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Evaluation of Metabolism-Associated Proteins in Abdominal Aortic Aneurysm

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Keywords
Abdominal aortic aneurysm · Irisin · Ghrelin · Follistatin · Activin A

Abstract
Introduction: Abdominal aortic aneurysm (AAA) development is inversely associated with diabetes mellitus. Targeting glucose metabolism seems to be a beneficial strategy for preventing AAA growth. Several metabolism-related factors are associated with AAA development. This study aimed to analyse the expression of the so far unstudied proteins irisin, follistatin, activin A, and ghrelin (ligand and receptor) in human and murine aneurysmal tissue, to assess the involvement of these pathways in AAA. ***Methods:*** Gene and protein expression was evaluated in aneurysmal and control tissue samples, by qPCR and immunohistochemistry. Vascular smooth muscle cells (VSMCs) were studied in vitro for expression. Circulating levels of the proteins of interest in human plasma samples were evaluated by ELISA. ***Results:*** In human aneurysmal tissue, the proteins of interest were predominantly expressed by VSMCs in neovessels. Expression by human VSMCs was confirmed in vitro. In human plasma, the concentration of irisin was higher in AAA (+/− diabetes) compared to controls. Patients with AAA and type 2 diabetes treated with metformin had lower levels of follistatin and ghrelin. ***Conclusion:*** This study demonstrates irisin, follistatin, and ghrelin as interesting proteins to be studied in the context of the observed negative association between AAA development and type 2 diabetes.

Introduction

The development of an abdominal aortic aneurysm (AAA) is characterized by the weakening of the aortic wall, caused by inflammation and degeneration of connective tissue proteins and smooth muscle cell (SMC) loss, followed by irreversible vessel widening and risk for rupture [1]. Interestingly, patients with type 2 diabetes mellitus (DM2) exhibit a lower risk of developing AAA than non-diabetic individuals [2]. Data from multiple observational studies suggest that this may be partly explained by the antidiabetic drug metformin (Met) [3]. The relationship between AAA, DM2, and Met highlights the relevance of dysregulated metabolic pathways in disease development. We and others have shown that there is a dysfunction in vascular metabolism in AAA and that treatment with drugs interfering with glucose metabolism results in a lower incidence and slower growth of experimental AAA, which is, in part,

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dependent on effects in the perivascular adipose tissue (PVAT) [4–6]. However, the precise mechanisms underlying the relationship between metabolic alterations and AAA remain elusive. Studies have reported that adipokines responsible for insulin-sensitizing and anti-inflammatory effects, such as adiponectin, leptin, and resistin, are associated with AAA [7]. Furthermore, elevated levels of adiponectin prevent AAA development in experimental models [8]. The effect of Met on inflammation and vessel wall degradation is likely mediated through alteration of metabolic peptides involved in glucose metabolism. Currently, important metabolism-related proteins have not yet been studied in the context of AAA, despite their association with vascular function and DM2.

In this paper, we study four of these proteins: irisin, follistatin, activin A, and ghrelin. Irisin is a glycoprotein cleaved from the transmembrane protein fibronectin type III domain-containing protein 5 (FNDC5), whose expression is regulated by PGC1-α [9, 10]. Its role in maintaining SMC phenotype [11] and its reduced blood concentration in DM2 patients [12] make it an interesting target to study in AAA. Follistatin is a glycoprotein expressed ubiquitously, involved in cellular signal transmission [13]. It is present in areas of abnormal SMC proliferation [14] and is associated with an increased risk of DM2 [15], which make it a relevant target to explore in the context of metabolic alterations in AAA. Many effects of follistatin are promoted by binding with high affinity to activin A, a proinflammatory cytokine of the TGF-β superfamily [14, 16]. Activin A is a dimer of β-subunits of inhibin A. It regulates glucose metabolism, promotes the proliferation of vascular SMCs (VSMCs), and inhibits the proliferation of endothelial cells, effects which are neutralized by follistatin [17, 18]. Ghrelin is a small peptide hormone produced mainly by the stomach and duodenum, but also by the vasculature in lower concentrations [19]. It prevents vascular remodelling in pulmonary vasculature [20] and is associated with DM2, with lower circulating levels present in the DM2 patients, compared with the non-diabetic population [21]. Ghrelin exerts its effects through the ghrelin receptor, also known as the growth hormone secretagogue receptor 1a, whose expression has been detected in the vasculature [22]. This study aimed to identify and quantify the expression of irisin, follistatin, activin A, and ghrelin ligand and its receptor, metabolism-related proteins associated with vascular function, and glucose metabolism, in aorta tissues and plasma from human and murine AAA and respective controls.

Methods

Human Material

Aortic samples from non-aneurysmal organ donors without clinical or macroscopic signs of aortic atherosclerosis or aneurysms and from AAA patients were collected at Linköping University Hospital, Sweden, between January 1, 2012, and December 31, 2016. Inclusion criteria for AAA were an abdominal aortic diameter superior to 30 mm. Biopsies were fixed in 4% formaldehyde for immunohistochemical analysis or placed in RNAlater (Ambion, Austin, TX, USA) overnight at 4°C and then stored at −80°C for RNA isolation, as previously described [23]. Patient samples used for qPCR included cDNA isolated from nine non-aneurysmal aortas (seven males and two females, age 45 ± 13.4 years); eight AAA with intima, media, and adventitia layers (referred to as whole AAA, eight males, 72.9 ± 5.7 years); seven AAA with layers including intima and media (six males, one female, 70.7 ± 5.2 years); ten AAA with layer from adventitia (six males, three females, one unknown gender, 70.4 ± 5.2 years); and eight AAA samples from PVAT layers (eight males, 70.8 ± 5.1). Media was separated from adventitia under microscope using forceps. Periaortic vascular adipose tissue was collected from the outside of the AAA in similar manner. Samples for different layers of AAA were obtained from non-matched patients. Gene expression was also analysed in isolated layers of the aorta (intima/media, adventitia, and PVAT) where tissue was available.

EDTA plasma was collected from a group of control individuals without AAA (20 males, 65 ± 0.0 years old), non-diabetic patients with AAA (20 males, age 72.6 ± 7.5 years), patients with AAA and DM2 (32 males and 13 females, age 74.5 ± 7.4 years), and patients with AAA and DM2 treated with Met (53 males and 17 females, age 70.7 ± 6.0 years), from a screening program at Uppsala University Hospital, Sweden, between January 1, 2009, and December 31, 2019. Baseline parameters are described in Table 1.

All AAA participants gave written informed consent to the study, which was approved by the regional ethical review board in Linköping (Regionala etikprövningsnämnden i Linköping, Approval No. M123-07) and Uppsala (Regionala etikprövningsnämnden i Uppsala, Approval No. 2007/052), Sweden. The study protocol conforms to the ethical guidelines of the 2013 Declaration of Helsinki.

Murine Material

AAA was induced in eight to twelve-week-old male apolipoprotein E deficient mice (Taconic) on normal chow, using 1 μg/min/kg angiotensin II (Cat. No. A9525, Sigma-Aldrich, USA) secreted from a subcutaneous

Table 1. Baseline parameters for patients with AAA and controls

Clinical characteristics	Control (*n* = 20)	AAA (*n* = 21)	AAA + DM (*n* = 43)	AAA + DM + Met (*n* = 59)
Age, years	65±0	72.8±7.4	74.3±7.3	70.5±5.6
Female, *n*	0	0	13 (30%)	11 (19%)
BMI, kg/m^2	28.1±7.9 (*n* = 19)	27.1±2.5 (*n* = 11)	27.8±5.5 (*n* = 28)	29.3±4.9 (*n* = 48)
Never a smoker, *n*	9 (45%)	3 (14%)	7 (16%)	5 (8%)
Former smoker, *n*	11 (55%)	14 (67%)	26 (60%)	33 (56%)
Active smoker, *n*		4 (19%)	8 (19%)	21 (36%)
Statins, *n*	6 (30%)	8 (38%)	19 (44%) Unknown (3/43)	43 (73%) Unknown (1/59)
Aortic diameter (SD), mm	18.2±2.0 (*n* = 20)	47.8±11.6 (*n* = 21)	43.2±12.5 (*n* = 42)	38.5 10.3 (*n* = 54)

AAA, abdominal aortic aneurysm; DM, diabetes mellitus; Met, metformin.

implanted osmotic mini-pump (Model 1004, Alzet, USA) for 28 days, or saline for controls, as previously described [6]. Mice were anesthetized using 2% isoflurane and received buprenorphine (0.05 mg/kg) post-operation twice a day for 48 h. To reduce the number of animals used for experimental purposes, tissue from mice induced for other projects was reused in this project.

Aneurysm development was confirmed by ultrasound. At the end of the study, suprarenal aorta was collected and divided into two parts, one for histology analysis and one for qPCR. Mice were euthanized by cervical dislocation, under anaesthesia. Ruptured aortas were not included for analysis. Experiments were approved by the local Ethical Committees in Linköping (Linköpings djurförsöksetiska nämnd, Approval No. 973) and Uppsala (Uppsala djurförsöksetiska nämnd, Approval No. 5.8.18-09456), Sweden.

Immunohistochemistry

Aortic samples from 4 AAA patients and two non-aneurysmal aortas were cut into 4-µm sections and deparaffinized in xylene, followed by decreasing concentrations of ethanol. Samples were boiled in 20 min in 5% Diva Decloaker (Biocare Medical, USA) for antigen retrieval and then blocked for endogenous peroxidase in 3% hydrogen peroxide for 5 min. Normal horse serum (20%) was used for blocking. Sections were incubated with primary antibodies against FNDC5 (diluted 1:200, rabbit polyclonal, #23995-I-AP, Proteintech, UK); follistatin (diluted 1:300, rabbit polyclonal, #PA5-114319, Thermo Fisher Scientific, USA); activin A (diluted 1:50, goat polyclonal, #PA5-47004, Thermo Fisher Scientific, USA); ghrelin (diluted 1:150, mouse monoclonal, #H00051738-M01, Novus Biologicals, UK); ghrelin receptor (diluted 1:150, rabbit polyclonal, #NBP2-16655, Novus Biologicals, UK); and actin α-smooth muscle (diluted 1:5,000, mouse monoclonal, #A5228, Sigma-Aldrich, USA).

Given that irisin is the soluble form of the transmembrane protein FNDC5, protein expression in tissue was analysed by targeting the full-length transmembrane protein FNDC5. Negative controls were performed for all antibodies by omitting the primary antibody. Detection was performed with HRP-conjugated anti-rabbit or anti-mouse secondary antibodies (diluted 1:1,500, Vector Laboratories, USA).

Cell Culture

Human aortic smooth muscle cells (#CC-2571, Lonza Group Ltd, Switzerland) were grown in recommended supplemented media (#CC-3182, Lonza Group Ltd, Switzerland) and stimulated with 20 ng/mL IFN-γ for 4, 24, and 48 h, according to previous studies. Each condition and its respective control were run in triplicate.

RT-qPCR

Aortic tissue was homogenized, and RNA was extracted with RNeasy columns using a standard protocol from the manufacturer (Cat. No. 74104, Qiagen, Germany). RNA was reverse transcribed using cDNA synthesis III kit (Cat. No 11752250, Invitrogen, USA). Semi-quantitative PCR was performed using TaqMan Universal PCR Master Mix (Cat. No 4304437, Thermo Fisher Scientific, USA). The following human probes were used: FNDC5 Hs00401006_m1; follistatin Hs00246256_m1; inhibin A Hs01081598_m1; ghrelin Hs01074053_m1; and ghrelin receptor Hs01026313_m1. The following mouse probes were used: FNDC5 Mm01181543_m1; follistatin

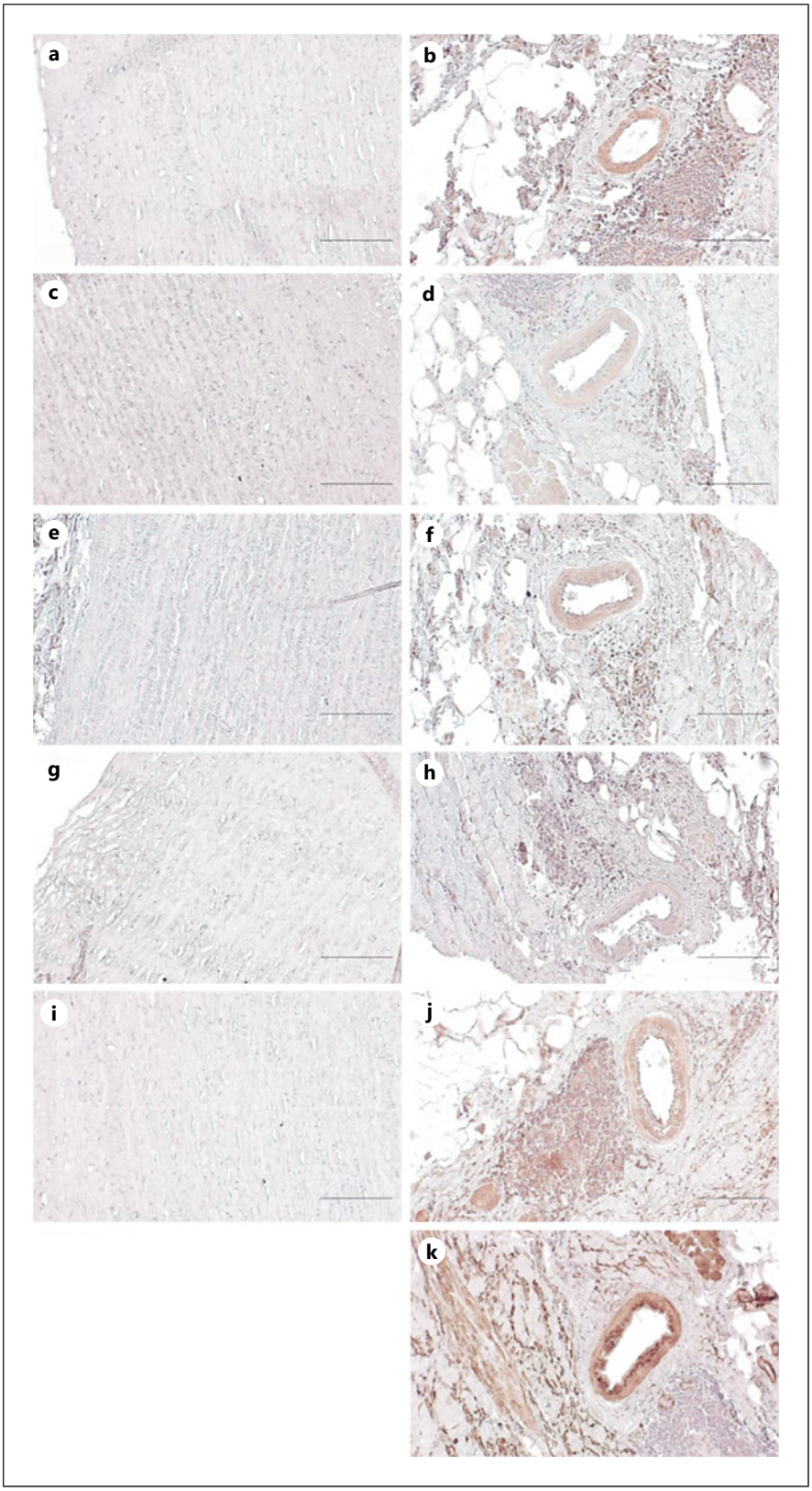


Fig. 1. Representative immunohistochemical detection of FNDC5 (**a**, **b**), follistatin (**c**, **d**), activin A (**e**, **f**), ghrelin (**g**, **h**), ghrelin receptor (**i**, **j**), and α-actin (**k**) in non-aneurysmal control aorta (**a**, **c**, **e**, **g**, **i**) and AAA (**b**, **d**, **f**, **h**, **j**, **k**), at ×200 magnification. Scale bar corresponds to 10 μm.

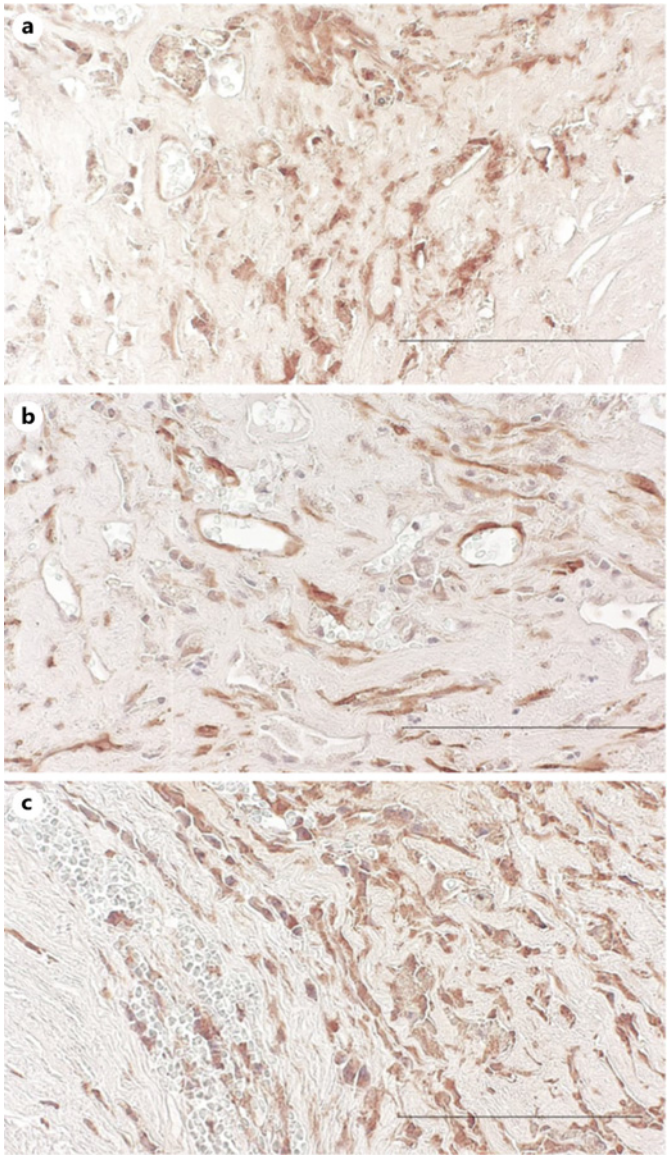


Fig. 2. Representative immunohistochemical detection of activin A (**a**), ghrelin receptor (**b**), and α-actin (**c**) in AAA, at ×400 magnification. Scale bar corresponds to 10 μm.

Mm00514982_m1; and inhibin A Mm00434339_m1. TATA-box binding protein (TBP, Mm00446973 for mice and Hs00427620 for human) was used as a housekeeping gene for normalization of the results.

FNDC5 expression was analysed in tissues from a different cohort of mice than follistatin and inhibin A. At gene expression level, FNDC5 was analysed, instead of irisin and inhibin A instead of activin A since FNDC5 is the gene coding for the full-length transmembrane protein from which irisin is cleaved and inhibin A is the gene coding for the two subunits that form activin A.

ELISA

Commercially available ELISA kits were used to analyse circulating levels of irisin (#EKX-99X4SI-96, Nordic Biosite, Sweden), follistatin (Cat. No. #EHFST, Thermo Fisher Scientific, USA), activin A (Cat. No #EHACTIVINA, Thermo Fisher Scientific, USA), and ghrelin ligand (Cat. No #BMS2192, Thermo Fisher Scientific, USA) according to the manufacturer's standard protocol.

Statistical Analysis

The statistical analyses were performed using GraphPad Prism 10 (GraphPad Software, USA). Data are reported as mean ± standard error of the mean. Differences between the groups were analysed by Mann-Whitney U test and Kruskal-Wallis test, followed by Dunn's multiple comparisons test. p values <0.05 were considered statistically significant.

Results

Protein Expression and Localization in Aortic Tissue

To determine if FNDC5, follistatin, activin A, and ghrelin ligand and receptor were expressed in the aorta, immunohistochemical analysis was performed on sections from non-aneurysmal controls and AAA samples. The proteins in the study show a similar pattern of expression. While no signal was detected in the control aortas, there was expression of all factors in SMC of supporting vessels and adjacent areas of mononuclear cell infiltrates in the AAA samples (shown in Fig. 1). Noteworthy, activin A and ghrelin receptor expression were also identified in SMC of the media layer (shown in Fig. 2). SMCs were identified by the expression of α-actin and their appearance.

RNA Expression in Human and Mouse

Analysis of mRNA expression in whole tissue from non-aneurysmal and aneurysmal aortas allowed to detect the expression of FNDC5, follistatin, inhibin A, and ghrelin. Expression of ghrelin receptor was not detected in either group. While no significant differences were found in mRNA expression between non-aneurysmal controls and AAA samples, some differences were detected between the different layers of the aorta. FNDC5 was more expressed in the PVAT than in the intima/media (shown in Fig. 3a). Follistatin (shown in Fig. 3b) and inhibin A (shown in Fig. 3c), on the contrary, had lower expression in the PVAT, compared to the intima/media.

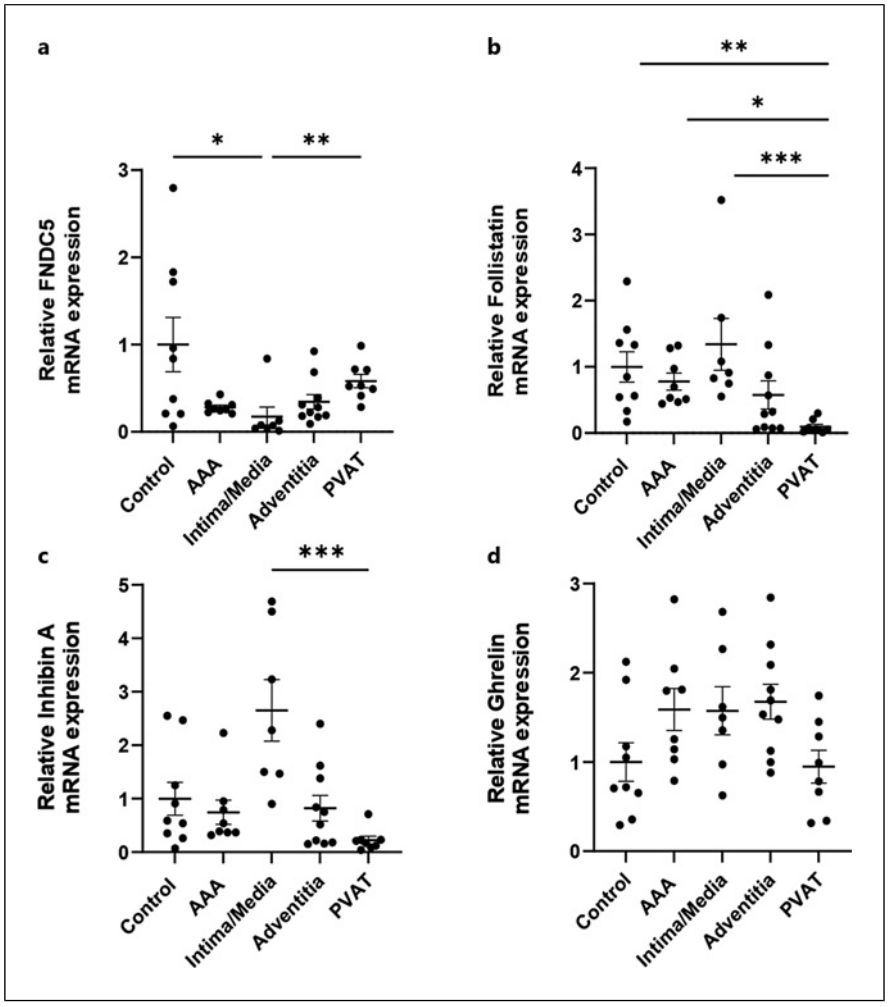


Fig. 3. Relative human mRNA expression. FNDC5 (**a**), follistatin (**b**), inhibin A (**c**), and ghrelin (**d**) in non-aneurysmal aorta (control, $n = 9$), AAA whole tissue (AAA, $n = 9$), intima/media layer of AAA ($n = 7$), adventitia layer of AAA ($n = 10$), and PVAT ($n = 8$) layer of AAA. Data are presented as mean ± SEM. Gene expression normalized to TPB. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. SEM, standard error of the mean; TPB, TATA-box binding protein.

Analysis of mRNA expression in the AngII mouse model of AAA was performed to confirm the data observed in human tissue. Expression of FNDC5 (shown in Fig. 4a) and inhibin A (shown in Fig. 4c) did not differ between controls and AAA samples, similar as observed in human samples. Follistatin (shown in Fig. 4b), however, was induced 2.5-fold in AngII-treated mice.

Expression in Cultured VSMC

To confirm expression in VSMCs and if the expression is inducible, these cells were cultured and stimulated with IFN-γ (i.e., found in elevated levels in AAA). Baseline mRNA expression of FNDC5, follistatin, and inhibin A was detected in unstimulated cells (control). No difference in FNDC5 expression was detected (shown in Fig. 4d). After 24 h, IFN-γ induced a 4.6-fold increase in follistatin mRNA expression (shown in Fig. 4e). At 48 h, IFN-γ induced a 3.5-fold decrease in inhibin A (shown in Fig. 4f). Ghrelin mRNA expression was not detected in the unstimulated nor the stimulated cultured VSMCs.

Protein Expression in Plasma

Met treatment is associated with reduced aneurysm growth in diabetes mellitus (DM) patients with AAA, and it has been shown to affect the plasma concentration of the proteins in study. Expression of irisin, follistatin, activin A, and ghrelin was measured in samples from non-aneurysmal patients (control), AAA patients without diabetes (AAA), AAA patients with diabetes (AAA + DM), and AAA patients with diabetes and Met treatment (AAA + DM + Met). Circulating levels of irisin were measured in two different cohorts of samples. In cohort 1 (shown in Fig. 5a), significantly higher levels of irisin were detected in the AAA (101.1 ± 30.83 ng/mL, $n = 20$) and AAA + DM groups (115.4 ± 30.61 ng/mL, $n = 10$),

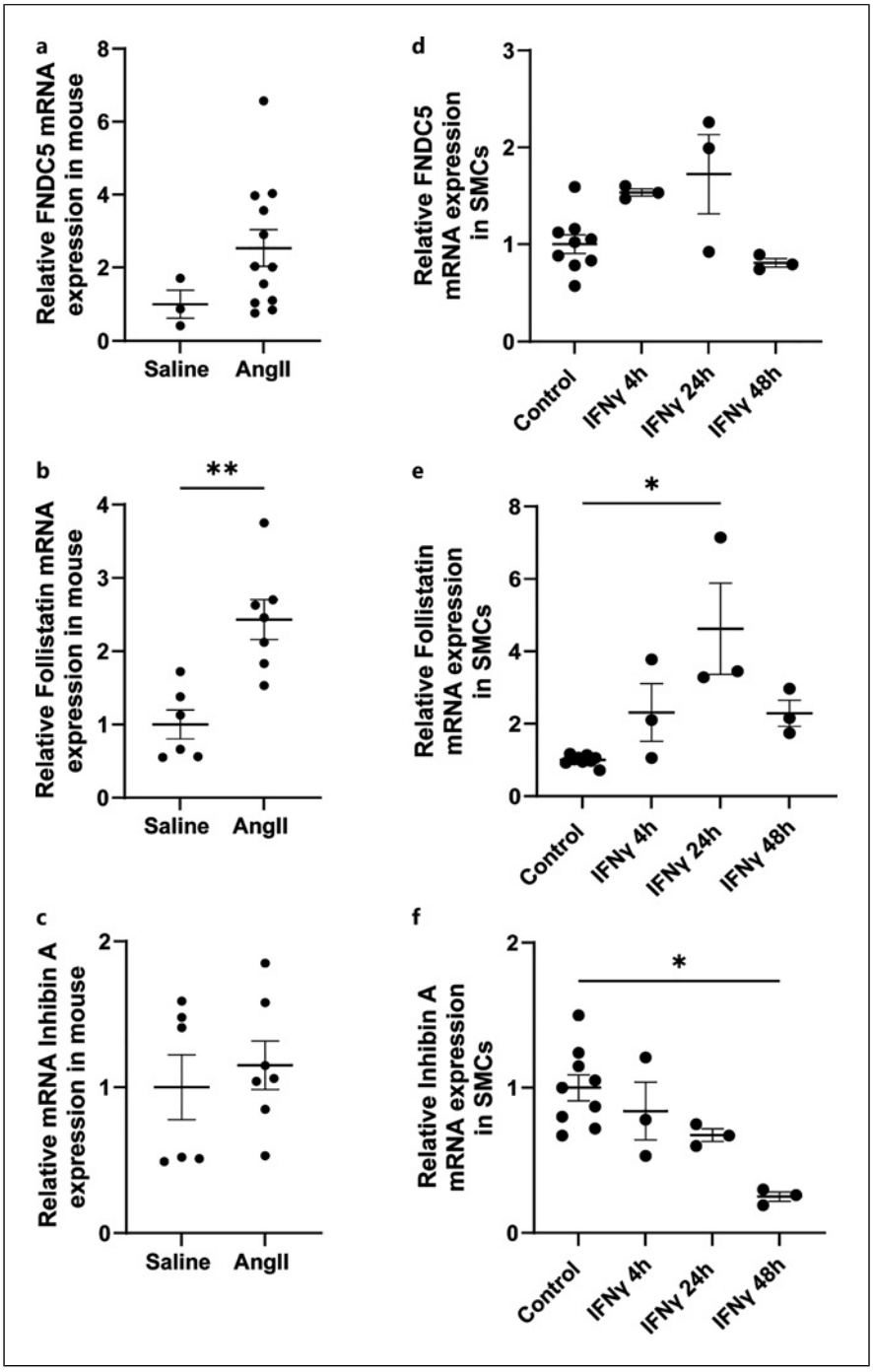


Fig. 4. Relative mouse mRNA expression. FNDC5 (**a**), follistatin (**b**), and inhibin A (**c**) in non-aneurysmal aorta (saline, n = 3–6) and AAAs (AngII) from mice induced for AAA using AngII (n = 7–12). Relative mRNA expression of FNDC5 (**d**), follistatin (**e**), and inhibin A (**f**) in cultured AoSMCs stimulated with IFN-γ (n = 3/time point) or vehicle as control (n = 9). Data are presented as mean ± SEM. Gene expression normalized to TPB. *p < 0.05, **p < 0.01. AoSMCs, aortic smooth muscle cells; SEM, standard error of the mean; TPB, TATA-box binding protein.

compared to the control group (74.71 ± 10.38 ng/mL, n = 18). In cohort 2 (shown in Fig. 5b), although expression of irisin in the AAA + DM + Met group (61.89 ± 21.90 ng/mL, n = 33) tends to be lower compared to the AAA + DM group (86.60 ± 49.54 ng/mL, n = 28), this difference did not achieve statistical significance (p = 0.072). Circulating levels of follistatin (shown in Fig. 5c) were found to be significantly lower in the AAA + DM + Met group (14 ± 4.5 ng/mL, n = 17), compared to the control (26 ± 19.0 ng/mL, n = 18) and AAA + DM group (31 ± 19.8 ng/mL, n = 20). Circulating concentration of activin A (shown in Fig. 5d) did not differ between groups. Circulating levels of ghrelin (Fig. 5e) were significantly lower in the AAA + DM + Met group (1,103 ± 584.0 pg/mL,

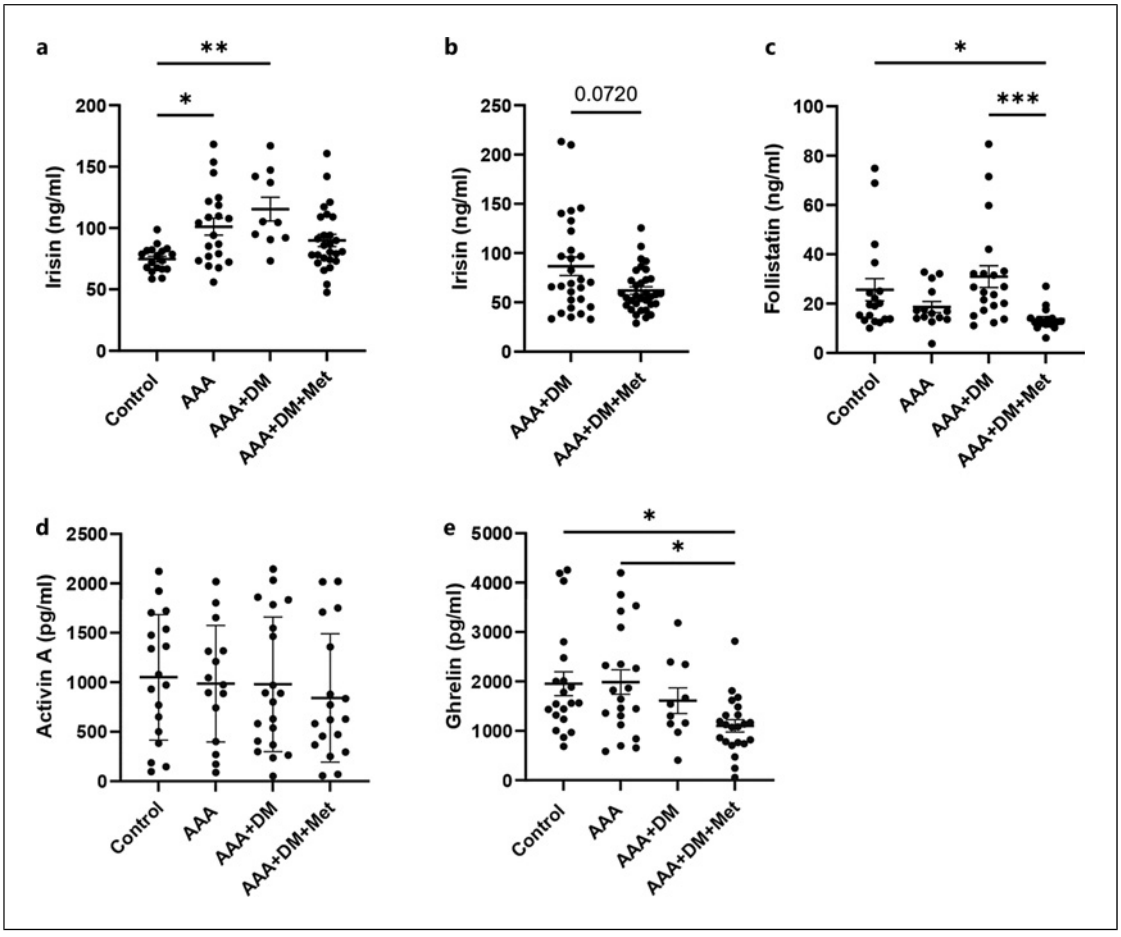


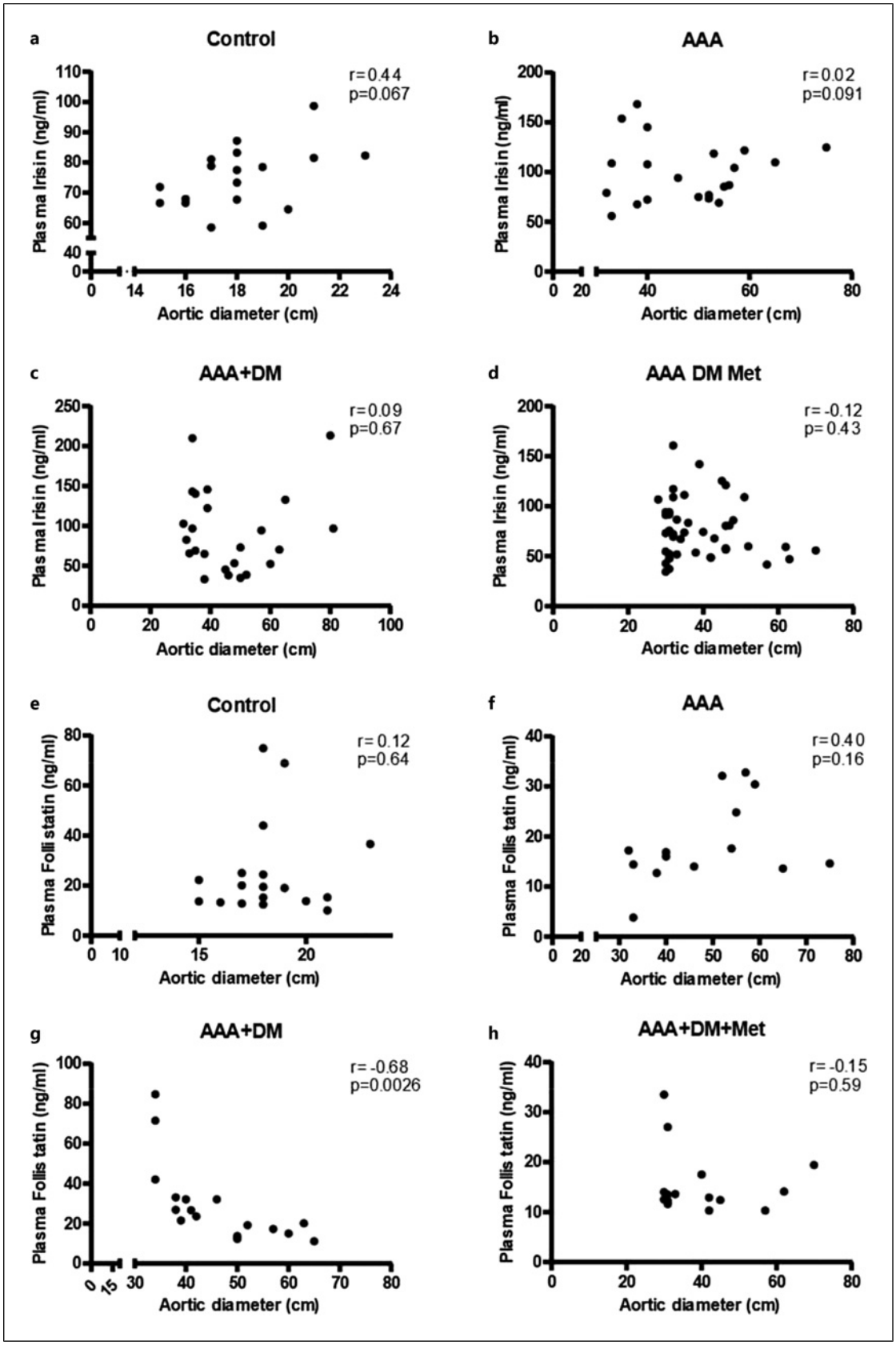
Fig. 5. Circulating levels of proteins of interest. Irisin (**a**, **b**), follistatin (**c**), activin A (**d**), and ghrelin (**e**) in non-aneurysmal individuals (control, n = 18–20), patients with AAA (n = 14–20), patients with AAA + DM (n = 10–28), and patients with AAA + DM+ Met (n = 17–33), as analysed by commercially available ELISA kits. Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. SEM, standard error of the mean.

n = 22), compared to the control (1,954 ± 1,082 pg/mL, n = 20) and AAA group (1,987 ± 1,101 pg/mL, n = 20). There was not any association between plasma protein concentration and aortic diameter (shown in Fig. 6).

Discussion

The importance of metabolism in AAA development is evidenced by the negative relationship between AAA and DM2 and Met [3]. In fact, Met is the most promising drug for AAA pharmacological treatment, currently undergoing clinical trials [24]. Nonetheless, the mechanisms behind DM2 and Met's protective effect against AAA are not completely elucidated. Among several factors, we have identified four potentially involved metabolisms-related proteins, namely, irisin, follistatin, activin A, and ghrelin, for further evaluation. This study aimed to be a first description of gene and protein expression at tissue and plasma levels of the selected factors in the intersection of AAA and DM2. We have shown that (1) irisin, follistatin, activin A, and ghrelin are expressed in aneurysmal tissue, by SMCs of neovessels, and, to some extent, in areas of mononuclear cell infiltrates; (2) gene expression of these proteins is, in general, not altered in human and experimental AAA; and (3) circulating levels of follistatin, ghrelin, and potentially irisin are lower in patients with AAA and DM2 treated with Met.

The exact pathological mechanisms of AAA development and the role of metabolism are not fully elucidated. Protein expression of irisin, follistatin, activin A,



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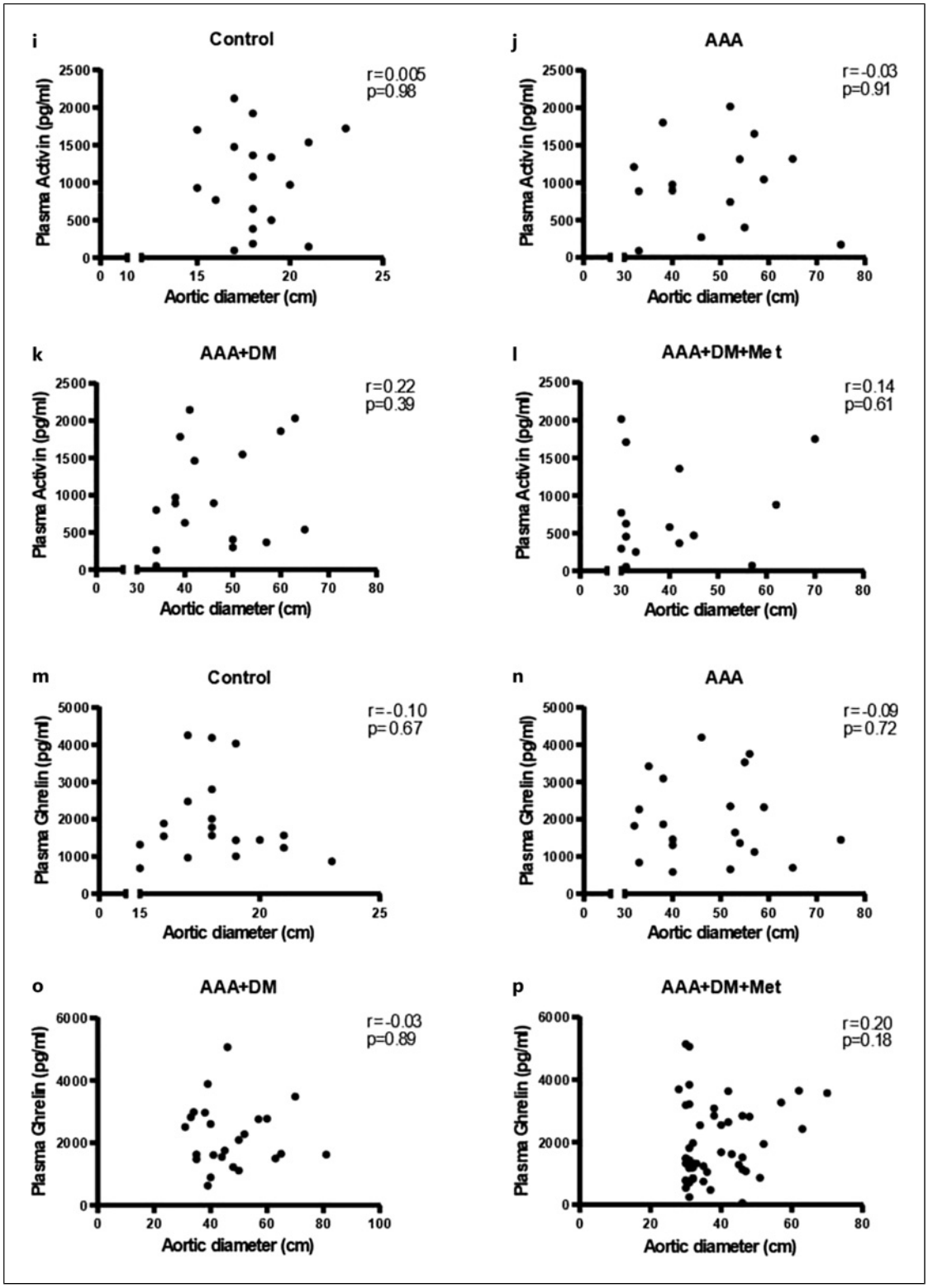

Fig. 6. Correlation analysis between aortic diameter and plasma concentration of the proteins of interest. **a–d** Irisin. **e–h** Follistatin. **i–l** Activin A. **m–p** Ghrelin.

and ghrelin (ligand and receptor) was detected in SMC and in areas of mononuclear cell infiltrates, most likely leukocytes, in aneurysmal tissue, but not in healthy aortas. This indicates a potential association of irisin, follistatin, activin A, and ghrelin in AAA development. Leukocytes can potentially interfere in the expression of these proteins by SMCs. More specific stainings are needed to clarify which mononuclear cells constitute these cells. Also, α-actin, used to localize the VSMC, can also be expressed by endothelial cells. We cannot exclude that also endothelial cells could express these factors. In vitro, we observed that stimulation of aortic smooth muscle cell with IFN-γ (a cytokine produced by several leukocytes, including T cells) resulted in an increased expression of follistatin after 24 h and decreased expression of activin after 48 h. To fully understand the mechanisms in regulation and expression of these proteins, other more detailed studies are needed.

Interestingly, when the expression of these proteins is analysed at gene expression level, mRNA expression can be detected in both controls and aneurysmal samples. At circulating protein level, these proteins were identified in plasma from patients with aneurysms and controls, with varying levels between patients versus controls, partly depending on Met treatment. The discrepancy between gene expression, tissue, and plasma expression could indicate that these factors are regulated differently at translational, transcriptional, and secretion levels.

The differences found in circulating plasma levels are an interesting finding. Despite observing that irisin, follistatin, and ghrelin are lower in diabetic patients with AAA treated with Met, we could not find any correlation between any of these factors and aortic diameter. This should be taken with caution, however, since the number of participants in each group was low, which is a general limitation of the study. Sex as biological variable was not able to be addressed due to limited number of females. It is intriguing to hypothesize that these factors can be mediators in the mechanism of action of Met. Nevertheless, the differences observed can also be a consequence of the Met treatment. Lower circulating levels could potentially reflect a lower number of supporting neovessels, responsible for releasing these factors. As shown in Table 1, the baseline parameters for patients with AAA +/− Met and controls differed in some aspects, including statin treatment. As this analysis is based on clinical cohorts where patients and controls are likely to receive different treatments based on, e.g., comorbidities, these baseline differences are to be expected. Future analysis of circulating protein expression within randomized trials where subjects with AAA are randomized to Met treatment versus no Met will be of interest to assess, as the randomization can abolish the risk for biased differences between groups.

An additional limitation of the current study is the discrepancy between results in murine and human AAA, which could partly depend on that animal models do not always mimic the human disease completely. For instance, the AngII model results in suprarenal aneurysms contrary to infrarenal in humans. In our previous comparisons between the porcine elastase and AngII models in mice and human aneurysms [5], we conclude that the AngII model better resembles the chronic phase of aneurysm development and the elastase model the acute phase, when it comes to metabolism-related pathways. Unfortunately, all mice aortas available were used for RNA analysis and therefore we were not able to confirm human histology results in the mice. As the sample numbers are low, the results should be interpreted with caution and be regarded as descriptive and hypothesis-generating.

In conclusion, our results show that irisin, follistatin, activin A, and ghrelin are expressed by neovessels in the aneurysmal aortic wall and that patients with AAA and DM2 treated with Met have lower circulating levels of these proteins. Further functional studies in mice are warranted to better understand the role of these proteins in the pathogenesis of AAA.

Acknowledgment

We thank Toste Länne for help with collection of samples.

Statement of Ethics

All AAA participants gave written informed consent to participate in the study, which was approved by the regional ethical review board in Linköping (Regionala etikprövningsnämnden i Linköping, Approval No. M123-07) and Uppsala (Regionala etikprövningsnämnden i Uppsala, Approval No. 2007/052), Sweden. The study protocol conforms to the ethical guidelines of the 2013 Declaration of Helsinki. Animal experiments were approved by the local Ethical Committees in Linköping (Linköpings djurförsöksetiska nämnd, Approval No. 973) and Uppsala (Uppsala djurförsöksetiska nämnd, Approval No. 5.8.18-09456), Sweden.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

D.W., A.B.R., A.W., and K.M. designed the study. D.W. and A.B.R. prepared the main manuscript, and all co-authors were involved in finalizing the manuscript. A.B.R., N.C., E.T., J.J., and D.W. performed laboratory work and statistical analysis. A.W. and K.M. provided clinical insights and took responsibility for patient data and follow-up. All authors were involved in the interpretation of data and read and approved the final manuscript.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author Dick Wågsäter (email: Dick.Wagsater@mcb.uu.se).

References

1 Sakalihasan N, Michel JB, Katsargyris A, Kuivaniemi H, Defraigne JO, Nchimi A, et al. Abdominal aortic aneurysms. Nat Rev Dis Primer. 2018;4(1):34. https://doi.org/10.1038/s41572-018-0030-7
2 Xiong J, Wu Z, Chen C, Wei Y, Guo W. Association between diabetes and prevalence and growth rate of abdominal aortic aneurysms: a meta-analysis. Int J Cardiol. 2016;221:484–95. https://doi.org/10.1016/j.ijcard.2016.07.016
3 Unosson J, Wågsäter D, Bjarnegård N, De Basso R, Welander M, Mani K, et al. Metformin prescription associated with reduced abdominal aortic aneurysm growth rate and reduced chemokine expression in a Swedish cohort. Ann Vasc Surg. 2021;70:425–33. https://doi.org/10.1016/j.avsg.2020.06.039
4 Gabrielson M, Vorkapic E, Folkesson M, Welander M, Matussek A, Dimberg J, et al. Altered PPARγ coactivator-1 alpha expression in abdominal aortic aneurysm: possible effects on mitochondrial biogenesis. J Vasc Res. 2016;53(1–2):17–26. https://doi.org/10.1159/000446653
5 Gäbel G, Northoff BH, Balboa A, Becirovic-Agic M, Petri M, Busch A, et al. Parallel murine and human aortic wall genomics reveals metabolic reprogramming as key driver of abdominal aortic aneurysm progression. J Am Heart Assoc. 2021;10(17):e020231. https://doi.org/10.1161/JAHA.120.020231
6 Kunath A, Unosson J, Friederich-Persson M, Bjarnegård N, Becirovic-Agic M, Björck M, et al. Inhibition of angiotensin-induced aortic aneurysm by metformin in apolipoprotein E–deficient mice. JVS Vasc Sci. 2021;2:33–42. https://doi.org/10.1016/j.jvssci.2020.11.031
7 Thanigaimani S, Golledge J. Role of adipokines and perivascular adipose tissue in abdominal aortic aneurysm: a systematic review and meta-analysis of animal and human observational studies. Front Endocrinol. 2021;12:618434. https://doi.org/10.3389/fendo.2021.618434
8 Wågsäter D, Vorkapic E, Van Stijn CMW, Kim J, Lusis AJ, Eriksson P, et al. Elevated adiponectin levels suppress perivascular and aortic inflammation and prevent AngII-induced advanced abdominal aortic aneurysms. Sci Rep. 2016;6(1):31414. https://doi.org/10.1038/srep31414
9 Fu J, Li F, Tang Y, Cai L, Zeng C, Yang Y, et al. The emerging role of irisin in cardiovascular diseases. J Am Heart Assoc. 2021;10(20):e022453. https://doi.org/10.1161/JAHA.121.022453
10 Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012;481(7382):463–8. https://doi.org/10.1038/nature10777
11 Song H, Xu J, Lv N, Zhang Y, Wu F, Li H, et al. Irisin reverses platelet derived growth factor-BB-induced vascular smooth muscle cells phenotype modulation through STAT3 signaling pathway. Biochem Biophys Res Commun. 2016;479(2):139–45. https://doi.org/10.1016/j.bbrc.2016.07.052
12 Song R, Zhao X, ZhangWang DR, Feng Y, Feng Y. Lower levels of irisin in patients with type 2 diabetes mellitus: a meta-analysis. Diabetes Res Clin Pract. 2021;175:108788. https://doi.org/10.1016/j.diabres.2021.108788
13 Parfenova OK, Kukes VG, Grishin DV. Follistatin-Like proteins: structure, functions and biomedical importance. Biomedicines. 2021;9(8):999. https://doi.org/10.3390/biomedicines9080999
14 Inoue S, Orimo A, Hosoi T, Matsuse T, Hashimoto M, Yamada R, et al. Expression of follistatin, an activin-binding protein, in vascular smooth muscle cells and arteriosclerotic lesions. Arterioscler Thromb. 1993;13(12):1859–64. https://doi.org/10.1161/01.atv.13.12.1859
15 Wu C, Borné Y, Gao R, López Rodriguez M, Roell WC, Wilson JM, et al. Elevated circulating follistatin associates with an increased risk of type 2 diabetes. Nat Commun. 2021;12(1):6486. https://doi.org/10.1038/s41467-021-26536-w
16 Sugino H, Sugino K, Hashimoto O, Shoji H, Nakamura T. Follistatin and its role as an activin-binding protein. J Med Investig. 1997;44(1–2):1–14.
17 Hashimoto O, Funaba M. Activin in glucose metabolism. Vitam Horm. 2011;85:217–34. https://doi.org/10.1016/B978-0-12-385961-7.00011-1
18 Namwanje M, Brown CW. Activins and inhibins: roles in development, physiology, and disease. Cold Spring Harb Perspect Biol. 2016;8(7):a021881. https://doi.org/10.1101/cshperspect.a021881
19 Pearson JT, Shirai M, Sukumaran V, Du CK, Tsuchimochi H, Sonobe T, et al. Ghrelin and vascular protection. Vasc Biol. 2019;1(1):H97–102. https://doi.org/10.1530/VB-19-0024
20 XuZhu YJJ, ChengJiang FKW, Gu WZ, ShenWu ZYD, Liang L, Du LZ. Ghrelin ameliorates hypoxia-induced pulmonary hypertension via phospho-GSK3 β/β-catenin signaling in neonatal rats. J Mol Endocrinol. 2011;47(1):33–43. https://doi.org/10.1530/JME-10-0143
21 Pöykkö SM, Kellokoski E, Hörkkö S, Kauma H, Kesäniemi YA, Ukkola O. Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes. 2003;52(10):2546–53. https://doi.org/10.2337/diabetes.52.10.2546
22 Kleinz M, Maguire J, Skepper J, Davenport A. Functional and immunocytochemical evidence for a role of ghrelin and des-octanoyl ghrelin in the regulation of vascular tone in man. Cardiovasc Res. 2006;69(1):227–35. https://doi.org/10.1016/j.cardiores.2005.09.001
23 Bengts S, Shamoun L, Kunath A, Appelgren D, Welander M, Björck M, et al. Altered IL-32 signaling in abdominal aortic aneurysm. J Vasc Res. 2020;57(4):236–44. https://doi.org/10.1159/000507667
24 Wanhainen A, Unosson J, Mani K, Gottsäter A; MAAAGI Trial Investigators; Björck M. The metformin for abdominal aortic aneurysm growth inhibition (MAAAGI) trial. Eur J Vasc Endovasc Surg. 2021;61(4):710–1. https://doi.org/10.1016/j.ejvs.2020.11.048