# Improvements in the Ecological and Nutritional Aspects of Down's Syndrome

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**Research**

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# Abstract

**Background and aims:** Down's syndrome patients are suffering from such gastrointestinal and metabolic complications which affect their prolongation for survival and this could be attributed to the malnutrition system. To reduce the risk factors for mortality, the paper has focused on the assessment of the socioeconomic, clinical, physical, biophysiological, and biochemical characteristics of them which can be affected by the type of nutrition system, toxicity, and ecological footprint.

**Methods:** Patients were males with trisomy 21 diagnosed by karyotype test and assessed by clinical examinations. The clinical observations, medical interventions, and oral diseases associated with DS have been defined and oral treatment is explored. Samples collected from different biofluids. The physicochemical analyses of the biomatrix samples were performed and these properties had compared to findings of healthy males and age-matched controls. In specific, trace elements which could be originated from environmental resources were assessed in saliva, blood, urine, and hair.

**Results:** Duraphat application was proved effective for their oral treatment and saliva was the optimum biomarker for detecting malnutrition. The patients were hypersensitive to Cu while the Mn content in blood and hair was considered an expression to the degree of epileptic condition and chronic seizure development. The ecological footprint was 5.6 gha and carbon footprint was recognised in food poverty habits. These can be reduced by eating more plant-based proteins and fibre-rich foods with low saturated fats and sodium.

**Conclusions:** The current findings provide an up-to-date reference for expected developmental outcomes in children with DS in terms of biophysicochemistry. The genetically sensitive intervention is affected by heredity factor and sensitivity to toxics. Down's syndrome is encouraged to live green-hipster life. Besides, doctors are recommended to order the physicochemical analyses for early detection of this special intervention.

**WHO Clinical Trial Registry:** Study ID number: DRKS00014074

Title page of the study: Down's syndrome in children males – biochemical characterisations in different media using non-randomised trial and systematic study

Website of Clinical Trial Registry: http://www.drks.de/drks_web/navigate.do?navigationId=trial.HTML&TRIAL_ID=DRKS00014074


# Background

Down's syndrome (DS) subjects represent an unsolved biological/clinical paradox [1, 2] which could be combined with nutritional diseases [3] and largely affected by the quality of the environment and environmental protection. In an almost unlimited number of pathologic conditions like the reproductive and neuropathology (as the development of neurofibrillary tangles in neurons and the deposition of

amyloid beta-protein), there is no study yet has elucidated the age-related biophysicochemical transformation and its association with the metabolism (incl. alterations in the basal and regulation of energy metabolism) occurred in disability development (DD). For instance, the allowable minerals concentrations and the impacting effects of vitamins and electrolytes on the brain have not been studied in DS. In addition, we do not know yet, how elements can affect the biochemical pathways? Or which can enhance the neurotransmitter system? We think heavy metals are triggered by nutritional system, environmental factors, pathological condition, or alterations in genotype.

With limited resources, Siqueira et al. [4] expected that there is an alteration in the secretory pathways of ducts and acinar cells of salivary glands among DS children due to differences in salivary electrolytes levels. The vast amounts of salivary essential chemical elements can be related to the type of nutritional system including the forms of nutrients. However, no paper has mentioned yet the safe foods and the possible elements that DS needs in meal. Even though, only one team [3] has designed some nutritional formulas for DS without proper justifications.

Still doctors are used to prescribing foods replicate formulas that the advocates of nutrition actually employ; irrespective of their patients' body requirements considering that all the disorders needs are coincidental. In addition, most of doctors do not really consider that trisomy 21 (Fig. 1S) alters the overall metabolism and both the physical and biochemical properties of the biofluids, enough to account for differences in DS pertaining food and the psychology of the patients.

As soon as, nutritional status estimation involving the assessment of the major nutritional categories, environmental effects and anthropometric parameters worth much concerns from scientists, so the current study has investigated the following: birth's distributions among single births and twins and their relations to mother's age, socioeconomic, essential dental indices, clinical observations, physical and biochemical characteristics of DS biosamples which can be affected by the type of nutrition system and toxicity.

For the first time, biophysiochemical assessment in blood, urine, hair, and saliva (whole and parotid) has been performed and compared between DS children and healthy age- and sex- matched individuals. The broad-scale analysis has involved new environmental measurements ever used in intellectual development (ID) or DS reports as full saliva indices, colour, electrical conductivity (EC), salinity, total dissolved solids (TDS), total suspended solids (TSS), turbidity, total nitrogen (TN), major alkaline and alkaline-earth ions, aluminium (Al), silicon (Si), and some heavy metals. Consequently, novel outcomes are presented in this report: (1) Finding new therapies for oral environment, (2) Discriminative tabulation of the best and worst foods for DS hygiene, (3) Defining the maladaptive food choices, and (4) Estimating the associated ecological footprint (EF).

# Materials And Methods

# Study design and clinical trial

Non-randomised controlled trial and systemic study (DRKS00014074) has examined the biophysiochemical characteristics as possible indicators to the nature of DS nutrition system and toxicity. This work was utterly executed in the Faculty of Dental Medicine, Damascus University in 2015–2017.

# Primary and secondary outcomes

Primary outcomes were defined as the changes in biophysiological and biochemical measurements and the primary timepoint was 30-days post commencement of intervention. Samples given for physiochemical analysis were collected from 14–55 days. While secondary outcomes comprised the changes in cognitive and functional scores from baseline to 65-days post commencement of intervention.

# Data collection - Patients selection and categorisation

Exclusion criteria - None of the Controls group had systemic diseases or any local infection before 3 months and did not also take any medication for at least 6 months before sample collection. Also, DS children with congenital oligodontia and delayed eruption (more than 1 year.) or with the history of antibiotics, anticholinergic, antihistaminic and antipsychotic therapy two weeks prior to sample collection were excluded. Treatments with vitamin D, aspirin, or herbal medicine were stopped before a month of sample collection.

Inclusion criteria - Participants (Males; DS: $N$ = 71, age: 2–15 yrs., mean age ($\mu_{age}$) ± standard deviation (σ): 8.6 ± 5.9 yrs.; Controls: $N$ = 74, age: 2–15 yrs., 8.2 ± 4.15 yrs.) had been chosen on the basis that all of them were permanent residences in Damascus and living far from plaster dust sources of any possible adjacent construction sites, far from the industrial zone (incl. concrete plants) and they were non-smokers (all kinds of tobacco) from non-smoker families. As a biomarker of exposure to tobacco smoke components and fibrosis transmembrane regulator function, thiocyanate ions in saliva and serum were determined (Table 7S). Candidates with concentrations of thiocyanate higher than 80 mg $SCN^-$/L (1 mg $SCN^-$/L = 17.2 μM $SCN^-$/L) saliva or 3.00 mg $SCN^-$/L serum were excluded from the study. The whole procedure was reported as consensus findings including further recommendations of the National Committee of Environmental Health Sciences (NCEHS) expert panel. Patients were euthyroid at the time of study as their thyroid-stimulating hormone (TSH) and free thyroxine hormone (FT4) were within the normal range.

The important conditions of inclusion criteria were highlighted in the patient's medical record. Parents, caregivers, and doctors (paediatrics and gynaecologists) of DS participants and their mothers (since pregnancy) were interviewed (SI; Ps. 21 and 22). Copies of paediatric reports of children (since birthhood) and the gynaecology reports of the mothers before giving births and after giving births were gathered and totally reviewed.

Baseline data, gathered at the start of the study have included standard demographic data, information on nutrition system and the nutrition therapy (if any). So, the developmental and family history, clinical observation, physiology, symptomology, medical (such as oral, mental, maladaptive behaviour, and challenging behaviour) remarks and the indicators to malnutrition were studied and saved in the Supplementary Information (SI). The EF was assessed by $CO_2$ Rechner.

# Questionnaire and anthropometry

The questionnaires (SI; Ps. 18–20) were designed and the anthropometric study (incl. methods and measurements) was screened, discussed and saved in the SI. The socio-economic data were determined with the use of Hollingshead Four-Factor Index of Social Status. The physical activity (PhA)-Questionnaire section of the National Health and Nutrition Examination Survey (NHANES) III was adapted to assess the participants' regular PhA habits. The intensity of each specific activity was estimated using the Ainsworth Compendium for Physical Activities. Moderate to vigorous physical activity (MVPhA) was defined as any PhA $\geq$ 3.5 METs (SI, P. 42). The Block Screening Questionnaire for Fat Intake was implemented to calculate a dietary fat score and estimate the percentage of dietary fat intake of the total dietary intake. The Behavioural Risk Factor Surveillance System, Fruit and Vegetable Module, was used to calculate the fruit and vegetable score and assess the mean number of fruits and vegetables eaten per day. Questionnaire scoring procedures used were those recommended in the Dietary Assessment Resource Manual. The food frequency questionnaires were administered as an interview with the participant and the participant's direct care provider present to assist with the questions as needed. Each variable was screened for missing data, outliers, and normal distribution. The results were tabulated.

The physical and anthropometry methods used were presented in SI (Ps. 19, 44, and 45).

# Sample collection

Patients' dental indices (SI; Ps. 24 and 25) in addition to the physical and biochemical analyses (incl. novel analyses) are compared with the corresponding data of samples of controls.

Each participant (DS and healthy) gave at least 40 mL, 10 mL, and 5 mL of saliva (SI; Ps. 45–50), blood and serum (SI; Ps. 50–52), and urine (SI; P. 52) in sequence, in addition to 0.1 g hair (SI; P. 53). The implemented analytical techniques and instrumentations used in this paper were saved in Table 7S.

Further, sweat samples were gathered from every participant to measure the salinity [6].

Blood test for leukaemia was completed by giving a sample of 1 mL. The following tests were assayed: white blood cells (WBCs), haemoglobin (Hb), packed-cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mercury (Hg). The diffusion of cytoplasmic stains of monocytes and macrophages of the positive results of tests were scanned under magnification × 540.

To check if Al is a genetic or environmental factor in DS, we checked the possible sources of pollution with this element in their areas of living and we tried to find any possible correlations between biological Al in DS and mother's age. In addition, we asked parents of children having twenty higher values of Al to contact the relatives of children inviting them to participate in this study by giving us saliva samples (at least 1 mL for each) to test Al. The number of those new participants reached 67.

The mineral contents in biofluids can be compared with previous studies saved in the Supplementary Material files (SM-A and SM-B).

# Safety considerations

Samples handled as potentially infectious and general guidelines for work with acids had been respected.

# Meal challenge

On study days, the micronutrient and macronutrient composition of most popular foods consumed by DS participants like bread, milk, eggs, chicken, veal, canned tuna, canned sardines and other canned foods as diced tomato, soup, potatoes, pumpkin, and fruit are tested and processed using the professional software Nutrilog (Marans, France). Then, the statistical correlations between mineral composition in biosamples and food were defined.

# Drugs and treatments

Every DS patient had a panoramic radiograph and oral assessment was performed. Diazepam was used as a drug for sedation on few occasions that this required. Topical applications of stannous ($SnF_2$, 10 mM) or acidulated phosphate fluoride solution (NaF-$H_3PO_4$, 0.5–1.23%) either a professional prophylaxis using a very fine cleaning agent or supervised self-cleaning were achieved (SI; Ps. 30–32). Enhanced feeding list for oral health improvement was tabulated. Patients with hypothyroidism had given Eltroxin, 50–200 mg/day. The degree of hypothyroidism has been estimated by the ratio of saliva $I^-$ : plasma $I^-$, the lower is the worse.

# Statistical analysis

Mann-Whitney U test used to statistically compare the groups, with significant set at $P$ 0.05. Statistical analysis was performed with SPSS, version 16.0.2 (SPSS Benelux BV, Gorinchem, The Netherlands) and Evalkit, version 3.1 (Tilburg, The Netherlands) that used for regression procedure according to Bablock and Passing.

# Patient involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for design or implementation of the study. No patients were asked to advice on interpretation or writing up of results. And now, there are no plans to disseminate the results of the research to study participants or the relevant patient community.

# Code availability

Code for food data analyses involving micronutrients and macronutrients is provided in the Nutrients Availability data repository (https://github.com/Stevensbrightmann/Nutrients.git). Other analysis codes that support the findings of the study are available from the corresponding author on reasonable request.

## Results

# Oral health - a reference to malnutrition

Has conventional food any effects on the oral conditions of DS? The present study showed that children with DS had poor oral hygiene and gingival health and much greater prevalence of periodontal diseases than controls (SI; Ps. 26–28). There were no significant differences ($P$ 0.01) in sulcus bleeding index (SBI) scores between groups, irrespective of dentition. The Quigley-Hein index (QHI) of bacterial plaque was increased by 20.6% in DS children more than controls (Table 2S), referring to periodontal problems and microbial inflammatory diseases induced by bacterial plaque. The periodontal destructions are characterised by the formation of deep periodontal pockets, associated to increased quantities of bacterial plaque and intense gingival inflammation. Oral ulceration (SI; P. 26 and Table 2S) was observed also in DS, which was painful and caused significant difficulties in eating and as a result could contribute to slow healing (SI; Ps. 40–42, and Table 5S). At its worst, this caused significant difficulties in eating and drinking (SI; Ps. 41 and 42) which refer to the need of special diet (SI; P. 77 and Table 3S).

# Saliva physiochemical characteristics

The structural characterisations of whole saliva and parotid saliva such as ash weight, SFR, $CO_2$, glucose, total protein, TN, total phosphorous (TP), IgA secretory, $SCN^-$, $CN^-$, EC, TDS, salinity, turbidity, colour, viscosity, and surface tension were presented in Tables 1 and 2, respectively.

Table 1
Structural characterisation of whole saliva in Down's syndrome (Level 2) compared to controls (Level 1); mean ($\mu$) ± standard deviation ($\sigma$); repetition ($n$) = 5

| Level | Ash wt., mg/dL | SFR, mL/min | $CO_2$, mg/dL | Glucose, mg/dL | Total protein, g/L | TN, mg/dL [(a)] | TP, mg/dL |
|---|---|---|---|---|---|---|---|
| 1 | 238 ± 32 | 0.46 ± 0.17 | 34.0 ± 12.0 | 0.92 ± 0.26 | 0.94 ± 0.28 | 72.5 ± 12.1 | 82.4 ± 16.7 |
| 2 | 465 ± 63 | 0.22 ± 0.10 | 15.7 ± 7.87 | 2.34 ± 1.02 | 3.67 ± 0.91 | 68.3 ± 23.9 | 155 ± 42.0 |
| *P*-value | 0.012 | 0.006 | 0.005 | 0.018 | 0.001 | 0.013 | 0.001 |

Level 1: IgA secretory [142–159 mg/dL], $SCN^-$ [17.7–60.5 mg $SCN^-$/L] (in serum: 0.65–2.28 mg $SCN^-$/L), $CN^-$ [0.09–0.63 mg $CN^-$/L], $Cl^-$ [540–696 mg $Cl^-$/L], EC [4,600-5,400 µS/cm], TDS [3270–4865 mg/L], Salinity [0.662–1.490 g/L], Turbidity [0.9–4.4 NTU], Colour [2–4 Hazen units], Viscosity (η) [1.19–1.35 cP], and Surface tension (γ) [46.7–48.2 mN/m]

Level 2: IgA secretory [84–116 mg/dL], $SCN^-$ [16.8–57.1 mg $SCN^-$/L] (in serum: 0.63–2.15 mg $SCN^-$/L), $CN^-$ [0.07–0.42 mg $CN^-$/L], $Cl^-$ [623–968 mg $Cl^-$/L], EC [9,800 – 12,425 µS/cm], TDS [5419–8102 mg/L], Salinity [1.152–2.619 g/L], Turbidity [6.4–9.5 NTU], Colour [6–8 Hazen units], η [1.01–1.12 cP], γ [32.1–36.9 mN/m], and *Mutans streptococci* colonies (66.2%) showed high values of CFU/mL ( 1,000,000 *S. mutans*)

Lower limit of detection (LOD) values for TN, TP, $SCN^-$, $CN^-$, EC, TDS, Salinity, Turbidity, and Colour are: 0.001 mg/dL, 0.01 µg/mL, 0.08 mg/L, 0.01 mg/L, 0.74 µS/cm, 9.14 mg/L, 2.80 mg/L, 0.056 NTU, and 0.17 Hazen units, respectively

Differences of distributions in the two groups (patient-control) are presented as critical values for Mann-Whitney U test; level of significance: 5% ($P$ = 0.05)

[(a)] N-non protein (Controls: 11.8 ± 3.57 mg/dL and DS: 10.5 ± 2.20 mg/dL)

Table 2
Structural characterisation of parotid saliva in Down's syndrome (Level 2) compared to controls (Level 1); $\mu \pm \sigma$; $n = 5$

| Level | Ash wt., mg/dL | SFR, mL/min | $CO_2$, mg/dL | Glucose, mg/dL | Total protein, g/L | TN, mg/dL[(b)] | TP, µg/mL |
|---|---|---|---|---|---|---|---|
| 1 | 99 ± 15 | 0.09 ± 0.02 | 40.2 ± 16.4 | 0.17 ± 0.05 | 0.11 ± 0.07 | 62.4 ± 18.1 | 48.1 ± 0.13 |
| 2 | 235 ± 39 | 0.03 ± 0.01 | 48.0 ± 19.0 | 0.42 ± 0.16 | 0.19 ± 0.12 | 54.1 ± 16.5 | 33.8 ± 18.4 |
| *P*-value | 0.009 | 0.001 | 0.001 | 0.002 | 0.001 | 0.003 | 0.006 |

Level 1: $SCN^-$ [14.1–51.3 mg $SCN^-$/L], $CN^-$ [0.07–0.58 mg $CN^-$/L], $Cl^-$ [479–682 mg $Cl^-$/L], EC [810–1750 µS/cm], TDS [1330–1907 mg/L], Salinity [0.264–0.582 g/L], Turbidity [0.3–1.8 NTU], Colour [1–2 Hazen units], η [0.98–1.03 cP], and γ [38.5–41.1 mN/m]

Level 2: $SCN^-$ [13.8–47.7 mg $SCN^-$/L], $CN^-$ [0.06–0.31 mg $CN^-$/L], $Cl^-$ [583–908 mg $Cl^-$/L], EC [2100–2900 µS/cm], TDS [2201–3321 mg/L], Salinity [0.472–1.069 g/L], Turbidity [6.4–9.5 NTU], Colour [6–8 Hazen units], η [0.76–0.85 cP], and γ [25.9–30.2 mN/m]

LODs for TN, TP, $SCN^-$, $CN^-$, EC, TDS, Salinity, Turbidity, and Colour are: 0.001 mg/dL, 0.01 µg/mL, 0.08 mg/L, 0.01 mg/L, 0.74 µS/cm, 9.14 mg/L, 2.80 mg/L, 0.056 NTU, and 0.17 Hazen units, respectively

(b) N-non protein (Controls: 49.0 ± 12.6 mg/dL, DS: 43.9 ± 10.6 mg/dL)

Differences of distributions in the two groups (patient-control) are presented as critical values for Mann-Whitney U test; level of significance: 5% ($P = 0.05$)

Further, pH, alkaline and alkaline-earth elements of whole saliva and parotid saliva were viewed in Tables 3 and 4, respectively.

Table 3
pH, alkaline and alkaline-earth elements of whole saliva in Down's syndrome (Level 2) compared with controls (Level 1); $\mu \pm \sigma$; $n = 5$

| Level | pH [c] | Na, µg/Ml | K, µg/mL | Mg, µg/mL | Ca, µg/mL | Ba, µg/L | Sr, µg/L |
|---|---|---|---|---|---|---|---|
| 1 | 6.95 ± 0.72 | 87 ± 23.4 | 1142 ± 158 | 5.17 ± 0.46 | 73.9 ± 23.6 | 3.41 ± 0.88 | 6.69 ± 2.11 |
| 2 | 7.72 ± 0.85 | 154 ± 47 | 810 ± 112 | 12.0 ± 2.3 [d] | 152.0 ± 34.2 [d] | 68.2 ± 9.70 | 22.6 ± 5.38 |
| *P*-value | 0.002 | 0.008 | 0.046 | 0.005 | 0.002 | 0.007 | 0.006 |
| $r^2$ | | 0.9985 | 0.9999 | 0.9990 | 0.9990 | 0.9998 | 0.9999 |
| *RSD* | | 3.18 | 0.82 | 2.40 | 3.60 | 5.64 | 1.47 |
| LOD | 0.1 | 0.12 | 0.14 | 1.22 | 0.84 | 0.10 | 0.50 |
| LOQ | 0.3 | 0.39 | 0.43 | 4.50 | 6.10 | 0.37 | 1.43 |
| L | | 1.02–5.75 | 1.07-50.0 | 5.10–24.3 | 6.20–59.0 | 0.41–229 | 2.06–237 |
| R | | 102.2 | 98.4 | 95.7 | 94.7 | 96.2 | 98.4 |

(c) Plague pH (controls): 5.76 ± 0.32; Plague pH (DS): 7.03 ± 0.58 ($t = 2.94$, $P = 0.001$)

(d) DS Ca and Mg were decreased in hair 42% and 27%, respectively compared with controls

Limit of quantitation (LOQ); Linearity (L); Recovery (R, %)

Differences of distributions in the two groups (patient-control) are presented as critical values for Mann-Whitney U test; level of significance: 5% ($P = 0.05$)

Table 4
pH, alkaline and alkaline-earth elements of parotid saliva in Down's syndrome (Level 2) compared with controls (Level 1); $\mu \pm \sigma$; $n = 5$

| Level | pH | Na, µg/Ml | K, µg/mL | Mg, µg/mL | Ca, µg/mL | Ba, µg/L | Sr, µg/L |
|---|---|---|---|---|---|---|---|
| 1 | 6.52 ± 0.68 | 105 ± 32.2 | 973 ± 125 | 3.15 ± 0.11 | 26.3 ± 10.6 | 12.7 ± 0.98 | 5.63 ± 1.47 |
| 2 | 7.35 ± 0.81 | 197 ± 64.0 | 687 ± 104 | 4.01 ± 0.72 | 64.0 ± 7.90 | 26.3 ± 3.96 | 17.9 ± 5.80 |
| *P*-value | 0.002 | 0.005 | 0.027 | 0.001 | 0.001 | 0.002 | 0.003 |

Differences of distributions in the two groups (patient-control) are presented as critical values for Mann-Whitney U test; level of significance: 5% ($P = 0.05$)

Heavy metals, aluminium and silicon in whole saliva and parotid saliva were obtained in Tables 5 and 6, respectively.

Table 5
Heavy metals, aluminium and silicon in whole saliva of patients with Down's syndrome (Level 2) compared with controls (Level 1); $\mu \pm \sigma$; concentrations expressed as µg/L; $n = 5$

| Level | Mo | Mn | Cr | Ti | Cu | Zn | Fe | Al | Si |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.29 ± 0.47 | 8.41 ± 2.13 | ≤ LOQ | ≤ LOQ | 484 ± 108 | 674 ± 119 | 629 ± 160 | ≤ LOQ | 108 ± 49 |
| 2 | 1.20 ± 0.53 | 3.59 ± 0.53 | 36.2 ± 14.8 | ≤ LOQ | 936 ± 246 | 54.6 ± 21.4 | 550 ± 78 | **1140 ± 178** | 160 ± 65 |
| *P*-value | 0.001 | 0.001 | 0.001 | 0.017 | 0.002 | 0.001 | 0.005 | - | 0.073 |
| $r^2$ | 0.9973 | 0.9982 | 0.9996 | 0.9973 | 0.9998 | 0.9994 | 0.9989 | 0.9965 | 0.9962 |
| *RSD* | 7.24 | 3.32 | 2.63 | 1.17 | 2.39 | 0.98 | 1.13 | 7.51 | 9.06 |
| LOD | 0.12 | 0.10 | 1.22 | 0.69 | 3.37 | 0.50 | 0.70 | 1.08 | 22.4 |
| LOQ | 0.37 | 0.31 | 4.15 | 2.34 | 11.5 | 1.70 | 2.30 | 3.55 | 74.8 |
| L | 0.50–60.0 | 0.65-37.0 | 5.20–16.8 | 2.55–87.4 | 13.2–110 | 3.07–42.8 | 5.00-108 | 18.4–193 | 93.7–224 |
| R | 95.2 | 93.7 | 99.4 | 102.6 | 97.8 | 101.4 | 100.8 | 102.0 | 107.3 |

Differences of distributions in the two groups (patient-control) are presented as critical values for Mann-Whitney U test; level of significance: 5% ($P = 0.05$)

Bovine Liver SRM 1577b, mussel tissue SRM 2976, poplar leaves GBW07604 and freeze-dried urine SRM 2670a were used in the assessments of *RSD* and R of the following elements in this order: Fe, Ti, Si, and some metals (Mo, Mn, Cr, Cu, Zn, and Al), respectively

LOD and LOQ were calculated from the standard deviations (σ) of the response for 1% $HNO_3$ using criteria of three times the standard deviation and ten times the standard deviation, respectively [5, 6]

Table 6
Heavy metals, aluminium, and silicon in parotid saliva from patients with Down's syndrome compared to controls; $\mu \pm \sigma$; concentrations expressed as µg/L; $n = 5$

| Level | Mo | Mn | Cr | Ti | Cu | Zn | Fe | Al | Si |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.08 ± 0.32 | 6.15 ± 1.28 | ≤ LOQ | ≤ LOQ | 127 ± 44.0 | 130 ± 29 | 69 ± 13.4 | ≤ LOQ | 90 ± 21 |
| 2 | 0.94 ± 0.10 | 1.52 ± 0.16 | 26.2 ± 9.43 | ≤ LOQ | 560 ± 81 | 47.3 ± 8.57 | 24.7 ± 3.48 | **285 ± 40.2** | ≤ LOQ |
| *P*-value | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

# Chemical characteristics of blood, urine, and hair

With limited studies of the extent of electrolytes in biological media other than saliva (especially in DS), we analysed the contents of Al, Si, and heavy metals in blood, urine, and hair (Table 7; supplemented data kept in Table 9S).

Table 7
Heavy metals, aluminium and silicon levels in biological samples from the study population ($n$ = 5)

| Level | Mo | Mn | Cr | Ti | Cu | Zn | Fe | Al | Si |
|---|---|---|---|---|---|---|---|---|---|
| Blood (µg/L) [e] | | | | | Blood (µg/mL) | | | | |
| 1 | 0.59 ± 0.14 | 3.74 ± 1.19 | ≤ LOQ | ≤ LOQ | 0.39 ± 0.18 | 0.88 ± 0.13 | 0.42 ± 0.19 | ≤ LOQ | ≤ LOQ |
| 2 | 0.56 ± 0.17 | 0.46 ± 0.71 | 50.1 ± 13.1 | ≤ LOQ | 1.70 ± 0.53 | 0.25 ± 0.12 | 0.20 ± 0.03 | 1.14 ± 0.47 | 0.54 ± 0.12 |
| 95th | 0.56–0.57 | 0.45–3.11 | 0.01–4.04 | - | 0.43–1.62 | 0.25–0.81 | 0.11–0.38 | 0.01–0.86 | 0.01–0.30 |
| Urine (µg/g creatinine) [f–j] | | | | | Urine (µg/mg creatinine) | | | | |
| 1 | 0.26 ± 0.14 | 2.56 ± 0.75 | 3.4 ± 0.90 | ≤ LOQ | 36.4 ± 9.72 | 0.32 ± 0.19 | 0.19 ± 0.07 | 1.24 ± 0.48 | 0.39 ± 0.12 |
| 2 | 0.27 ± 0.19 | 8.64 ± 1.38 | 0.97 ± 0.73 | ≤ LOQ | 0.76 ± 0.68 | 0.77 ± 0.14 | 37.6 ± 12.1 | 0.17 ± 0.03 | 0.15 ± 0.02 |
| 95th | 0.26–0.27 | 2.72–7.95 | 0.80–3.26 | - | 0.97–15.8 | 0.41–0.72 | 0.23–28.9 | 0.19–1.21 | 0.15–0.34 |
| Hair (µg/g) | | | | | Hair (µg/mg) | | | | |
| 1 | 1.82 ± 0.10 | 5.07 ± 3.22 | 3.07 ± 0.09 | ≤ LOQ | 2.01 ± 0.64 | 0.28 ± 0.08 | 16.2 ± 3.98 | 0.56 ± 0.04 | 0.11 ± 0.07 |
| 2 | 1.75 ± 0.49 | 0.85 ± 0.75 | 70.8 ± 13.6 | ≤ LOQ | 64.5 ± 9.31 | 0.09 ± 0.01 | 2.46 ± 0.79 | 0.43 ± 0.12 | 8.46 ± 5.17 |
| 95th | 1.75–1.80 | 0.04–7.05 | 0.10–14.3 | - | 1.29–20.8 | 0.01–0.36 | 4.35–14.9 | 0.44–0.51 | 0.11–8.34 |

| Level | Mo | Mn | Cr | Ti | Cu | Zn | Fe | Al | Si |
|---|---|---|---|---|---|---|---|---|---|
| Level 1: Controls (*N* = 74), Level 2: DS (*N* = 71); 5–95th percentiles: 95th | | | | | | | | | |
| (e) pH (controls): 7.32 ± 0.89; pH (DS): 6.80 ± 0.74 (*t* = 2.29, *P* = 0.003) | | | | | | | | | |
| (f) pH (controls): 6.43 ± 0.66; pH (DS): 7.16 ± 0.75 (*t* = 2.46, *P* = 0.015) | | | | | | | | | |
| (g) Urinary concentrations of heavy metals were adjusted for creatinine levels to reduce inter-individual variation of the measurements | | | | | | | | | |
| (h) Hydroxyproline (OHP)/Creat. (DS: 0.023 ± 0.003 mg/mg, Controls: 0.012 ± 0.002 mg/mg, *P* = 0.000); Fasting urinary OHP and creatinine (Creat.) were assayed by an OHP kit and Jaffe's colorimetric method, correspondingly | | | | | | | | | |
| (i) Ca/Creat. (DS: 0.050 ± 0.010 mg/mg, Controls: 0.043 ± 0.004 mg/mg; *P* = 0.000) | | | | | | | | | |
| (j) No acute kidney injury (AKI) observed and no thrombotic thrombocytopenic purpura (TTP) registered – But still a wealth of secrets on the mechanisms involved about the gamut of electrolytes of DS urine is worthy further investigations which will enhance renal physiology. So that, we suggest studying a pathophysiological characterisation of DS patients in order to enable next generation scientists to target mechanistically the right drug and best meal for DS "a potential sponge-toxics" body, at the right time | | | | | | | | | |

Major possible sources of the sharing elements in this study and their normal levels in healthy men are presented in Table 12S.

# Previous trials – Non-controlled designed nutritional formulas

Thiel and Fowkes [3] were designed a nutritional formula for DS patients and this capsulated: Vitamin A (Retinol: $C_{20}H_{30}O$), vitamin B1 (thiamine: $C_{12}H_{17}N_4OS^+$), folate (folic acid and vitamin B9: $C_{19}H_{19}N_7O_6$), vitamin B12 (cobalamin: $C_{63}H_{88}CoN_{14}O_{14}P$), vitamin C (L-ascorbic acid: $C_6H_8O_6$), iron (Fe), magnesium (Mg), manganese (Mn), zinc (Zn), carnitine (β-hydroxy-γ-*N*-trimethylaminobutyric acid), 3-hydroxy-4-*N*,*N*,*N*-trimethylaminobutyrate: $C_7H_{15}NO_3$), carnosine (β-alanyl-L-histidine: $C_9H_{14}N_4O_3$), choline (2-hydroxy-N,N,N-trimethylethan-1-aminium: $C_5H_{14}NO^+$), and serine (2-amino-3-hydroxypropanoic: $C_3H_7NO_3$). The same team had also suggested adding excess amounts of copper (Cu), cysteine (2-amino-3-sulphhydrylpropanoic acid: $C_3H_7NO_2S$), and superoxide dismutase enzyme (SOD, EC 1.15.1.1, which is already high in DS!) for no justified reasons. This team had also suggested adding excess amounts of Cu and cysteine, with no explanation or any justification of this formula.

DS good health is directly linked to the behaviour which is affected by the use of nutritional supplements, vitamins, minerals, and environmental quality. However, the vitamin-mineral regimens supposedly work regardless of the cause of the brain dysfunction. So, it is hard to believe the suggested regimens in this section can have such a general beneficial effect on brain or are related to the intelligence. Therefore, environmental factors had received our attention in this work.

# Macronutrients consumption

The biochemical analyses in the preliminary stage (6-months before starting the current work) have showed a well-absorption of the biochemical contents of the pills and capsules. Nonetheless, no adverse clinical or biochemical effects were found. Conventional nutritional supplements have not enhanced any specific abilities that contribute to overall global intelligence. Thus, the design of the current experiments in this work has avoided thyroid medication.

The DS consumption of macronutrients were assessed and compared with the recommended dietary intakes in Table 8.

Table 8
Dietary macro-nutriment consumption compared with the recommended dietary intake

| Nutrient | $\mu \pm \sigma$ | Recommended Dietary Intake |
|---|---|---|
| Protein (g) [k,l] | 89 ± 3.03 | - |
| Lipid (g) | 75 ± 3.31 | - |
| CH (g) | 305 ± 8.24 | - |
| Vitamin D (µg) | 6.31 ± 0.97 | 15.0 |
| Ca (mg) | 592 ± 184 | 1200 |
| K (mg) | 2350 ± 506 | 4700 |
| Na (mg) | 5218 ± 813 | 1500 |
| P (mg) | 1103 ± 169 | 1250 |
| (k) The rise in protein supply is associated with an increase in total energy intake (TEI) | | |
| (l) It is optional to elevate protein intake from 1.5 g/kg/day to at least 2.0-2.5 g/kg/day [7] | | |

Table 8 displayed the quantitative and qualitative daily intakes of macronutrients, bone-related vitamin D, and basic minerals. Fat, protein, and CH intakes were not adequate. The protein loss could be assigned to the blisters which affect the protein synthesis for tissue repair and inflammatory processes. Fats are beneficial in this perspective, as volume-for-volume they deliver more than twice the calories of proteins and CH. A positive correlation between DS- total body fat (TBF) mass and CH tolerance was observed.

# Statistical correlations between DS mineral composition and food

The statistical correlations between basic consumed foods and minerals composition in DS biological material are presented in Table 9. These can prevent DS adverse health effects.

Table 9
Spearman correlation of metals concentrations in different biological samples of DS with food consumption

| Element | Sample | Bread | Milk (m) | Eggs | Chicken | Veal | Canned tuna | Canned sardines | Other canned food |
|---|---|---|---|---|---|---|---|---|---|
| Mo | Saliva | | 0.227 | | | | | | |
| | Blood | | | 0.183 | | | | | |
| | Urine | | | | | | | | 0.129 |
| | Hair | | | | | | | | |
| Mn | Saliva | 0.119 | | 0.102 | | | | | -0.164 |
| | Blood | | | | 0.135(n) | | | | |
| | Urine | | | | | | 0.150(n) | 0.137(n) | |
| | Hair | | | | | 0.107 | 0.162 | 0.185 | |
| Cr | Saliva | 0.186 | | 0.220 | 0.151 | | | | -0.130 |
| | Blood | | | | | | | | |
| | Urine | | | | | | | | 0.118 |
| | Hair | | | | | 0.129 | | | |
| Ti | Saliva | | | | | | | | |
| | Blood | | | | | | | | |
| | Urine | | | | | | | | |
| | Hair | | | | | | | | |
| Cu | Saliva | 0.121 | | | | | 0.140 | 0.146 | 0.135 |
| | Blood | | | | | | | | |
| | Urine | | | | | | | | |
| | Hair | | | | | | 0.131 | | |
| Zn | Saliva | 0.177 | 0.162 | | | | | | |
| | Blood | | | | 0.276(n) | | | | |

| Element | Sample | Bread | Milk (m) | Eggs | Chicken | Veal | Canned tuna | Canned sardines | Other canned food |
|---|---|---|---|---|---|---|---|---|---|
| | Urine | | | | | -0.171 | | 0.120(n) | |
| | Hair | | | | | | 0.248 | | |
| Fe | Saliva | 0.165 | | 0.189 | | | | | |
| | Blood | 0.262 | | 0.414 | | | 0.207 | | |
| | Urine | | | | | | | | -0.253 |
| | Hair | | | | 0.140 | | | | |
| Al | Saliva | | | | | | 0.294 | | -0.167 |
| | Blood | | | | | | | | 0.121 |
| | Urine | | | | | | | | |
| | Hair | | | | | | | | |
| Si | Saliva | 0.144 | | | | | | | |
| | Blood | | | | | | | | |
| | Urine | | | | | | | | |
| | Hair | | | | | | | | |

(m) Interestingly, patients were taken milk-based Ca for increasing the bioavailability of Ca due to the presence of other nutrients such as proteins fragments as seen in Table 8 and non-protein nitrogen which can increase bone modelling

(n) $P < 0.01$

Blank cells indicate lack of statistically significant correlation. $P < 0.05$ if the cell has not assigned with (n)

Nutritional status may have an effect on the biological content of minerals. The associations of the elements in blood, urine, and hair with basic meals are presented in the following series (Table 9): Cr = Cu > Mn > Al = Fe = Zn > Mo = Si > Ti, Mn = Zn > Cr = Fe = Mo > Al = Cu = Si = Ti, Mn > Cr = Cu = Fe = Zn > Al = Mo = Si = Ti, respectively. Toxicokinetic differences among the studied biological samples may account for the lack of correlations found for the selected metals in these matrixes. Blood and urine levels have reflected recent exposures especially of Cr, Cu, Al, and Si, in addition to, Mo, Mn, Zn, and Fe, correspondingly, contrary to hair content particularly of Cr, Cu, and Si which may refer to past exposures.

Table 9 has also presented different associations between saliva and its metallic content and both bread and eggs. These associations were expressed in the following series: Cr = Cu > Mn > Al = Fe = Zn > Mo = Si

> Ti. Hence, saliva can be defined as a primer bioindicator for malnutrition.

# Challenges in in vitro findings

*Turbidity of saliva* – In DS saliva, *streptococcus pneumonia* and *haemophilus influenza* had been observed in both whole saliva and parotid saliva and their media were more turbid than normal subject's saliva. Turbidity of saliva was significantly correlated to C-reactive protein (CRP) (Table 9S; $r = 0.95$, $P < 0.001$). As a suggestion, trans fats should be avoided (SI; P. 77).

*Salinity of saliva and sweat* – DS patients achieved higher salinity in saliva than controls (Tables 1 and 2), which is caused due to the recurrent chronic idiopathic of the salivary glands. It is also dependent upon functional derangement of the digestive apparatus. Higher DS saline saliva is associated with the deficient (or depraved appetite), a thick yellow or brownish fur, nausea, pain and heaviness in the right side (clearly, referring to liver problem), thirst, constipation, headache, confused vision, and singing in the ears. These new observations guided us to estimate for the first time the salt content in DS sweat and comparing these findings with the corresponding values of the controls. Using pilocarpine ($C_{11}H_{16}N_2O_2$) as sweat stimulant, the osmolality of DS was larger (214 mM/kg) than these for controls (85 mM/kg). This means that DS body fluids are roughly more saline than normal individuals, which could be due to metabolic imbalance.

*Viscosity of saliva* – This may influence the development of caries, that was significantly lower in DS children saliva than in controls group (Tables 1 and 2).

*Saliva CO $_2$* – The whole saliva concentrations of $CO_2$ were significantly ($P < 0.05$) lower in DS than controls, however, the situation was insignificantly reversed in parotid saliva attributed to the large range. Moreover, the whole and parotid saliva mean concentrations of $CO_2$ were not varied enormously. Expectedly, $CO_2$ concentrations were directly correlated with salivary pH levels. Carbonic acid ($H_2CO_3$) formed of $CO_2$ in breath in salivary water is a key mediator in mineralisation. Initially, it would dissolve food and enamel mineral but also break down and readily release the same. Moreover, the $CO_2$ and water may enter the salivary glands either from the blood or may be formed in the glands by aerobic respiration [8]. Subsequently, the $H^+$ ions are conveyed to the blood while $Na^+$ ions from the blood are transferred to the salivary glands and secreted with the $HCO_3^-$ ions. This series of events may be the mechanism accounting for the rise in pH, $Na^+$, and $HCO_3^-$ levels ($HCO_3^-$ concentrations were not presented in the current paper) with decreases in secretion rate. Thus, an increase in carbonic anhydrase (CA, EC 4.2.1.1) activity could be the factor responsible for the electrolyte increase.

*SFR and glucose* – SFR was found directly proportional to $CO_2$ levels in this study. DS subjects secreted substantially lower overall salivary constituents into the oral cavity due to the slow SFR. However, the low level of SFR in parotid saliva may primarily refer to detective secretion from the submandibular, lingual or mucus glands. Besides, the low SFR in DS whole saliva suggested a reduction in clearance of sugar (DS

glucose was 2.5 times that of controls; Tables 1 and 2) may attributed to the PhA (1.24 ± 0.22 h/day) and increasing the risk of oral disease (SI; Ps. 26–29, 67 and Table 2S). Remarkably, we also found diabetes among the relatives of DS individuals, which refers to a genetic linkage that could be existed between the tendency for nondisjunction during meiosis (induced by Hg, lead (Pb), and tin (Sn); data not shown in this paper) and the tendency to develop diabetes.

*Saliva total proteins* – Salivary proteins have many functions, among them, the bacterial aggregation, oxidation of hydrogen peroxide ($H_2O_2$), antiviral, antimicrobial, and antifungal activity. A total protein was significantly increased in DS whole saliva and parotid saliva than in controls. Total protein was found to correlate positively and strongly highly significant with plaque index (PI) ($r = 0.98$, $P < 0.001$) and gingival index (GI) ($r = 0.96$, $P < 0.001$). These correlations proved that total proteins had collaborated so far in the inhibition of mineral precipitation and remineralisation.

*TP of saliva* – To the best of our knowledge, the factors which regulate the hydroxyapatite (HAP: $Ca_5(PO_4)_3(OH)$) balance are free calcium and phosphate ions. Phosphorous and calcium are directly related to caries incidence, the maturation or remineralisation of enamel, and calculus formation. The mean phosphorous concentration in saliva of the study group was more than that of the control group (1.88 times for the whole saliva) and this difference between the study and controls group was found to be highly significant ($P = 0.001$). This limit was increased dramatically with increasing age ($t = 2.015$, $df = 41$, $P < 0.01$). Albeit, TP-DS parotid saliva was significantly decreased 1.42 times that of controls (Table 2).

*IgA of saliva* – Secretory IgA (sIgA) in saliva is a local defence factor against caries. IgA antibodies may neutralise extracellular enzymes and reduce the initial adherence of bacteria by inhibiting sucrose ($C_{12}H_{22}O_{11}$)-independent or sucrose-dependent *streptococcal* accumulation on tooth surfaces. A negative correlation between sIgA level and caries prevalence has been detected in this work. Total salivary IgA was lower in DS than in controls, but the difference was not statistically significant. Therefore, we suggest that detection of salivary sIgA levels may serve as a simple predictor of the susceptibility or resistance of DS individuals to caries formation.

*TN of saliva* – Ammonia ($NH_3$) production from the metabolism of urea ($CH_4N_2O$) (Controls: 44 ± 8 mg/dL, $t = 12.315$, $df = 65$; DS: 57 ± 11 mg/dL, $t = 9.218$, $df = 65$; $P < 0.001$) by urease (EC 3.5.1.5) enzymes of oral bacteria has moderated plaque acidification which generally could inhibit dental caries. However, it is noticeable here that the decrease in salivary TN was correlated to the low DS dental caries, the event that worth more research.

*Alkaline and alkaline-earth of saliva* – Sodium and potassium have seen to play a role in the regulation of SFR. The increasing of alkaline and alkaline-earth ions concentrations except $K^+$ ions were perhaps due to maturation or remineralisation of enamel and calculus formation (Table 2S). The alkali medium of DS saliva was enforced by lower secretion of whole saliva (73.9%) and insignificantly raising of $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Ba^{2+}$, and $Sr^{2+}$ by 48.4% (53.3% in parotid saliva), 51.4% (58.9% in parotid saliva), 56.9% (21.4% in

parotid saliva), 95.0% (51.7% in parotid saliva), 70.4% (68.5% in parotid saliva), respectively (Tables 3 and 4). The differences between the study and control groups were found to be significant ($P < 0.01$). These cations are expected to increase with the halogen ions in saliva (Tables 1 and 2) which refers again to saline nature of DS saliva. We think $Na^+$ ions are reciprocated through the low primary acinar secretion apparently by both active and passive processes. The effect produced by duct cells has led to $Na^+$ removal. More to the point, $Na^+$ ions have been increased owing to the lack of active transport mechanism at the end of the excretory ducts. A positive correlation was found between $Na^+$ of DS saliva and salivary buffering capacity (SBC). Contrary to these findings, $K^+$ was decreased in DS saliva (whole and parotid), suggesting that there is an alteration in the metabolism of the duct and/or acinar cells of salivary glands of DS children. However, statistically significant ($P < 0.01$) and negative correlations were found between these alkali and earth-alkali concentrations and DMFT indices (i.e., -4.556 for DMFT and $[Ca^{2+}]$, -3.211 for DMFT and $[Mg^{2+}]$). Remarkably, $K^+$ showed a negative correlation with dental caries which was not statistically significant ($P > 0.01$), whereas $Na^+$ showed a positive correlation with dental caries.

*Saliva pH* – In whole saliva, $CO_2$ and SFR exhibited negative correlations with pH, contrary to Mg and TN. In parotid saliva, TN was negatively correlated with pH, opposing to $CO_2$ and SFR which were positively correlated with pH.

The relatively higher pH of DS saliva caused by the following factors:

-Parotid saliva contained higher levels of non-specific esterase (Fig. 2S) and sodium bicarbonate ($NaHCO_3$). These caused a change in the amount of CA which is a responsible for the distributions of the cellular and secretory elements of these glands. Notably, CA increases the production of carbonic acid ($H_2CO_3$) from 200 $hr^{-1}$ to 600,000 $sec^{-1}$ [8].
-Glandular CA contribution as a catalysing agent in the reaction of $CO_2$ and $H_2O$ gives $H_2CO_3$. This acid spontaneously dissociates into $H^+$ and $HCO_3^-$. Most of the $H^+$ ions attach to Hb and other proteins (Table 9S), minimising the change in blood pH. On the other hand, the $CO_2$ and $H_2O$ possibly diffuse into the plasma which can enter the salivary glands either from the blood or be formed in the glands by aerobic respiration [8].
-Metabolic alterations due to instabilities of salivary enzymes in patients with DS.

Subsequently, $H^+$ ions likely convey to blood (see the lower level of pH of blood of DS patients in Table 7), while, $Ca^{2+}$ and $Mg^{2+}$ transfer from blood to salivary glands (Tables 3 and 4) and secret with $HCO_3^-$ ions. This series of events probably forms the mechanism accounting for the rise in pH, $Ca^{2+}$ and $Mg^{2+}$ levels in saliva with the decrease in secretion rate.

*Silicon in saliva* – Silica plays the role of the substrate for the nucleation but does not inhibit the conversion of the precursors to HAP. More Si (probably $Si^{4+}$) ions were detected in the whole saliva but

little amount found in the parotid saliva of the controls, possibly as remaining traces result from the mouth rinse with sodium fluorosilicate ($Na_2SiF_6$) (SI; P. 32).

*Aluminium in biosamples* – Similar to individuals with young senile dementia of the Alzheimer's type, DS appeared to have increased absorption of Al. Absorbed Al was excreted in the biological samples (Tables 5–7) without being absorbed systemically. But the question is Al a causal genetic or environmental factor in DS? The answer is that we did not detect any specific environmental factors and no significant correlations among mother's age and their children's biological Al were observed. Therefore, we gave more space to study the Al genetic effect. To elucidate this factor, we analysed saliva in different relatives to DS individuals. Among first-degree relatives (brothers and sisters), second-degree relatives (uncles and aunts), and third-degree relatives (cousins), Al concentrations in saliva were 54.8 ± 2.33 µg/L, 28.2 ± 1.67 µg/L, and 10.8 ± 1.09 µg/L, respectively. However, no significant differences in the incidence rate have been observed. Here, it is noteworthy to suggest doing more researches on possible effects on microtubule defect, brain enzymatic systems, neurotoxicity, reproductive, developmental effects, and neurobehavioral immunological following inhalation of Al.

*Heavy metals (Ti, Cr, Mn, Fe, Zn, Cu, and Mo)* – Generally, studies which quantified transition metals in biofluids (SM-A), gave only limited information on the metal's subsequent biological effects, especially for the case of saliva which is continuously produced, washed, and swallowed. So that, after a deep literature survey, it can be clearly seen that there is an inadequate availability of data concerning the association of DS biological heavy metals to patients' health.

In the current study, heavy metals are presented in trace amounts in biological matrixes.

*Titanium* analysis in biological materials has not referred to any implications.

*Chromium* released to the DS' saliva, blood and hair was above the average dietary intake (Table 12S) which refers to a possible toxicity with this element. Taking into consideration that it cannot be excluded that even nontoxic concentrations of Cr could be sufficient to induce biological effects in cells (as oral mucosa). Exclusively, Cr was not related to pH but probably may cause a bitter taste in the mouth which was confirmed by DS patients. From our experience, Cr has impacted sugar metabolism through its role in the uptake of insulin (Table 9S) which also causes losses of Cr in urine (Table 7). Cr also possibly aided in lowering the low-density lipoprotein cholesterol ($C_{27}H_{45}OH$) (LDL-C) and raising low-density lipoprotein cholesterol (HDL-C) (Table 9S).

*Manganese* is an essential element in many metabolic pathways in very limited amounts (Tables 5–7). Our results found Mn exposure has increased Fe concentration in DS biological fluids more than controls. Thus, we can say: “Mn exposure or in dose may inhibit DS-Fe absorption”.

*Iron* is reduced in DS saliva which may be expressed with the ineffective removal of plaque (see PI and QHI in Table 2S) and debris (see DI-S in Table 2S) from DS teeth. Therefore, it can be deduced that the decreased constricting power of pharyngeal musculature and dysphagia (sideropenic dysphagia) in DS is

attributed to the reduction in the amount of Fe in their biological materials as saliva, blood, and hair. As a result, an introducing supplemental Fe for the adequate production of red blood cells and for increasing muscles masses of DS patients is highly suggested.

*Zinc* is an essential element for many body functions, including enzyme activity, gene expression, intestinal epithelial regeneration, male reproductive system and a variety of immune mechanisms. $Zn^{2+}$ ions (like many ions as $Mg^{2+}$) are moved to the salivary fluid by passive transport and play a part of the cytosolic copper-zinc SOD enzyme. $Zn^{2+}$ has negatively correlated to DMFT and dental indices which were statistically insignificant ($P > 0.01$), so dietary $Zn^{2+}$ can reduce the susceptibility to dental caries in some critical conditions. On the other hand, the lower limits of Zn in DS patients can be handled by taking Zn-containing supplements, as zinc gluconate ($C_{12}H_{22}O_{14}Zn$) that holds very little amounts of cadmium (Cd). However, many Zn products contain Cd, this is because Zn and Cd are chemically similar and often occur together in nature. The amount of Zn supplement must be balanced, taking into consideration that exposure to high levels of Cd over a long time can lead to kidney failure.

*Copper* is a metal that occurs naturally in many foods (Table 9), including vegetables, legumes, nuts, grains, fruits, shellfish, avocado, beef, and animal organs (i.e., liver and kidney). Cu shortage in urine could be a cause of seizures (11.3% of DS), since epileptics often exhibit that. However, from the best of our knowledge with DS patients, Cu supplements (especially with Zn intakes) is not recommended since we realised our patients had hypersensitive to Cu and showed more slow growth ratios with Cu-supplement. In a short experiment, we gave DS children ($N = 10$) 14–28 mg Zn/day and found that all of them had developed Cu deficiency for an unknown reason. Tables 5–7 showed higher Cu concentrations in DS children's whole saliva, parotid saliva, and other biological matrices in comparison with controls. Moreover, we found that Zn-supplementation has decreased the higher level of Cu-DS. For this point, more specific studies shedding light on DS liver health in function to Cu oral supplementation and the inverse relationship between Zn (and maybe Fe) and DS patients' Cu-diet (as Zn/Cu = 2: 1, 5: 1, and 15: 1) should be designed for next study.

*Molybdenum* is an essential catalyst for enzymes (xanthine oxidase (XO): EC 1.17.3.2, sulphite oxidase: EC 1.8.3.1, and aldehyde oxidase (AO): EC 1.2.3.1) helping to metabolise fats and carbohydrates (CH) and facilitate the breakdown of certain amino acids (AA) in the body. Its role is important to the health. In a short experiment, higher decrease in Mo concentration (despite the soil of the living areas of the patients showed relatively high concentrations of Mo; data not shown) in four DS children ($N = 4$, 5.63%, three of them have oesophageal cancer family history) was registered which may refer to genetic (genetic sulphite oxidase) deficiency or/and nutritional deficiencies of Mo (Table 9), that could result from the inability to form Mo coenzyme (unknown reason). Those children were suffered from seizures ($N = 4/11$), opisthotonos (5/11), and lens dislocation (8/11). They have been given ammonium molybdate ($(NH_4)_6Mo_7O_{24}$) 300 mcg/day IV which caused dramatic recovery, taking the benefit from the Mo's antioxidant properties that helped to break down toxins in the body. However, Cu in biological fluids (i.e., saliva and blood) had decreased when this subgroup of DS patients ($N = 4$) were given

tetrathiomolybdate ($H_8N_2MoS_4$). By turn, the other DS patients showed normal contents of Mo since enamel contains high amounts of Mo which assisted them in lessening tooth decay. Therefore, it was not exigent to supplement those patients an extra dose of Mo.

# Ecological footprint

Fig. 1 proves that food accounts for 15.3% of a household carbon footprint (CF), the higher portion was observed in lower-income households.

The analysis showed that the EF, CF, and CF share of total EF are 5.6 global hectares (gha), 8.8 $CO_2$ tonnes/year, and 54% $CO_2$, respectively. The reduction of these numbers requires direct action to lessen waste and energy use. Thus, to enhance the ecological lifestyle of DS patients, the following recommendations should be addressed:

(1) Governments via city leaders are encouraged to initiate sustainability policies as by adopting urban planning and development strategies to manage the resources depicted in Fig. 1.
(2) Healthcare professionals are encouraged to use telemedicine to reduce the CF of DS healthcare.
(3) Encouraging parents to use renewable energy.
(4) Accessing to family planning by controlling family size to create a sustainable future for those patients, this can largely improve long-term DS footprint.
(5) DS females' rights should be supported and strictly protected.
(6) Controlling diet and cutting food waste which are powerful sustainability levers.
(7) Lessening the amount of meat intake (SI; Ps. 40, 77, and 78) as it is a major source of greenhouse gas emissions (GGEs), along with its other issues as animal welfare, water-use, and land degradation. So that can reduce the DS-EF by enjoin vegetarian-friendly meal days.
(8) Eating more plant-based protein (i.e., beans, legumes, nuts, tofu, and seeds) than animal sources, can cut GGEs. However, these products are rich with phenylalanine (Phe) (Recommended daily limit (RDL): 25 mg/kg body weight or 11 mg/pound) should be controlled.
(9) Eating more fibre-rich foods with low saturated fats (SI; Ps. 36 and 77) and Na, can improve the digestive health, serve in balancing the gut bacteria, and protect against heart disease, colorectal cancer, and diabetes.
(10) Living the green-hipster life. Leafy green as seaweed can be suggested as an environmentally-friendly nutrition for DS.

## Discussion

# Electrolyte imbalance and nutritional deficiency

Nutritional deficiency was revealed with the meagre quantities of some minerals in blood as Zn compared to controls which can hamper DNA synthesis and affect the metabolic degree of SOD and

$H_2O_2$, this could be attributed to the impaired cellular immunity. Here, we think the high SOD activity could be ascribed to the localisation of this gene in chromosome 21 and as a result of the failure in the antioxidant system in DS subjects. Zn is necessary for growth, wound healing, and immune function. Therefore, the oral Zn supplementation (zinc gluconate or 650 mg sulphate/day) can compensate this declination. However, when the quantities of Zn and albumin blood are extremely low, caloric and protein intake must be increased alongside Zn supplementation. Besides, due to the interactions of Zn and Fe during absorption, those elements can be supplemented at different times of the day.

Fe-deficiency maybe caused by the blood loss through blisters, ulceration, and desquamation of the gastrointestinal mucosa. The resultant Fe malabsorption is frequently associated to the poor wound healing, breathlessness, and reduced tolerance to PhA. The progressive decline in Hb levels (Table 9S) is also perceived in compliant subjects taking oral Fe supplementation associated with vitamin C to enhance intestinal Fe absorption. Further, it seems that DS patients tended to consume less Fe than the recommended dietary allowance. This could be assigned to the trouble in chewing and swallowing meat (which contains the more easily absorbed heme form of Fe) and suppressed erythropoiesis due to inflammatory cytokines. The low concentrations of total Fe in blood and hair have associated with the increased ferritin (Ft) and transferrin (Tf) in serum (DS: 188 ± 121 ng/mL, Controls: 65 ± 53 ng/mL, $P$ = 0.129; DS: 1.73 ± 0.58 ng/mL, Controls: 1.36 ± 0.45 mg/mL, $P$ = 0.106, respectively) and decreased TfR (DS: 3.96 ± 0.62 µg/mL, Controls: 5.42 ± 1.37 µg/mL, $P$ = 0.000). The levels of serum Ft, Tf, and TfR are involved in iron homeostasis. These parameters were also assessed to investigate the possible detrimental effect of Mn exposure on systemic iron metabolism.

We gave more attention to Mn as there is no study has investigated the time-dose relationship between this element exposure and its concentration in the body fluids with no reliable biological indicator (or biomarker) has been established to evaluate its exposure. Since Mn exposure alters Fe metabolism, we hypothesised that changes in serum concentrations of Fe-related proteins may be used as the potential indicator(s) for Mn exposure. Mn may compete with Fe for the [Fe-S] cluster in the active centre of Fe regulatory proteins (IRPs). Such a competition, while suppressing IRP's enzymatic catalytic function, would increase the protein's ability to bind to mRNAs encoding TfR and Ft, which in concert with an enhanced transport of Fe at brain barriers may promote the cellular overload of Fe in brain, subsequently leading to Fe-initiated neuronal oxidative damage. The low levels of Mn in blood and hair could be ascribed to the epileptic condition and chronic seizure.

Albeit, we think other metal ions concentrations have attained the highest values in hair since they may bind to the sulphur of the keratin presents in hair matrix (see the higher levels of Cu, Cr, and Si in hair; Table 7). The lower levels of metals in blood and urine refer to a recent exposure.

# Improper medication vs. safe diet

Before this study, most of our patients were used intermittently thyroid medication and some doctors have randomly suggested valproate (or some other anti-epileptic drugs as neuropsin inhibitors,

carbamazine, gabapentin, topiramate, lamotrigine, and vigabatrin) for DS to treat epilepsy, bipolar disorder, migraine headache, and seizure (partial and generalised). Nonetheless, we noticed that this type of medication has lowered the level of carnitine ($C_7H_{15}NO_3$; which plays a vivid role in energy production) in blood between 60–72% (free carnitine [7.36–10.5 μM/L] and total carnitine [12.2–17.5 μM/L]). Thus, we initiated parents of DS patients during this type of treatment feeding their children rich sources of L-carnitine as red meat, poultry, fish, and dairy products rather than focusing on vegetables and grains. These meals are also rich with choline (can increase the plasma choline) which can treat the intractable epilepsy (selenium is a triggering factor should be checked) of some DS patients and in common the presynaptic cortical cholinergic deficiency and the extensive loss of choline acetyltransferase. Interestingly, DS patients who ate these meals rich of phosphatidylcholine for a month (e.g., a meal of red meat or fish twice a week) have shown improved EEG pattern (dominant component within 8 Hz band of the basic rhythm reached maximum in its appearance rate) but with a requisite to control the levels of Phe and tyrosine.

Some doctors used ACTH to regulate steroid hormone cortisol. Here, we tracked this procedure and found that this kind of therapy has lowered homocarnosine (known as anticonvulsant agent) levels, the event that obviously can oppose the $H_2O_2$-mediated Cu, Zn-SOD fragmentation, and antiglycation. Thus, it can reduce brain damage. More to the point, we suggest to check and control serine protease neurospin, EC-SOD, and Se in plasma. For hypotonia treatment, tryptophan supplementation is ordinarily prescribed.

Further, doctors have prescribed some unplanned nutritional supplements, which did not show us any remarkable influence on the status of children. Patients had generally feeding problems and frequent infectious diseases since their infancy, which might be attributed to low Ca intake in such circumstances and inadequate bone mineralisation. The low levels of Ca and vitamin D intakes (Table 8) can obviously lead to bone fragility. On the other hand, the low level of 25-hydroxyvitamin D in serum could be ascribed to the presence of skin blisters (ratio of blisters to body surface area (BSA): 20% BSA = 0.30, 40% BSA = 0.30, 100% BSA = 0.97) and bandages in some patients which reduce their exposures to sunlight and interfere with cutaneous vitamin D production. Taking into consideration, the higher BSA affected, the greater the nutrient loss and energy requirement. Patients had insufficient intakes for vitamin D, Ca, K, and, P, contrariwise to sodium. Thus, rich meals with Ca and K minerals are suggested where those elements are directly involved in bone remodelling. In addition, NaCl should be checked out individually since a high NaCl diet plays a major role in urine Ca excretion and bone resorption.

By hand, TEI, total energy expenditure (TEE), and total energy balance (TEB) were low (Table 9S). Thus, it is highly important to feed DS patients softer food with a high energy content.

Remarkably, with the available unprotecting diets (Approximately, fat (16 ± 3%), protein (19 ± 2%), and CH (65 ± 5%)) consumed, DS patients had shown dry, chapped lips as do those with actinic chelits, which could be caused from too much sun exposure and malnutrition. They had further complained from gastroesophageal reflux, poor sucking and swallowing skills. Among DS patients, deficient nutrient uptakes made foodstuff choices and food intolerance or malabsorption linked with slow metabolic rates.

Typically, most of DS disorders (after giving samples) in this study were treated with prescription drugs (even still there are no efficacious biomedical treatments for CNS-associated strength) and many of these caused unwanted side effects. Therefore, we recommend here alternative or complementary nutritional rich meals with:

(1) **AA** which can easily be converted into neurotransmitters that alleviate such symptoms (Fig. 3S) as depression and other mental disorders. Accordingly, this potential remedy can overcome the adverse effects of given drugs. (AA in blood can be assayed every six month)
(2) **Vitamin A**, can be used to play a vivid role as a part in the immunological defect and treat hyperkeratotic lesions allied to nutrient loss, dry skin, and blepharitis. (Vitamin A in blood and plasma retinol can be measured once a year)
(3) **Ca and vitamin D** (associated with intensive psychomotor programmes) must be started since early childhood. (Ca and vitamin D in blood can be assayed at least a year)

# Conclusions

The current study is unique in its broad spectrum of biophysical and biochemical measurements in various biofluids and the developmental outcomes were registered. The biological study defined a positive correlation between TBF and CH. Na and K ions were responsible on SFR regulation. Foods were poor with vitamin D, Ca, K, and, P, the wrong way round to Na. The strength of the bitter taste was associated with the intensity of Cr toxicity. The toxicity study revealed also that DS individuals living in the same city with controls are affected with recent exposures with Si, Cr, Cu, Fe, Mn, Mo, Zn, and Al, in addition to past exposures with Si, Cr, and Cu. Mashed meals with high energy rates encompassing especial ingredients rich with kale, parsley, spinach, sweet potatoes, organic eggs, fruits, millet, and little amount of meat, in addition to clean water are suggested.

Despite, nutrients were introduced in perfect forms to be digested and absorbed, but at the same time, their consumed diets were with fair content of nutrients and laden with simple CH and dairy products. Unmanaged dietary restriction can disturb a DS growth and bone health, leading to nutritional deficiency and failure to thrive. The report regulated the nutritional system as by giving patients organic vegetables which have many of the nutrients needed for the body to remain healthy and strong and for the brain to be active and alert. But it suggested to avoid breads, sugars, pastas and most cereals contain simple CHs which can grow yeast in the intestinal tract and result in confused thinking, slow digestion and constipation which allows toxins to leak into the gut through the small intestine.

Macro- and Micro- nutrients are associated with immune functions and risk of morbidity, which in turn affect growth.

Further, it is highly recommended to improve the medical care, functional status, nutritional system, and environmental conditions for DS patients to secure them a better life which may extend their ages.

In extension, we are going to investigate if the excretion of isotopes (i.e., $Ca^{43}/Ca^{46}$, $Ba^{135}/Ba^{138}$, $Fe^{57}/Fe^{58}$, $Cu^{65}/Cu^{63}$, $Cd^{112}/Cd^{114}$, $Pb^{206}/Pb^{208}$ and $Zn^{66}/Zn^{68}$) in saliva and/or other biological matrixes as urine can be collaborated in the calculation of the average energy utilisation or the estimate energy requirements over several days.

This research is providing data for understanding neurodegeneration and for testing treatment that will hopefully, and importantly benefit DS patients.

# Abbreviations

| | |
|---|---|
| AA | Amino Acids |
| AKI | Acute Kidney Injury |
| CA | Carbonic Anhydrase |
| CF | Carbon Footprint |
| CH | Carbohydrates |
| CRP | C-Reactive Protein |
| DD | Disability Development |
| DI-S | Simplified Debris Index |
| DMFT | Decay, Missing, and Filled Tooth |
| DS | Down's Syndrome |
| EC | Electrical Conductivity |
| EF | Ecological Footprint |
| FT | Ferritin |
| FT4 | Free Thyroxine Hormone |
| GGE | Greenhouse Gas Emission |
| gha | Global Hectares |
| GI | Gingival Index |
| Hb | Haemoglobin |
| HAP | Hydroxyapatite |
| HDL-C | High-Density Lipoprotein Cholesterol |
| ID | Intellectual Development |
| IRPs | Fe Regulatory Proteins |
| LDL-C | Low-Density Lipoprotein Cholesterol |
| LOD | Lower Limit of Detection |
| LOQ | Limit of Quantitation |
| MCH | Mean Corpuscular Haemoglobin |
| MCHC | Mean Corpuscular Haemoglobin Concentration |
| MCV | Mean Corpuscular Volume |
| MVPhA | Moderate to Vigorous Physical Activity |

| | |
|---|---|
| NCEHS | National Committee of Environmental Health Sciences |
| NHANES | National Health and Nutrition Examination Survey |
| OHP | Hydroxyproline |
| PCV | Packed-Cell Volume |
| PhA | Physical Activity |
| Phe | Phenylalanine |
| PI | Plaque Index |
| QHI | Quigley-Hein Index |
| RDL | Recommended Daily Limit |
| SBC | Salivary Buffering Capacity |
| SBI | Sulcus Bleeding Index |
| SFR | Saliva Flow Rate |
| SOD | Superoxide Dismutase |
| TBF | Total Body Fat |
| TDS | Total Dissolved Solids |
| TEB | Total Energy Balance |
| TEE | Total Energy Expenditure |
| TEI | Total Energy Intake |
| TF | Transferrin |
| TfR | Transferrin Receptor |
| TN | Total Nitrogen |
| TP | Total Phosphorous |
| TSH | Thyroid Stimulating Hormone |
| TSS | Total Suspended Solids |
| TTP | Thrombotic Thrombocytopenic Purpura |
| WBCs | White Blood Cells |

# Declarations

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# Authors' contributions

CRedit roles: LA and MAJ. Conception and design: LA. Literature search: MAJ. Study selection, data extraction and quality assessment: LA and MAJ. Investigation: MAJ. Materials: MAJ. Resources: MAJ. Methodology: LA and MAJ. Drafting of manuscript: LA. Validation: LA. Data and analysis guaranty: LA. Medical guaranty: MAJ. Acquisition and analysis of data: LA and MAJ. Analysis and interpretation of data: LA and MAJ. Study supervision and mentorship: LA. Drafting the manuscript for important intellectual content: LA and MAJ.

All authors critically revised the article and gave final approval of this version to be published.

# Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work, no financial relationships with any organisations that might have an interest in the submitted work in the previous three years, and no other relationships or activities that could appear to have influenced the submitted work.

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# Availability of data and materials

The datasets analysed during the current study are publicly available and more details can be available from the corresponding author on reasonable request.

Sharing data including symptomology, clinical, and medical observations, challenging behaviour, psychology, oral prophylaxis and dental assessment, and detailed methods used for physical and biochemical analyses are presented in the Supplementary Information file. The Supplementary Material-A (SM-A) and Supplementary Material-B (SM-B) involve two tables for elemental comparison. Supplementary data related to this article can be found at https://doi.org/

# Ethics approval and consent to participate

The study was performed in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki; Good Clinical Practice) for experiments involving humans and the International Organization for Standardisation of standard 14155. The protocol was approved by the institutional ethics committees (Ethics registration: EC/FD/1538) at the Faculty of Dental Medicine/Damascus University. Parents of healthy and DS patients gave written informed consents. Completeness and quality of data were assured by 100% source document verification. An independent data monitoring committee adjudicated all adverse clinical events. Subsequently, individuals' proforma were prepared to gather adequate information from case sheets of patients and their mothers including symptomatology and laboratory investigation.

# Consent for publication

Not applicable.

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# Figures

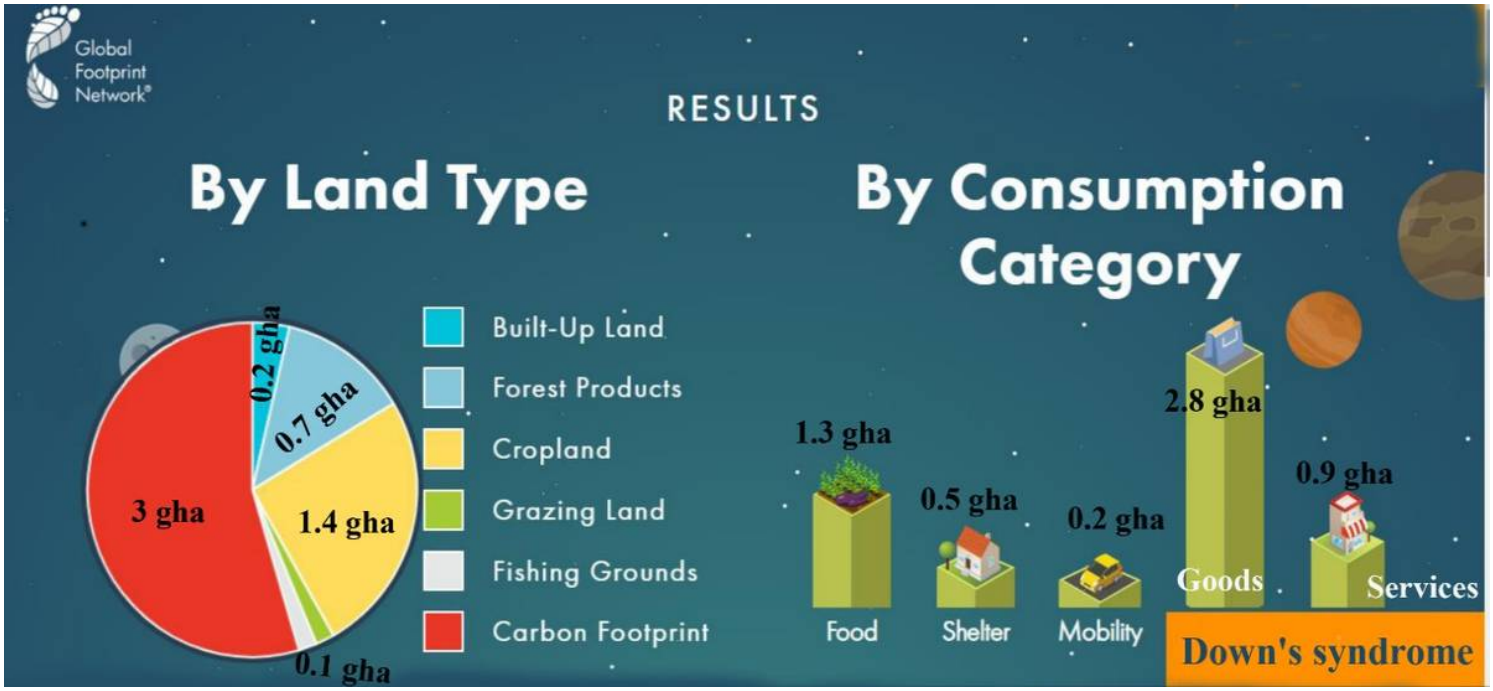


**Figure 1**

Ecological footprint of down's syndrome

# Supplementary Files

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