 0.1</td>
<td>1.15 ± 0.1</td>
<td>0.91 ± 0.2</td>
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<td>Glomerular Surface (µm²)</td>
<td>2593.0 ± 274.0</td>
<td>2687.0 ± 134.3</td>
<td>2415.0 ± 163.6</td>
<td>2610.0 ± 81.4</td>
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<tr>
<td>Nuclei Number of glomerular cells</td>
<td>30.4 ± 1.6</td>
<td>29.1 ± 2.3</td>
<td>23.6 ± 2.2</td>
<td>26.9 ± 2.7</td>
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<td>Collagen Deposition (arbitrary units)</td>
<td>2.2 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>1.7 ± 0.2</td>
<td>1.2 ± 0.1</td>
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<tr>
<td>Mesangial Matrix (arbitrary units)</td>
<td>1.3 ± 0.07</td>
<td>2.2 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>1.9 ± 0.2</td>
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</table>
Figure S8. Effect of high (35%) fat diet on nociception (A) paw withdrawal latency (seconds) to thermal stimulus and (B) paw withdrawal threshold (g) to mechanical stimulus, where n=12-17. *p<0.05 compared with respective normal diet control. ###p<0.01 and ####p<0.001 compared with respective WT counterpart.
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Figure S9. The effect of a HFD on circulating levels of (A) KC, (B) TNF, (C) IFN and (D) endothelin-1 in plasma taken from WT and TRPV1KO mice fed either a normal or HFD from 3-15 weeks of age, n=6-8. Analysis of mean ± s.e.m. by ANOVA + Bonferroni’s t-test showed no significant difference. Plasma taken from WT and TRPV1KO mice fed either a normal or HFD was analysed for endothelin-1 (ET-1).