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RESEARCH ARTICLE

Biomarkers of age effect on renal function in Down syndrome

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Abstract

Objective: To assess differences in kidney function between Down syndrome (DS) individuals and a control group related to aging.

Methods: Creatinine (Cr) and specific gravity (SG) were assessed by Spectrophotometric and refractometric assays in urine samples of 103 individuals with DS and 82 age-matched controls.

Results: Significantly lower levels of Cr and SG were found in DS after puberty. Significant correlations were found between SG and age as well as between Cr and SG in DS and controls ($p \leq 0.05$).

Conclusions: Premature aging in kidneys of DS patients could lead to an impaired renal function.

Keywords: Down syndrome, premature aging, creatinine, specific gravity, urine

Introduction

Down syndrome (DS) or trisomy 21 is one of the most important human congenital diseases, occurring in 1 out of 700–1000 live births (Hook, 1981). Clinical symptoms were first described by John Langdon Down in 1866, but the association with one extra copy of chromosome 21 was first reported by Lejeune (1959). DS patients present different morphological characteristics such as short stature, obesity and bilateral epicanthic eyefolds. Furthermore, growth retardation has been noted during life. The syndrome is as well associated with mental retardation, *elevated oxidative stress*, congenital heart disease, immune system disorders, digestive problems, thyroid dysfunction, an increased risk of leukaemia, Alzheimer disease and premature aging.

Aging is a natural process that involves physical and physiological changes leading to a loss of different biological functions. These changes can affect, directly or indirectly, the kidney. It is well known that renal function declines with age (Wiggins, 2011). Two of the most useful indicators of renal function are urinary creatinine (Cr) and specific gravity (SG) (Vionescu et al., 2002, Nyman et al., 2006). Cr is a normal endogenous end-product of human metabolism. This non-proteic organic nitrogen compound is formed by spontaneous dehydration of creatine and phospho-creatine from muscle metabolism. Cr is cleared from the body through the kidney mainly by glomerular filtration and to a lesser extent by active secretion from blood through the renal tubules (Boeninger et al., 1993). In addition to be a renal function indicator, Cr has been used as a muscle mass index (Keshaviah et al., 1994) or as an adjustment parameter for urine dilution. Urine SG is a unitless measure of the ratio between the density of urine and the density of water, and it is also used to assess the hydration status of an individual (Kavouras, 2002). Moreover, SG assessment is a fast, simple and inexpensive method to estimate the concentration or dilution ability of the kidneys (Vionescu et al., 2002).

An impaired renal function may lead to a reduced excretion of Cr (Wyss and Kaddurah, 2000). Several works reported significantly reduced Cr clearance and higher levels of Cr in plasma samples of DS patients (Coburn et al., 1967; Nishida et al., 1979). Coburn et al. (1967) suggested that a lower Cr clearance in DS may indicate that excretion by glomerular filtration is slightly impaired in these subjects. Moreover, different renal anomalies have been described in DS (Gupta et al., 1991; Mercer et al., 2004; Malaga et al., 2005) which could induce chronic renal disease in some cases leading to a diminished

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excretion of Cr. However, it seems that the incidence of renal failures in DS population does not differ from the incidence within the rest of population (Malaga et al., 2005). Furthermore, there is no evidence in literature about urine SG related to this concern.

The use of urine samples provides many advantages in population studies as their collection is non-invasive, has limited infectious disease risk to participants and researchers and provides enough volume for multiple assays and future research. Ease of collection is the major advantage for spot urine samples instead of 24 h urine voids, since 24 h collection can be cumbersome and often improper or incomplete, especially in children, and it may bias the result. Furthermore, Cr of first morning urine samples is very useful because it is not affected by practice of exercise and food intake; therefore, it has little day-to-day variation.

Since premature aging is a known feature in DS the aim of this work was to analyze a possible impaired renal function in these patients compared to a normal population using urinary levels of Cr and SG as indicators. Furthermore, factors such as gender, meat intake and thyroid function were also analyzed in order to gather new information about renal function in this common genetic disorders.

Materials and methods

Study population

We examined 103 individuals with DS (49 males and 54 females), aged from 1 to 57 years. The recruitment of children and young people was performed out of students of a kindergarten and primary and secondary special school in Community of Madrid (Spain). The adults were members of institutions for the mentally retarded (Down and non-Down) in the Community of Madrid.

In all individuals, DS diagnosis was confirmed by a cytogenetic analysis, which identified 96 subjects with regular trisomy 21, 5 individuals with Robertsonian translocation trisomy t(q21q21) and 2 subjects with mosaicism. Chromosome analysis was performed on cultured peripheral blood lymphocytes. Three different handing techniques were used to examine the chromosomes in detail: GTG, CBG and RHG bands.

The control group comprised 82 healthy and non-smokers subjects (33 males and 49 females), aged from 5 to 59 years, with a normal diploid complement of chromosomes. *Control subjects were biological non-Down siblings living in the same household in order to avoid environmental variations such as diet.*

Both groups (DS and controls) were divided into five age groups: Group I from 1 to 9 years ($n = 23$), Group II from 10 to 15 years ($n = 28$), Group III from 16 to 25 years ($n = 59$), Group IV from 26 to 40 years ($n = 40$) and Group V more than 40 years ($n = 35$). These groups represent respectively: infancy, adolescence pre-menarque (based on females), early adulthood, adulthood and senescence (based on DS individuals according to the classification of Bittles et al. Participants were given a questionnaire to obtain the following information: a) sociodemographic parameters: age and gender; b) frequency of meat intake per week; and c) treatment for hypothyroidism. 31 patients with DS were receiving medical treatment with levothyroxine for hypothyroidism (4 patients in age group I, 5 in age group II, 10 in age group III, 8 in age group IV and 4 in age group V).

Each participant or a legal guardian signed an informed consent form detailing the analysis and handing of the data. The study was approved by the Ethic Committee of Superior Council of Scientific Investigation in Spain.

Urine sample collection

First morning urine samples were collected in a sterile flask covered with aluminium foil to keep out stray light. Cr was analyzed within 2 h of the collection and 1-mL aliquots were frozen at–20°C until SG determination. Samples presented neither signal of hematuria nor hemolisis.

Reagents

Creatinine was purchased from Fluka (Buchs, Switzerland), picric acid and hydrochloric acid was purchased from Probus (Barcelona, Spain) and sodium hydroxide was purchased from Panreac (Madrid, Spain). All reagents were of analytical grade and deionized water was obtained from a Milli-RO water system (Millipore, Bedford, MA).

Biochemical determinations

Cr was assayed according to the method described by Varley and Gowenlock which is based on the Jaffé reaction (Jaffé, 1986). A calibration curve was performed with known Cr concentrations in order to calculate final concentration of Cr in urine samples. A UVmini-1240 Shimadzu spectrophotometer (Shimadzu, Tokyo, Japan) was used to read absorbance at 500 nm.

After urine samples were thawed at 20°C, SG determination was monitored using a RL2 Polskie Zaklady Optyczne hand refractometer (PZO, Warszawa, Poland) (refraction index units range: 1.333 to 1.349). A unit converter table (Reichert, USA) was used to convert refraction index units to SG units.

Statistical analysis

Outcome variables were Cr concentration (expressed in mmol/L) and SG units (expressed in arbitrary units). Results are expressed as mean (standard deviation). Kolmogorov–Smirnov test was used to analyze the normal distribution of urinary Cr concentration and urinary SG in each population. Analysis of variance (ANOVA) and least significant difference (LSD) *post hoc* test were used to analyze differences in both variables between DS and controls related to age groups, gender and meat intake as well as between DS treated or un-treated for hypothyroidism. Mann–Whitney's *U* test was used to analyze differences between age groups in DS treated or

un-treated for hypothyroidism. Correlation analyses were performed using Pearson's (product-moment) correlation test between Cr, SG and age. Lineal regression tests were performed for significant correlations. Significant differences were considered when $p<0.05$. Data were analyzed with SPSS version 17.0 statistical software (SPSS Inc., Chicago, IL).

Results

Normal frequencies were observed for the different causes of DS in our population as those established by Hook (1982). We did not consider any analysis between different causes of DS, due to the reduced number of individuals with trisomy 21 by Robertsonian translocation ($n=6$) and mosaic trisomy 21 ($n=2$), and we therefore performed the rest of comparison as just one group. Normal distributions were accomplished for Cr and SG in DS and control populations. When levels of Cr and SG were compared for all ages between DS and control group significant differences were found for both Cr: 10.9 (5.7) mmol/L vs. 16.1 (7.0) mmol/L in DS and controls, respectively, $p<0.05$; and SG: 1.019 (0.006) vs. 1.022 (0.006) in DS and controls respectively, $p<0.05$.

Table 1 reports Cr and SG values in both groups stratified by gender and age group. As expected, significant differences were observed between DS and control group in both genders, but they were not found between males and females within each population. In addition, significant differences were found between DS and control group for Cr in age groups III, IV and V, and for SG in age groups IV and V. The comparison of the parameters studied between age groups within each population (Table 1) revealed significant differences for Cr in the control group and for Cr and SG in the DS group.

No significant correlation was found between Cr and age neither in DS group nor in control group. Nevertheless, a significant negative correlation was found between SG and age in DS ($r=-0.379$, $p<0.01$) and in controls ($r=-0.243$, $p<0.05$). Furthermore, a significant positive correlation was found between Cr and SG in DS ($r=0.637$; $p<0.01$) and in controls ($r=0.648$, $p<0.01$). When correlations analyses were performed by age group, the correlation between Cr and SG was not significant only in the age group I in both populations. Figure 1 shows the lineal regression models of the significant correlations.

When the influence of meat intake was analyzed, no significant differences were found for urinary levels of Cr and SG in any age group neither in DS nor in controls ($p>0.05$ for all).

Patients with DS who were receiving medical treatment with levothyroxine for hypothyroidism showed slightly reduced levels of Cr and SG than those without treatment, however differences were only significant for Cr levels: 8.6 (4.1) mmol/L vs. 11.8 (5.9) mmol/L; $p<0.01$. Furthermore, when the influence of levothyroxine treatment is analyzed in each age group, significant differences only appear in the age group III (Table 2).

Table 1. Urinary levels of creatinine (Cr) and specific gravity (SG) in control and DS population according to gender and age groups. Values are expressed as Mean (standard deviation).

Population		n	Cr (mmol/L)	SG (unitless)
	Gender			
	Males	49	11.6 (5.8)†	1.020 (0.006)
	Females	54	10.2 (5.6)‡	1.019 (0.006)‡
	Age group			
DS	I (1–9 years)	14	7.2 (2.6)§	1.023 (0.005)#
	II (10–15 years)	17	11.6 (6.6)	1.020 (0.005)#
	III (16–25 years)	37	12.4 (5.8)**	1.021 (0.006)#
	IV (26–40 years)	19	10.3 (4.8)**	1.016 (0.005)**
	V (>40 years)	16	10.2 (6.3)*	1.016 (0.004)*
	Gender			
	Males	33	17.0 (7.8)	1.022 (0.007)
	Females	49	15.4 (6.5)	1.022 (0.006)
	Age group			
Control	I (1–9 years)	9	9.7 (3.4)¶	1.024 (0.006)
	II (10–15 years)	11	14.2 (6.3)	1.024 (0.007)
	III (16–25 years)	22	17.8 (5.6)	1.022 (0.006)
	IV (26–40 years)	21	18.9 (8.2)	1.023 (0.007)
	V (>40 years)	19	14.9 (6.9)	1.020 (0.005)

†Significant differences ($p<0.05$) between DS and controls in males.
‡Significant differences ($p<0.05$) between DS and controls in females.
*Significant differences ($p<0.05$) between DS and controls in the same age group.
**Significant differences ($p<0.01$) between DS and controls in the same age group.
§Significant differences ($p<0.05$) in DS respect to age groups II and III.
#Significant differences ($p<0.05$) in DS respect to age groups IV and V.
¶Significant differences ($p<0.05$) in controls respect to age groups III and IV.

Besides, when DS patients receiving medical treatment with levothyroxine were excluded, significant differences between DS and controls were still observed.

Discussion

It is well known that Cr and SG can be used as renal function indicators (Vionescu et al., 2002; Nyman et al., 2006) and that urinary Cr excretion is decreased in the elderly (Musso et al., 2007). As aging is an irreversible process that involves many organs, including kidney, we evaluated urinary levels of Cr and SG in DS patients to assess if their premature aging can affect their renal function before than in a healthy population.

Our results showed that DS patients have lower levels of urinary Cr than controls in the age range from 16 to 40 years. Because Cr is the product of the breakdown of creatine, found primarily in muscle mass, a good correlation between lean body mass and Cr elimination has been previously observed (Forbes et al., 1976). *The lean body mass of the participants, was not included in this study since Allison et al. (1995) and Luke et al. (1996) found no differences in fat-free mass between DS and control individuals*

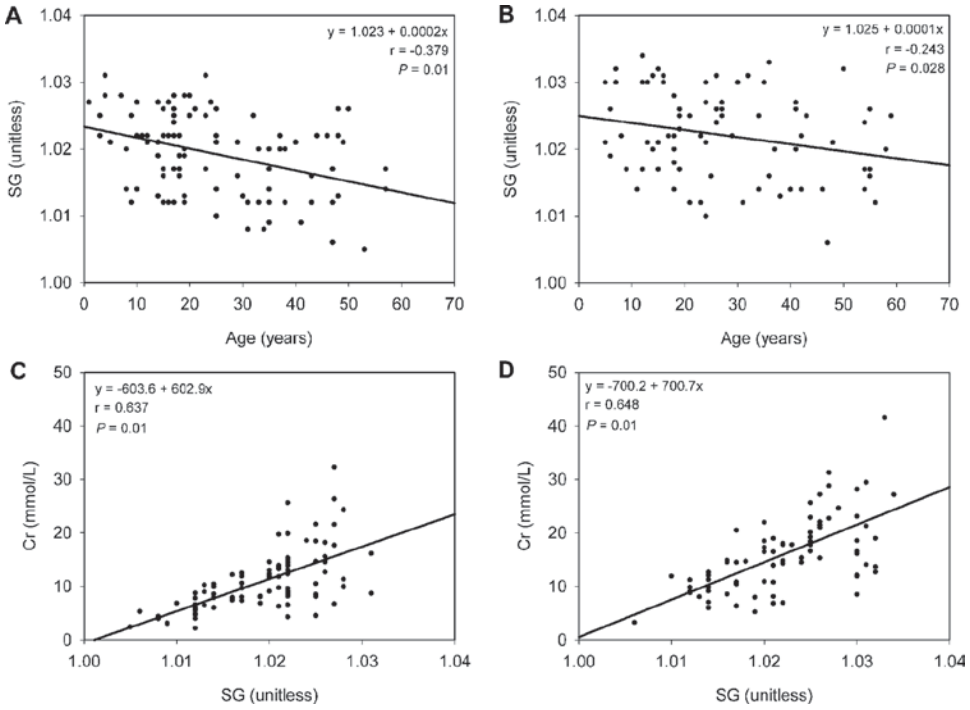

Figure 1. Lineal regression models of significant correlations. (A) Specific gravity (SG) versus age in DS; (B) SG versus age in controls; (C) Creatinine (Cr) versus SG in DS; (D) Cr versus SG in controls.

Table 2. Urinary levels of creatinine (Cr) and specific gravity (SG) in patients with Down syndrome (DS) who were receiving levothyroxine treatment for hypothyroidism (DS with hypothyroidism) and those without hypothyroidism diagnosed. Values expressed as mean (standard deviation).

	DS with hypothyroidism			DS without hypothyroidism		
Age group	*n*	Cr (mmol/L)	SG (unitless)	*n*	Cr (mmol/L)	SG (unitless)
I (1–9 years)	4	6.2 (1.8)	1.022 (0.008)	10	7.5 (2.9)	1.024 (0.005)
II (10–15 years)	5	9.3 (3.1)	1.020 (0.003)	12	12.6 (7.6)	1.020 (0.005)
III (16–25 years)	10	8.0 (3.3)*	1.018 (0.005)	27	14.0 (5.7)	1.022 (0.006)
IV (26–40 years)	8	9.6 (5.4)	1.015 (0.006)	11	10.8 (4.5)	1.016 (0.005)
V (>40 years)	4	9.5 (6.3)	1.015 (0.008)	12	10.4 (6.6)	1.016 (0.007)

*$p < 0.05$ (significantly different) from the DS without hypothyroidism group.

neither in children nor in adults. A reduced metabolic rate in DS individuals could be another explanation for these results. It may lead to a lower production of Cr and consequently to a lower urinary excretion of the metabolite. *Actually, senile sarcopenia (decrease in Cr production) has been reported in elderly healthy individuals (Musso and Oreopoulos, 2011), which could occur prematurely in DS population. Nevertheless there are controversial results about that in DS (Allison et al., 1995; Fernhall et al., 2005).*

But the fact is that DS individuals suffer from elevated oxidative stress (Campos et al., 2011) leading to premature aging. This premature aging involves several changes in different physical and physiological aspects, such as premature graying of the hair, hair loss and wrinkling of the skin (Lott and Lai, 1982); premature menopause in women (Coppus et al., 2010); vision and hearing impairments (Van Buggenhout et al., 1999) or higher risk to develop thyroid disease (Korsager et al., 1978). Although it seems that ceruloplasmin activity, the main antioxidant in the kidney, was not found to decrease significantly with age (Tórsdóttir G et al., 2001) decreasing urinary excretion of Cr and lower levels of SG suggest an impaired renal function.

It is important to remark that this premature aging in kidney of DS individuals could be reflected in the differences obtained in this work between both groups, mainly due to differences after puberty (age groups III, IV and V), since urinary Cr values decreased prematurely in DS. Moreover this premature aging could also explain the significant decrease observed in DS for SG in age group IV and V compared to controls, since a general decline in glomerular filtration rate and muscularity have been noted in the old age (Alessio et al., 1985). Although a Cr reabsorption in the renal tubules of healthy old persons has been reported (Musso et al., 2009) the differences found in both Cr and SG (which includes all metabolites excreted in urine) strengthen the hypothesis of an impaired renal function in elderly DS patients. Furthermore, as urinary dilution capability is decreased in aged people (Musso and Oreopoulos, 2011), lower values of urinary Cr and SG in DS patients denote a more diluted urine.

The differences observed in Cr within each population between children and adults could be due partly to differences in lean muscle mass, as it has been reported for healthy populations by Barr et al., 2005.

Correlation coefficients of 0.637 and 0.648 were found between Cr and SG in DS and controls, respectively (Figure 1), showing that the two parameters only partly express the same phenomenon. Since correlation coefficients were similar in DS and control groups mechanisms explaining levels of Cr and SG could be the same or very similar in both populations. Moreover, not significant correlation between Cr and SG was found in the age group I neither in DS nor in controls. This could be due to several factors such as muscle mass, which increases as children grow, being urinary Cr excretion dependent on the muscle mass of the subjects (Remer et al., 2002). Furthermore, significant differences between DS and controls were found in Cr levels but not in SG in the age group III.

Attending to gender, significantly lower values of Cr and SG have been reported in healthy females compared to males (Carrieri et al., 2001; Barr et al., 2005; Suwazono et al., 2005). However, we observed no significant differences with respect to gender in Cr and SG for both populations, although levels of urinary Cr were slightly lower in females in both populations (Table 1).

Other factors may also need attention concerning deviations of variability in urinary Cr. Increase as well as reduction in dietary intake of meat is known to modify urinary Cr levels. Meat contains creatine as well as traces of Cr. Absorbed dietary creatine can be accumulated in the body over time and lead to a gradual increase in Cr excretion (Hoberman et al., 1948). Moreover, cooking meat converts creatine to Cr, so high-meat diet can contribute to increase the urinary levels of Cr. However, in this work no significant differences were found for urinary levels of Cr when weekly frequency of meat intake was analyzed neither in DS nor in controls.

Finally, hypothyroidism is the most frequent thyroid abnormality in DS (Fort et al., 1984), with a frequency in our study of 30%. It is well known that levels of Cr are influenced by thyroid hormones. Thus, hypothyroidism enhances serum Cr levels due to decreased Cr clearance with decreased glomerular filtration rate and increased production of Cr (Lafayette et al., 1994). Previous works reported reduced Cr clearance in non-Down patients with hypothyroidism, but normal values were obtained when they were treated with thyroid hormones (Montenegro et al., 1996). However, we found that DS patients receiving levothyroxine for thyroid dysfunction showed slightly decreased levels of Cr in all age groups, which were significantly lower in the age group III (from 16 to 25 years), than DS patients without diagnosed hypothyroidism. We suggest that factors involving a decrease excretion of Cr in hypothyroidism may be different in DS than in non-Down individuals and could also explain, almost in part, the decreased levels of Cr found in DS patients without diagnosed thyroid disorders. In fact, mild plasma TSH elevation in almost all young DS children has been reported by van Trotsenburg et al. (2005) and symptoms of hypothyroidism are often wrongly attributed to DS itself.

In addition, some abnormalities reported in DS are related to thyroid function: 1) decreased levels of selenium (Nevé et al., 1983) which acts as antioxidant protecting the thyrocyte from peroxides 2) an impairment in the activity of phenylalanine hydroxylase (Shaposhnikov et al., 1979), which converts the phenylalanine in tyrosine, and 3) overexpression of DYRK1A kinase (Dowjat et al., 2007), which could reduces availability of tyrosine. Thus, medical treatment of thyroid dysfunction in DS subjects does not normalize Cr excretion. Since SG levels were also lower in DS patients receiving levothyroxine than DS without medical treatment in the age group III, we suggest that the results obtained could be due to a renal dysfunction caused by hypothyroidism in DS, however further investigation is required to ascertain the mechanisms underlying these results.

In conclusion, our results showed lowered urinary levels of Cr in DS in the age range from 16 to 59 years, as well as lowered SG in the age range from 26 to 59 years. *These decreased levels could be due to the characteristically elevated oxidative stress observed in DS that leads to premature aging also affecting the kidney. Thus, a possible impaired renal function may demand more clinical attention in order to control any other related problems.* Furthermore, decreased Cr levels in DS individuals treated with levothyroxine suggests that hypothyroidism could affect the urinary excretion of Cr in this pathology as the treatment with this medicine seems not to return Cr levels to its normal state in DS. *Moreover, as Cr is used as an adjustment parameter in many biochemical determinations we recommend to take this differences into consideration for DS studies designs. We also recommend further research in renal function of DS individuals by using new urinary biomarkers, like cystatin C and glomerular and tubular damage markers since there is no evidence in current literature.*

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to patients selection. Á.C. was responsible for the study design, oversaw, and finalized the report. This research was supported by Fundación Inocente, Inocente.

Declaration of interest

The authors have no conflict of interests.

References

Alessio L, Berlin A, Dell'Orto A, Toffoletto F, Ghezzi I. (1985). Reliability of urinary creatinine as a parameter used to adjust values of urinary biological indicators. *Int Arch Occup Environ Health* 55:99–106.

Allison DB, Gomez JE, Heshka S, Babbitt RL, Geliebter A, Kreibich K, Heymsfield SB. (1995). Decreased resting metabolic rate among persons with Down Syndrome. *Int J Obes Relat Metab Disord* 19:858–861.

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. (2005). Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect* 113:192–200.

Bittles AH, Bower C, Hussain R, Glasson EJ. (2007). The four ages of Down syndrome. *Eur J Public Health* 17:221–225.

Boeniger MF, Lowry LK, Rosenberg J. (1993). Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. *Am Ind Hyg Assoc J* 54:615–627.

Campos C, Guzmán R, López-Fernández E, Casado A. (2011). Evaluation of urinary biomarkers of oxidative/nitrosative stress in adolescents and adults with Down syndrome. *Biochim Biophys Acta* 1812:760–768.

Carrieri M, Trevisan A, Bartolucci GB. (2001). Adjustment to concentration-dilution of spot urine samples: correlation between specific gravity and creatinine. *Int Arch Occup Environ Health* 74:63–67.

Coburn SP, Seidenberg M, Mertz ET. (1967). Clearance of uric acid, urea, and creatinine in Down's syndrome. *J Appl Physiol* 23:579–580.

Coppus AM, Evenhuis HM, Verberne GJ, Visser FE, Eikelenboom P, van Gool WA, Janssens AC, van Duijn CM. (2010). Early age at menopause is associated with increased risk of dementia and mortality in women with Down syndrome. *J Alzheimers Dis* 19:545–550.

Dowjat WK, Adayev T, Kuchna I, Nowicki K, Palminiello S, Hwang YW, Wegiel J. (2007). Trisomy-driven overexpression of DYRK1A kinase in the brain of subjects with Down syndrome. *Neurosci Lett* 413:77–81.

Down JLH. (1866). Observations on an ethnic classification of idiots. *Clinical Lecture Reports, London Hospital*. 3:259–262.

Fernhall B, Figueroa A, Collier S, Goulopoulou S, Giannopoulou I, Baynard T. (2005). Resting metabolic rate is not reduced in obese adults with Down syndrome. *Ment Retard* 43:391–400.

Forbes GB, Bruining GJ. (1976). Urinary creatinine excretion and lean body mass. *Am J Clin Nutr* 29:1359–1366.

Fort P, Lifshitz F, Bellisario R, Davis J, Lanes R, Pugliese M, Richman R, Post EM, David R. (1984). Abnormalities of thyroid function in infants with Down syndrome. *J Pediatr* 104:545–549.

Gupta SK, Venkataseshan VS, Churg J. (1991). Mesangiocapillary glomerulonephritis in Down's syndrome. *Am J Nephrol* 11:112–117.

Hoberman HD, Sims EA, Peters JH. (1948). Creatine and creatinine metabolism in the normal male adult studied with the aid of isotopic nitrogen. *J Biol Chem* 172:45–58.

Hook EB. (1981). Down's syndrome-frequency in human populations and factors pertinent to variation in rates. In: de la Cruzz FF, Gerald PS, editors. *Trisomy 21 (Down syndrome): research perspectives*. Baltimore: University Park Press; p. 3–68.

Hook EG. (1982). Epidemiology of Down syndrome. In: Pueschel SM, Rynders JE, editors. *Down syndrome Advances in biomedicine and Behavioral Sciences*. Cambridge, Massachusetts: Ware Press.

Jaffé M. (1986). Über den niederschlag, welchen pikrinsäure in normalen harn erzeugt und über eine neue reaktion des kreatinins. *Z Physiol Chem* 10:391–400.

Kavouras SA. (2002). Assessing hydration status. *Curr Opin Clin Nutr Metab Care* 5:519–524.

Keshaviah PR, Nolph KD, Moore HL, Prowant B, Emerson PF, Meyer M, Twardowski ZJ, Khanna R, Ponferrada L, Collins A. (1994). Lean body mass estimation by creatinine kinetics. *J Am Soc Nephrol* 4:1475–1485.

Korsager S, Chatham EM, Ostergaard Kristensen HP. (1978). Thyroid function tests in adults with Down's syndrome. *Acta Endocrinol* 88:48–54.

Lafayette RA, Costa ME, King AJ. (1994). Increased serum creatinine in the absence of renal failure in profound hypothyroidism. *Am J Med* 96:298–299.

Lejeune J, Gautier M, Turpin R. (1959). Etude des chromosomes somatiques de neuf enfants mongoliens. *CR Hebd Acad Sci* 248:1721–1722.

Lott IT, Lai F. (1982). Dementia in Down's syndrome: observations from a neurology clinic. *Appl Res Ment Retard* 3:233–239.

Luke A, Sutton M, Schoeller DA, Roizen NJ. (1996). Nutrient intake and obesity in prepubescent children with Down syndrome. *J Am Diet Assoc* 96:1262–1267.

Málaga S, Pardo R, Málaga I, Orejas G, Fernández-Toral J. (2005). Renal involvement in Down syndrome. *Pediatr Nephrol* 20:614–617.

Mercer ES, Broecker B, Smith EA, Kirsch AJ, Scherz HC, A Massad C. (2004). Urological manifestations of Down syndrome. *J Urol* 171:1250–1253.

Montenegro J, González O, Saracho R, Aguirre R, González O, Martínez I. (1996). Changes in renal function in primary hypothyroidism. *Am J Kidney Dis* 27:195–198.

Musso CG, Macías Nuñez JF, Oreopoulos DG. (2007). Physiological similarities and differences between renal aging and chronic renal disease. *J Nephrol* 20:586–587.

Musso CG, Michelángelo H, Vilas M, Reynaldi J, Martinez B, Algranati L, Macías Núñez JF. (2009). Creatinine reabsorption by the aged kidney. *Int Urol Nephrol* 41:727–731.

Musso CG, Oreopoulos DG. (2011). Aging and physiological changes of the kidneys including changes in glomerular filtration rate. *Nephron Physiol* 119 Suppl 1:p1–p5.

Nève J, Sinet PM, Molle L, Nicole A. (1983). Selenium, zinc and copper in Down's syndrome (trisomy 21): blood levels and relations with glutathione peroxidase and superoxide dismutase. *Clin Chim Acta* 133:209–214.

Nishida Y, Akaoka I, Kobayashi M, Maruki K, Oshima Y. (1979). Renal impairment in urate excretion in patients with Down's syndrome. *J Rheumatol* 6:103–107.

Nyman U, Björk J, Sterner G, Bäck SE, Carlson J, Lindström V, Bakoush O, Grubb A. (2006). Standardization of p-creatinine assays and use of lean body mass allow improved prediction of calculated glomerular filtration rate in adults: a new equation. *Scand J Clin Lab Invest* 66:451–468.

Remer T, Neubert A, Maser-Gluth C. (2002). Anthropometry-based reference values for 24-h urinary creatinine excretion during growth and their use in endocrine and nutritional research. *Am J Clin Nutr* 75:561–569.

Rubin SS, Rimmer JH, Chicoine B, Braddock D, McGuire DE. (1998). Overweight prevalence in persons with Down syndrome. *Ment Retard* 36:175–181.

Shaposhnikov AM, Khal'chitskii SE, Shvarts EI. (1979). [Disorders of phenylalanine and tyrosine metabolism in Down's syndrome]. *Vopr Med Khim* 25:15–19.

Suwazono Y, Akesson A, Alfvén T, Järup L, Vahter M. (2005). Creatinine versus specific gravity-adjusted urinary cadmium concentrations. *Biomarkers* 10:117–126.

Tórsdóttir G, Kristinsson J, Hreidarsson S, Snaedal J, Jóhannesson T. (2001). Copper, ceruloplasmin and superoxide dismutase (SOD1) in patients with Down's syndrome. *Pharmacol Toxicol* 89:320–325.

Van Buggenhout GJ, Trommelen JC, Schoenmaker A, De Bal C, Verbeek JJ, Smeets DF, Ropers HH, Devriendt K, Hamel BC, Fryns JP. (1999). Down syndrome in a population of elderly mentally retarded patients: genetic-diagnostic survey and implications for medical care. *Am J Med Genet* 85:376–384.

van Trotsenburg AS, Vulsma T, van Rozenburg-Marres SL, van Baar AL, Ridder JC, Heymans HS, Tijssen JG, de Vijlder JJ. (2005). The effect of thyroxine treatment started in the neonatal period on development and growth of two-year-old Down syndrome children: a randomized clinical trial. *J Clin Endocrinol Metab* 90:3304–3311.

Varley H, Gowenlock AH, Bell M. 1980. *Non-protein nitrogen, urea, urate, creatine and creatinine. Practical Clinical Biochemistry*. London: William Heinemann Medical Books Ltd.; p. 484–485.

Voinescu GC, Shoemaker M, Moore H, Khanna R, Nolph KD. (2002). The relationship between urine osmolality and specific gravity. *Am J Med Sci* 323:39–42.

Wiggins J. (2011). Why do our kidneys get old? *Nephron Exp Nephrol* 119 Suppl 1:e1–e5.

Wyss M, Kaddurah-Daouk R. (2000). Creatine and creatinine metabolism. *Physiol Rev* 80:1107–1213.