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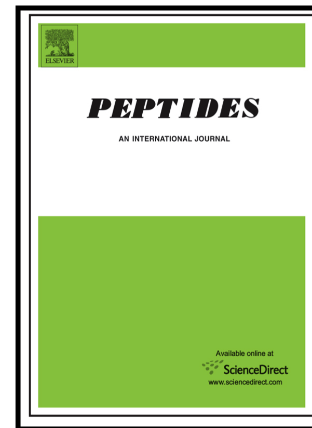

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Ac3IV, a V1a and V1b receptor selective vasopressin analogue, protects against hydrocortisone-induced changes in pancreatic islet cell lineage

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Abstract

The Avpr1a (V1a) and Avpr1b (V1b) receptor selective, vasopressin (AVP) analogue, Ac3IV has been shown to improve metabolism and pancreatic islet structure in diabetes and insulin resistance. The present study further investigates these actions by assessing the ability of Ac3IV to protect against pancreatic islet architectural disturbances induced by hydrocortisone

(HC) treatment in transgenic *Ins1*$^{\text{Cre/+}}$;*Rosa26-eYFP* mice, that possess beta-cell lineage tracing capabilities. HC intervention increased ($p < 0.001$) energy intake but reduced ($p < 0.01$) body weight gain, with no impact of Ac3IV. All HC mice had reduced ($p < 0.05$) circulating glucose, but plasma insulin and glucagon concentrations remained unchanged. However, HC mice presented with increased ($p < 0.001$) pancreatic insulin content, which was further augmented by Ac3IV. In addition, Ac3IV treatment countered HC-induced increases in islet-, beta- and alpha-cell areas ($p < 0.01$), as well as promoting islet number towards control levels. This was accompanied by reduced ($p < 0.05$) beta-cell growth, but enhanced ($p < 0.001$) alpha-cell proliferation. There were no changes in islet cell apoptotic rates in any of the groups of HC mice, but co-expression of CK19 with insulin in pancreatic ductal cells was reduced by Ac3IV. Assessment of beta-cell lineage revealed that Ac3IV partially protected against HC-mediated de-differentiation of mature beta-cells, whilst also decreasing ($p < 0.01$) beta- to alpha-cell transdifferentiation. Our data indicate that sustained activation of V1a and V1b receptors exerts positive islet cell transition effects to help retain beta-cell identity in HC mice.

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Introduction

Arginine vasopressin (AVP; also referred to as antidiuretic hormone (ADH)) is a nine amino acid residue hormone secreted by the pituitary gland, with a well characterised physiological role in osmotic regulation mediated via Avpr2 (V2) receptors [1]. However, other functional

AVP receptors, namely Avpr1a (V1a) and Avpr1b (V1b), have recently been demonstrated on pancreatic islet beta-cells associated with prominent stimulation of insulin secretion [2]. This, together with suggested local synthesis of intra-islet AVP [2], has led to the postulation that this hormone plays an important role in metabolism. In harmony with this, V1a and V1b receptors are also found on metabolically active tissues where they appear to exert positive actions [3]. Taken together, it appears that appropriate modulation of AVP receptors could be harnessed for the treatment of metabolic diseases.

In support of our postulate, a recently characterised enzymatically stable AVP analogue, namely Ac3IV, has been demonstrated to induce prominent antidiabetic effects, including notable benefits on pancreatic islet architecture, following sustained administration in high fat fed (HFF) mice [4]. Significantly, Ac3IV was shown to be selective for V1a and V1b receptors, and lacked activity at V2 receptors that are responsible for the effects of AVP on the kidney and water retention [4]. Benefits of Ac3IV on islet architecture in HFF mice were linked to positive actions on islet cell proliferation and apoptosis [4], in agreement with previous *in vitro* observations using AVP [2]. Further investigation under conditions of streptozotocin (STZ)-induced beta-cell loss using transgenic mice with islet-cell lineage tracing capabilities revealed encouraging effects of Ac3IV on the transition of both islet alpha- and beta-cells [5]. Thus, sustained activation of V1a and V1b receptors by Ac3IV in diabetes is confirmed to lead to improvements in pancreatic islet morphology as a result of dual benefits on islet cell turnover as well as alpha- and beta-cell transdifferentiation [4,5].

The process of islet cell transdifferentiation involves the transitioning of mature alpha- or beta-cells to their opposite cell-type, thought to help compensate for loss of either cell population [6–8]. In this regard, it is a deficit in beta-cell mass that is often the underlying pathophysiological feature in human forms of diabetes [9], with altered transdifferentiation implicated in this process [10–14]. Ac3IV has already been demonstrated

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to positively influence islet cell transitioning in insulin-deficient STZ diabetes, leading to preserved beta-cell area [5]. However, the impact of Ac3IV on transdifferentiation under situations of adaptive pancreatic beta-cell expansion is unknown. Thus, the most prominent form of diabetes in humans, namely type 2 diabetes, is often associated with initial insulin resistance and beta-cell adaption, prior to onset of beta-cell loss and overt diabetes phenotype [6,9,15]. Therefore, in the current study we have sought to identify whether islet cell transitioning plays a role in hydrocortisone (HC) induced islet adaptation [16], and if Ac3IV treatment can positively impact upon this.

Fully characterised transgenic *Ins1$^{Cre/+}$;Rosa26-eYFP* mice were employed [17–21], a murine model allowing for beta-cell lineage tracing through recombination of a fluorescent marker in the *Ins1* promoter gene of these mice. Pancreatic islet adaptations were induced by 10-days HC administration, and subsequent ability of pharmacological upregulation of V1a and V1b receptor pathways by Ac3IV to protect against the detrimental effects of HC investigated. Thus, the primary objective of the current study was to assess the impact of Ac3IV on changes in pancreatic islet cell lineage in HC treated mice. Our data reveal that sustained V1a and V1b receptor signalling helps to reverse the islet adaptations induced by HC.

Materials and Methods

Peptides

The enzymatically stable, V1a and V1b selective, AVP analogue, Ac3IV (Ac-CYIQNCPRG-NH_2) [4], was obtained from Synpeptide Co. Ltd. (Shanghai, China) at 95% purity. Additional peptide characterisation relating to confirmation of purity and identity was conducted in-house by HPLC and MALDI–ToF MS, as described previously [22].

Animals

Full details of the generation and characterisation of transgenic *Ins1*$^{Cre/+}$;*Rosa26-eYFP C57BL/6* mice are detailed by Thorens *et al*. (2015) [17]. Mice were bred in-house with PCR employed to confirm genotype as previously described by our laboratory [18,21]. Experiments were carried out under the UK Animals (Scientific Procedures) Act 1986 & EU Directive 2010/63EU and approved by the University of Ulster Animal Welfare and Ethical Review Body (AWERB). Animals were used at 12-14 weeks of age and were maintained in an environmentally controlled unit at 22 ± 2 °C with a 12 h dark and light cycle and given *ad libitum* access to standard rodent maintenance diet (10 % fat, 30 % protein and 60 % carbohydrate; Trouw Nutrition, Northwich, UK) and drinking water.

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Experimental protocols

Insulin-resistance was induced in *Ins1*$^{Cre/+}$;*Rosa26-eYFP* mice via daily HC injection (70 mg/kg body weight, i.p.) for 10 days (Fig. 1). This model has previously been employed to examine the impact of related compounds in the setting of HC induced pancreatic islet adaptation [23-25]. At baseline, day -3, all animals received twice daily injection of saline (0.9 % (w/v) NaCl) to acclimatise them to the treatment regimen, and were then matched for body weight and allocated into groups (n=6) on day 1 (Fig 1). Two days prior to the first HC injection, groups of mice (n=6) then continued on twice daily (09:00 and 17:00 h) treatment with saline vehicle or received twice daily Ac3IV (25 nmol/kg bw) instead, for the remainder of the study duration (Fig. 1). The peptide dosing regimen was chosen based on previous positive observations with Ac3IV, or related peptides, in insulin resistant and diabetic mice [4,5,26]. Body weight, cumulative food and fluid intake were assessed at regular intervals. To facilitate assessment of cumulative food and fluid intake all mice were singly housed. At the end of the treatment period, non-fasting blood glucose, plasma insulin and glucagon

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concentrations were determined. At termination, animals were sacrificed by lethal inhalation of CO_2 followed by cervical dislocation. Pancreatic tissues were then excised, divided longitudinally, and processed for either determination of pancreatic hormone content following acid/ethanol protein extraction or fixed in 4% PFA for 48 h at 4 °C for histological analysis [22].

Immunohistochemistry

Fixed tissues were processed and embedded in paraffin wax blocks using an automated tissue processor (Leica TP1020, Leica Microsystems) and 5 μm sections cut on a microtome (Shandon Finesse 325, Thermo Scientific). Slides were dewaxed by immersion in xylene and rehydrated through a series of ethanol solutions of reducing concentration (100–50%) at 10% intervals. Heat-mediated antigen retrieval was then carried out in citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0). Sections were blocked in 4% BSA solution before 4°C overnight incubation with appropriate primary antibodies including insulin (1:400; Abcam, ab6995), glucagon (1:400; raised in-house, PCA2/4), GFP (1:1000; Abcam, ab5450), Ki-67 (1:500; Abcam, ab15580) or CK19 (1:500; Abcam, ab15463). In this respect, CK19 is normally expressed in the pancreatic exocrine, with such cells shown to be capable of developing into endocrine insulin-positive islet cells [27]. The specificity of these antibodies has been confirmed in our previous studies [5,18-25]. Slides were then rinsed in PBS and incubated for 45 min at 37°C with appropriate Alexa Fluor secondary antibodies (1:400; Invitrogen, Alexa Fluor 498 or 594, Invitrogen). Slides were finally incubated with DAPI for 15 min at 37°C, and then mounted for imaging using a fluorescent microscope (Olympus model BX51) fitted with DAPI (350 nm) FITC (488 nm) and TRITC (594 nm) filters and a DP70 camera adapter system [18].

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Image analysis

Islet parameters, including islet, beta- and alpha-cell areas, were analysed using the Cell^{F} imaging software and the closed loop polygon tool (Olympus Soft Imaging Solutions). For transdifferentiation cells expressing insulin with no GFP (insulin^{+ve}, GFP^{-ve} cells), cells expressing GFP without insulin (insulin^{-ve}, GFP^{+ve} cells) along with cells co-expressing GFP and glucagon (glucagon^{+ve}, GFP^{+ve} cells) were analysed, as appropriate. For ductal cell transitioning, islet and ductal cells co-expressing CK19 and insulin (CK19^{+ve}, insulin^{+ve}) were assessed. Islet cell apoptosis was determined using co-expression of TUNEL stain with either insulin or glucagon. Similarly, islet cell proliferation was also assessed using Ki-67 staining and co-expression with either insulin or glucagon. To note, for all co-staining procedures, cell number and not cell area was assessed (Figs 4&5). All cell counts were determined in a blinded manner with >50 islets analysed per treatment group, with sections selected for analysis at intervals of every 10 sections.

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Biochemical analyses

Blood samples were collected from the cut tail vein of non-anaesthetised mice. Blood glucose was measured using a portable Ascencia Contour blood glucose meter (Bayer Healthcare, Newbury, Berkshire, UK). For plasma insulin and glucagon, blood was collected in chilled fluoride/heparin coated microcentrifuge tubes (Sarstedt, Numbrecht, Germany) and centrifuged using a Beckman micro-centrifuge (Beckman Instruments, Galway, Ireland) for 10 min at 12,000 rpm. Plasma was removed and stored at −20 °C, until required for analysis. For hormone content, snap frozen pancreatic tissues were homogenised in acid/ethanol (75% (v/v) ethanol, distilled water and 1.5% (v/v) 12 M HCl) and protein extracted in a pH neutral TRIS buffer, with protein content determined using Bradford reagent (Sigma-Aldrich). Plasma and pancreatic insulin content were determined by an in-house insulin

radioimmunoassay (RIA) [28], whilst plasma and pancreatic glucagon content were assessed by a commercially available ELISA kit (glucagon chemiluminescent assay, EZGLU-30K, Millipore) following the manufacturer's guidelines.

Statistics

Data were analysed using GraphPad PRISM 8.0, with data presented as mean ± SEM. Comparative analyses between groups of mice were carried out using a one-way ANOVA with a Bonferroni *post hoc* test or a two-way repeated measures ANOVA with a Tukey *post hoc* test, as appropriate. Results were deemed significant if $p < 0.05$.

Results

Effects of Ac3IV on body weight, food intake, glucose, insulin and pancreatic hormone content in HC treated *Ins1$^{Cre/+}$; Rosa26-eYFP* mice

All HC treated animals presented with significant ($p < 0.01$) body weight loss at the end of the 12-day treatment period, that was marginally reversed by Ac3IV (Fig. 2A). Interestingly, despite body weight loss, cumulative energy intake was increased ($p < 0.001$) in all HC mice on days 1-4, and in Ac3IV treated HC mice on days 5, 6 and 12 ($p < 0.05$ to $p < 0.001$; Fig. 2B). On day 12, all HC mice had reduced ($p < 0.05$ to $p < 0.01$) circulating glucose levels (Fig. 2C), but there were no obvious changes in plasma insulin or glucagon concentrations between the different groups of mice (Fig. 2D&E). Calculation of non-fasted blood glucose to plasma insulin ratios as a rough approximation of insulin sensitivity gave values of 2.53 ± 0.32, 1.81 ± 0.21 and 1.92 ± 0.26 for control, HC and HC/Ac3IV mice, respectively. This is suggestive of insulin resistance in the HC-treated group but failed to reach statistical significance ($p < 0.08$). HC intervention increased pancreatic insulin ($p < 0.001$), which was

further augmented by concurrent Ac3IV therapy (Fig. 2F), with pancreatic glucagon content remaining unchanged in all groups of mice (Fig. 2G).

Effects of Ac3IV on pancreatic islet morphology in HC treated *Ins1$^{Cre/+}$;Rosa26-eYFP* mice

Representative images of pancreatic islets co-stained for insulin and glucagon from each group of mice are provided in Figure 3A. As expected, HC increased ($p < 0.01$) islet area, as a result of increased ($p < 0.01$) beta- and alpha-cell areas (Fig. 3B-D). Ac3IV largely protected against HC-induced pancreatic islet morphological changes (Fig. 3B-D), but beta-cell area was elevated ($p < 0.05$) in these mice when compared to controls, although still significantly reduced ($p < 0.05$) compared to HC mice (Fig. 3C). In addition, Ac3IV fully reversed the increased ($p < 0.05$) number of islets/mm^2 resulting from HC administration (Fig. 3E).

Effects of Ac3IV on alpha and beta-cell proliferation and apoptosis, as well as ductal cell transdifferentiation, in HC treated *Ins1$^{Cre/+}$;Rosa26-eYFP* mice

Ac3IV significantly ($p < 0.05$) decreased beta-cell proliferation when compared to HC control mice (Fig. 4A). Both HC administration alone ($p < 0.05$), and concurrent Ac3IV treatment ($p < 0.001$), increased alpha-cell proliferation (Fig. 4B). Alpha- and beta-cell apoptotic rates were not different between all groups of mice (Fig. 4C&D). There were no obvious changes in the number of CK19^{+ve}, insulin^{+ve} stained cells within islets (Fig. 4E), but Ac3IV reduced ($p < 0.05$) co-expression of CK19 with insulin in pancreatic ductal cells (Fig. 4F).

Effects of Ac3IV on beta- to alpha-cell transdifferentiation in HC treated *Ins1$^{Cre/+}$;Rosa26-eYFP* mice

Representative images of pancreatic islets co-stained for GFP with either insulin or glucagon from each group of mice are depicted in Figure 5A&B. HC administration has no effect of the number of insulin^{+ve}, GFP^{-ve} islet cells, but numbers were significantly elevated ($p < 0.001$) by Ac3IV treatment (Fig. 5C). The number of GFP^{+ve} cells that were negative for insulin, but positive for glucagon, were both increased ($p < 0.001$) by HC intervention (Fig. 5D&E). Whilst Ac3IC restored the number of glucagon^{+ve}, GFP^{+ve} islet cells to control levels (Fig. 5E), insulin^{-ve}, GFP^{+ve} cell numbers were still elevated ($p < 0.05$) in this group compared to controls, but importantly reduced ($p < 0.05$) when compared to HC control mice (Fig. 5D).

Discussion

AVP has previously been demonstrated to play an important role in pancreatic beta-cell function and survival [2,4,26], with the V1a, V1b receptor selective AVP analogue, Ac3IV, displaying good antidiabetic efficacy in preclinical models of diabetes and obesity, eliciting comparable benefits to the clinically approved GLP-1 mimetic exendin-4 [4,5,26]. In terms of underlying mechanisms, Ac3IV is known to positively influence islet-cell transdifferentiation events under STZ-induced beta-cell stress [5], a biological action also shared by the incretin enhancer drugs [18,26].

In the present study, Ac3IV evoked similar benefits on islet cell transition in HC-treated mice, despite the contrasting aetiology and phenotype compared with the other models. Thus, STZ-diabetic mice exhibit beta-cell destruction and severe insulin deficiency that resulted in overt hyperglycaemia [5,29], whereas the current HC mice displayed characteristic beta-cell expansion often linked to insulin resistance, but less dramatic effects

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on metabolism [16,30]. Thus, HC mice did not present with hyperglycaemia, thereby presenting without the need for beta-cell hyperactivity and hyperinsulinaemia, despite a notable elevation of pancreatic insulin content as well as accompanying beta-cell expansion. However, in this respect it should be acknowledged that in humans, hydrocortisone treatment is typically associated with truncal obesity, progression of impaired glucose tolerance and ultimately towards overt type 2 diabetes over time [31]. In contrast, the current study was undertaken in young, metabolically healthy mice for a relatively short period of time, enabling sufficient increase in beta-cell function to slightly lower blood glucose in response to 10 day hydrocortisone injection. Although, our observations of increased pancreatic islet area and number, as well as beta-cell area, in HC treated mice agree well with other reports [32].

Given the established actions of V1a receptor activation to suppress appetite [33], relative lack of effect of Ac3IV on body weight and food intake in HC mice might appear somewhat unexpected [34]. This could partly be a consequence of the short duration of treatment, but it is more likely to reflect the fact that HC alone resulted in significant weight loss and that effects of Ac3IV on energy homeostasis were superseded by counterregulatory mechanisms. Despite previous reports of a glucagonotropic role for AVP [35], plasma and pancreatic glucagon levels remained unchanged in all groups of mice, yet distinct elevations of alpha-cell proliferation in HC mice were further augmented by Ac3IV. This is perhaps surprising given that alpha-cell area was decreased towards control levels in Ac3IV mice without change in alpha-cell apoptosis. However, changes in circulating glucagon might only be anticipated if blood glucose levels fall below normal [5], which would seem unlikely in the current setting. In that regard, alpha-cells are suggested to be progenitors for mature beta-cells [36], and observations related to changes in islet-cell area could be linked to benefits of Ac3IV on islet cell transition events in HC mice. Consistent with this view, marked increases

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of insulin^{+ve}, GFP^{-ve} and decreases in glucagon^{+ve}, GFP^{+ve} staining cell populations were observed in Ac3IV-treated mice. The insulinotropic effects of AVP are believed to be partly mediated through modulation of glucagon signalling and related paracrine effects on beta-cells [4,35], which could also be a factor in relation to changes in alpha-cell turnover.

As previously reported [18], HC-induced pancreatic islet cell transitioning events associated with a loss of beta-cell identity. Although beta-cell de-differentiation was clearly evidenced by increased insulin^{-ve}, GFP^{+ve} staining, classic adaptive elevations in islet and beta-cell areas were still evident in HC mice [18,37]. Thus, islet expansion in HC mice is most likely a result of altered cell turnover rather than changes in transdifferentiation, corresponding to our observed increases of alpha- and beta-cell proliferation in these mice. The current scenario where Ac3IV helps to retain beta-cell identity, but in parallel reverses expansion of beta-cell area in HC mice is not easily explainable on first examination. However, a second plausible route for islet enlargement in HC mice could relate to pancreatic ductal precursor cell morphogenesis towards an islet cell phenotype [5,38]. Thus, co-expression with insulin in pancreatic ductal cells was markedly reduced in Ac3IV mice, and the AVP analogue also curbed increases in islet numbers in HC mice [37]. Therefore, following steroid treatment, pancreatic islet adaptations may be more dependent on exocrine ductal cell morphogenesis or islet cell proliferation, rather than endocrine cell transdifferentiation events. In contrast, previous investigations with Ac3IV in STZ-diabetic mice revealed that ductal and intra-islet co-expression of CK-19 and insulin was increased by sustained V1a and V1b receptor activation [5], pointing towards phenotype-specific benefits of Ac3IV. Thus, Ac3IV reverses the detrimental islet and metabolic defects of diabetes induction irrespective of disease aetiology [2,4,5,26].

Improvements in glycaemic status are known to prevent beta-to alpha-cell transdifferentiation as well as reversing beta-cell de-differentiation [39]. Since HC mice did

not present with hyperglycaemia, this pathway is ruled out for Ac3IV-induced changes in islet cell transdifferentiation. As a result, it would also be interesting to establish if insulin^{+ve} cells transitioning from an alpha-cell phenotype represent fully mature, functioning beta-cells [39], and quantification of transcription factors such as Pdx1, MafA and Nkx6-1 would be useful in this regard [40]. On the other hand, it would also be helpful to know whether former beta-cells that are now positive for glucagon still retain beta-cell glucose sensing behaviour whilst secreting glucagon instead.

Notably, effects of the clinically approved incretin enhancer drugs, liraglutide and sitagliptin, on islet cell transdifferentiation have previously been reported to be negligible in HC mice, with more prominent effects on islet cell proliferation and apoptosis observed [18]. This might suggest complementary effects of V1a, V1b and GLP-1 receptor signalling, as has been suggested previously [4]. In agreement, AVP can augment GLP-1 secretion from enteroendocrine L-cells [41], with similarities existing between the cell signalling pathways activated by both hormones [42].

Taken together, the present study clearly confirms that V1a and V1b receptor activation exerts positive effects on islet cell transition events in HC mice, using advanced cell lineage tracing technologies. Despite this, there are also some minor limitations that should be acknowledged. Through necessity of design, all animals received three daily i.p. injections for a period of 10 days, which might be considered to induce a mild stress response [43]. However, none of the mice exhibited outward signs of stress and we employed an injection acclimation period as well as habituating mice to routine handling. The Ac3IV treatment period of 12 days is relatively short, but obvious Ac3IV-induced alterations in pancreatic islet architecture were noted, confirming applicability of our chosen treatment regimen. Indeed, a comparable treatment period has been successfully utilised for other studies evaluating drug-induced changes of pancreatic islet cell lineage in insulin resistant

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and diabetic rodents [23-25]. It would also have been useful to directly assess insulin sensitivity in the HC mice, although early studies have shown that chronic treatment with this steroid rarely results in hyperglycaemia or notable insulin resistance, unless the mice are aged [44]. In addition, we are unable to determine the exact role of changes in islet-cell turnover and ductal cell transdifferentiation, in relation to positively altered islet cell transition events, for the overall benefits of Ac3IV in HC treated mice. Although technically demanding, triple staining of GFP alongside both glucagon and insulin would have been useful to further corroborate the observed Ac3IV evoked changes on islet cell lineage.

In conclusion, the present study has highlighted the potential of the V1a, V1b selective AVP analogue, Ac3IV, as a novel agent with benefits on pancreatic islet structure linked to positive effects on islet cell turnover and beta- to alpha-cell transdifferentiation. Together with our previous studies using animal models of diabetes with different aetiologies [2,4,5,26,42], therapies targeting V1a and V1b receptors merit further consideration for future exploration as a new class of potentially useful antidiabetic drugs.

Conflict of interest

All authors declare no conflict of interest.

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Figure Legends

Figure 1. Experimental timeline for *Ins1$^{Cre/+}$;Rosa26-eYFP* transgenic mice studies. *Ins1$^{Cre/+}$;Rosa26-eYFP* mice (n=6) received twice daily treatment with saline vehicle (0.9% NaCl) or Ac3IV (25 nmol/kg bw, i.p) 2 days prior to daily administration of HC (70 mg/kg bw, i.p) for 10 days.

Figure 2. Effects of Ac3IV on body weight, blood glucose, energy intake as well as circulating and pancreatic insulin and glucagon in HC treated *Ins1$^{Cre/+}$;Rosa26-eYFP* mice. Body weight change (A) and cumulative energy intake (B) were measured during twice daily treatment with saline vehicle (0.9% NaCl) or Ac3IV (25 nmol/kg bw, i.p) for 12 days in HC treated *Ins1$^{Cre/+}$;Rosa26-eYFP* transgenic mice. Terminal non-fasted blood glucose (C) plasma and pancreatic insulin (D,F) as well as glucagon (E,G) levels were assessed on day 12. Values are mean ± SEM for n=6 mice. *P<0.05, **P<0.01, ***P<0.001 compared to control mice. $^{\Delta\Delta}$P<0.01 compared to HC saline control.

Figure 3. Effects of Ac3IV on pancreatic morphology in HC treated *Ins1$^{Cre/+}$;Rosa26-eYFP* mice. Parameters were assessed following 12 days twice-daily treatment with saline (0.9% NaCl) vehicle or Ac3IV (25 nmol/kg bw, i.p) in HC treated *Ins1$^{Cre/+}$;Rosa26-eYFP* transgenic mice. Representative images (40X) of stained islets are provided in panel A. Islet (B), beta- (C) and alpha-cell areas (D) as well as number of islets/mm^2 of pancreas (E) were assessed using Cell^F imaging software and the closed loop polygon tool. Values are mean ±

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SEM for n=6 mice. *P<0.05, **P<0.01 compared to control mice. $^{\Delta}$P<0.05 and $^{\Delta\Delta}$P<0.01 compared to HC saline control.

Figure 4. Effects of Ac3IV on islet cell turnover and expression of ductal cell markers in in HC treated *Ins1$^{Cre/+}$;Rosa26-eYFP* mice. Parameters were assessed following 12 days twice-daily treatment with saline (0.9% NaCl) vehicle or Ac3IV (25 nmol/kg bw, i.p) in HC treated *Ins1$^{Cre/+}$;Rosa26-eYFP* transgenic mice. Beta- and alpha-cell proliferation (A,B) and apoptosis (C,D) were assessed by Ki-67 or TUNEL co-staining with insulin/glucagon, respectively. (E,F) CK-19 co-localisation with insulin in both pancreatic islet (E) and ductal (F) cells was also assessed. Values are mean ± SEM for n=6 mice. *P<0.05 and ***P<0.001 compared to control mice. $^{\Delta}$P<0.05 compared to HC saline control.

Figure 5. Effects of Ac3IV on pancreatic islet cell lineage in HC treated *Ins1$^{Cre/+}$;Rosa26-eYFP* mice. Parameters were assessed following 12 days, twice-daily treatment with saline (0.9% NaCl) vehicle or Ac3IV (25 nmol/kg bw, i.p) in HC treated insulin-resistant *Ins1$^{Cre/+}$;Rosa26-eYFP* transgenic mice. Representative images (40X) of pancreatic islets depicting co-localisation of GFP (green) with either insulin or glucagon (red) are shown in panels A&B. Numbers of insulin^{+ve}, GFP^{-ve} (C), insulin^{-ve}, GFP^{+ve} (D) and glucagon^{+ve}, GFP^{+ve} (E) islet cells were assessed utilising the cell counting function in Cell^{F} imaging software. Values are mean ± SEM for n=6 mice. *P<0.05 and ***P<0.001 compared to control mice. $^{\Delta}$P<0.05 and $^{\Delta\Delta\Delta}$P<0.01 compared to HC saline control.

Fig 1

Figure 1

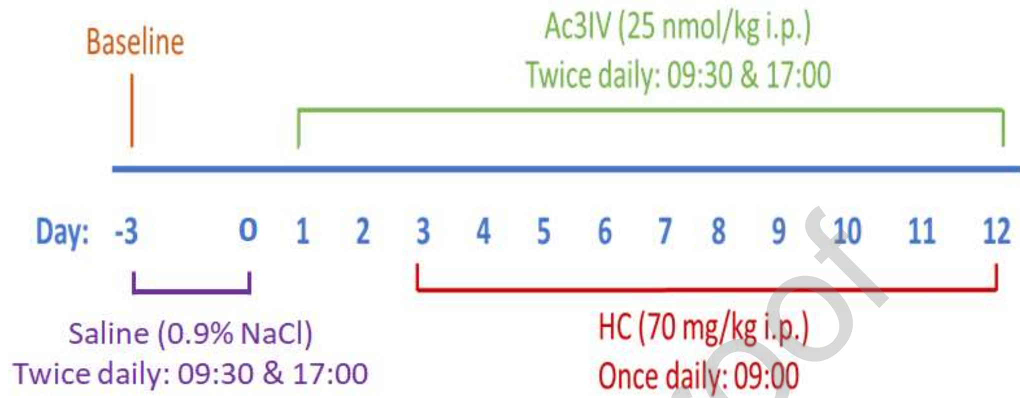

Figure 2

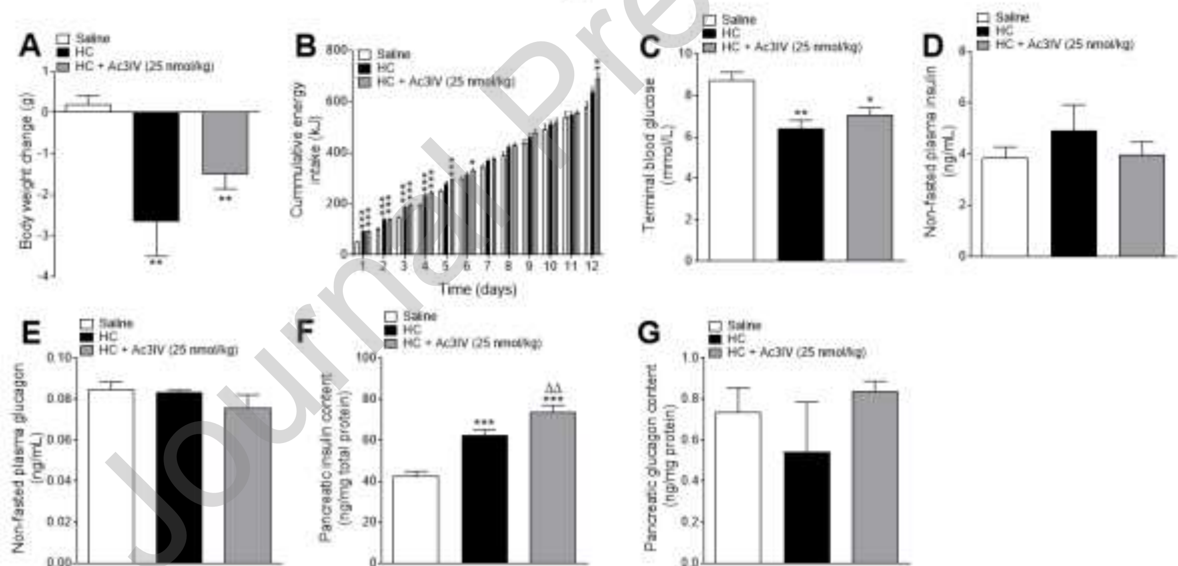

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Figure 3

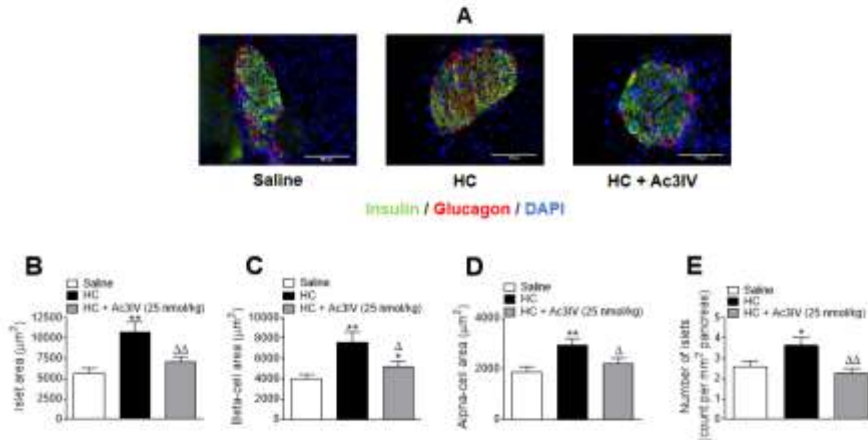


Figure 4

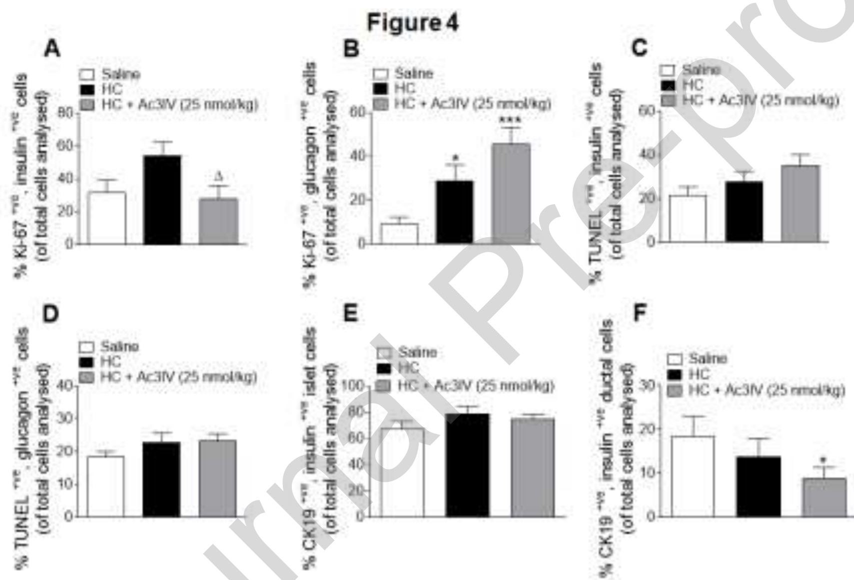
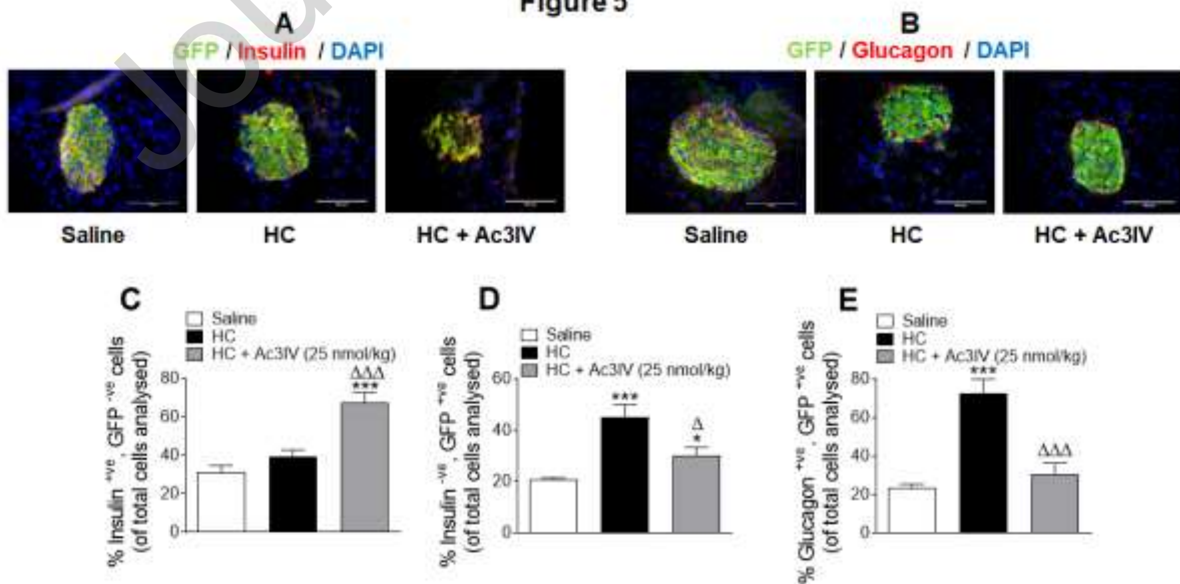


Figure 5



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CRediT authorship contribution statement

Shruti Mohan Methodology, Validation, Data Curation, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization. **Ryan Lafferty:** Methodology, Validation, Data Curation, Formal analysis, Investigation, Writing - Review & Editing. **Peter Flatt:** Conceptualization, Methodology, Validation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. **R. Charlotte Moffett**: Validation, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. **Nigel Irwin:** Conceptualization, Methodology, Validation, Writing - Original Draft, Writing - Review & Editing.

Highlights

- Ac3IV is a V1a and V1b receptor selective vasopressin analogue and has been shown to improve metabolism
- In this study, Ac3IV exerted positive islet structure effects in insulin resistant hydrocortisone (HC) mice
- Islet benefits of Ac3IV were linked to islet cell transition events that retained beta-cell identity
- Ac3IV also promoted positive effects on islet cell turnover in HC mice

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