

Influence of thyroid state on cardiac and renal capillary density and glomerular morphology in rats

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Abstract

The purpose was to analyse the cardiac and renal capillary density and glomerular morphology resulting from a chronic excess or deficiency of thyroid hormones (THs) in rats. We performed histopathological, morphometrical and immunohistochemical analyses in hypothyroid and hyperthyroid rats to evaluate the density of mesenteric, renal and cardiac vessels at 4 weeks after induction of thyroid disorders. The main angiogenic factors in plasma, heart and kidney were measured as possible mediators of vascular changes. Mesenteric vessel branching was augmented and decreased in hyper- and hypothyroid rats respectively. The numerical density of CD31-positive capillaries was higher in left and right ventricles and in cortical and medullary kidney from both hyper- and hypothyroid rats vs controls. Numbers of podocytes and glomeruli per square millimetre were similar among groups. Glomerular area and percentage mesangium were greater in the hyperthyroid vs control or hypothyroid groups. No morphological renal lesions were observed in any group. Vascularisation of the mesenteric bed is related to TH levels, but an increased capillarity was observed in heart and kidney in both thyroid disorders. This increase may be produced by higher tissue levels of angiogenic factors in hypothyroid rats, whereas haemodynamic factors would predominate in hyperthyroid rats. Our results also indicate that the renal dysfunctions of thyroid disorders are not related to cortical or medullary microvascular rarefaction and that the proteinuria of hyperthyroidism is not secondary to a podocyte deficit. Finally, TH or its analogues may be useful to increase capillarity in renal diseases associated with microvascular rarefaction.

Key Words

- ▶ CD31
- ▶ Nestin
- ▶ Immunohistochemical
- ▶ Angiogenic Factors
- ▶ Hyperthyroidism
- ▶ Hypothyroidism

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Introduction

Thyroid hormone (TH) can induce cardiac hypertrophy and angiogenesis in several animal models (Chilian *et al.* 1985, Weiss & Grover 1987, Breisch *et al.* 1989, Tomanek *et al.* 1998), and TH and the TH analogue 3,5-diiodothyropropionic acid (DITPA) have been reported to have pro-angiogenic effects in the heart under physiological conditions (Weiss

& Grover 1987, Wang *et al.* 2003) and after myocardial infarction (Tomanek *et al.* 1998, Zheng *et al.* 2004).

The reduction in myocardial arterioles observed in hypothyroid rats was found to be prevented by thyroxine (T₄; Liu *et al.* 2008, 2009) or DITPA (Liu *et al.* 2009) administration, and the microvascular rarefaction of left

ventricle produced by ascending aortic constriction was restored in mice with chronic tri-iodothyronine (T₃) administration, which had no effect on the capillary density in control mice (Makino *et al.* 2009). Greater capillarity was observed in soleus and gastrocnemius muscles of hyperthyroid rats vs controls (Capo & Sillau 1983), and TH analogues were found to generate new arterial buds and increase the number of vessels per muscle fibre in a rabbit model of hind-limb ischaemia (El Eter *et al.* 2007). It has also been reported that TH provides an important support for the neovascularisation required by tumour masses (Yalcin *et al.* 2010).

Various angiogenic factors have been implicated in TH-induced angiogenesis. DITPA administration increased the protein expression of vascular endothelial growth factor (VEGF)₁₆₄, VEGF₁₈₈, basic fibroblast growth factor (bFGF or FGF2), angiopoietin-1 and Tie-2 (Wang *et al.* 2003) and bFGF in neovascularisation around infarction areas (Zheng *et al.* 2004). More recently, T₄ treatment restored poor platelet plasma VEGF levels in hypothyroid patients to levels in euthyroid controls, but the blood vessel density was not reported (Dedecjus *et al.* 2007).

Although TH and analogues are pro-angiogenic in various models, as reported earlier, their effects at the microvascular level remain controversial, with reports of unchanged (Wachtlova *et al.* 1985, Breisch *et al.* 1989, Heron & Rakusan 1994) or even reduced (Gerdes *et al.* 1979, Weiss & Grover 1987, Tomanek *et al.* 1995, Anjos-Ramos *et al.* 2006) cardiac capillary density in hyperthyroid rats. Cardiac capillary density was found to be increased in hypothyroid rats (Heron & Rakusan 1994) and rabbits (Tomanek *et al.* 1993). However, no data have been published on the relationship between TH and renal capillary density. With this background, the objective of this study was to analyse the effects of a chronic excess or deficiency of circulating TH on cardiac and renal capillary densities and on glomerular morphology by means of macroscopic morphology, histopathology and immunohistochemistry. A further objective was to explore possible explanations for the changes observed by measuring the main angiogenic factors in plasma and tissues (heart and kidney).

Materials and methods

Animals

Male Wistar rats born and raised in the experimental animal service of the University of Granada were used. Experiments were performed according to European

Union guidelines for the ethical care of animals. Rats initially weighing 250–280 g were maintained on standard chow and tap water *ad libitum* except where stated. The animals were divided into three groups: control, hyperthyroid and hypothyroid rats. Hyperthyroidism was induced by injecting s.c. T₄ (75 µg/rat per day), while hypothyroidism was induced by the continuous administration of 0.03% methimazole via drinking water. These treatments were administered for 4 weeks. Tail systolic blood pressure (SBP) and heart rate (HR) were recorded using tail-cuff plethysmography in unanaesthetised rats (LE 5001-Pressure Meter, Letica SA, Barcelona, Spain).

Experimental protocol

When the experimental period was completed, rats were anaesthetised with thiobutobarbital (100 mg/kg i.p., Inactin, Research Biochemicals International, Natick, MA, USA) and maintained at 37 °C on a servo-controlled heated rodent operating table. A tracheostomy was performed, and polyethylene PE-240 tubing was inserted in the trachea. Left femoral vein and artery were cannulated with PE-50 tubing. The vein was used for the infusion, and the artery was connected to a pressure transducer (MacLab, AD Instruments, Hastings, UK) for BP measurements. In order to maintain a euvolemic state, 1% albumin dissolved in isotonic NaCl solution was i.v. infused at 2 ml/h. A midline incision was made. The kidney was placed in a metal holder to eliminate respiratory movements and the left renal artery was isolated. A perivascular blood flow probe (1RB) was placed around the renal artery for renal blood flow (RBF) measurement with a T106 flowmeter (Transonic Systems, Inc., Ithaca, NY, USA). Direct BP and HR were recorded continuously for 60 min with a sampling frequency of 400/s (McLab, AD Instruments). SBP, HR and RBF were measured for 60 min, and results over the last 30 min were averaged for inter-group comparisons. The mesenteric vascular bed was then exposed on a white plastic surface (2×2 cm) and photographed at a constant distance (Olympus Optical Company, Ltd., Tokyo, Japan, SP-800UZ) to analyse vessel branching. Vessel branching was measured by counting the number of branches with a common origin on the white surface. Blood samples drawn via femoral catheter were used to determine plasma variables. Finally, the rats were killed by exsanguination; the heart, kidneys and aorta were removed; and the heart and kidneys were then weighed.

Histopathological study

Samples from the kidney, heart and thoracic aorta of the rats were fixed in buffered 10% formaldehyde and then paraffin embedded for conventional morphology study. Longitudinal kidney and transversal ventricular heart and thoracic aorta sections were stained with haematoxylin and eosin and with periodic acid-Schiff stain. The morphological study was done in blinded fashion on 4 µm sections with light microscopy, using the most appropriate stain for each lesion. Values were calculated semiquantitatively using a four-point scale (0, absence; 1, mild (<10% of vessel, tubules or glomeruli involved); 2, moderate (10–25%); 3, severe (>25%)).

The morphometrical analysis of glomeruli was performed automatically using the Fibrosis HR program, as previously reported by our group (Masseroli *et al.* 1998). The aortic morphometry was carried out with the ImageJ 1.44 program for Windows (Java image software in public domain: <http://rsb.info.nih.gov/ij/>).

For immunohistochemical analysis, paraffin-embedded heart and kidney sections on the same slide were dewaxed, hydrated and heat treated in 1 mM EDTA buffer for antigenic unmasking in a PT module (Thermo Fisher Scientific, Fremont, CA, USA) at 95 °C for 20 min. Sections were incubated for 30 min at room temperature with anti-CD31 PECAM-1 (M20 clone, diluted 1:50) to identify the numerical vascular density in kidney and heart or with anti-nestin (polyclonal, diluted 1:100) to identify newly formed blood vessels and glomerular podocytes. Immunohistochemical staining was done with an automatic immunostainer (Autostainer480,

Thermo Fisher Scientific) using the polymer peroxidase-based method, followed by development with diaminobenzidine. All reagents and antibodies were purchased from the same company (Master Diagnóstica, Granada, Spain). The number of positive glomeruli and cortical and medullary vessels per square millimetre and the number of podocytes and glomerular capillaries per glomerulus were counted using a millimetre scale in the eyepiece of a BH2 microscope with 40× objective (Olympus Optical Company, Ltd.).

Angiogenic factors

The recognised angiogenic factors (Fett *et al.* 1985, Risau & Flamme 1995, Nakamura *et al.* 2006) VEGF and angiogenin were measured in plasma and all these factors plus HIF-1α in the heart and kidney. Hearts and kidneys were homogenised in 50 mM HCl-Tris (pH 7.4) containing 1% Triton X-100 and centrifuged for 15 min at 1000 g. VEGF was analysed by Luminex x-MAP technology with a kit purchased from Millipore (Billerica, MA, USA), while bFGF, angiogenin and HIF-1α were measured by ELISA with kits purchased from Cusabio (Wuhan, China). Tissue protein was determined with the DC Protein Assay kit (Bio-Rad).

Statistical analyses

One-way ANOVA was used to compare variables at the end of the experiments. When the overall ANOVA was significant, pairwise comparisons were performed using Bonferroni's method. The non-parametric Kruskal–Wallis

Table 1 Biological variables in control, hypothyroid (methimazole-treated, 0.03% in drinking water) and hyperthyroid (T₄-treated, 75 µg/rat per day s.c.) rats 5 weeks after thyroid disorder induction

Groups	Hypothyroid	Control	Hyperthyroid
Morphological variables			
Body weight (g)	256 ± 4.0 [‡]	373 ± 7.2	303 ± 4.7*
Kidney weight (mg)	579 ± 0.18 [‡]	915 ± 29	868 ± 0.22
Kidney/body weight (mg/g)	2.26 ± 0.06 [†]	2.45 ± 0.07	2.87 ± 0.10 [‡]
Heart weight (g)	514 ± 22 [‡]	930 ± 31	843 ± 16
Heart/body weight (mg/g)	2.00 ± 0.07 [‡]	2.49 ± 0.07	2.78 ± 0.07 [†]
Haemodynamic variables			
SBP (mmHg)	117 ± 4.0 [‡]	130 ± 3.1	156 ± 1.9 [‡]
HR (bpm)	352 ± 6.5 [†]	403 ± 9.9	487 ± 17.6 [‡]
RBF (ml/min)	1.60 ± 0.07 [‡]	2.80 ± 0.19	3.60 ± 0.10 [‡]
Relative RBF (ml/min per g)	2.77 ± 0.12	3.08 ± 0.24	4.15 ± 0.10 [‡]
Thyroid hormone levels			
T ₄ (µg/dl)	0.5 ± 0.3 [‡]	4.6 ± 0.3	41 ± 5 [‡]
T ₃ (ng/dl)	6 ± 2.2 [‡]	78 ± 3.2	204 ± 9 [‡]

*P < 0.05; [†]P < 0.01; [‡]P < 0.001 vs controls.

test and Mann–Whitney *U* test were used for immunohistochemical and morphometrical variables. SPSS–Windows 15.0 programme (SPSS, Inc., Chicago, IL, USA) was used for the analyses. $P < 0.05$ was considered significant in all tests.

Results

Biological variables

Table 1 exhibits the values obtained for variables known to be affected by TH excess or deficiency. At the end of the 4-week study period, the body weight was significantly lower in the hyperthyroid and hypothyroid groups than in the controls. In comparison to values in controls, the kidney weight, kidney-to-body weight ratio, heart weight, heart-to-body weight ratio, SBP, HR, absolute RBF and FT₃ and FT₄ values were significantly higher in hyperthyroid rats and significantly lower in hypothyroid rats. RBF relative to kidney weight was significantly increased in hyperthyroid rats, but the reduction in hypothyroid rats (vs controls) did not reach statistical significance.

Macroscopic morphology

Figure 1 depicts representative panoramic images of sections of aorta, heart and kidney from all groups.

Mesenteric vessel branching was significantly increased in hyperthyroid rats and significantly decreased in hypothyroid rats (Fig. 2 and Table 2).

Histopathological, morphometrical and immunohistochemical results

The numerical density of CD31-positive capillaries was higher in left and right ventricles from both hypo- and hyperthyroid rats vs controls (Fig. 3). There was no significant difference ($P = 0.226$, Kruskal–Wallis test) in either thyroid disorder group vs controls in vascular nestin expression (index of newly formed vessels) in the ventricular mass (Fig. 3). The aortic wall area was larger and the aortic wall/lumen ratio higher in the hyperthyroid rat group, although significance, was only reached for the ratio (Table 2).

No morphological renal lesions (glomerular, tubulointerstitial or vascular lesions in renal parenchyma) were observed at 4 weeks of treatment in any group (control, hyperthyroid or hypothyroid groups).

The experimental groups showed no significant differences compared with controls in number of podocytes and in number of glomeruli per square millimetre (Table 2). The number of capillaries per glomerulus was higher in hyper- and hypothyroid groups than in controls, although the difference did not quite reach statistical

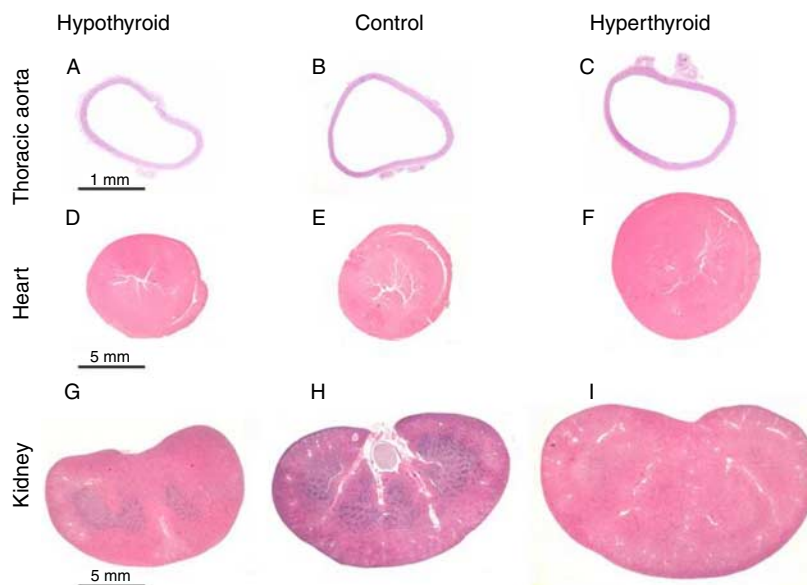
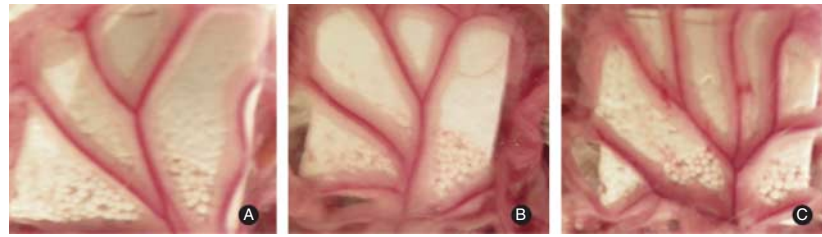


Figure 1

Haematoxylin–eosin staining of representative sections from whole aorta (top), ventricular heart (medium) and kidney (bottom) at low magnification. Note increase and decrease in size of heart and kidney sections of

hyperthyroid and hypothyroid rats respectively. Scale bars = 1 and 5 mm. Full colour version of this figure available via <http://dx.doi.org/10.1530/JOE-12-0208>.

**Figure 2**

Representative images of mesenteric arteriolar branching in the groups. Note decrease and increase in arteriolar branching number in hypo (A)- and hyperthyroid (C) groups respectively in comparison with

controls (B). Full colour version of this figure available via <http://dx.doi.org/10.1530/JOE-12-0208>.

significance. The glomerular area was larger in the hyperthyroid group than in the control and hypothyroid groups, and the percentage of mesangium was higher in the hyperthyroid group than in the control group (Table 2).

Immunohistochemistry study with CD31 antibody revealed that the numerical density of cortical and medullary capillaries was higher in kidneys from hyperthyroid and hypothyroid rats ($P=0.01$ and $P=0.001$ Kruskal–Wallis test respectively) than in those from controls (Figs 3 and 4). In addition, the number of nestin-positive newly formed capillaries per unit area in total renal mass was higher in the hyperthyroid group (Figs 3 and 4; $P=0.013$ Kruskal–Wallis test).

Plasma and tissue angiogenic factors

In comparison to controls, plasma angiogenin was markedly lower in the hypothyroid group (Table 3). Plasma VEGF was similar among all groups, and no bFGF was detected in any group. There were no differences in cardiac angiogenic factors between the hyperthyroid and control groups, whereas bFGF and HIF-1 α were significantly higher in hypothyroid animals than in controls. In comparison to controls and plasma angiogenin, bFGF and VEGF were higher in those from hypothyroid rats.

Discussion

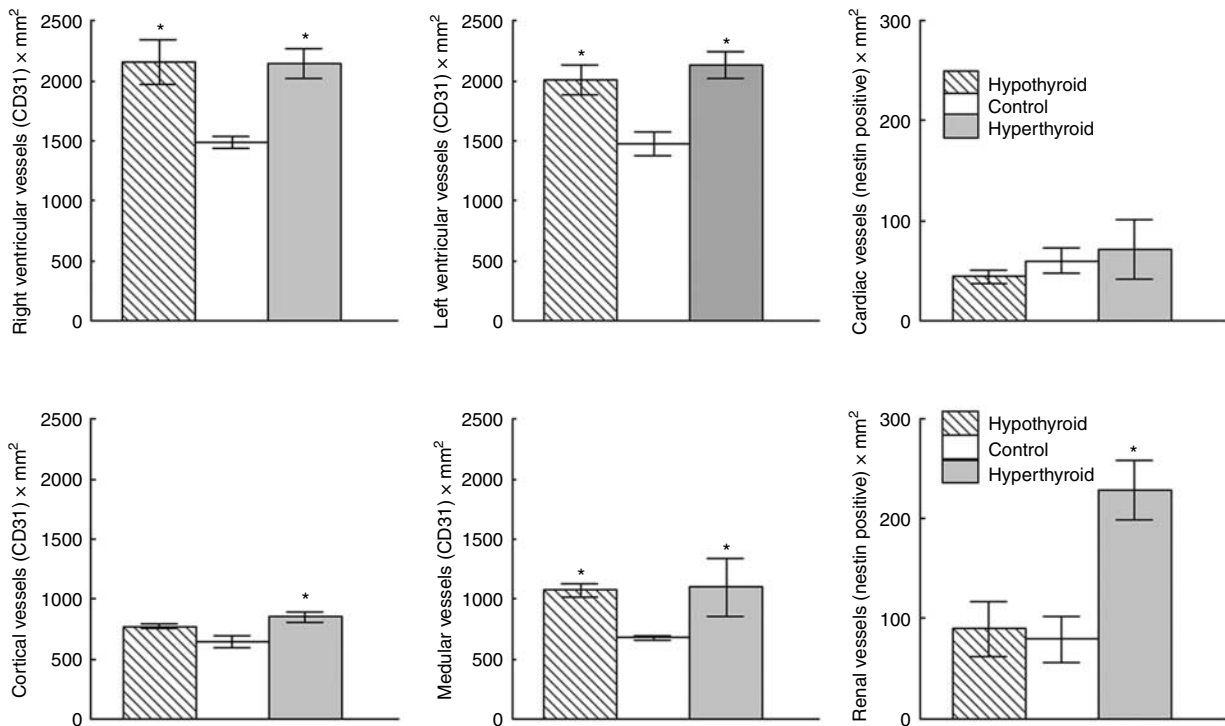
Observations of the vascular branching of the mesenteric bed in this study indicate that TH excess is associated with augmented angiogenesis and that hypothyroidism courses with vascular rarefaction. These observations are in agreement with previous studies in other vascular beds (Capo & Sillau 1983, Liu *et al.* 2008, 2010). However, at the microvascular level, the increased numerical density of CD31-positive capillaries in the heart and kidney of both hyperthyroid and hypothyroid animals does not conform to the usual differential pattern in these disorders. Results in mesenteric vessels were distinct from those in heart and kidney, which may be attributable to differences in the tissues and/or in the size of the vasculature. We discuss below possible causes of the changes detected in vascular contents and their potential role in the cardiovascular and renal abnormalities of thyroid disorders.

Results show increased cardiac and renal capillarity and augmented vascularisation of the mesenteric bed in hyperthyroid rats. These changes cannot be explained by the main angiogenic factors measured in the plasma, heart and kidney. This would contrast with the stimulating effects on angiogenic factors evoked by T₄ in other experimental protocols in cell cultures (Davis *et al.*

Table 2 Histopathological, morphometrical and immunohistochemical variables. Values are expressed as mean \pm s.d. Mann–Whitney *U* test

Groups	Hypothyroid	Control	Hyperthyroid
Number of glomeruli (mm ²)	16.48 \pm 5.22	12.48 \pm 2.96	12.31 \pm 5.64
Glomerular area (μ m ²)	33 621.26 \pm 5565.18 [§]	38 042.12 \pm 6849.74	50 153.21 \pm 10 901.23 [‡]
Capillary/glomerulus	33.62 \pm 8.79	26.62 \pm 7.32	33.33 \pm 5.04
Number of podocytes/glomerulus	8.00 \pm 0.75	8.83 \pm 1.29	9.01 \pm 0.97
Mesangium (%)	56.42 \pm 4.85	51.55 \pm 4.19	57.96 \pm 2.88 [†]
Aortic wall/lumen relationship	6.91 \pm 1.46	6.27 \pm 0.69	8.47 \pm 0.88 [*]
Mesenteric arteriolar branching	3.88 \pm 0.22 [*]	4.57 \pm 0.18	6.67 \pm 0.58 [*]

* $P<0.01$ vs control rats; [†] $P<0.05$, [‡] $P<0.01$ control vs hyperthyroid rats; [§] $P<0.01$ hypothyroid vs hyperthyroid rats.

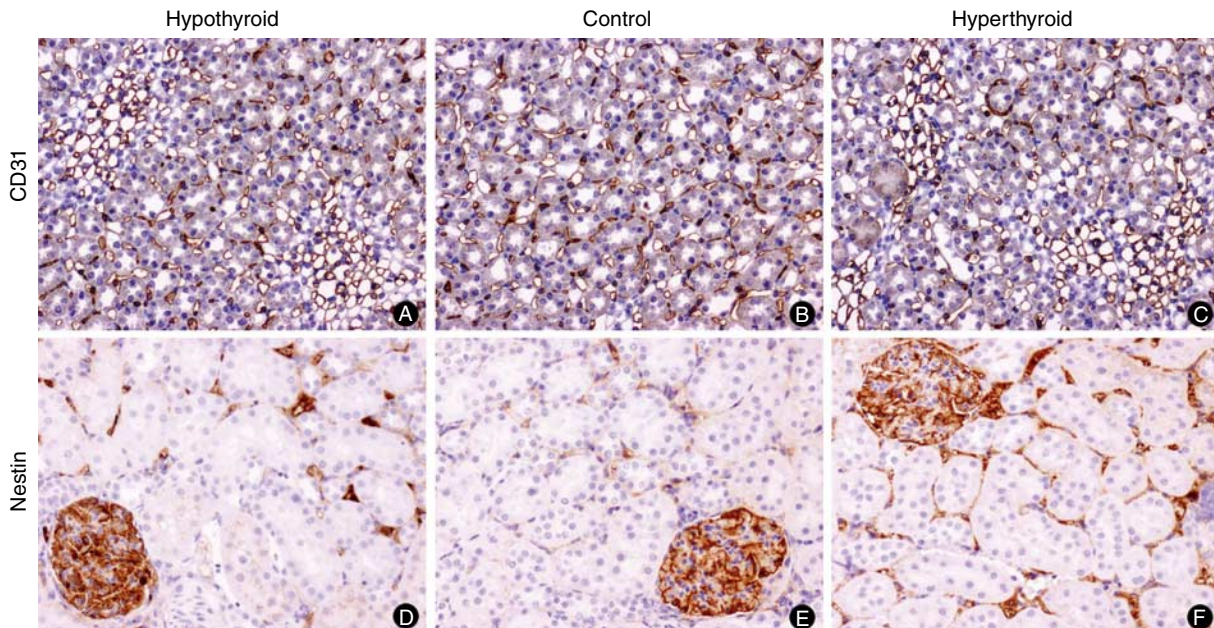
**Figure 3**

CD31-positive vessels in the cardiac ventricles, renal cortex and medulla and nestin-positive vessels in heart and kidney from the three groups. * $P < 0.05$ compared with the control group.

2004, 2008) or short-term studies (Jiang *et al.* 2008, Zhang *et al.* 2010). These discrepancies can be due to the adaptive response that occurs when T_4 is administered *in vivo* in long-term treatments. Hyperthyroidism augments cardiac output, tissue blood flow and oxygen availability (Vargas *et al.* 2006). This hyperdynamic circulation increases the shear stress or stretch, which induces angiogenesis in models of volume overload (Chen *et al.* 1994) and in endothelial cells subjected to cyclic stretch (Zheng *et al.* 2001). These haemodynamic and metabolic factors prevent the increase in HIF-1 α and hence the triggering of an angiogenic response mediated by VEGF, bFGF or angiogenin.

The greater cardiac capillary density in the hypothyroid group is in agreement with previous findings in rats (Heron & Rakusan 1994, Savinova *et al.* 2011) and rabbits (Tomanek *et al.* 1993). The cardiac capillary response to hypothyroidism is thought to be similar to that of bradycardially paced hearts, in which capillary densities are increased (Tomanek *et al.* 1993, Lei *et al.* 2004). The reduction in myocyte diameter may be responsible for this increase, given that the hypothyroidism-induced myocardial atrophy would bring capillaries closer together, thereby increasing the number of capillaries per square

millimetre of tissue. Savinova *et al.* (2011) reported an increased capillary density in the cardiac ventricle of thyroidectomised (TX) female rats that correlated with LV weight when controls and TX rats were pooled in a common regression line. However, TX rats showed a reduction of around 28% in LV weight/body weight ratio and an increase of around 66% in myocardial capillary density. Moreover, the LV weight/body weight ratio was restored at 72 h after T_3 administration, whereas the capillary density remained 29.6% higher than in controls. Our results show that the renal and cardiac atrophy produced a reduction of 7.75% in heart weight/body weight ratio and 19.6% in kidney weight/body weight ratio and an increase in capillary density of 36.6% in the heart and 59.2% in the kidney (medulla), clearly above expected values based on the reductions in cardiac and renal mass. Heron & Rakusan (1994) also found a higher than expected capillary density relative to the myocyte cross-sectional area in hypothyroid rats, evidencing capillary proliferation in their atrophic hearts. According to these findings, an absolute increase in capillary density takes place in hypothyroid hearts, which may result from an increased cardiac production of angiogenic factors (bFGF and HIF-1 α), as observed in bradycardia (Lei *et al.* 2004).

**Figure 4**

Representative images of differential immunohistochemical expression of CD31 and nestin in kidney. Note the larger number of medullary CD31- and cortical nestin-positive vessels (brown deposits) in the hyperthyroid group in comparison with the control group. In the hypothyroid group, there is

also a slight increase in the number of CD31- and nestin-positive vessels (original magnification $\times 20$, polymer peroxidase-based method). Full colour version of this figure available via <http://dx.doi.org/10.1530/JOE-12-0208>.

By contrast, plasma angiogenin, one of the most potent angiogenic factors (Nakamura *et al.* 2006), was markedly reduced in hypothyroid rats. This decrease, together with the vasoconstriction secondary to reduced metabolic needs (Liu *et al.* 2010), may participate in reducing the number of blood vessels in the mesenteric vascular bed.

Insufficient angiogenesis or loss of peritubular and glomerular capillaries has been observed in renal disease models (Kang *et al.* 2001, Basile 2004), indicating that small vessels may play a role in modulating renal injury. It has also been suggested that microvascular plasticity is an active process in renal disease (Lerman & Chade 2009).

Table 3 Angiogenic factors in plasma, heart and kidney. Data are expressed as mean \pm s.e.m.

Groups	Hypothyroid	Control	Hyperthyroid
Plasma			
Angiogenin (ng/ml)	29.0 \pm 6.7 [†]	195.7 \pm 16.4	167.6 \pm 28.5
bFGF (pg/ml)	–	–	–
VEGF (pg/ml)	12.4 \pm 2.2	11.2 \pm 1.7	10.0 \pm 1.2
HIF-1 α (pg/ml)	–	–	–
Heart			
Angiogenin (ng/mg prot)	6.29 \pm 1.1	5.86 \pm 1.1	4.04 \pm 1.1
bFGF (pg/mg prot)	20.6 \pm 3.3*	11.0 \pm 2.6	7.71 \pm 0.67
VEGF (pg/mg prot)	32.2 \pm 4.9	22.3 \pm 3.3	16.0 \pm 2.7
HIF-1 α (pg/mg prot)	304.3 \pm 45.2*	138.6 \pm 36.5	182.6 \pm 52.9
Kidney			
Angiogenin (ng/mg prot)	8.16 \pm 0.46 [†]	5.54 \pm 0.57	5.46 \pm 0.68
bFGF (pg/mg prot)	93.0 \pm 13.0*	59.0 \pm 8.62	55.5 \pm 11.1
VEGF (pg/mg prot)	54.5 \pm 14.5 [†]	13.4 \pm 4.91	9.80 \pm 2.61
HIF-1 α (pg/mg prot)	3.59 \pm 0.23	3.26 \pm 0.27	3.27 \pm 0.21

* $P < 0.05$, [†] $P < 0.01$ compared with the control group. bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; HIF-1 α , hypoxia-inducible factor 1 α .

We found a higher numerical density of capillaries in kidneys from both hyperthyroid and hypothyroid rats than in kidneys from control animals. Furthermore, the number of newly formed nestin-positive capillaries per unit area was higher in the hyperthyroid rats vs controls. In large vessels, the endothelial expression of nestin, which is associated with newly formed capillaries, is related to adaptations to dynamic changes in the vascular network (Mokrý *et al.* 2008). The higher shear stress produced by the greater RBF in the hyperthyroid group may play a role in their increased capillary formation. By contrast, higher renal levels of angiogenin, bFGF and VEGF may participate in the increased renal capillary density in the hypothyroid group. Taken together, these results suggest that the renal functional abnormalities of thyroid disorders are not associated with cortical or medullary vascular rarefaction.

No data have been published on glomerular morphology in thyroid disorders. The experimental groups showed no significant differences vs controls in number of podocytes and in number of glomeruli per square millimetre, but the glomerular area was greater in the hyperthyroid group with respect to the control and to the hypothyroid groups. The percentage of mesangium was also higher in the hyperthyroid group than in the control group. The absence of morphological renal lesions in the hyperthyroid or hypothyroid rats is in agreement with previous findings of no glomerular, tubulointerstitial or vascular lesions in renal parenchyma of hyperthyroid or hypothyroid rats treated for 6 weeks (Perez-Abud *et al.* 2011).

The number of podocytes per glomerulus was evaluated in this study in order to seek a possible explanation for the proteinuria reported in hyperthyroid rats (Vargas *et al.* 2006) and Graves' disease patients (Weetman *et al.* 1985). This is because podocytes play an essential role in the permeability of the glomerular barrier and are the principal target of glomerulopathies and proteinuric diseases (Mathieson 2009). However, the results obtained show that the number of podocytes per glomerulus was not influenced by TH excess or deficiency, indicating that hyperthyroidism-induced proteinuria is not secondary to a reduction in the number of podocytes.

In summary, this study shows that hyperthyroidism produces an augmented vascularisation of the mesenteric bed, whereas hypothyroidism induces vascular rarefaction. However, immunohistochemical studies revealed increased capillarity in heart ventricles and in renal cortex and medulla in both thyroid disorders. These findings can be explained by tissue levels of angiogenic factors in hypothyroid rats and by metabolic and haemodynamic

changes in hyperthyroid rats. The number of podocytes in glomeruli was not influenced by TH excess or deficiency. We propose that the increased renal capillarity in hypothyroidism may play a protective role against renal injury and that TH or TH analogues may be useful to increase vascularity in renal diseases that course with microvascular rarefaction.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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