Perinatal L-Arginine and Antioxidant Supplements Reduce Adult Blood Pressure in Spontaneously Hypertensive Rats

Simona Racasan, Branko Braam, Dione M. van der Giezen, Roel Goldschmeding, Peter Boer, Hein A. Koomans, Jaap A. Joles

Abstract—Embryo cross-transplantation and cross-fostering between spontaneously hypertensive rats (SHR) and normotensive rats (WKY) suggest that perinatal environment modulates the genetically determined phenotype. In SHR the balance between NO and reactive oxygen species (ROS) is disturbed. We hypothesized that increasing NO and diminishing ROS in perinatal life would ameliorate hypertension in adult SHR. Pregnant SHR and WKY and their offspring received L-arginine plus antioxidants (vitamin C, vitamin E, and taurine) during the last 2 weeks of pregnancy and until either 4 or 8 weeks after birth. Systolic blood pressure (SBP) and urinary excretion of protein, nitrates (NO_3⁻), and thiobarbituric acid reactive substances (TBARS) were measured. At 48 weeks of age rats were euthanized for glomerular counts. Perinatal supplements reduced SBP persistently in SHR and prevented the SBP increase observed in aging WKY. Initially NO_3⁻ excretion was lower and TBARS excretion higher in SHR than WKY. There was a direct effect on NO_3⁻ excretion in supplemented pregnant SHR and their offspring, but no increase was observed after stopping the supplements. TBARS excretion was only depressed up to 14 weeks by the supplements despite persistent differences in SBP. Consistent effects on nephron number were absent. Mild proteinuria, present in control SHR at 48 weeks, was prevented in all supplemented rats. Perinatal supplementation of NO substrate and antioxidants results in persistent reduction of SBP and renal protection in SHR, although effects on NO_3⁻ and TBARS were only transient. This suggests a critical role for perinatal pro- and antioxidant balance in programming BP later in life. (Hypertension. 2004;44:83-88.)

Key Words: diet ■ hypertension, renal ■ oxidative stress ■ nitric oxide

Epidemiological studies indicate that perinatal factors such as placental insufficiency and reduced birth weight can increase blood pressure (BP) later in life.1,2 In animal models there is ample evidence to support this, although a common denominator has not been found. Both embryo cross-transferring and cross-fostering studies between spontaneously hypertensive rats (SHR) and normotensive rats (WKY) resulted in reduced BP in SHR offspring,3,4 suggesting that either nongenetic causal factors leading to hypertension or consequences of hypertension itself are transmitted to the neonates. Most studies have focused on maneuvers such as restriction of maternal nutrition5,6 to simulate the Barker hypothesis that offset the potential benefit of transient BP reduction of maternal nutrition and lactation is unknown. Hence we first studied whether the imbalance between NO and ROS is already present in young SHR blunts the increase in BP for up to 21 weeks after stopping the supplements.

Transient inhibition of the renin-angiotensin system in young SHR blunts the increase in BP for up to 21 weeks after cessation of treatment.12 However, effects on BP were small and usually waned over time. We found that blocking the renin-angiotensin system in SHR in the last 2 weeks of pregnancy and the first 2 months after birth initially reduced BP. However, there were gross malformations in intrarenal arteries, subsequent development of malignant hypertension, and early death.13 Thus, perinatal interventions in the renin-angiotensin system result in deleterious effects on development that offset the potential benefit of transient BP reduction. Therefore, our second aim was to determine whether the...
perinatal supplements would permanently reduce BP without inducing pathological changes.

Nephrogenesis in the rat occurs in the last 2 weeks of pregnancy and the first 2 weeks after birth. Brenner proposed that a reduction in nephron number is germane to essential hypertension. Lower nephron numbers in SHR versus WKY have been documented, but this is not a consistent finding, and crossbreeding experiments could not reveal an association between glomerular number and BP in the F2-generation. Nevertheless it is possible that an early reveal an association between glomerular number and BP in the offspring. Thus our final aim was to monitor whether the perinatal supplements would prevent development of renal damage at this age.

The SHR is relatively resistant to hypertension-induced renal damage, which appears only from 1 year of age. Thus our final aim was to assess whether providing the dietary supplements increased nephron number.

Methods

Breeding and Supplement Protocol

SHR and WKY rats (Iffa-Credo, France), kept under standard conditions, were fed nonsynthetic rodent chow (Special Diets Services, Witham, Essex, UK). Sentinel animals, housed under the same conditions, were regularly monitored for infections. The Utrecht University Board for studies in experimental animals approved the protocol.

Adult SHR and WKY females were mated with adult males from the same strain. From day 7 of gestation, females and their offspring, until 4 or 8 weeks of age, received either tap water and regular chow (CON) or L-arginine (20 g/L), vitamin C (594 mg/L), and taurine (25 g/L) in drinking water and vitamin E (9 g/kg) in food (perinatal supplements). This combination of dietary supplements was chosen because it conceivably influences both arms of the NO/ROS balance and because of known BP lowering effects in adult SHR. Supplement intakes per 100 g body weight per day at 4 weeks, calculated from water and food intake, were as follows: L-arginine (360 to 470 mg); vitamin C (11 to 14 mg); taurine (450 to 588 mg); and vitamin E (113 to 178 mg). In WKY we studied females supplemented until 4 and 8 weeks of age and males supplemented until 8 weeks of age.

In SHR dams, systolic BP (SBP) was measured by tail-cuff (IITC, San Diego, Calif) before mating and repeatedly during pregnancy. During the last week of pregnancy, urine was collected for 16 hours. From day 7 of gestation, females and their offspring, until 4 or 8 weeks of age, received either tap water and regular chow (CON) or L-arginine (20 g/L), vitamin C (594 mg/L), and taurine (25 g/L) in drinking water and vitamin E (9 g/kg) in food (perinatal supplements). This combination of dietary supplements was chosen because it conceivably influences both arms of the NO/ROS balance and because of known BP lowering effects in adult SHR. Supplement intakes per 100 g body weight per day at 4 weeks, calculated from water and food intake, were as follows: L-arginine (360 to 470 mg); vitamin C (11 to 14 mg); taurine (450 to 588 mg); and vitamin E (113 to 178 mg). In WKY we studied females supplemented until 4 and 8 weeks of age and males supplemented until 8 weeks of age. In SHR dams, systolic BP (SBP) was measured by tail-cuff (IITC, San Diego, Calif) before mating and repeatedly during pregnancy. During the last week of pregnancy, urine was collected for 16 hours. Pups were weaned at 4 weeks. The number of pups and litters per group are indicated in the Table.

In the offspring, SBP was measured by tail-cuff at 8 weeks of age and thereafter every 6 weeks until euthanasia at 48 weeks. Mean arterial pressure (MAP) was measured directly under pentobarbital anesthesia at 10 weeks of age in SHR CON males (n=8) and SHR males supplemented until 8 weeks (n=8). Rats were placed in metabolism cages without food, but free access to water, for 24 hours at 8, 14, and 20 weeks of age for determination of urinary protein, NOx (N-hydroxy NO2), and thiobarbituric acid reactive substances (TBARS) excretion, and at 48 weeks for protein excretion. Thus the group supplemented until 8 weeks was exposed to vitamin C, L-arginine, and taurine during urine collection at 8 weeks. Immediately thereafter the supplements were withdrawn. Urine was collected on antibiotics (A9909; Sigma, St. Louis, Mo) to prevent formation of NO metabolites and stored at -80°C. At 48 weeks rats were anesthetized with pentobarbital and exsanguinated; the right kidney was excised, sliced, and immersion-fixed in phosphate-buffered formaldehyde (4%).

Analyses

Proteinuria was measured with Coomassie blue. Plasma creatinine was determined colorimetrically (Sigma). Urinary NO3 concentration was determined by fluorometric quantification of nitrate content (Cayman Chemicals, Ann Arbor, Mich). Lipid peroxidation in urine was determined by measurement of TBARS.

Morphology

Paraffin sections were stained with periodic acid-Schiff. Protein content was measured as percentage of renal cortex, was measured using Video Plan (Kontron Elektronik, Germany). The methodology of glomerular counting can be found in an online supplement available at http://www.hypertensionaha.org.

Calculations and Statistics

Data are expressed as mean±SEM. One-way ANOVA and 2-way ANOVA for repeated measurements were applied where appropriate. The Student-Newman-Keuls test was used post-hoc (P<0.05).

Results

Development

The supplements did not have consistent effects on development. All litters were carried to full gestation and litter size was not affected (pups/litter SHR CON 9±1, SHR perinatal supplements 8±1, WKY CON 8±1, WKY perinatal supplements 9±3). There were no evident developmental defects, and survival in all groups was 100% at 48 weeks. Perinatal supplements resulted in mild but permanent lower body weights in male and female SHR; however, in WKY, supplemented rats tended to have higher body weights at 48 weeks (Table).

NOx and TBARS Excretion

Judging by urinary excretions of stable NO metabolites and TBARS, the balance between NO and ROS was shifted toward ROS in young SHR and favorably influenced during and for ≈10 weeks after dietary supplementation. Pregnant rats receiving the supplements had significantly increased NOx excretion as compared with nonsupplemented pregnant SHR (161±14 versus 100±5 μmol/mmol creatinine, P<0.05), but there was no effect on TBARS excretion (2.26±0.04 versus 2.12±0.16 μmol/mmol creatinine). At 8 weeks of age in both males and females NOx excretion was lower in SHR than in WKY (Figure 1). Generally NOx excretion in the offspring tended to decrease between 8 and 20 weeks, in accordance with literature. Supplements in male and female SHR at 8 weeks were accompanied by a higher value of NOx excretion. This was clearly a direct effect because in SHR supplemented until 4 weeks NOx excretion at 8 weeks was not increased. Moreover, at 14 weeks NOx excretion was reduced in male SHR supplemented until 8 weeks and in both supplemented groups of female SHR. There were no differences in NOx excretion between CON and both perinatally supplemented groups at 20 weeks despite the differences in SBP. Thus, after they were discontinued, the supplements tended to temporarily reduce, rather than permanently enhance, NO production. TBARS excretion was similar in female and male WKY, but 3- and 4-fold higher in male and female SHR, respectively (Figure 2). TBARS excretion tended to decrease between 8 and 20 weeks in SHR, but to increase in WKY, so
that by 20 weeks of age there were no longer any strain differences present. Importantly, TBARS excretion was decreased in the group treated until 4 weeks at 8 weeks of age (males and females) and 14 weeks (males), and in the male group treated until 8 weeks at 14 weeks of age. TBARS excretion in male and female SHR supplemented until 8 weeks was omitted because addition of vitamin C in vitro showed that high vitamin C levels in urine falsely increased this measurement. The effects of perinatal supplements on TBARS excretion were transient and had disappeared by 20 weeks in all supplemented SHR irrespective of gender or duration of supplementation. Nevertheless, it appears that after stopping the supplements TBARS excretion remained low for at least 10 more weeks.

**Systolic Blood Pressure**

In the last days of pregnancy SHR display a marked decrease in BP,25 both in CON and supplemented rats; however, the supplements had no significant effect (Figure 3). Our most striking finding was the persistent lowering of SBP by the perinatal supplements in SHR. At 8 weeks of age supplemented male SHR offspring had significantly lower SBP than CON male SHR (Figure 4). After stopping the supplements this effect persisted, which was first confirmed by direct measurement of MAP in 10-week-old male SHR supplemented for 8 weeks (124±4 mm Hg) versus 10-week-old male SHR CON (147±4 mm Hg; P<0.05). SBP increased between 8 and 14 weeks, but this occurred in parallel in CON male SHR, and the difference between the groups persisted until euthanasia at 48 weeks. At 8 weeks the male SHR supplemented until 4 weeks showed little decrease in SBP, however from 14 to 38 weeks the SBP was similar to that in male SHR supplemented until 8 weeks and, hence, considerably lower than in SHR CON, after which the difference waned. At 8 weeks supplemented female SHR offspring had slightly, but significantly, lower SBP than CON female SHR, a difference that was maintained up to euthanasia at 48 weeks in both groups of perinatally treated females. In WKY CON, BP increased slowly from 8 weeks to 48 weeks in both males and females. These increases were prevented by the perinatal

---

![Figure 1](image1.png)  
**Figure 1.** NOx excretion in control male and female WKY (■), and SHR (●) and perinatally treated SHR until 4 weeks (■) and until 8 weeks (●) at 8, 14, and 20 weeks. #P<0.05 vs WKY control; *P<0.05 vs SHR control; $P<0.05 vs SHR supplement until 8 weeks; &P<0.05 vs 8 weeks in the same group.

![Figure 2](image2.png)  
**Figure 2.** TBARS excretion in control male and female WKY (■), and SHR (●) and perinatally treated SHR until 4 weeks (■) and until 8 weeks (●) at 8, 14, and 20 weeks. #P<0.05 vs WKY control; *P<0.05 vs SHR control; $P<0.05 vs SHR supplement until 8 weeks; &P<0.05 vs 8 weeks in the same group.
supplements, and significant differences were found after 26 weeks.

**Glomerular Number, Proteinuria, Plasma Creatinine, and Renal Morphology**

Glomerular density in male and female SHR CON was not significantly lower than in male and female WKYCON, respectively (Table). Only female SHR supplemented until 4 weeks had a significantly higher glomerular density. None of the groups had proteinuria from 8 to 20 weeks. However, by 48 weeks, SHR CON had mild proteinuria, which was prevented by the perinatal supplements (Figure 5). Plasma creatinine was similar in all groups at 48 weeks. There were no effects of perinatal supplements on proteinuria and plasma creatinine in WKY, and no renal vascular abnormalities in any of the supplemented rats. The lower tubular protein cast area in supplemented SHR reflects the protection against the incipient increase in proteinuria observed in SHR CON (Figure 5). Casts occurred predominantly in the juxtamedullary zone.

**Discussion**

Essential hypertension is considered to be the result of interaction between genetic and environmental factors. The “programming hypothesis” proposes that adverse influences during development increase BP later in life. In the present study we hypothesized that an altered balance between NO and ROS is an important determinant of hypertension in SHR. We therefore exposed pregnant SHR and their offspring to a combination of antioxidants and excess NOS substrate during the critical period of nephrogenesis. The main finding of our study was that dietary perinatal supplements persistently lowered BP and protected against renal damage in adult male and female SHR despite cessation of treatment at an early age.

A BP-lowering effect of the perinatal supplements was first measured at 8 weeks and was maintained up to 48 weeks. At 8 weeks of age, SBP was lower in the 8-week group in which the measurement was performed close to the cessation of the supplements, when, judging by the high NOx excretion, these rats were still under the direct effect of the supplements, as compared with the group supplemented until 4 weeks. This difference in NOx excretion was no longer present at later time points, suggesting that in SHR particularly the increase of BP later in life (after aged 8 weeks) is amenable to the beneficial effects of these perinatal supplements. Interestingly, perinatal supplements also appeared to reduce the slight increase in BP characteristic of aging WKY, which has been shown to be susceptible to L-arginine plus angiotensin-converting enzyme inhibition, suggesting that effects of the perinatal supplements on BP regulation in later life are not only manifest in the SHR strain.

As mentioned previously, the urinary NOx excretion suggests that NO production was at control SHR levels or even during development increase BP later in life. In the present study we hypothesized that an altered balance between NO and ROS is an important determinant of hypertension in SHR. We therefore exposed pregnant SHR and their offspring to a combination of antioxidants and excess NOS substrate during the critical period of nephrogenesis. The main finding of our study was that dietary perinatal supplements persistently lowered BP and protected against renal damage in adult male and female SHR despite cessation of treatment at an early age.

A BP-lowering effect of the perinatal supplements was first measured at 8 weeks and was maintained up to 48 weeks. At 8 weeks of age, SBP was lower in the 8-week group in which the measurement was performed close to the cessation of the supplements, when, judging by the high NOx excretion, these rats were still under the direct effect of the supplements, as compared with the group supplemented until 4 weeks. This difference in NOx excretion was no longer present at later time points, suggesting that in SHR particularly the increase of BP later in life (after aged 8 weeks) is amenable to the beneficial effects of these perinatal supplements. Interestingly, perinatal supplements also appeared to reduce the slight increase in BP characteristic of aging WKY, which has been shown to be susceptible to L-arginine plus angiotensin-converting enzyme inhibition, suggesting that effects of the perinatal supplements on BP regulation in later life are not only manifest in the SHR strain.

As mentioned previously, the urinary NOx excretion suggests that NO production was at control SHR levels or even during development increase BP later in life. In the present study we hypothesized that an altered balance between NO and ROS is an important determinant of hypertension in SHR. We therefore exposed pregnant SHR and their offspring to a combination of antioxidants and excess NOS substrate during the critical period of nephrogenesis. The main finding of our study was that dietary perinatal supplements persistently lowered BP and protected against renal damage in adult male and female SHR despite cessation of treatment at an early age.

A BP-lowering effect of the perinatal supplements was first measured at 8 weeks and was maintained up to 48 weeks. At 8 weeks of age, SBP was lower in the 8-week group in which the measurement was performed close to the cessation of the supplements, when, judging by the high NOx excretion, these rats were still under the direct effect of the supplements, as compared with the group supplemented until 4 weeks. This difference in NOx excretion was no longer present at later time points, suggesting that in SHR particularly the increase of BP later in life (after aged 8 weeks) is amenable to the beneficial effects of these perinatal supplements. Interestingly, perinatal supplements also appeared to reduce the slight increase in BP characteristic of aging WKY, which has been shown to be susceptible to L-arginine plus angiotensin-converting enzyme inhibition, suggesting that effects of the perinatal supplements on BP regulation in later life are not only manifest in the SHR strain.

As mentioned previously, the urinary NOx excretion suggests that NO production was at control SHR levels or even...
slightly lower once the supplements were stopped. TBARS excretion was much higher in control SHR than WKY rats, whereas treated groups had significantly lower urinary TBARS than SHR controls at 8 weeks. This was not solely due to a lower BP because in that case the difference would probably have persisted. Overall the NO, and TBARS excretion data suggest that the main effect of the supplements was less ROS, which resulted in a temporary shift in the NO-ROS balance toward NO for a few weeks. However, by 20 weeks all the differences in NO, and TBARS excretion between WKY, SHR, and perinatally treated SHR had disappeared. Nevertheless the differences in BP persisted, suggesting that although in our adult offspring differences in BP were no longer determined by differences in the NO-ROS balance, a temporary shift in NO-ROS balance away from ROS can have a permanent impact on BP control. It is possible that the supplements have a direct antihypertensive effect because at 8 weeks of age, SBP was lower in the SHR supplemented until 8 weeks than in SHR CON or SHR supplemented until 4 weeks. If such a direct antihypertensive effect was already present in very early life this could conceivably have ameliorated secondary changes in the developing kidneys that underlie full-blown hypertension in adult life. However, in the absence of information on perinatal BP regulation, mechanisms remain unclear. Nonetheless, the implications of our findings are of potential importance.

Besides a permanent shift in NO-ROS balance, other factors such as growth, glomerular number, and maternal BP also do not conclusively link the perinatal supplements with BP in later life. Intrauterine growth retardation has been associated with higher BP in adult life. This risk is further increased by rapid catch-up of body weight early in life. In our study we unexpectedly found lower body weight at an early age in supplemented offspring of both strains, but there was only catch-up of growth in the WKY. Nevertheless both strains showed persistent decrease in SBP. Thus, perinatal supplements did not appear to act on SBP by influencing body growth.

Maternal protein restriction in normotensive rats leads to a lower number of glomeruli in conjunction with hypertension and low-birth-weight infants have reduced glomerular number. Available data concerning glomerular number in SHR is conflicting; some studies do not indicate lower glomerular number as compared with WKY, whereas others find 15% to 20% less glomeruli. The increased glomerular number in the 4-week perinatally supplemented SHR female offspring, resulting in a mean number exceeding that in normotensive WKY rats, might be one mechanism by which shifted NO/ROS balance in a crucial period results in persistently lower BP in SHR. Certainly when factored for lower body weight, and consequently extracellular volume, it is conceivable that with this group the increased complement of nephrons may have improved sodium handling and BP control. However, this increase was not found in the other groups with equally reduced SBP and, hence, cannot be the entire explanation.

Finally, maternal BP in SHR tended to be slightly reduced by the supplements in the second trimester of pregnancy. Because embryo transplantation from SHR to WKY is known to reduce BP in the offspring, maternal BP certainly could play a role in protection. But even if placental hemodynamics play a role, this cannot entirely explain our findings, because cross-suckling of SHR pups by WKY mothers also reduces BP in the offspring. BP in the perinatally supplemented SHR was intermediate between the values in nonsupplemented SHR and WKY. Recently BP lowering has been achieved in young adult SHR using intracardiac injection at 5 days of age of viral particles containing angiotensin type-1 receptor antisense cDNA. Although the latter approach had a comparable intermediate effect, application to a highly prevalent condition such as hypertension is much less feasible than dietary supplementation with nonprescription compounds such as vitamins and amino acids. Moreover, an intact renin-angiotensin system is a prerequisite for normal renal development. We found that blocking the renin-angiotensin system in SHR over the same period as in the present study induced gross malformations in intrarenal arteries, resulting in subsequent malignant hypertension and early death. Such changes were not observed in the present study. Thus prenatal administration of dietary supplements would appear to be a more prudent approach in human hypertension than directly targeting the neonatal renin-angiotensin system.

Male offspring of protein-deprived rat dams already have proteinuria at 8 weeks of age, and survival of all offspring was 69% versus 100% in controls at 11 months, progressing to 44% versus 93% by 18 months. In contrast, 8-week-old control SHR did not have proteinuria, and survival at 11 months was 100%, more closely resembling essential hypertension where proteinuria and mortality in adolescents is uncommon. In that sense, the findings are also not the direct reverse of the protein-deprivation studies and appear to follow a different paradigm. Supplements similar to ours, when administered to SHR from prenatal life until 24 weeks of age, reduced SBP but not proteinuria. At the end of our study, at 48 weeks of age, whereas kidney function was normal and glomerulosclerosis was absent, a mild increase in protein excretion and tubular protein cast area was present in CON SHR. These changes were entirely prevented by the supplements, possibly as a result of life-long lowering in BP.

In conclusion, perinatal dietary supplementation with L-arginine and antioxidants, presumably by temporarily modifying the balance between NO and ROS toward NO, persistently attenuates the development of hypertension and results in renoprotective effects in aging SHR.

**Perspectives**

Our findings underline the importance of perinatal environmental factors for development of BP regulation later in life. Because we used dietary supplements, one could speculate that improvement in maternal redox status by modifying nutrition during pregnancy and possibly lactation, as well as reducing other sources of oxidative stress, might prove beneficial in the long run in preventing development of hypertension and thus sparing life-long antihypertensive treatment.
Acknowledgments

The Dutch Kidney Foundation supported this study (C99.1823). S.R. received an ISN Fellowship. B.B. is a fellow of the Royal Netherlands Academy of Arts and Sciences. We thank Paula Martens and Nel Willeke for expert laboratory assistance.

References

Perinatal L-Arginine and Antioxidant Supplements Reduce Adult Blood Pressure in Spontaneously Hypertensive Rats
Simona Racasan, Branko Braam, Dionne M. van der Giezen, Roel Goldschmeding, Peter Boer, Hein A. Koomans and Jaap A. Joles

Hypertension. 2004;44:83-88; originally published online June 7, 2004; doi: 10.1161/01.HYP.0000133251.40322.20

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/44/1/83

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2004/06/24/01.HYP.0000133251.40322.20.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
Perinatal L-arginine and antioxidant supplements reduce adult blood pressure in SHR

Simona Racasan, Branko Braam, Dionne M. van der Giezen, Roel Goldschmeding, Peter Boer, Hein A. Koomans, and Jaap A. Joles

Correspondence:

Jaap A. Joles, D.V.M., Ph.D.
Department of Nephrology and Hypertension (Room F03.226)
University Medical Center
Heidelberglaan 100
P.O. Box 85500
3508 GA Utrecht
The Netherlands

Telephone:  #31 30 2507329
Fax:  #31 302543492

E-mail:J.A.Joles@med.uu.nl
Glomeruli were counted at magnification 40x, by applying a grid on randomly chosen fields, and expressed as number of glomeruli/mm³ (n), calculated by the formula n=G/(F x A x (D+T)), where G is the number of glomeruli counted in 300 fields, F the number of fields counted, A the grid area, D the average glomerular tuft diameter, and T the section thickness (0.004 mm)¹. Glomerular counts were done on single sections, so that absolute numbers should not be directly compared with studies using more advanced technology ². However, because it was our aim to identify differences between groups, we believe that within the study our approach was valid.
REFERENCES
