Supplementary Information

Improvements in the ecological and nutritional aspects of Down's syndrome

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Abbreviations

Acute Kidney Injury	AKI
Acute Megakaryoblastic Leukaemia	AMKL
Adiponectin	ADIPOQ
Adrenocorticotropic Hormone	ACTH
Amelocemental Junction	AML
Amino Acids	AA
Angiotensinogen	AGT
Assessing Carried, Absent and Obturated Teeth Index	CAO
Assisted Reproductive Techniques	ART
Bilivierdin Reductase A	BLVRA
Blood Glucose	BG
Body Mass Index	BMI
Body Surface Area	BSA
Bone Mineral Density	BMD
Brockport Physical Fitness Test	BPFT
Bruininks-Oseretsky Test Motor Proficiency	BOTMP

Calculus Index	Cal-I
Carbohydrate	CH
Carbon Footprint	CF
Central Nervous System	CNS
Chronic Marginal Periodontitis	CMP
Clinical Attachment Loss	CAL
Confidence Interval	CI
Congenital Adrenal Hyperplasia	CAH
C-Reactive Protein	CRP
Creatinine	CR
C-Terminal Telopeptide	CTx
Decayed, Missing and Filled Teeth Index	DMFT
Dehydroepiandrosterone Sulphate	DHEA-S
Diastolic Blood Pressure	DBP
Disability Development	DD
Down's Syndrome	DS
Dual-Energy X-ray Absorption	DXA

Early Myoclonic Encephalopathy	EME
Ecological Footprint	EF
Electroencephalographic	EEG
Environmental Sensitivity	ES
Environmental Sustainability	ES
Femtolitre	fL
Ferritin	Ft
Follicle Stimulating Hormone	FSH
Frozen Embryo Transfer	FET
Gamete Intrafallopian Transfer	GIFT
General Health Questionnaire	GHQ
Generalised Bacterial Gingivitis	GBG
Gingival Index	GI
Glucose Oxidase	GOx
Haematocrit	Ht or HCT
Haemoglobin	Hb or Hgb
Health Related Quality of Life	HRQoL

High-Density Lipoprotein Cholesterol	HDL-C
Homocysteine	HCY
Horseradish Peroxidase	HRP
Human Chrorionic Gonadotropin	hCG
Hydroxyproline	OHP
Intellectual Development	ID
Interleukin-1 Receptor Antagonist	IL-1RA
Interleukin-6	IL-6
Interleukin-10	IL-10
Intima-Media Thickness	IMT
In Vitro Fertilisation-Embryo Transfer	IVF-ET
Iron Regulatory Proteins	IRPs
Low-Density Lipoprotein Cholesterol	LDL-C
Luminal Diameter	LD
Luteinising Hormone	LH
Mean Corpuscular Haemoglobin	MCH
Mean Corpuscular Haemoglobin Concentration	MCHC

Mean Corpuscular Volume	MCV
Milli-International Units	mIU
Moderate to Vigorous Physical Activity	MVPhA
National Health and Nutrition Examination Survey	NHANES
National Oral Health Survey	NOHS
Non-Protein Nitrogen	NPN
Odds Ratio	OR
Orthopantomography	OPT
Packed-Cell Volume	PCV
Parathormone	PTH
Patient Care Dataset System	PCDS
Perceived Positive Change	PPC
Phenylalanine	Phe
Physical Activity	PhA
Plaque Index	PI
Polycyclic Aromatic Hydrocarbons	PAHs
Posteroanterior	PA

Procollagen Type 1 N Propeptide	P1NP
Protein Phosphatase 1 Regulatory Subunit 1A	PPP1R1A
Public Health Paediatric Endocrinology Service	PHPES
Questionnaire for Children's Health Related Quality of Life	QCHRQoL
Quigley-Hein Index	QHI
Recommended Daily Limit	RDL
Salivary Buffering Capacity	SBC
Sense of Coherence	SOC
Short-Term Memory	STM
Simplified Debris Index	DI-S
Social Capital	SC
Standard Difference	SD
Standard Error	SE
Standard Error of Difference	SED
Sulcus Bleeding Index	SBI
Superoxide Dismutase Enzyme	SOD
Systolic Blood Pressure	SBP

Thrombotic Thrombocytopenic Purpura	TTP
Thyroid Hormone	TH
Thyroid Peroxidase	TPO
Thyroid Stimulating Hormone	TSH
Thyrotropin Releasing Hormone	TRH
Thyroxine Free Serum	Free T_4
Total Body Fat	TBF
Total Energy Balance	TEB
Total Energy Expenditure	TEE
Total Energy Intake	TEI
Transferrin	Tf
Transferrin Receptor	TfR
Transient Myeloproliferative Disorder	TMD
Triglyceride	TG
Triiodothyronine Free Serum	Free T_3
Tumour Necrosis Factor- α	hsTNF-α
Waist Circumference	WC
Wall Cross-Sectional Area	WCSA

White Blood Cells	WBCs
Xaa-Pro Aminopeptidase 1	XPNPEP1
Zygote Intrafallopian Transfer	ZIFT

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1. Background

1.1. Down's syndrome spreading-out

Nearly 83 million of the world's population is estimated to be mentally handicapped [1,2]. According to an article published on Children's Health on NBC News website, eight million documented yearly, suffering from some types of birth defects [3]. A particular congenital birth defect that relates close to home is Down's syndrome (DS). In addition, one of the leading causes of intellectual development (ID) disorders recognised today is DS [4] which has mostly frequent chromosomal aberration in males [5].

1.2. DS definition and diagnosis

DS is an aneuploidy syndrome, a genetic disease mainly characterised by intellectual disability, caused by a trisomy of chromosome 21 (21q22), and often associated with thyroid malfunction and multiple clinical dysfunctions. Trisomy 21 affects virtually every organ system and results in the complex clinical presentation of DS [6,7]. Patterns of differences are now recognised as patients' age and these patterns bring about new opportunities for disease prevention and treatment [8,9]. DS is occurred in about one in every 700 live new-borns [10]. Systemic manifestations in DS are common, such as recurrent respiratory infections, congenital heart defects, immunologic disorders, and hypothyroidism [11]. Out of different malignant conditions, some DS patients can develop acute leukaemia [12].

1.3. Saliva

1.3.1. General functionalisation

Saliva as one of the major biofluids, not only lubricates the oral tissue, making oral functions such as speaking, eating, and swallowing possible, but also protects teeth and oral mucosal surfaces, in different ways [13], playing a critical role in the prevention or reversal of the caries process.

1.3.2. Saliva a competitive biofluid

Recently, researchers [14-19] had proposed the possibility of using saliva as an alternative to blood in diagnosing and monitoring of diseases.

Saliva provides minerals and proteins that maintain supersaturation of such ions as alkaline and alkaline-earth in the plaque fluid [18]. Most commonly used laboratory diagnostic procedures involve analyses of the cellular and chemical constituents of blood. Despite, saliva offers some distinct advantages over the other biological fluids and is accepted matrix for biomonitoring heavy metals exposure in occupational and environmental toxicology [18,19].

For a large-scale risk assessment, chemical elements contents in saliva provide several advantages in clinical chemistry. First, saliva is secreted by salivary glands, containing ingredients of body extracellular fluids. Interestingly, essential metal ions are not merely passively diffused from glands to saliva, but rather actively transported into it. Thus, in theory, the chemistry of saliva differs from that of serum, which provides an additional means for biochemical assessment of toxic exposure in humans. Second, the sample of saliva can be readily obtained; the operation is simple and the procedure proves to be readily acceptable to our study subjects. Third, collection of saliva is non-invasive and can be done in any location even on the work site. Finally, saliva samples are convenient for storage and transport, and stable for elemental analysis.

Consequently, collection and evaluation of the secretions from individual salivary glands are primarily used for detection of gland-specific pathology, i.e., infection and obstruction. Thereby, physical and biochemical assessment of parotid saliva is highly suggested and has been involved in this work.

1.3.3. Previous studies of saliva characterisation of DS and current challenges

Previous studies showed that oral cavity, palate and maxilla are small compared to the mandible [20]. In addition, some showed a delay in the eruption of deciduous and permanent dentitions as well as agenesis of teeth [21], and a high prevalence of periodontal disease [22].

The understanding of salivary function in promoting DS healthy oral condition has become a topic of major importance for the nowadays-oral clinicians. In order to comprehend the role of each salivary component in oral cavity homeostasis, it is crucial to perceive how the changes of these components or their absences may be linked with pathological conditions and the nutrition system [23]. However, knowledge integration between DS saliva and oral pathology is far from being complete. Therefore, it is of critical importance to establish which salivation patterns and concentration ranges of each salivary component are to be considered as normal in order for the clinician to diagnose altered salivary phenotypes possibly linked to pathological systemic or oral conditions.

As a result, the knowledge of the effect of DS on salivary functions remains equivocal, although, there are unsatisfactory studies have been conducted in this regard. It is currently essential to study whether the salivary indices, physical and biochemical characteristics would be altered in DS even little is known about dental issues and the periodontal diseases caused by poor oral hygiene in DS.

1.4. Climate change and environmental risk

Climate change is a major peril to DS health while healthcare professionals are yet far from accepting their responsibility for taking action to mitigate this threat, as by addressing the environmental sustainability (ES) in their medical works. DS patients need several million pounds of minerals, metals, and fuel in their lifetime, however, some of these sources are noxious. To date, environmental factors have not received the research attention that they warrant. Even though, heavy metal contamination can be accumulated in the environment and

living organisms leading to long term toxic effects to DS patients. In this connection, we think that chemical elements could be equated on a host of environmental and subject characteristics which may influence for instance DS' IQ test scores.

But, what are the DS demands on nature? And what is the quantity of nature it takes to support them? Ecological footprints (EFs) are the measure of consumption. On the demand side, the EF measures the ecological assets that a given population requires to produce the natural resources it consumes (including plant-based food and fibre products, livestock and fish products, timber and other forest products, and space for urban infrastructure) and to absorb its waste.

Since there is no study has focused on the carbon footprint (CF) of patients in general, and in specific with a primary care setting, so we decided to study the EF of DS to be a useful reference for not even mentally handicapped but can launch further similar studies for other syndromes.

2. Developmental and family history

2.1. Chromosomal anomalies

Figure 1S showed the chromosome translocation and clarified two chromosomes 21 (48 million nucleotides) had attached to chromosome 14 (3.5% of total DNA in cells). The molecular mechanism likely involved the extra copy of chromosome 21 but which genes and pathways were responsible, need further more research. The mechanism probably did not involve mutations to the gene GATA1 (haematopoietic progenitor) that were seen in two other haematological abnormalities of DS, transient myeloproliferative disorder (TMD) and acute megakaryoblastic leukaemia (AMKL) of DS (Leukaemia was remarkably found in a high rate between the relatives to DS patients even many DS patients are not!). Therefore, the

tendencies for meiotic nondisjunction and for developing leukaemia might also be genetically considered.

Fig. 1S. DS chromosomes translocation.

2.2. General DS's anomalies

The features of those patients were found: short stature, rounded and some had flattened faces, oblique eyes, small noses with flattening tips, protruding large tongues, short necks, and strabismus.

2.3. Questionnaires

Toxicity and nutritional assessments based on the anthropometric, clinical, biophysiochemical, and dietary investigations are used to determine the environmental sensitivity (ES) and nutritional status of DS individuals.

Parents provided information about their child's age, child's race, ethnicity, developmental history, major medical diagnoses, education level, and income in the form of a questionnaire. Information on co-occurring or a history of major medical diagnoses was obtained by asking parents what current and/or past major medical diagnoses had been given?

A dietary survey was carried out from a food questionnaire corresponding to a data collection of 7 days. Parents and children were informed of the collection procedures and were invited to write down the nature and quantity (through household balance) of any solid or liquid food consumed during 7 consecutive days. Parents were asked to document as much as possible of their children usual eating habits during the study period. This questionnaire consists of columns for each day with a breakdown for each meal of the day (breakfast, snack, lunch, afternoon snack, dinner, snack, etc.). These records were processed using the professional software Nutrilog (Marans, France), which displays the nutrient analysis of any food or combination of selected foods, to obtain the average nutrient intake.

A physical activity (PhA) questionnaire was used to quantify the average daily energy expenditure. It consisted of the collection of PhA data that included details on the frequency, duration and relative intensity for each activity type that contributes to energy expenditure. With the help of parents and caregivers, participants recorded all their daily activities during seven days, indicating the times of the beginning and the end of each activity. The PhA ratio values corresponding to ' and 30 sedentary (e.g., school, TV, video games, meals, passive transport, options) or physical activities (e.g., walking, training, competition) at three intensity levels were registered. From the questionnaire, it was possible to estimate the daily energy expenditure of each participant.

To assess the EF, with the help of DS parents, the following interview was completed and data managed by CO_2 Rechner:

How often does he (DS) eat animal-based products?

How much of the food that his eat is unprocessed, unpackaged or locally grown?

Which housing type best describes his home?

- Freestanding, no running water
- Freestanding, running water
- Multi-storey apartment
- Duplex, row house or building within 2-4 housing units
- Luxury condominium

What material is his house constructed with?

How many people live in his household?

What is the size of his home?

Do they have electricity at home?

How energy efficient at home?

What percentage of his home's electricity comes from renewable sources?

Compared to their neighbours, how much trash does the DS family generate?

How far does he travel by car or motorcycle each week?

What is the average fuel economy of the vehicles he uses most often?

When travels by car, how often does he carpool?

How far does he travel on public transportation each week?

How many hours does he fly each year?

2.4. Standard of living

The standard of living was 39.4% poor, 43.6% average, and 17.0% wealthy. Social capital (SC), sense of coherence (SOC), general health questionnaire (GHQ), and perceived positive change (PPC) had been estimated as the following: 17.8±3.93, 39.6±7.52, 14.9±5.30, and 36.0±5.72, respectively.

2.5. DS in births

The paediatric reports of children (since birthhood) and the gynaecology reports of the mothers before giving births and after giving births were reviewed.

According to these archives, DS was found slightly less (16.9%) in twins than in single births (*N* = 59). The risk of knock-on implications of more children being born with congenital abnormalities was higher between monozygotic (identical) twins than in dizygous (*N* = 2). This research highlighted the possible relation between monochorionic pregnancies and DS births. Monochorionic twins were monozygotic (*N* = 10/71; 14.1%) who shared the same placenta and occurred in 0.3% of all pregnancies in the society (registered intervention by an expert gynaecologist who performed 18-obstetric operations for mothers of DS offspring).

There was a possibility that assisted reproductive techniques (ART) might cause an independent risk of congenital abnormality because of increasing maternal age (children's births distribution *vs*. maternal age; Table 1S) and the use of both of ART and the increasing rates of multiple pregnancies.

Table 1S

Birth distribution in relation to mother's age.

Mother's age, yrs.	Number of DS births
Under 20	1
20-29	2
30-34	3
35-39	5
40-45	11

Over 45	49
Unemployed/homemakers'	mothers (38.0%) and most were married (90.2%). Available social support was low (i.e., only 26.8% of mothers had a place to ask for care of their children with DS, and 9.86% of mothers could use social support to rest/act themselves)

ART was included *in vitro* fertilisation-embryo transfer (IVF-ET), gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT), and frozen embryo transfer (FET). These techniques also could be applied to oocyte donation and gestational carriers. Approximately 99% of ART cycles performed were IVF-ET. Check et al. [24] proved that IVF-ET can help many couples conceive successfully. However, ART might be recommended when other treatments (such as intrauterine insemination) had not been successful or when there was severe male factor infertility, severe endometriosis or tubal obstruction.

2.6. Challenges of data collection concerning research logistics and procedures, and our recommendations

With the absence of some patient's medical history files or the validity of patient's health data and/or other electronic health record history infographic, it is necessary to establish a patient care dataset (PCDS) system and an adequate coding in every hospital and clinic to save up the records for all children with DS and ease the circulation of data and information. On a large scale, there is also a need for national register, so that coordination and planning of treatments will receive the appropriate recognition.

3. Clinical observations, heterogeneous symptomatology, medical remarks, and characterisations

Health-related interventions including oral change outcomes and drugs, behaviour, and dietary interventions were examined.

3.1. Patients recruitment

Participants living in a metropolitan area were recruited for the study which was utterly executed in the Faculty of Dental Medicine, Damascus University in 8/52015-28/9/2017. Each individual guided prior to respond to our instructions.

DS patients were in a general with low resistance caused by immunodeficiency. The main immunological defect occurred in the thymus-dependent system could result in a reduced number of mature T cells and a relatively large proportion of immature cells. According to Whittingham et al. [25] the immune system is under stress.

Medical assessment was divided into three parts: a behavioural self-administered questionnaire, oral health assessment, and biophysiochemical assessment. The behavioural questionnaire was administered to the respondents with disability development (DD) by the

research team and they were also asked about personal and demographic information. For the subjects with more severe disabilities, information was provided by a caregiver.

Each child gave at least 40 mL, 10 mL, 5 mL of saliva, blood, and urine in succession, in addition to 0.1 g hair.

3.2. Dental study

3.2.1. Oral health assessment

The oral health assessment followed the protocol used in the National Oral Health Survey (NOHS) which was based on the WHO guidelines. Parents gave information about dental practices (incl. period since last dental visit, number of dental visits in the last 12 months, and number of times teeth brushed in a day) and type of dental services used (private or public). The oral health assessment was carried out by a single dental surgeon with experience in the dental examination of people with DD. The examination covered oral mucosal pathology, malocclusion, periodontal disease, dental caries, and treatment needs.

Patients' dental indices in addition to the physical and biochemical results (incl. the outcomes of the novel analyses in this field presented in the current study) are compared with the corresponding outcomes of control samples.

3.2.1.1. Evaluation of dental indices

To determine oral health status of the children (Controls: $N = 74$ and DS: $N = 71$), the oral region of each participant was first examined, and the decayed, missing and filled teeth (DMFT) index was calculated and recorded according to Klein et al. [26]. DMFT index measures the amount of permanent tooth decayed, missing, and filled in individual's mouth, ranging from 0 to 32. A panoramic X-ray study was scanned.

Comparisons were made between the two groups for every calculated index. The clinical attachment loss (CAL) was used to assess the chronic periodontitis and has been evaluated

through periodontal probing, appreciating the depth of the pockets and also the degree of recession, at the level of every test tooth in at least 6 sites (B, L, MB, ML, DL). The reference element for evaluating the attachment loss was amelocemental junction (AML) expressing the distance between the bottom of the sulcus/pocket in millimetres [27,28]. The Rx exam, useful in appreciating the importance of attachment loss was performed on retro-dento-alveolar clichés in isometric and orthoradial incidence or through orthopantomography (OPT) [27]. The periodontal destructions are characterised by the formation of deep periodontal pockets, associated to the indicated quantities of bacterial plaque and intense gingival inflammation using Quigley-Hein index of bacterial plaque (QHI) index [29]. Observations corresponding to each individual were recorded on the odontogram, with the determination of carried, absent and obturated teeth (CAO) index [30]. Oral hygiene was rated based on: (i) clinical examination of the calculus index (Cal-I) [31], (ii) Sulcus bleeding index (SBI) [31], (iii) O'Leary plaque index (PI) [32] used to measure the amount of plaque, and (iv) the periodontal condition evaluated by gingival bleeding index (GBI) [33]. Bacterial plaque [29] was dyed and sampled with a blunt-tipped dental probe, sliding the latter along the gingival sulcus at four points per tooth, and evaluating all surfaces. The presence or absence of plaque was estimated, regardless of its amount, the corresponding index (PI) [32] was obtained as a percentage on summing the results then dividing by the total number of points explored. The simplified debris index (DI-S) [31] was based on numerical determinations representing the amount of debris found on six preselected tooth surfaces: the buccal/labial surfaces of the maxillary right first molar (tooth 16), the maxillary right central incisor (tooth 11), the maxillary left first molar (tooth 26), the mandibular left central incisor (tooth 31), and the lingual surfaces of the mandibular left first molar (tooth 36) and the mandibular right first molar (tooth 46).

3.2.1.2. Periodontal study results

We clearly noticed a growing of eruptions, prevalence of congenitally missing teeth, and clinical crowding of the teeth among DS children (especially < 6 yrs.), smaller sizes of peg-shaped and pointed teeth, missing teeth mainly upper incisor, increasing of interdentally spacing with age even when there was no hypodontia, mouth breathing, imbalanced occlusal forces, bruxism, bifid uvula and cleft palate, chronic superficial periodontitis, gingivitis, poor access to oral health, and increased cariogenic diet. These findings met some of the observations of Cogulu et al. [34] and Castilho et al. [35]. Acute necrotising ulcerative gingivitis prevailed with patients in the puberty (10-15 yrs.). The prevalence of the periodontal disease was higher for children over 10 with more noticeable effects on the mandibular incisors and the maxillary molars.

The weak oral conditions are attributed to mental retardation, subgingival plaque composition, immune/inflammatory responses, and malnutrition. The periodontal destructions (Table 2S) refer to the low degree of nutritional response. Nonetheless, there is no cure for DS and therefore treatment of oral ulcers that are associated with DS is palliative. All of these observations were attributed to low powers of concentration, lack of manual coordination, physical inability to adequately clean the oral cavity, and lack of motor skills.

Table 2S

Periodontal parameters in patients with DS; mean ($\bar{x}$)±standard deviation (σ); $n = 3$.

Dental index	Controls ($N = 74$)	DS ($N = 71$)	P
CAL	0.52±0.48	1.42±0.51	< 0.001
CAO	5.17±3.72	4.83±3.65	< 0.001

Cal-I	0.42±0.29	0.77±0.53	0.006
CMP	16.2	67.6	< 0.001
DI-S	0.62±0.45	1.83±0.85	0.084
DMFT	1.02±0.67	0.82±0.59	0.034
GBG	75.7	32.4	< 0.001
GI	1.16±0.35	1.81±0.92	0.001
PI	0.96±0.12	1.72±0.68	< 0.001
QHI	2.74±2.51	3.45±3.09	< 0.001
SBI	0.30±0.11	0.36±0.12	0.072
Aggressive periodontitis	8.10	0.00	< 0.001
Number of carious teeth	0.20±0.48	0.90±1.05	0.012
Number of extracted teeth (%)	63.9	64.7	0.007
Oral ulcers (number/month)	1.20±0.30	5.20±3.70	0.002

Chronic marginal periodontitis (CMP), generalised bacterial gingivitis (GBG, %), and gingival index (GI)

Besides, DS patients had significantly higher Cal-I, GI, and PI scores than controls ($P < 0.01$) which may attribute to the poor utilisation of dental services: Lack of knowledge about good oral hygiene practices among caretakers and concerned authorities, lack of motivation, low priority given to dental care in the society, lack of facility for early and regular oral health check-up and prompt treatment, and cost of treatment. The higher GBG in DS children met with the findings of Sakellari et al. [36] who showed higher levels of periodontopathic bacteria including *porphyromonas gingivalis* and *tannerella forsythensis (Bacteroides forsythus).* The increasing of CAL in DS issue referred to the severity of the accompanied states, is characterised by bone loss in the coronal third of the root and moderate. Bone loss is occurred in the middle third of the root and advanced when in the

apical third of the root length [37]. Precocious expulsion of the teeth, loss of alveolar bone measured by orthopantomography, being found in 73.3% of patients. CAO teeth index was significantly decreased in DS which reflects the efficacy of increasing the frequency and efficiency of assisted brushing. The DI-S was insignificantly increased 3 times that of controls, expressing the ineffective removal of plaque and debris from DS teeth when using a toothbrush. Hence, optimal oral hygiene and the selection of proper diet are of particular importance in DS patients in order to limit their buccal disease onset.

Quite clearly the composition and physiology of saliva are factors which may influence oral health and therefore they need to be considered in any programme investigating the aetiology of dental disease, particularly where the disease prevalence differs from that usually expected. Thus, the composition of saliva (especially electrolyte levels and nitrogenous metabolites in whole saliva) is highly requested and thus has been studied in this report.

3.2.1.3. Caries

Dental caries is fundamentally a diet bacterial disease [38]. Here, we noticed that the ingestion of non-sticky refined sugar at meals, sticky refined sugar at meals, and sticky refined sugar between meals, would affect dental caries. The sugar was presented as sucrose, bread, chocolate, caramel and toffee, and the control group was receiving a low carbohydrate (CH), high fat diet virtually free of sugar. The difference in caries prevalence among the two Levels (DS and controls) can be attributed to several factors i.e., socio-economic (determined with the Hollingshead Four-Factor Index of Social Status), diet, oral hygiene, and specific morphology or to immune protection caused by the elevated salivary *S. mutans* specific *IgA* (serotype g and c) (See the footnote of Table 1).

Caries experience in both deciduous and permanent dentition was significantly lower than controls. Analysis (Table 2S) revealed that individuals with DS had lower dental caries

(16.9%; majority < 6 yrs.) than those in control group (combined odds ratio (OR) 0.32; 95% confidence interval (CI) 0.19 to 0.46). These results were backed up with the lower DMFT of DS patients (0.82±0.69; standard difference (SD) -0.23; standard error (SE) 0.10; -0.39 < 95% CI < -0.01). A difference in eruption times of DS patients was noticed 1-2 yrs. later than that of controls. The missing teeth were upper incisor (87%), peg-shaped teeth (9%), and pointed teeth (4%).

Diet counselling achieved through the time of this study has stressed on sucrose intake restriction which had resulted a significant improvement in a dental health.

3.2.2. Oral prophylaxis and dental hygiene

Although dental treatment need was not high, these children and their parents had had dental health education including oral hygiene instruction, in order to improve DS overall oral health.

The results showed that patients' basic needs like oral prophylaxis, restorations and extractions were similar and required more care from the parents and community-based dental team. The reduced caries prevalence in DS children could be associated with parents' greater concern about their children health care. Parents of this group were advised to regularly examine their children's oral which helped us identifying unusual tooth formation and patterns of the eruption. A panoramic radiograph was scanned more than individual films to determine whether teeth had congenitally missing. And with much care, primary teeth were likely maintained, considering placing space maintainers where teeth were missing. Regular scaling was of great benefit, the ultrasonic scaler was being a useful and well-received adjunct. The plaque was removed as thoroughly as possible without damaging either the hard or soft tissues. Roll tooth brushing and mechanical cleaning procedures for example flossing (especially when the spaces between teeth were closed) were the most readily available and effective ways of controlling dental plaque. Provided these aids had been

conducted correctly and regularly. The brush used was soft multi-tufted nylon with rounded tips. The size of the brush was appropriate for the individual's mouth. The electric toothbrush was a useful and effective tool. Thus, we recommend using the electric toothbrush, if DS-service provider/assistant lacks the perceptual-motor skill to utilise a manual brush. The use of unwaxed dental floss had a prominent place in an effective plaque control programme. The use of a McKesson mouth prop or other simple devices to steady the mandible was then helpful.

3.2.3. Oral therapy

Diazepam (*7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepine-2-one*, $C_{16}H_{13}ClN_2O$) orally or by intravenous injection, was preferably verified via an antecubital fossa vein. It was a useful drug for sedation on the few occasions that this required. During semi-annual recall appointments, our patients had received topical applications of stannous (SnF_2, 10 mM) or acidulated phosphate fluoride solution ($NaF\text{-}H_3PO_4$, 0.5-1.23%) either a professional prophylaxis using a very fine cleaning agent or supervised self-cleaning.

Oral treatment had successfully been offered and carried out as follows,

a. Regular examination,
b. Prophylaxis and scaling,
c. Orthodontic assessment, and
d. Extraction of over-retained deciduous teeth.

In more details, 83% (*N* = 59) of the DS children received treatment and 52.1% (*N* = 37) of them obtained more than one treatment. These treatments were composed of dental examination 77.5% (*N* = 55), teeth cleaned or polished 33.8% (*N* = 24), fluoride treatment 16.9% (*N* = 12), oral hygiene instruction 14.1% (*N* = 10), dental filling or crown 12.7% (*N* =

9), tooth extracted 11.3% (*N* = 8), orthodontic treatment 8.5% (*N* = 6), gum treatment 1.4% (*N* = 1), and other 7% (*N* = 5).

3.2.3.1. Therapeutic fluoride supplements

The child with DS who had rampant caries or one that was not progressing adequately under a basic fluoride treatment placed on a more intensive therapeutic programme. This was indicated for only a short time until oral hygiene and diet improved or even that would be for an extended period. Those regimens were in addition to the basic systemic supplement had also topical in effect, the patient was not to swallow or ingest the given doses.

3.2.3.2. Accepted therapeutic regimes

These included:

i. Daily rinsing with 0.055% NaF solution;
ii. Weekly rinsing with 0.1-0.2% NaF solution; and
iii. Brushing with either 0.5-1.23% acidulated phosphate five times a year.

Prescription of these potentially excessive fluoride regimes was specifically considered carefully in younger patients and monitored closely because of potential causing fluorosis.

Fluoride varnishes used to prolong the exposure of the surface of the tooth to the active agent, thus increasing the amount permanently retained. Varnishes applied directly to the tooth surface without prior etching. Two products were used:

a. Duraphat was prepared by 5% NaF in a natural colophonium base and adhered to tooth surfaces even when wet with saliva. The later was dispensed in a concentration of 50 mg NaF per mL, yielding 22.6 mg F^- per mL. This procedure was hardened to a yellow-brown coating,

b. Fluro-Protector is a polyurethane (R_1R_2NHCOO, R_1, and R_2 are alkyl radicals)-based lacquer consisting of 0.7% F^- by weight in a 5% difluorosilane (F_2H_2Si). It was of a lower pH and F^- content than Duraphat and more transparent and colourless.

Duraphat has proved as a quickest and easiest agent for this application between other topical fluoride techniques. So, clinically after many experiments, we recommend using a 0.4 mL of Duraphat as a dosage for pre-school DS children and 0.6 mL with repeated applications at 6 monthly intervals for school children. More details are found in our WHO Clinical Trial Registry: DRKS00014074.

3.2.4. Nutrition recommendation for oral health

Parents were advised to maintain their children's good daily oral hygiene, adopt preventive measures such as topical fluoride and sealants, and emphasise on noncariogenic (or neutral) foods (Table 3S) and beverages as snacks.

Table 3S

List of cariogenic and noncariogenic foods.

Cariogenic	Noncariogenic (soft lumps or finely mashed)
Biscuits	Bread
Buns	Cereals
Cakes	Cheese
Chocolate	Dry fruit
Confection	Fresh corn
Flavoured/sweetened milk	Fresh fruit

Fruit pies	Low-sugar breakfast
Fruit syrup	Milk
Ice cream	Pasta
Jams	Peanut
Jellies	Popcorn
Pastries	Rice
Puddings	Sandwich
Sugar	Sugar-free confectionery
Sugared breakfast cereal	Sugar-free drink
Sugared soft drink	Sweetened yoghurt
	Toast
	Unsweetened/artificially
	Vegetables (raw/boiled/frozen)
	Water

3.3. General clinical characterisation and physiology

DS children were of short neck, exhibited hypotonia, ligamentous laxity, poor postural control, and perceptual motor difficulties (Table 4S). Interestingly, it could be determined that all of the DS participants were more successful in tasks involving the comprehension of emotions than in their production (Table 4S). This finding indicated that the level of their intellectual disabilities influences the ability to express emotions and the cognitive deficits, such as problems in maintaining attention, planning, keeping up, self-regulating and being flexible. The aerobic capacity, muscular strength and functional capacity of DS individuals

were poor especially at earlier age (< 10 yrs.), who affected passively the fitness test performance as Bruininks-Oseretsky Test Motor Proficiency (BOTMP) and Brockport Physical Fitness Test (BPFT) (Data were related to the epidemiology, thus, they had not been presented in this paper). These results refer to the importance to extend our study to involve both of the physiological and biochemical tests.

DS patients exceptionally tended to adopt lower speed and larger step width when they perceived instability. In a period 7-9 yrs. old, they suffered from trip-related fallings. Further, we also noticed some clinical features as small ears, conductive hearing loss, upslanting palpebral fissures, epicanthal folds, iris Brushfield spots, protruding tongue (Abnormal tongue movement could be attributed to the pocketing food (packaging)), 5th finger clinodactyly, and single transverse palmar creases. DS individuals may also have congenital heart defects, Hirschsprung disease, duodenal atresia, early Alzheimer disease (some medical reports of those patients have referred to higher levels of Abeta protein aggregated extracellularly in amyloid plaques, suggesting dementia), and leukaemia (N = 2/71; mean values: white blood cells (WBCs) 1.4 cells/µL, haemoglobin (Hb) 9.83 g/dL, packed-cell volume (PCV) 37 %, mean corpuscular volume (MCV) 69 fL, mean corpuscular haemoglobin (MCH) 28 pg, mean corpuscular haemoglobin concentration (MCHC) 32 g/dL, and Hg 2.93 µg/L; Figure 2S). Even leukaemia is not heredity, but we have our fears about a possible relation includes leukaemia of DS relatives (especially for the second-degree relatives) where we found 1/4 and 3/7 affected states between uncles and aunts of those two patients, respectively. In addition, surprisingly, those children and their families are accustomed to eat both freshwater fish and saltwater fish, 2-3 times a week. With a great passion fuelled with our curiosity, we listed the species of those fish found on their tables and these were: (1) Fish farming sources: *Acanthobrama tricolor*, *Astatotilapia flaviijosephi*, *Levantine barbell*, and *Tristramella magdelainae* and (2) Fresh fish: *Apogon nigripinnis*,

Hymenocephalus italic, *Micromesistius poutassou*, *Nettastoma melanurum*, *Scarus ghobban*, and *Upeneus moluccensis*. This refers that genetic condition of DS could be affected with the heredity factor and can be sensitive to the toxicity.

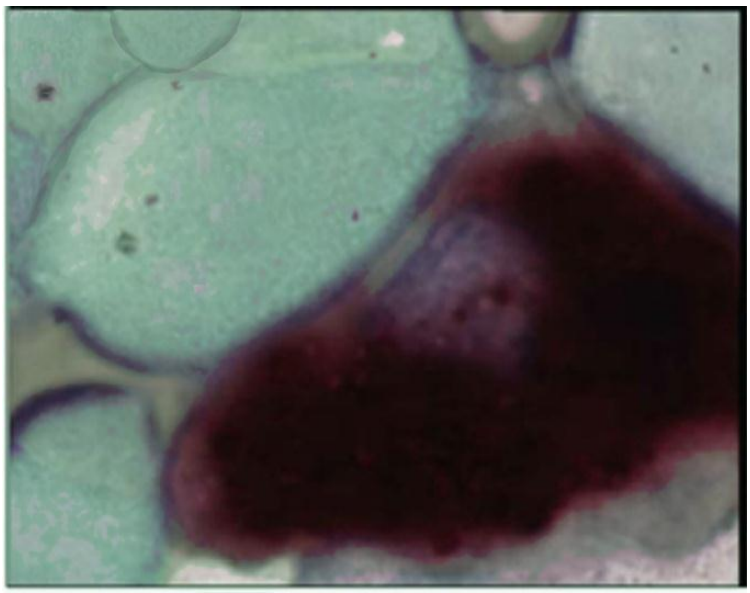

Fig. 2S. DS - Non-specific esterase pattern with cytocentrifuge mononuclear of blood smears under magnification × 540, illustrates a diffusion of cytoplasmic stains of monocytes and macrophages.

Seizures occurred in 11.3% of individuals affected with DS. All major seizure types have been associated with reflex epilepsies and infantile spasms at early ages. According to the medical sheet of the patients with seizures, no significant differences among seizure treatments have been reported. At early infancy onset, two epileptic seizures states were found between all the studied cases. Two infants had become inactive and hypotonic since the seizures began.

Moreover, each subject's disability was confirmed by a psychological assessment [39]. There was an absence of psychomotor development (Table 4S) and the appearance of neurological symptoms. The two states were associated with early myoclonic encephalopathy

(EME) and specific electroencephalographic (EEG) pattern. A prominent erratic myoclonia and metabolic aetiologies were observed in these states. Their families showed an autosomal recessive form of EME which enabled the identification of missense mutation in the gene encoding the mitochondrial glutamate/proton "symporter" GC1. However, the identification of mutant GC1 as an aetiology of EME had emphasised the importance of mitochondrial component of glutamate in normal brain function (control bands) and also presented cognitive deterioration. The epileptic spasms were effectively responded to adrenocorticotropic hormone (ACTH), glucocorticoids, and vigabatrin. EEG showed distinctive electroclinical characteristics as large amplitude nonsynchronous waves. The electrographic seizure activity lasted longer than that in controls. In other four states, DS children (6 and 7 yrs.) have shown significantly more severe histological abnormalities. All of the DS individuals were snoring which can be explained by the fact that they breathe through mouth due to their nasal obstruction.

The incidence of congenital cardiac defects was about 60.6%, halved after 3-months (twice-daily) of eating fibre-rich foods with low saturated fats and sodium meal.

We also noticed in the early years a prevalent lesion of the *ostium primum* defect (a form of atrial septal defect), while the ventricular septal defect predominated in the puberty.

Table 4S

Health related quality of life (HRQoL) in parents of children with DS' age: results of the Questionnaire for Children's Health Related Quality of Life (QCHRQoL) at preadolescent's age band 6–10 years versus child's age band 11–15 years, $n = 3$.

	6-10 yrs. ($N = 25$)	11-15 yrs. ($N = 25$)	*P*	*D*
Gross motor functioning	76.0±21.6	79.2±19.3	0.201	0.18
Fine motor functioning	78.3±7.11	78.0±8.01	0.637	-0.03
Cognitive functioning	59.1±27.2	66.3±23.8	0.030	0.25
Sleep	62.2±28.4	57.4±26.7	0.209	-0.17
Pain	58.0±26.8	61.5±22.9	0.331	0.16
Social functioning	66.3±22.7	69.4±20.3	0.224	0.20
Daily activities	67.8±26.4	73.8±19.5	0.130	0.23
Sexuality	73.4±21.8	70.2±24.6	0.247	-0.12
Vitality	49.6±24.9	54.3±20.2	0.169	0.24
Positive emotions	57.7±16.3	54.9±20.4	0.435	-0.11
Depressive emotions	72.5±17.6	69.1±15.8	0.657	0.03
Aggressiveness	77.9±15.2	81.6±14.6	0.024	0.24

Results are reported as mean ($\overline{x}$)±standard deviation (σ). Higher scores indicate better HRQoL. Comparison was executed by paired *t* tests. To correct for multiple testing, the critical *P* value of $P < 0.05$ was adjusted by

the number of domains within QCHRQoL: $P < 0.004$ (0.05/12). The data concern parents (50 mothers and 50 fathers) who fully completed the QCHRQoL at both time points

Further, in connection to sexual development, we noted micropenises, decreased sperm count, cryptorchidism, and ambiguous genitalia.

3.4. Mental health

Still, there are two major questions: how information can be treated with DS children? And how metabolism can affect the information?

We registered mental health concerns of DS patients as: general anxiety, repetitive and obsessive-compulsive behaviours; oppositional, impulsive, inattentive behaviours; sleep related difficulties; depression; autism spectrum conditions; and neuropsychological (i.e., hypotonia and cranial nerve dysfunction including cranial nerve XII (hypoglossal nerve) which is responsible on tongue movement) problems characterised by progressive loss of cognitive skills (Table 4S). The cognitive disorders had resulted in markedly compromised functional skills, including basic activities of self-care such as feeding and bathing.

Pathologically, the brains of DS had exhibited an increased number of astrocytes (star-shaped glial cells of the central nervous system (CNS)) and neuronal disorganisation within the hippocampus. Alterations in glutamate homeostasis secondary to dysfunctional astrocytes had been identified. These findings suggested that induced molecular abnormalities were sufficient to cause seizures.

3.5. HRQoL

Each child was administered the Wechsler Intelligence Scale for Children-Revised [40], followed by the Peabody Picture Vocabulary Test-Revised (Form L) [41]. During the second session, each child was administered form of BOTMP [42], followed by the Developmental Test of Visual-Motor Integration [43].

As we see from Table 4S, HRQoL does not vary significantly over the change in the age of DS child.

Factors such as relative challenges in expressive language (incl. vocabulary and grammar although receptive vocabulary appeared to be in line with non-verbal mental age) and phonological awareness (abilities and recording as rhyme detection and production), low morphosyntax development, weaknesses in verbal short-term memory (STM) combined with relative strengths in visuospatial STM, and comorbid health conditions (i.e., sleep difficulties (N = 54/71), leukaemia (N = 2/71), congenital heart defects (N = 43/71)) had contributed to the increased risk of maladaptive behaviours associated with DS.

3.5.1. Maladaptive behaviour

Maladaptive (i.e., daily activities and responsibilities including conceptual, practical, social skills, and repetitive behaviour) (more details can be seen in Figure 3S and Table 4S) and challenging behaviours (as inattention and non-compliance) were expressed in 25.4% of our DS studied. *These heterogeneous symptomatology, clinical observations, and significant diagnostic refer to the important need to identify new and more comparable biomarkers as chemical elements in biofluids before we move to develop certain substances use disorders or advance therapies related to DS.*

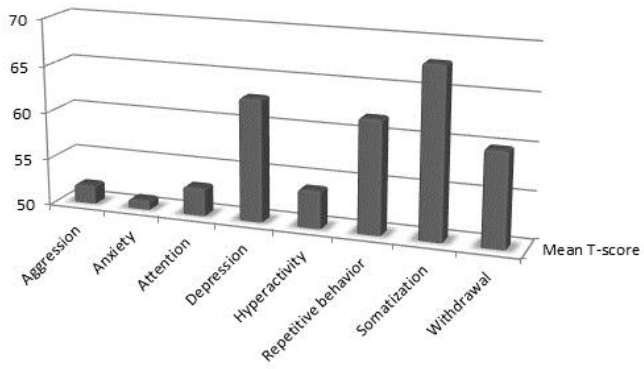

Fig. 3S. Maladaptive behaviour profile.

3.5.2. Challenging behaviours and packing: indicators to malnutrition

They had demonstrated an increased risk of challenging behaviours related to argumentativeness (67.6%), disobedience (74.6%), stubbornness (84.5%), depression (15.5%) and conduct problems (i.e., neurophysiologic, feeding difficulties and food pocketing or packing) (See Table 5S). These behavioural observations could be attributed to the specific nature of the nutritional system of those patients. Bread, pasta, and crackers were most commonly consumed texture of packed food. Non-smooth food textures (e.g., rice) and meat were the next most noticeable textures of packed food. The parents' descriptions of the food packing included describing the behaviour as being ‘chipmunk-like' (*N* = 14/71, 19.7%), as a result of over-stuffing one's mouth (*N* = 14/71, 19 .7%), and that food would also pack in their palate (*N* = 9/71, 12.7%). In this case, packing may refer to a possible imbalance between nutrient supply and nutrient requirement, impaired nutrient utilisation or uncontrolled reduction of body substance. Besides, we recognised that food packing had also caused an increase of the risk of tooth decay and cavities. Packing, in addition to poor sucking and swallowing skills, may lead to an increased risk of aspiration, malnutrition, choking, and even death.

Fifty-two parents (of DS patients, *N* = 26/71, 36.6%) specifically identified that meat was a very problematic food. Two thirds of individuals (*N* = 47/71, 66.2%) had trouble with

drinking water out of an open cup and/or controlling the speed of drinking. Only ten parents (of DS patients, N = 5/71, 7.04%) reported that their child/adult was able to eat hard vegetables and fruits as green pears and apples.

It seems that DS children with feeding problems may pack food because they do not have the oral motor skills to consume more textured food (e.g., ground beef) and packing occurred more with high textured food (e.g., bread) than low texture food (e.g., puree). Difficulties with chewing and swallowing have also been identified as severe oral motor skill deficits in this genetic condition. These difficulties could be due to cranial nerve abnormalities, which are a major feature of DS. Abnormalities of cranial nerve V and cranial nerves IX, X, and XI could contribute to dysphagia, abnormal chewing, packing and adverse feeding behaviours. Notably, the scarring process in mouth have resulted microstomia and ankyloglossi which reduced food consumption and caloric intake due to the difficulty in chewing.

On the other hand, one of our patients at the registration stage had severe consequences due to choking on food. These consequences were anoxic cerebral palsy and caused a fatality due to choking on a piece of broccoli. Feeding difficulties, in addition to breathing difficulties and reflux, were significantly prevalent in DS.

In addition, we found also hypogonadism (represents the major risk factor for osteoporosis in males), low muscular tone and strength due to malnutrition status, inactive lifestyle and inadequate participation in PhA (1.24±0.22 h/day). PhA could be a key factor in skeletal health which might be associated to the risk of developing osteoporosis. Because of reduced comprehension and shortened attention spans of subjects with mental retardation, questionnaires were designed for use of their brevity and their short directions. The PhA-Questionnaire section of the National Health and Nutrition Examination Survey (NHANES) III was adapted to assess the participants' regular PhA habits [44]. The duration of reported

activities was also determined. The PhA questionnaire was administered by the researchers of this study through interviews with the participants and the participants' direct care providers (who assisted with the questions as needed). The intensity of each specific activity was estimated using the Ainsworth Compendium for Physical Activities [45]. Moderate to vigorous physical activity (MVPhA) was defined as any PhA ≥ 3.5 METs [46].

Table 5S

Therapies employed for feeding difficulties.

Procedure	Count (%)
Feeding therapy use [(a)]	66 (93.0)
Speech language pathologist	55 (77.5)
Reflux medication use	52 (73.2)
Liquid or solid chaser use when eating	50 (70.4)
Past use of modified utensils when eating	44 (62.0)
Occupational therapist	41 (57.7)
Surgeries for eating and swallowing	32 (45.1)
Current use of modified utensils when eating	24 (33.8)
Therapy to reduce saliva (i.e., Botox therapy)	16 (22.5)
Have to remove food from cheeks when eating	9 (12.7)
Psychologist	8 (11.3)

[(a)] Individuals reported any past/current feeding therapy from one or more of the following therapists: speech language pathologist, occupational therapist, and psychologist

The Block Screening Questionnaire for Fat Intake was used to calculate a dietary fat score and to estimate the percentage of dietary fat intake of the total dietary intake [47]. The Behavioural Risk Factor Surveillance System, Fruit and Vegetable Module, was used to calculate the fruit and vegetable score and estimate the mean number of fruits and vegetables eaten per day [48]. Questionnaire scoring procedures used were those recommended in the Dietary Assessment Resource Manual [49]. 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 presented in Table 6S.

Table 6S

Results of food frequency questionnaires for child's age band 11–15 years (N = 25) of DS compared with healthy participants (N = 25), n = 3.

Variable	Kids with DS	Kids without DS	*P*
Dietary fat score [(b)]	24.2±5.19	19.60±4.36	0.002
Dietary fruit and vegetable score [(c)]	20.8±4.20	18.10±3.52	0.034
MVPhA score (minutes/week) [(d)]	162.4±274.9	308.2±356.3	0.011

[(b)] Score from the Block Screening Questionnaire for Fat Intake

[(c)] Score from the Behavioural Risk Factor Surveillance System, Fruit and Vegetable Module

[(d)] Variables were logarithmically transformed before analysis; nontransformed values are presented

The investigation showed that healthy control individuals are used to buy candy or/and snacks, independently. While, the children with DS scored higher for dietary fat, fruit, and vegetable consumption and participated in fewer minutes of MVPhA than the matched controls (Table 6S).

3.6. Physiology and biochemistry

Studies related to this experimental section are very scant and their results are inadequate.

3.6.1. Methods

3.6.1.1. Physical characterisation

To determine whether community-residing children with DS possess reduced atherosclerotic burden (estimated by a noninvasive intima-media thickness (IMT) assessment of the carotid artery), they have been compared with an appropriate control group of age-, gender-, and race-matched children without DS.

Participants dressed in lightweight clothing, weight was measured to the nearest 0.5 kg. Height was retained to the nearest 0.5 cm. Stature was reached using a standard stadiometer (Holtain, Ltd., Crymych, UK) and body mass (BM) using a balance-beam scale (SECA 709, Hamburg, Germany). Body mass index (BMI) was calculated by dividing the weight in kilograms by the height squared in meters squared. Total body fat (TBF) was assessed by dual-energy x-ray absorptiometry (DXA) (Prodigy, software version 6.7; GE Medical Systems, Madison, Wisconsin). Waist circumference (WC) was calculated to the nearest 0.1 cm with a cloth measuring tape. Bone mass density (BMD) of the lumbar vertebrae was measured in posteroanterior (PA) projection by DXA.

A standard ultrasound machine with a 7.5-MHz linear-array transducer was used to collect B-mode images of the left common carotid artery for assessing IMT. Participants were measured in the supine position with the head positioned at 45°. End-diastolic images (gated

off the R wave on electrocardiography) were collected and transmitted to a personal computer for off-line analysis. Electronic wall-tracking software was used to measure IMT on the far wall. Measurements were taken from the leading edge of the intima to the beginning of the adventitial layer. Luminal diameter (LD) and wall cross-sectional area (WCSA) were calculated. The same technician analysed all data in a blinded fashion.

3.6.1.2. Saliva collection

This research had selected the unstimulated whole saliva and parotid saliva for control and infectious samples. The whole saliva specimens were chosen and collected since this type of saliva predominates during most part of the day and considers more important for the maintenance of oral health, reflecting a physiological status of the oral cavity and of the entire body [18,19]. Besides, the subjects had also provided samples of parotid saliva. The volume of parotid saliva was collected in as few collecting sessions as was compatible with the rate of SFR and subject co-operation, 30 min is the maximum time allocated for any one meeting [50].

Out of the design box, a short experiment was carried out to indicate the statistical size of population. Salivary buffering capacity (SBC) was defined for controls (N =10) and DS (N = 10) groups, taking into consideration, the normal value for the probability level, difference of the means (D), standard deviations, and statistical size of the population which had been estimated.

Saliva samples (N = 145) were collected from male children. Each individual gave at least 40 mL of saliva in consecution during 14-55 days. The samples were collected in Salivette tubes (Sarstedt AG & Co., Nümbrecht, Oberbergischer Kreis, Germany) after overnight fasting [18]. The candidate, upon waking each time, had provided a sample exactly between 9.30 am and 12.30 pm to avoid circadian effects [18]. Individuals were instructed to refrain from eating, smoking, and drinking coffee and tea for 90 min prior to saliva collection.

During saliva collection in morning, each subject sat in a relaxed position with head in a slightly-inclined forward pose, allowing saliva to accumulate on the floor of mouth, considering that first few millilitres of saliva were discarded [19]. Then, resting whole saliva was collected for 10 min and every 1 min the volunteer expectorated oral fluid available in the mouth into 10 mL pre-weighed Eppendorf plastic tube.

First, unstimulated saliva samples (age: 2-15 yrs., mean age (μ_{age}) ± standard deviation (σ): 8.2 ± 4.15 yrs.) were selected from 74 healthy and non-diabetic young individuals (N = 74). None of this group (Controls) had systemic diseases or any local infection before 3 months and did not also take any medication for at least 6 months before saliva collection. Dental examinations were conducted by a paedodontist under natural light. Children with congenital oligodontia and delayed eruption (more than 1yr.) were excluded. All erupted teeth were evaluated according to the criteria recommended by World Health Organization (WHO) [51]. Second, DS children (N = 71) (age: 2-15 yrs., μ_{age} ± σ: 8.6 ± 5.9 yrs.) were patients chosen from the public health paediatric endocrinology service (PHPES). Children patients were trisomy 21 diagnosed by karyotype test and assessed by clinical examination. However, individuals with the history of antibiotics, anticholinergic, antihistaminic and antipsychotic therapy two weeks prior to saliva collection were excluded. In general, we gave high attention to the medications taken as with vitamin D, aspirin, or herbal medicine by the participants that could get in the way with ions metabolisms which can interfere for instance with Fe metabolism.

Parotid saliva was obtained from the subjects (DS and controls) using a modified Carlson-Crittenden device [50]. In general, saliva was collected under petroleum ether (A.R.) (Sigma-Aldrich Chemie Gmbh, Munich, Germany).

3.6.1.3. Saliva sample preparation for physical and biochemical assessments

The preparation of the sample was divided into the following stages:

Stage (1), 25 mL of sample was assigned for non-elemental analyses including total nitrogen (TN) and total phosphorous (TP) with 3 repetitions (n = 5) for each measurement. Direct analyses of viscosity (η), surface tension (γ), pH, cyanide (CN^-), thiocyanate (SCN^-), ash weight, TDS, TSS, EC, turbidity, colour, glucose, SFR, DMFT index, and saliva indices were performed for each sample. Dissolved CO_2 was measured using the micro-diffusion method of Conway [52]. Immediately after a sample collection, 2 mL of saliva was transferred to a standard Conway unit (67 mm dia.) and diffused for 60 min. TN in 1 mL of saliva was determined by the micro-Kjeldahl method. The sample was digested with cont. sulphuric acid (H_2SO_4) (PVS Chemicals Belgium NV, Ghent, Belgium) and salicylic acid (monohyroxybenzoic acid: $C_7H_6O_3$) (30 : l w/v) (Gurudev Marketing Private Ltd., Vadodara, India) with potassium sulphate (K_2SO_4) (Krishna Chemicals, Vatva, Ahmadabad, India), copper sulphate ($CuSO_4$) (Indian Platinum Pvt Ltd., MIDC, Mumbai, India) and selenium dioxide (SeO_2) (Changsha Santech Materials Co., Ltd., Hunan, China) at 120 $^{\circ}$C until the solution cleared. Titration of the boric acid (H_3BO_3) (Merck-Millipore Co., Massachusetts, USA) was carried out against 0.2 N sodium hydroxide (NaOH) (Sigma-Aldrich Chemie Gmbh, Munich, Germany) using a bromocresol green ($C_{21}H_{14}Br_4O_5S$) (Sigma-Aldrich Chemie Gmbh, Munich, Germany)/methyl red ($C_{15}H_{15}N_3O_2$) (Sigma-Aldrich Chemie Gmbh, Munich, Germany) indicators. Besides, TP was measured by the colorimetric method.

All the concentrations were expressed as mean (μ)±standard deviation (σ) with 95% CI.

Stage (2), 15 mL of sample was assigned for total proteins and elemental analyses (excepting TN and TP). Samples were treated with 20% trichloroacetic acid (TCAA: Cl_3CCOOH) (VWR, Radnor, PA, USA) (0.5 mL of 20% TCAA to 1 mL saliva sample) and centrifuged to remove proteins. The residual saliva sample was weighed, and taken to dryness under infrared lamps, then ashed at 400 $^{\circ}$C for 2 hrs; the ash weight was recorded (for protein assessment).

Stage (2A): Total proteins and immunoglobulin A (IgA) measurements, 10-14 g (to 1 mg) of the precipitate (sub-sample) of the residual ash was dissolved in 1 mL 10 M hydrochloric acid (HCl) (VWR, Radnor, PA, USA) and boiled for several min. to convert pyrophosphate ($P_2O_7^{4-}$) to orthophosphate (PO_4^{3-}), and made up to 20 mL. Then total protein was measured by the colorimetric method [53], non-protein nitrogen (NPN) [54] and IgA using Stone et al. [55] method.

Stage (2B): Na and K measurements in the effluent - the mixture (saliva after acid extraction with TCAA and HCl) was inserted the micro-centrifuge (Jack Chen Biologix Plastics Co., Ltd., Shanghai, China) and applied to a speed of 2500 rpm for 20 min. to take out food rests, bacteria, mucosal cells, microorganisms and desquamated cells from oral epithelium, and other extraneous particles. Then, each filtrate was passed through a 0.45 μm MF-Millipore (Merck-Millipore Co., Massachusetts, USA) to take away any existed higher-molecular-mass and sulphates (SO_4^{2-}) in the matrix of sample. The resulting solution was not diluted. Na and K were measured directly by flame-atomic emission spectroscopy (FAES).

Stage (2C), 1 mM NaOH solution was added to adjust pH of specimen to about 11 which made the mixture clearer and more transparent. Samples were divided into several portions to determine alkaline-earth (Mg, Ca, Sr, and Ba), transition metals (Ti, Cr, Mo, Mn, Fe, Cu, and Zn,), Al, and Si. Samples were stored in sealed Thermo Scientific Nalgene LDPE bottles away from direct sunlight at -20 $^{\circ}$C (-4 $^{\circ}$F) to avoid any significant change of enzyme activity (i.e., amylase) or if any still existed protein degradation. Immediately before analysis, samples were thawed at room temperature 25 $^{\circ}$C (77 $^{\circ}$F). Ca and Mg were assayed by validated methods [18,19] using ISE apparatus that equipped with calcium selective electrode based on solid-state PVC polymer and phenylene bis (ditolyl) phosphine oxide as ionophore, whereas magnesium selective electrode was based on tetraphenylborate salt of Mg-4,7-diphenyl-1,10-phenanthroline (1 : 3) complex in *o*-nitrophenyloctyl ether ($C_{14}H_{21}NO_3$) in

PVC matrix. Besides, 1 mL aliquot was taken and diluted with 9 mL of 1% nitric acid (HNO_3) (v/v, > 18 Ωcm^{-2}) (Merck-Millipore Co., Massachusetts, USA) in order to analyse Ba and Sr by ICP-MS.

Mo, Mn, Cr, and Ti were measured by standard addition method using GF-AAS. However, Al, Si, Fe, Cu, and Zn were tested by direct method using GF-AAS technique.

3.6.1.4. Chemical characterisation (Saliva)

In order to estimate the sample size, the SBC (as proved an important bioindicator) of both of the DS (11–15 yrs.) and the controls (11–15 yrs.) had been determined for ten individuals (N =10) of each group.

Determination of sample size (N)

The significance level (α) = 5 %

β = 20%

Power of the test (1- β) = 80%

$$N = \frac{2\times [Z_\alpha (\sigma)]^2}{d^2}$$

$$= \frac{2\times [2\times (0.54)]^2}{(0.18)^2}$$

$$= 72$$

Where,

Z_α - Normal value for the probability level = 2 at 5% level;

N - Sample size

σ - Standard deviation = 0.54

d - Difference in the means = 0.18

As a result, 71 subjects with DS and 74 healthy subjects are involved in this study. Saliva was gathered from each subject in order to assess the difference in the physicochemical properties between DS saliva and saliva controls.

pH method of analysis

pH of saliva sample is dependent on the level of dissolved carbon dioxide (CO_2) thus for a true pH value and to avoid any time-relating pH changes or loss of CO_2, the degree of acidity was measured immediately after each sample collection, using a hand-held pH meter. Every day during the time of this research, pH meter had been calibrated with reference buffers of pH 4.0 and 7.0.

Glucose assessment

Patients were asked to wash their mouths with tap water and spit two or three times, after which they informed to spit the saliva pooled in their mouths for the following 10 min. into the sterile sample collection container.

Saliva samples were centrifuged and glucose was estimated in the supernatant saliva by the glucose oxidase (GOx) method using 4-aminophenazone ($C_{11}H_{13}N_3O$) as oxygen acceptor [39].

3.6.1.5. Chemical characterisation (Blood and serum)

All blood draws were made in the morning after an overnight fast (12 hrs.). Participants and care providers were phoned the night before testing and again in the morning before the testing session to remind them of the 12 hrs. fast. To lessen the age effect on the biochemical

and physical measurements, we decided to measure these parameters in a narrow band (N = 25, [11-15] yrs.) for matched groups.

Cholesterol profiles, triglycerides (TG), and glucose levels were determined by colorimetric reflectance spectrophotometry. C-reactive protein (CRP) was analysed with an ultrasensitive assay using rate nephelometry. Insulin was determined by chemiluminescent immunoassay. Homocysteine (HCY) was determined through a fluorescence polarisation immunoassay. Seated auscultatory blood pressure was measured with a mercury sphygmomanometer according to the guidelines established by the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.

Plasma samples were obtained by centrifugation (1800 × g for 20 min.), conveyed into coded plastic tubes, promptly ice-covered and kept at -20 $^{\circ}$C until analysis. Adiponectin (ADIPOQ), tumour necrosis factor- α (hsTNF-α), interleukin-6 (IL-6), and leptin were assayed using a commercial immune-enzymatic kit (Quantikine by R&D Systems, Minneapolis, MN, USA) with the corresponding minimum detectable dose of sensitivity, 0.25 ng/mL, 0.12 pg/mL, 0.70 pg/mL, and 6.20 ng/ mL.

Hormonal status is important for adolescence, the critical period for bone mineralisation. Thus, follicle stimulating hormone (FSH), luteinising hormone (LH), total testosterone, dehydroepiandrosterone sulphate (DHEA-S), and 17-OH progesterone were measured accordingly, by radioimmunoassay.

Parathormone (PTH) was analysed by MM-PTH radioimmunoassay kit with 5% and 10% of intra-assay and inter-assay CV, correspondingly.

Serum ferritin (Ft) levels were determined by using an ELISA method. The ELISA quantitation kit was purchased from Ramco Laboratories (Houston, TX, Catalog #S-22). The assay procedure followed the instructions by the manufacturer. Briefly, the sera were diluted 10-fold with sample diluent supplied with the assay kit. The diluted samples were pipetted to

the wells pre-coated with polyclonal anti-human Ft antibody. Following addition of horseradish peroxidase (HRP) conjugated secondary antibody, the reaction mixtures were incubated, washed, and the absorbance at 490 nm recorded. The concentrations of serum Ft were calculated from a standard curve derived from the same procedure using purified human Ft. Serum concentrations of transferrin receptor (TfR) were determined by using the similar ELISA test kit purchased from Ramco Laboratories (Catalog #TF-94). The experimental procedure followed the instructions by the manufacturer. The sera were diluted 100-fold with the sample diluent. The absorbance was determined at 450 nm and the concentrations of TfR were estimated from a standard curve using human TfR as the standard. Serum levels of transferrin (Tf) were also determined by an ELISA kit (Montgomery, TX; Catalog #E80-128). The assay procedure followed the instructions by the manufacturer. Serum samples were diluted 20,000-fold prior to assay. The absorbance was read at 490 nm and converted to calculate the serum concentrations using purified human Tf as the standard.

Elemental analysis - Because values did not follow a normal distribution, 5–95th percentiles of the data of each element and biological material was presented coupled with mean and standard deviation. The elements were measured using graphite furnace-atomic absorption spectroscopy (GF-AAS) and the statistical analyses were performed using SPSS. The magnitude of the correlation between metal concentration in the biological matrixes was assessed by the Spearman rank correlation test.

3.6.1.6. Chemical characterisation (Urine)

Urine samples were collected on morning from all subjects using sterile urine containers, portioned into 1 ml aliquots to avoid freeze/thaw cycles in repeated experiments of the same sample, and stored at -20 $^{\circ}$C for further use. The label-free mass spectrometry-based profiling has been performed for urinary proteomic study. Moreover, the chemical elements have been determined by GF-AAS. Appropriate matrix modifiers were used for the selected metal

studied and prepared in 0.2% (v/v) HNO_3 and 0.1% Triton X-100. Prior dilution of each sample was critical in order to obtain the best results. Urinary concentrations of the selected metals were adjusted for creatinine levels to reduce inter-individual variation of urinary measurements.

3.6.1.7. Chemical characterisation (Hair)

Hair is a more stable matrix than blood and urine in collection, transportation and does not show storage alterations for the period of sampling and analysis. Therefore, hair testing has substantially assisted in monitoring excessive DS exposure to toxics, which may prove its suitability for use in this prospective pilot study for identifying DS population at increased risk in contaminated civilian areas. So that, hair could be a useful indicator for contamination rather than nutrition.

Participant gave hair samples (approximately 0.1 g) which were washed with double-distilled water and neutral soap. The length varied between 0.4 and 2.5 cm. The hair samples were kept in acid pre-cleaned polyethylene (PE) containers and the hair was washed via ultrasonic cleaning in a non-ionic detergent (Triton X-100, Merck, Darmstadt, Germany) solution, and then the detergent was removed by copious rinse with Milli-Q water and washed by ultrasonic cleaning in an ethanol solution (Merck, Darmstadt, Germany), and again with Milli-Q water. The cleaned hair was dried at 70 °C overnight. The hair was ground to a fine powder using a manual agate mortar and then after addition of 1 mL of HNO_3 (Merck), 0.5 mL of HCl (Merck), 2mL of H2O2 (Merck) and 2 mL of H_2O were digested during 30 min in a microwave oven Multiwave 3000 (Anton Parr, Graz, Austria). Chemical elements are measured by GF-AAS.

3.6.1.8. Physiochemical analyses protocols

Table 7S describes the instruments used and the referenced methods implemented for the biophysical and biochemical analyses.

Table 7S

The adopted analytical techniques and instrumentations used in this paper.

Analyte	Instrument	Referenced methods
Ash weight	-	ASTM E1755-01 (see also: http://www.nrel.gov/docs/gen/fy08/42622.pdf)
Glucose ($C_6H_{12}O_6$)	DR 1900 (Hach, Colorado, USA)	[19] (see also: https://secure.megazyme.com/files/Booklet/K-GLOX_DATA.pdf)
Carbon dioxide (CO_2)	-	[52]
Total protein	DR 1900 (Hach, Colorado, USA)	[53]
IgA	Polystyrene Elisa plate	[55]
Saliva flow rate (SFR)	Graduated syringes	[56]
Viscosity (η)	Ostwald-type capillary viscometer	[57]
Surface tension (γ)	Micro capillary rise apparatus	[58,59]
Colour	DR 1900 (Hach, Colorado, USA)	APHA
Electrical conductivity	MP-4 Portable Meter (Hach, Colorado,	ASTM D1125

(EC)	USA)	
pH	CP-411 pH-Meter (Analyiso GmbH, Greifswald, Germany)	ASTM D1293
Sodium (Na) and potassium (K)	Jenway PFP7 flame photometer (Jenway Gransmore Green Felsted, Essex, UK)	ASTM D1428
Calcium (Ca) and magnesium (Mg)	Microlyte 6 analyser (Thermo Fisher Scientific Oy, Vantaa, Finland)	[19]
Strontium (Sr) and barium (Ba)	ICP-MS, Thermo Elemental, (Thermo Fisher Scientific, Waltham, MA, USA)	[60]
Aluminium (Al)	Acid extractable by the preliminary treatment, GTA-novAA 400 P (Analytik Jena AG, Jena, Germany)	[61] (See also: ASTM D857)
Silicon (Si)	GTA-novAA 400 P (Analytik Jena AG, Jena, Germany)	[62]
Molybdenum (Mo), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), chromium (Cr), titanium (Ti), and silicon (Si)	GTA-novAA 400 P (Analytik Jena AG, Jena, Germany)	ASTM D3919
Cyanide (CN^-)	DR 1900 (Hach, Colorado, USA)	ASTM D2036-09(2015)

Thiocyanate (SCN^-)	DR 1900 (Hach, Colorado, USA)	ASTM D4193-08(2013)e1
Salinity	MP-4 Portable Meter (Hach, Colorado, USA)	-
Total dissolved solids (TDS)	MP-4 Portable Meter (Hach, Colorado, USA)	ASTM D5
NPN	-	[54]
Total nitrogen (TN)	Micro Kjeldahl Apparatus (Labconco, Kansas, MO, USA)	ASTM D8083 - 16
Total phosphorous (TP)	Spectron CA72TP (Endress+Hauser AG, Reinach BL, Switzerland)	ASTM D515
Total suspended solids (TSS)	DR 1900 (Hach, Colorado, USA)	ASTM D5907
Turbidity	2100Q IS (Hach, Colorado, USA)	ASTM D1889

3.6.2. Results

3.6.2.1. Salivary buffering capacity vs. physiooral indicators

The difference of the SBC of the controls and DS is considered to be not statistically significant (Table 8S). Besides, a negative correlation was found between SBC and dental caries (which is lower in DS). No significant difference in SBC and salivary pH, but a lower SFR (Table 1) in DS subjects.

Table 8S

SBC of the controls (*N* =10) and DS (*N* = 10).

SBC											$\bar{x}$	σ	SED	*P*
Control	3.7	3.5	3.4	3.4	3.2	3.2	3.1	3.0	2.9	2.7	3.21	0.30	0.20	0.37
DS	3.9	3.8	3.6	3.0	3.0	2.7	2.7	2.6	2.5	2.5	3.03	0.54		

SBC determined according to Dumont [63]; Two-tailed *P* value, σ: standard deviation, SED: Standard Error of Difference; CI = -0.231 to 0.591 and *t* value = 0.9194

The SFR fluctuations along with the ionic composition of saliva may also affect the chemical behaviour as metal ions and vitamins in oral cavity. For instance, the 19.7% (*N* = 14/71, *n* = 5, DS: 21.8±6.24 μg/dL; *N* = 69/71, *n* = 5, Controls: 38.6±10.2 μg/dL, OR = 5.12, *P* = 0.018) deficiency of vitamin A detected in DS compared with controls can be attributed to the SFR reduction in parotid saliva which supports the fact that DS patients have a sub-normal salivary secretion. But, how vitamin A malabsorption can affect the salivary gland function in DS?

3.6.2.2. General physiological and biochemical (blood and serum) characterisations

Table 9S proved a decreased bone biochemical markers, a diminished bone formation rate and reduced mechanical strength. In addition, what we clearly found in this study that DS patients had lower BMD and higher prevalence (43.7%) of hypothyroidism (patients received replacement therapy of Eltroxin, 50-200 mg/day) compared to controls due to inadequate weight bearing activity which had affected the skeleton. Hypothyroidism could adversely affect the patient's relationship with food, and accordingly appetite (evidenced by the high saline saliva; Tables 1 and 2) and in whole the nutritional status. The BMD mainly in the

trabecular could be attributed to the mineral changes than the peripheral skeleton which refers to malnutrition. BMD reliability was high (r = 0.99) and bias was approximately 1%. The low BMD in DS was correlated significantly with the decrease in bone formation markers compared to controls without DS but it was not clear for us whether this was due to specific effects of chromosome 21 genes (Figure 1S) or lifestyle factors.

Table 9S

Comparison of basic physiological and biochemical parameters between DS and healthy participants.

Variable	Kids with DS (N = 25)	Kids without DS (N = 25)	P
Descriptive variables			
Age (yrs.)	12.8±1.65	12.9 ± 1.54	0.518
IQ	54.0±12.0	92.0 ±17.0	0.693
Height (cm)	141.6±7.45	160.2±5.58	0.000
Stature (cm)	117.8±6.75	76.4±8.41	0.000
Weight (kg)	49.6±11.6	57.1±13.6	0.002
BMI (kg/m^2)	21.2±4.08	19.0±4.76	0.012
BMD of the lumbar Vertebrae (g/cm^2)	0.65±0.12	1.01±0.05	0.001
Carotid artery			
IMT (mm)	0.37±0.06	0.42±0.08	0.000
LD (mm)	5.12±0.63	5.46±0.71	0.129
WCSA (mm^2)	21.40±4.25	24.8±4.63	0.053
Fasting plasma levels			

WBCs (cells/µL)	6700±2300	8931±3210	0.101
Hb (g/dL)	10.5±1.09	13.2±1.81	0.153
Ht (%)	46.5±1.50	39.0±3.75	0.144
hCG (mIU/mL)	8.00±3.00	3.50±0.40	0.208
Na (mg/L) [(e)]	3620±148	3209±107	0.005
K (mg/L) [(f)]	107.3±36.5	173.6±23.8	0.019
Ca (mg/L)	77.2±6.40	90.0±0.40	0.001
P (mg/dL)	4.02±0.28	4.37±0.40	0.003
As (µg/L)	0.73±0.14	0.47±0.08	0.001
Hg (µg/L)	1.48±0.29	0.63±0.13	0.001
Pb (µg/L)	0.92±0.21	0.49±0.07	0.001
CR (mg/dL)	1.50±0.30	0.80±0.10	0.024
Serum P1NP (ng/mL)	1.60±1.40	18.2±3.80	0.701
Serum CTx (ng/mL)	0.30±0.10	0.40±0.10	0.522
HDL-C (mg/dL)	33.9±8.72	30.2±6.84	0.044
LDL-C (mg/dL)	75.2±15.9	77.8±19.6	0.719
TG (mg/dL)	84.9±37.0	70.2±36.3	0.034
Insulin (µU/mL)	7.31±6.43	6.69±4.94	0.502
BG (mg/dL)	79.0±10.6	60.2±6.77	0.684
CRP (mg/dL)	0.40±0.34	0.21±0.18	0.001
HCY (µmol/L)	6.07±1.24	6.22±1.37	0.573
SBP (mm Hg)	100.7±12.7	108.5±14.6	0.026
DBP (mm Hg)	51.6±8.86	63.2±7.73	0.001
Free T_3 (pg/mL)	2.41±0.80	2.96±2.07	0.062
Free T_4 (pg/mL)	1.17±0.23	1.53±0.58	0.057

ADIPOQ (ng/mL)	54.5±10.1	11.7±6.93	0.008
hsTNF-α (pg/mL)	16.2±8.6	4.29±0.54	0.000
IL-6 (pg/mL)	114±65	7.68±3.92	0.000
Leptin (ng/mL)	94.6±45.2	6.59±0.27	0.000
Total proteins (g/L)	42.3±8.27	78.4±1.20	0.068
Hormones			
FSH (mIU/mL)	7.71±3.54	6.83±2.92	0.000
LH (mIU/mL)	7.00±2.91	3.51±1.48	0.006
Testosterone (nmol/L)	28.2±12.5	32.8±11.6	0.001
17-OH progesterone (ng/mL)	3.27±3.06	1.10±0.39	0.038
DHEA-S (µg/mL)	3.38±1.28	3.02±1.27	0.002
PTH (pmol/L)	16.8±8.09	27.4±10.6	0.044
Body composition			
TBF (%)	25.4±6.85	21.8±7.62	0.007
WC (cm)	88.1±13.0	84.1±13.3	0.065
Energy balance			
TEI (kcal)	2211±286	2883±472	0.001
TEE (kcal)	2042±358	2500±559	0.001
TEB (kcal)	160±32	280±50	0.001

Systolic blood pressure (SBP), Triiodothyronine Free serum (Free T_3), Thyroxine Free serum (Free T_4), Total energy intake (TEI), Total energy expenditure (TEE), and Total energy balance (TEB)

(e) No Na-drugs taken as sodium zirconiumcyclosilicate (ZS-9: $(2Na \cdot H_2O \cdot 3H_4SiO_4 \cdot H_4ZrO_6)_n$)

(f) No cardiorenal diseases (incl. kidney) observed and no agents taken to control plasma of K^+

In this connection, weight-bearing exercise (referred by functional muscle-bone unit) and Ca intake can expectedly increase BMD. Still, there are three questions which need further research: Does low bone mass exacerbate muscular tone and strength? Does muscle strength impact on bone mineralisation? And is there any definite relation between BMD and osteoporotic cracks usually found in DS? Here, we suggest conducting specific functional tests to explore the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal interactions in DS.

The mean IMT of children with DS was significantly lower than the mean IMT of the matched control group. Stepwise linear regression resulted in only 2 significant predictors of IMT in the group of with DS. Male gender and weekly minutes of MVPhA (Table 6S) could be identified as significant predictors of IMT for children with DS. Stepwise linear regression resulted in 4 significant predictors of IMT in the control group without DS: fasting plasma insulin, age, fruit and vegetable score, and LDL-C, were all significant predictors of IMT in the group of without DS. The model variance accounted for approximately 66% of the IMT variance in the control group, indicating that the traditional risk factors are better predictors of IMT for subjects without DS compared to patients with DS. These results also indicate that non-traditional risk factors may be associated with IMT in patients with DS. The significant reduction in IMT values of DS patients along with differing predictors of IMT in patients with DS indicates that patients with DS possess a unique atherogenic model that differs from that of subjects without DS. However, the potential protective mechanisms against atherosclerosis in patients with DS require additional research. Examining this unique DS

model of atherosclerosis may identify protective factors that could be applied to protect against CVD. In this connection, although patients with DS had similar or worse (i.e., CRP and TG) CVD risk factors than individuals without DS, their IMT was still significantly lower (12%). Regression analysis revealed significant relations between CVD risk factors and IMT in controls (total prediction model: R^2 = 0.8019, Adjusted R^2 = 0.7627, P = 0.033); however, in patients with DS (total prediction model: R^2 = 0.3625, Adjusted R^2 = 0.3326, P = 0.028), these relations were not observed. Taken together, these data suggest that patients with DS may possess genetic factors that protect them, to some degree, against atherogenesis. Future studies are needed that examine the genes responsible for DS and atherogenesis.

Adipocytokines are bioactive mediators released from adipose tissue including adipocytes and other cells present in fat tissues. The high plasma content of ADIPOQ in DS is stressed by the production of anti-inflammatory mediators as interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1RA) or by blocking interferon-α production. The higher values of hsTNF-α and IL-6 in DS refer to the vessel dysfunction, while the higher leptin in those subjects reflects to an increased vulnerability to infectious or inflammatory diseases.

From the main text, the OHP/Creat. ratio was significantly higher than that of normal subjects, suggesting the DS patients have a high bone turnover.

DS-FSH was somewhat insignificantly higher than controls and LH was significantly greater for patients which refer to a partial gonadal deficiency. Additionally, there were not any differences in DHEA-S levels between the two groups, whereas 17-OH progesterone levels were significantly higher (P < 0:05) in DS referring to the genetic disorder congenital adrenal hyperplasia (CAH) if the 17-OH progesterone overpasses 3.15 ng/mL. Despite, there was no statistical difference of mean testosterone levels between the groups, the levels for DS patients were on the low side of the normal laboratory range. The PTH level was decreased

insignificantly (with exception of 4 patients, who had an extremely low values; ≤ 5 pmol/L but associated with no particular lower BMD) for DS patients even its contents were within the normal laboratory range.

HCY was decreased with non-epileptic seizures (seizures occurred in 11.3% of DS) who can be shot by L-acetyl-cysteine but on the opposite patients with epileptics have shown higher levels of HCY which points to the possible fact that HCY may provoke seizures. This likely due to the overexpression of cystathionine-β-synthase, which diverts HCY into cysteine, thus preventing it from being recycled (remethylated) into methionine within the S-adenosylmethionine cycle.

By way, there were also no differences in the cholesterol profiles, except that children with DS had higher TG than controls, while CRP and TBF were higher for children with DS compared to the matched control group. SBP and DBP were significantly lower in children with DS compared to the matched control group.

As we know, cations can operate as cationic exchangers. Cation-coupled Cl-cotransporters as Na^+ and K^+ maintain osmotic homeostasis and play a vivid role in immunity and cellular regulation, thus, we believe that they highly expressed in the CNS. Hence, biochemical (as proteomic) central and peripheral mechanisms can be involved in the development and maintenance of DS pathogenesis.

Ca and P in plasma did not differ significantly between the groups and Ca excretion in urine (as seen in the main text) was normal, even the elements in plasma were lower than controls. This was anticipated where in general, in unsophisticated cases of osteoporosis, the fairly slow rate of bone loss and the integrity of such of the regular homeostatic mechanism do not cause changes in those elements. On the other way, Ca deficiency (14.2%) can lead to

reduction in BMD (35.6%) by increasing bone desorption to preserve the level of ionised Ca in the extracellular fluid.

The higher levels of toxics (As, Pb, and Hg) found in serum made us tracking longitudinally the distribution of other heavy metals in basic biological fluids.

3.6.2.3. Urinary proteins and altered metabolites

Urinary proteomic is a novel tool for biomarker discovery in diseases associated with kidneys and portrays the pathological changes associated with the function of kidney and the urogenital tract for patients [64] as our case DS. The results indicate for the first time that DS is associated with up-regulation of hydroxyphenylacetate and uridine, and concomitant down-regulation in glutamine and phenylalanine (Phe) levels. The detection of bilivierdin reductase A (BLVRA) (an excellent cytoprotectant against oxidative stress and hypoxia) in urine (Table 10S) suggests an impaired function of cells and their membranes as a consequence of the oxidative damage and immunological dysfunction. On the other hand, it seems that BVR activity has been induced by the elevated serum quantity (and possibly in urine) of hsTNF-α (Table 9S). This can develop the NF-kB activation.

Table 10S

Potential protein biomarkers identified in urine with DS.

Protein name	Protein average mass (kDa)	Protein score	Number of matched peptides
Acylpyruvase FAHD1, mitochondrial	25.2	1315	3
Angiotensinogen (AGT)	50.0	712	10

BLVRA	33.5	356	5
Potassium/sodium hyperpolarisation-activated cyclic nucleotide-gated channel 3	86.4	156	4
Protein phosphatase 1 regulatory subunit 1A (PPP1R1A)	19.2	1604	6
Superoxide dismutase [Cu–Zn]	16.0	995	7
Xaa-Pro aminopeptidase 1 (XPNPEP1)	70.7	927	7

Table 11S shows that hydroxyphenylacetate and uridine were up-regulated, whereas glutamine and Phe were down-regulated in DS patients. This also refers to the potential oxidative damage in DS.

Table 11S

List of significantly altered urinary metabolites in DS patients.

ID	m/z	Neutral mass	Retention time (min)	Factor of change	Regulation
Creatine	133.4011	132.3790	0.65	1.7	Up
Creatinine	115.2097	114.1858	0.55	1.2	-
2,6 Dimethyl-	305.2622	304.2371	5.60	3	Up

heptanoyl carnitine					
Glucose (or other sugar)	182.8867		3.14	2.7	Down
Hydroxyphenyl acetate	154.6009	153.5674	2.62	2.6	Up
Indoline	121.2846	120.2637	2.86	1.8	Down
L-Glutamine	148.5219	146.0691	2.47	3.5	Down
3 or 7 methylxanthine	168.7370	167.7093	2.75	1.8	Down
Phe	167.7511	166.7294	2.31	1.7	Down
Uridine	247.5280	246.5096	2.58	Infinity	Up

3.6.2.4. Biochemical characteristics of saliva

The physical properties, amount, and composition of saliva are influenced by factors such as diet, time of day, and physic condition and those factors may possibly explain the variations in saliva composition.

The congenital oligodontia (observed between the excluded patients), delayed eruption, increased pH (more alkaline), decreased SFR (73.9%), and an increase of glucose concentration (60.7%) (Tables 1 and 2) have referred to the lower dental caries of DS patients.

Remarkably, the significant decrease in dental caries in the primary and permanent dentition of DS patients has accompanied with an increase in major salivary electrolytes (see Tables 1-6, especially EC values). These findings guided a relatively less restorative

treatment need than that seen in the normal childhood population due to the presence of less dental caries.

The increase of Ca^{2+} and TP in DS saliva as described in the main text, is expected to reduce enamel solubility; thus, decreasing incidence of dental caries (Table 2S). Salivary K^{+}, Na^{+}, and Mg^{2+} (Table 3) showed a positive but statistically insignificant correlation with dental caries ($P > 0.01$). Here, we think that not even salivary secretion but also substances secreted within saliva influence the strength caries attack. Hence, salivary composition is a key factor in determining of dental caries.

Compared to Siqueria et al. [65] the SFR, pH, Ca^{2+}, and Mg^{2+} of DS patients were higher than the corresponding findings of the Indian study by 9.7%, 4.4%, 93.9%, and 94.2%, respectively. However, it is reasonable to accept the alkaline phase of salivary DS medium as a major biochemical characteristic of this intervention.

At last, heavy metals have affected saliva, salivary glands, and oral health as a result of human exposure or due to nutritional status (Table 5S). But more studies in series are needed to realise the salivary heavy metals possibly the risk of oral carcinogenesis.

3.6.2.5. Prioritised biomonitoring matrices

Studies on saliva, blood, urine, and hair of subjects with DS are limited and less clear-cut, and there is no biological study has concerned a broad-scale of human biology like our research. For that reason, the distribution of Mo, Mn, Cr, Ti, Cu, Zn, Fe, Al, and Si levels in different biological fluids (saliva, whole blood, urine, and hair) in DS population has been discussed in the main text. DS biological monitoring has been focused on these matrices for the establishment of environmental limits of exposure of metals. So that, those media can be used as a vehicle of excretion of substances from the human body as of heavy metals.

Seemingly, the presence of roadside dust in hair and evaporation of sweat on hair has led to greater incorporation of toxic elements in hair (Table 7) via this exogenous route. Even, the toxics could be discarded by cleaning-up step, but generates another environmental issue. In fact, these levels are supposedly attached to the keratin molecules during the short period of hair formation which may correlate with ions blood concentrations (and maybe body stores). Moreover, lifestyle may also contribute to hair content of toxics in hair, since it is hard to avoid external contamination as artificial hair treatment (i.e., dyeing, breaching, waiving, deodorant). Thus with the results presented in the main text, we suggest saliva, blood, and urine as bioindicators for malnutrition. In addition, DS human hair can be suggested over blood and urine for environmental health survey.

3.6.3. Major possible sources of the micro- and macro- elements in body

To understand the potential sources of elements distribution and their concentrations in biological samples, the normal body containment of these elements obtained from the web have been archived in Table 12S.

Table 12S

Possible sources of the micro- and macro- minerals in healthy human body.

Element	Major possible sources	Levels in healthy human body	Web references
Al	1- Kitchen utensils, 2- Medicines such as antacids, 3- Cosmetics, and 4- Food (cocoa, herbs, nondairy creamer, nuts, pickle, potatoes, preserves, rhubarb, soup, spices, sugars, and tomatoes)	Bone: 4-27 mg/kg Liver: 3023 mg/kg Muscle: 0.7-28 mg/kg Daily dietary intake: 2.5 mg Total mass in average 70 kg human: 60 mg PEL: 15 mg/m^3 total particulate, 5 mg/m^3 respirable particulate.	https://www.atsdr.cdc.gov/toxprofiles/tp22-c2.pdf http://www.healthy.net/Health/Article/Aluminum/1958 https://www.atsdr.cdc.gov/toxprofiles/tp22-c6.pdf https://environmentalchemistry.com/yogi/periodic/Al.html
Ba	*1- Glassware,* *2- Additives for oils and fuels,* *3- Drinking water (about 2mg/L),* *4- Air (about 1.5 ng/kg), and* *5- Food (nuts and seafood)*	*Bone: 3-70 mg/kg* *Liver: 0.04-1.2 mg/kg* *Muscle: 0.09 mg/kg* *Daily dietary intake: 0.6-1.7 mg* *Total mass in average 70 kg human: 22 mg*	*http://www-jmg.ch.cam.ac.uk/data/weii/barium.html* *https://www.atsdr.cdc.gov/ToxProfiles/tp24-c1-b.pdf*

		PEL: 15 mg/m^3 total particulate, 0.5 mg/m^3 air	*http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf*
Ca	1- Water, 2- Food (cheese, fish, fruit, leafy greens, legumes, milk, yogurt, and seafood), and 3- Drinks (juice)	Bone: 170000 mg/kg Liver: 100-360 mg/kg Muscle: 140-700 mg/kg Daily dietary intake: 600-1400 mg Total mass in average 70 kg human: 1 Kg PEL: 15 mg/m^3 total particulate, 0.5 mg/m^3 air	http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf http://www-jmg.ch.cam.ac.uk/data/weii/calcium.html https://ods.od.nih.gov/factsheets/Calcium-HealthProfessional/
Cr	*1- Environmental (airborne emissions, asbestos lining erosion, contaminated landfill, effluents from chemical plants, and tobacco smoke), and* *2- Food (apples, bananas, beef, black pepper, butter, chicken, eggs, green peppers, molasses,*	*Bone: 0.1-0.33 mg/kg* *Liver: 0.02-3.3 mg/kg* *Muscle: 0.024-0.84 mg/kg* *Daily dietary intake: 0.01-1.2 mg* *Total mass in average 70 kg human: 14 mg*	*http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf* *http://www.nytimes.com/health/guides/nutrition/chromium-in-*

		oysters, spinach, and wheat)	*PEL (Cr^{6+}): 15 mg/m^3 total particulate, 0.5 mg/m^3 air*	*diet/overview.html https://www.atsdr.cdc.gov/csem/csem.asp?csem=10&po=5*
Cu	1-	Food (cashews, chocolate, legumes, meats, mushrooms, seafood, sesame seeds, and whole grains)	Bone: 1-26 mg/kg Liver: 30 mg/kg Muscle: 10 mg/kg Daily dietary intake: 0.50-6 mg Total mass in average 70 kg human: 72 mg PEL: 0.1 mg/m^3 total particulate, 1.0 mg/m^3 air	http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf http://www.whfoods.com/genpage.php?tname=nutrient&dbid=53
Fe	*1-*	*Food (beef, dried beans, dried fruits, egg yolks, iron-fortified cereals, oysters, poultry, salmon, tuna, and whole grains)*	*Bone: 3-380 mg/kg* *Liver: 250-12400 mg/kg* *Muscle: 180 mg/kg* *Daily dietary intake: 6-40 mg* *Total mass in average 70 kg human: 4.2 g* *PEL: n.a.*	*http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf* *https://medlineplus.gov/ency/article/002422.htm*

K	1-	Food (broccoli, burgers, chickens, cod, legumes, milk, nuts, potatoes, peas, red meat, salmon, soybean, tomatoes, veggie, and yoghurt)	Bone: 2100 mg/kg Liver: 16000 mg/kg Muscle: 16000 mg/kg Daily dietary intake: 1400-1700 mg Total mass in average 70 kg human: 140 g PEL: n.a.	http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf https://medlineplus.gov/ency/article/002413.htm
Mg	*1-*	*Food (apricots, bananas, dark-green, dried beans such as soybeans, baked beans, lentils and peanuts, green leafy vegetables, legumes, low-fat milk, nuts such as almonds and cashews, spinach whole grains, and yogurt)*	*Bone: 700-1800 mg/kg* *Liver: 590 mg/kg* *Muscle: 900 mg/kg* *Daily dietary intake: 250-380 mg* *Total mass in average 70 kg human: 19 g* *PEL: n.a.*	*http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf* *https://draxe.com/magnesium-deficient-top-10-magnesium-rich-foods-must-eating/*
Mn	1-	Food (almonds, bananas, beetroot, blackberries, brown rice, cloves, carrots, coconuts, cucumbers, egg yolk, figs, garlic, grapes, green beans, hazelnuts, kiwis, leeks, lettuce, molasses, mustard greens, oats, peppermint, pineapples,	Bone: 0.2-100 mg/kg Liver: 3.6-9.6 mg/kg Muscle: 0.2-2.3 mg/kg Daily dietary intake: 0.4-10 mg Total mass in average 70 kg human: 12 mg PEL: 0.1 mg/m^3 total	http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf https://www.inlifehealthcare.com/2015/03/02/7-essential-

	raspberries, rice, spinach, strawberries, tea, tofu, turmeric, watercress, whole wheat), 2- Ground water, surface water, and sewage, and 3- Pesticides	particulate, 1.0 mg/m^3 air	minerals-natural-food-sources/ https://www.organicfacts.net/health-benefits/minerals/health-benefits-of-manganese.html
Mo	*1- Food (bell peppers, celery, carrots, cucumber, dairy, dried peas, eggs, fennel, lentils, lima beans, oats, romaine lettuce, soybeans, tomatoes and yogurt)*	*Bone: 0.7 mg/kg* *Liver: 1.3-5.8 mg/kg* *Muscle: 0.018 mg/kg* *Daily dietary intake: 0.05-0.35 mg* *Total mass in average 70 kg human: 5 mg* *PEL: 10 mg/m^3 total particulate, 3 mg/m^3 air*	*http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf* *http://www.whfoods.com/genpage.php?tname=nutrient&dbid=128*
Na	1- Food (additives, baking soda, condiments, dairy foods, eggs, fish, meat, monosodium glutamate (MSG), poultry, pickled foods, olives, smoked meats, table salt, and various seasonings) and 2- Drinking water	Bone: 10000 mg/kg Liver: 2000-4000 mg/kg Muscle: 2600-7800 mg/kg Daily dietary intake: 2-15 g Total mass in average	http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf http://www.nytimes.com/health/guides/nutrition/sodium-in-

		70 kg human: 100 g PEL: n.a.	diet/overview.html
P	*1- Food (beans, cheese, fish, low fat dairy, meat, nuts, and seeds) and* *2- Fresh water*	*Bone: 67000-71000 mg/kg* *Liver: 3.0-8.5 mg/kg* *Muscle: 3000-8500 mg/kg* *Daily dietary intake: 900-19000 mg* *Total mass in average 70 kg human: 780 g* *PEL: 0.1 mg/m^3 total particulate, 0.1 mg/m^3 air*	*http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf* *https://www.healthaliciousness.com/articles/high-phosphorus-foods.php*
Si	1- Food (alfalfa, avocados, comfrey, cucumbers, dandelions, dark greens, equisetum arvensa, herbs, horsetail, hulls of wheat, lettuce, nettles, oats, onions, rice, strawberries, and sugar beet and cane pulp), 2- Drinking water and beverages, 3- Dust, and 4- Pharmaceuticals, cosmetics	Bone: 17 mg/kg Liver: 12-120 mg/kg Muscle: 100-200 mg/kg Daily dietary intake: 18-1200 mg Total mass in average 70 kg human: 1.0 g PEL: 15 mg/m^3 total particulate, 5 mg/m^3 air	http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf http://www-jmg.ch.cam.ac.uk/data/weii/silicon.html https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2658

	and medical implants and devices.		806/
Sr	*1- Industrial equipment,* *2- Nuclear reactors and atom bomb fallout,* *3- Burning coal and oil,* *4- Breathing air,* *5- Medical waste,* *6- Food (fish, leafy vegetables such as cabbage, and livestock), and* *7- Drinking water*	*Bone: 36-140 mg/kg* *Liver: 0.05-0.36 mg/kg* *Muscle: 0.12-0.35 mg/kg* *Daily dietary intake: 0.8-5.0 mg* *Total mass in average 70 kg human: 0.23 g* *PEL: 15 mg/m^3 total particulate, 3 mg/m^3 respirable particulate.*	*http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf* *http://www-jmg.ch.cam.ac.uk/data/weii/strontium.html* *https://www.atsdr.cdc.gov/phs/phs.asp?id=654&tid=120*
Ti	1- Bone-plates, screws and cranial plates for skull fractures, 2- Ceramics, and 3- Medicine	Bone: n.a. Liver: 1.2-4.7 mg/kg Muscle: 0.9-2.2 mg/kg Daily dietary intake: 0.8 mg PEL: 15 mg/m^3 total particulate, 10 mg/m^3 air Total mass in average 70 kg human: 20 mg	http://justonly.com/chemistry/pdfs/elemental_composition_human.pdf http://www.rawfoodexplained.com/minerals/the-minerals-in-the-body.html
Zn	*1- Food (Beef, beans, dairy*	*Bone: 75-150 mg/kg*	*http://justonly.com*

products, dark meat of a chicken, fish, fortified cereals, lamb, legumes, nuts, oysters, pork, poultry, red meat, seafood, whole grains, and yeast)	*Liver: 240 mg/kg* *Muscle: 240 mg/kg* *Daily dietary intake: 5-40 mg* *Total mass in average 70 kg human: 2.3 g* *PEL: 15 mg/m^3 total particulate, 5 mg/m^3 respirable particulate.*	*/chemistry/pdfs/elemental_composition_human.pdf* *http://www-jmg.ch.cam.ac.uk/data/weii/zinc.html* *https://medlineplus.gov/ency/article/002416.htm* *http://www.fitday.com/fitness-articles/nutrition/vitamins-minerals/the-importance-of-zinc-in-the-body.html*

4. Precautionary measures

It is important to check the iodine deficiency or excess and micronutrient imbalances every 6-months since they directly affect the normal functioning of the hypothalamic-pituitary thyroid axis, in addition to track the levels of thyroid hormone (TH), thyroid peroxidase (TPO), thyrotropin releasing hormone (TRH), and thyroid-stimulating

hormone (TSH) every year. The thyroxin supplementation can also be suggested to protect the brain from any damage.

5. Food avoidance and restriction

Considering the eating disturbance, nutrient-toxic effects, and the sensory characteristics of food, the following bearing-toxin foods should be avoided in excess amount:

I. Refined vegetable and seed oils (particularly if cooked) including corn, sunflower, safflower, soybean, and cottonseed oils which are rich in polyunsaturated omega-6 fats. Likewise, in especial issue with epileptic seizure and high HCY levels in blood (hyperhomocysteinemia), DS individuals are prone to endothelial cell injury causing inflammation in blood vessels, at these conditions, high intakes of linoleic acid should be evaded.

II. Plastic packed foods and beverages with bisphenol-A (recommended daily limit (RDL): 23 mcg/lb (50 mcg/kg) of body weight) as packaged foods and canned items, such as fish (which also rich with Hg; neurotoxin element), chicken, turkey, beans, vegetables, and bottled water. These products constitute a risk factor especially for high BMI and low insulin, thus should be exchanged with unprocessed foods. Herein, urinary bisphenol-A should be measured in DS one time a year.

III. Trans fats (i.e., baked goods, cakes, coffee creamer, cookies, crackers, fast food, frozen pies, frozen pizza, refrigerated dough products such as biscuits and cinnamon rolls (rich with coumarin; safety limit: 0.45 mg/lb (1 mg/kg)) sourced from Cassia and Ceylon, snacks as microwave popcorn, vegetable shortenings and some stick margarines) which can increase the higher levels of CRP in DS patients.

IV. Red meat (as beef, lamb, pork, and veal) when cooked can release toxic compounds as polycyclic aromatic hydrocarbons (PAHs). Thus, in connection to trisomy 21q22

living in a smoking environment, grilled or smoked meat at high temperatures which emits high concentration of volatile PAHs has a negative genetic effect and can be considered devastating.

V. Excess of added sugar as high fructose corn syrup “empty calories” especially with tumour growth as leukaemia is danger. However, sugar can be encouraged at limited addition levels which can release dopamine (a neurotransmitter in the brain) that stimulates reward pathways.

VI. Seafood (as bluefish, croaker, halibut, king mackerel, lobster (American and Maine), marlin, sea bass, shark, swordfish, tilefish, trout, tuna (ahi, fresh Bluefin, white albacore, and canned)) which is rich of Hg can damage the brain and nerves of DS and can impede the developing of brain and nervous system. Alternatively, the following two suggestions can be recommended: (A) Sea products (soft lumps or finely mashed) with lower Hg can be consumed no more than 6-oz/month: carp, cod (Alaskan), crab (dungness, blue, and snow), herring, mahi mahi, monkfish, perch (freshwater), skate, snapper, tuna (chunk light and fresh pacific albacore) and (B) Sea products (soft lumps or finely mashed) with very low contents of Hg can be consumed two 6-oz/week, at most: anchovies, butterfish, calamari (squid), catfish, clams, crab (king), crawfish/crayfish, flounder, haddock, hake, lobster (spiny/rock), oysters, perch (ocean), Pollock, salmon, sardines, scallops, shad (American), shrimp, sole, tilapia, and whitefish. (omega-3 fats and Hg of hair and blood can be measured once a year)

6. Future directions of nutrition psychology

Nature is their future! Concerning the rise in nutritional-related problems and reduced feeding time available during the day, future research should explore the efficacy of feeding therapy interventions on reducing packing in individuals with DS. In addition, future research

should examine whether altering the time it takes an individual with DS to finish eating a typical meal would be beneficial in reducing the problematic feeding behaviours. Caregiver anxiety and parents of individuals with DS collaborations who mostly experience feeding difficulties should be explored.

Natural foodstuffs are low in energy and nutrient, except bulky volumes are gulped. If the patient can swallow only liquid food and has little appetite (demonstrated by the high saline saliva; Tables 3 and 6), the nutrient content of meals should be condensed without growing their volume. Hence, parents and caregivers are encouraged to supplement high energy foods (olive oil, cheese, butter, mayonnaise…etc.) to regular meals or to buy commercial nutritional supplements with high energy and protein.

Biological terminology

- **Acute kidney injury (AKI):** is an abrupt loss of kidney function that develops within 7 days and its causes are numerous. Generally, it occurs because of damage to the kidney tissue caused by decreased kidney blood flow (kidney ischemia) from any cause (e.g., low blood pressure), exposure to substances harmful to the kidney, an inflammatory process in the kidney, or an obstruction of the urinary tract that impedes the flow of urine. AKI is diagnosed on the basis of characteristic laboratory findings, such as elevated blood urea nitrogen and creatinine, or inability of the kidneys to produce sufficient amounts of urine. AKI may lead to a number of complications, including metabolic acidosis, high potassium levels, uraemia, changes in body fluid balance, and effects on other organ systems, including death. People who have experienced AKI may have an increased risk of chronic kidney disease in the future. Management includes treatment of the underlying cause and supportive care, such as renal replacement therapy.

- **Adrenocorticotropic hormone (ACTH):** also known as corticotrophin is a polypeptide tropic hormone produced and secreted by the anterior pituitary gland. It is also used as a medication and diagnostic agent. It is an important component of the hypothalamic-pituitary-adrenal axis and is often produced in response to biological stress (along with its precursor corticotrophin-releasing hormone from the hypothalamus). Its principal effects are increased production and release of cortisol by the cortex of the adrenal gland. ACTH is also related to the circadian rhythm in many organisms. Deficiency of ACTH is a sign of secondary adrenal insufficiency (suppressed production of ACTH due to an impairment of the pituitary gland or hypothalamus, cf. hypopituitarism) or tertiary adrenal insufficiency (disease of the hypothalamus, with a decrease in the release of corticotrophin releasing hormone CRH). Conversely, chronically elevated ACTH levels occur in primary adrenal insufficiency (e.g. Addison's disease) when adrenal gland production of cortisol is chronically deficient. In Cushing's disease a pituitary tumour is the cause of elevated ACTH (from the anterior pituitary) and an excess of cortisol (hypercortisolism) – this constellation of signs and symptoms is known as Cushing's syndrome.

- **Aerobic capacity:** is the maximal amount of physiological work that an individual can do as measured by oxygen consumption. It is determined by a combination of aging and cardiovascular conditioning and is associated with the efficiency of oxygen extraction from the tissue. The maximum amount of O_2 in mL an athlete can use in one minute/kg of body weight. Generally, the higher the VO_2 max, the higher the anaerobic threshold and the faster an athlete can go in endurance competitions without fatigue. Training can increase VO_2 max by up to 20%, which can be determined by graded exercise testing. It is only of academic interest as O_2 consumption can’t be monitored during workouts. Factors affecting size and strength of heart, concentration of oxygen-carriers in blood

(haemoglobin), density of capillaries and mitochondrial in the muscles, and activity of aerobic enzymes; proper training enhances these factors and increases aerobic capacity.

- **Ambiguous genitalia:** is a rare condition in which an infant's external genitals don't appear to be clearly either male or female. In a baby with ambiguous genitalia, the genitals may not be well-formed or the baby may have characteristics of both sexes.
- **Angiotensinogen (AGT):** an α2-globulin precursor from which all other angiotensin peptides are derived. It is produced primarily by the liver, but AGT mRNA has also been detected in adipocytes, the kidney, regions of the brain, the adrenal gland, the heart and blood vessels.
- **Astrocytes:** they get their name because they are "star-shaped". They are the most abundant glial cells in the brain that are closely associated with neuronal synapses. They regulate the transmission of electrical impulses within the brain.
- **Auscultatory gap:** is a period of diminished or absent Korotkoff sounds during the manual measurement of blood pressure. The improper interpretation of this gap may lead to blood pressure monitoring errors: namely, an underestimation of systolic blood pressure and/or an overestimation of diastolic blood pressure.
- **Autism spectrum conditions (ASC):** is a lifelong disability that affects how someone sees the world, processes information, and relates to other people.
- **Autosomal DNA:** is a term used in genetic genealogy to describe DNA which is inherited from the autosomal chromosomes. An autosome is any of the numbered chromosomes, as opposed to the sex chromosomes. Humans have 22 pairs of autosomes and one pair of sex chromosomes (the X chromosome and the Y chromosome).
- **Autosome:** is a chromosome that is not an allosome (a sex chromosome). Autosomes appear in pairs whose members have the same form but differ from other pairs in

a diploid cell, whereas members of an allosome pair may differ from one another and thereby determine sex. The DNA in autosomes is collectively known as atDNA or auDNA. For example, humans have a diploid genome that usually contains 22 pairs of autosomes and one allosome pair (46 chromosomes total). The autosome pairs are labelled with numbers (1–22 in humans) roughly in order of their sizes in base pairs, while allosomes are labelled with their letters. By contrast, the allosome pair consists of two X chromosomes in females or one X and one Y chromosome in males. Unusual combinations of XYY, XXY, XXX, XXXX, XXXXX or XXYY, among other allosome combinations, are known to occur and usually cause developmental abnormalities. Autosomes still contain sexual determination genes even though they are not sex chromosomes. For example, the SRY gene on the Y chromosome encodes the transcription factor TDF and is vital for male sex determination during development. TDF functions by activating the SOX9 gene on chromosome 17, so mutations of the SOX9 gene can cause humans with a Y chromosome to develop as females. All human autosomes have been identified and mapped by extracting the chromosomes from a cell arrested in metaphase or prometaphase and then staining them with some sort of dye (most commonly, Giemsa). These chromosomes are typically viewed as karyograms for easy comparison. Clinical geneticists can compare the karyogram of an individual to a reference karyogram to discover the cytogenetic basis of certain phenotypes. For example, the karyogram of someone with Patau Syndrome would show that they possess three copies of chromosome 13. Karyograms and staining techniques can only detect large-scale disruptions to chromosomes—chromosomal aberrations smaller than a few million base pairs generally cannot be seen on a karyogram.

- **Biliverdin reductase** (**BVR**): An enzyme (EC 1.3.1.24) found in all tissues under normal conditions, but especially in reticulo-macrophages of the liver and spleen. BVR facilitates the conversion of biliverdin to bilirubin via the reduction of a double-bond between the second and third pyrrole ring into a single-bond.

  There are two isozymes, in humans, each encoded by its own gene, biliverdin reductase A (BLVRA) and biliverdin reductase B (BLVRB).

- **Biofluids:** Body fluid, bodily fluids or biofluids are liquids originating from inside the bodies of living people. They include fluids that are excreted or secreted from the body, and body water that normally is not.
- **Blood glucose (BG) test:** It measures the amount of a type of sugar, called glucose, in the blood. Glucose comes from carbohydrate foods. It is the main source of energy used by the body. Insulin is a hormone that helps the body's cells use glucose. Insulin is produced in the pancreas and released into the blood when the amount of glucose in the blood rises. Normally, blood glucose levels increase slightly after eating. This increase causes pancreas to release insulin so that blood glucose levels do not get too high. Blood glucose levels that remain high over time can damage eyes, kidneys, nerves, and blood vessels.
- **Body mass index (BMI):** or Quetelet index is a value derived from the mass (weight) and height of an individual. The BMI is defined as the body mass divided by the square of the body height, and is universally expressed in units of kg/m^2, resulting from mass in kilograms and height in meters. The BMI is an attempt to quantify the amount of tissue mass (muscle, fat, and bone) in an individual, and then categorise that person as *underweight*, *normal weight*, *overweight*, or *obese* based on that value. Commonly accepted BMI ranges are underweight: under 18.5 kg/m^2, normal weight: 18.5 to 25, overweight: 25 to 30, obese: over 30. People of Asian descent have different associations between BMI, percentage of body fat, and health risks than those of European descent,

with a higher risk of type 2 diabetes and cardiovascular disease at BMIs lower than the WHO cut-off point for overweight, 25 kg/m^2, although the cut-off for observed risk varies among different Asian populations.

- **Bone mineral density (BMD) test:** The test can identify osteoporosis, determine the risk for fractures (broken bones), and measure the response to osteoporosis treatment. The most widely recognized BMD test is called a central dual-energy x-ray absorptiometry, or central DXA test. It is painless—a bit like having an x-ray.
- **Brockport Physical Fitness Test (BPFT):** is a health-related, criterion-referenced test of fitness. The term health-related is used to distinguish objectives of this test battery from others that might be more appropriately related to skill or physical performance. The phrase criterion-referenced conveys that the standards for evaluation are based on values believed to have significance for an individual's health.
- **Chemiluminescent immunoassay:** is a variation of the standard enzyme immunoassay (EIA), which is a biochemical technique, used in immunology. They can also be used as diagnosis tools in medicine, as well as being in used in several other different industries for various applications.
- **Choline** ($C_5H_{14}NO$) is an essential nutrient that is naturally present in some foods and available as a dietary supplement. **Choline** is a source of methyl groups needed for many steps in metabolism. The body needs **choline** to synthesise phosphatidylcholine and sphingomyelin, two major phospholipids vital for cell membranes. The most common symptoms of choline deficiency are fatty liver and/or hemorrhagic kidney necrosis.
- **Chromosome 21 genes:** is the smallest human autosome, with 48 million nucleotides (the building material of DNA) representing about 1.5 percent of the total DNA in cells.

People without Down's syndrome have two copies of chromosome 21, while those with three copies of chromosome 21 have Down's syndrome, also called "trisomy 21".

- **Cryptorchidism:** is the absence of one or both testes from the scrotum. It is the most common birth defect of the male genital. About 3% of full-term and 30% of premature infant boys are born with at least one undescended testis. However, about 80% of cryptorchid testes descend by the first year of life (the majority within three months), making the true incidence of cryptorchidism around 1% overall. Cryptorchidism may develop after infancy, sometimes as late as young adulthood, but that is exceptional. It is distinct from monarchism, the condition of having only one testicle. The condition may occur on one or both sides; it more commonly affects the right testis.
- **Cotransporters:** are a subcategory of membrane transport proteins (transporters) that couple the favourable movement of one molecule with its concentration gradient and unfavourable movement of another molecule against its concentration gradient. They enable cotransport (secondary active transport) and include antiporters and symporters. In general, cotransporters consist of two out of the three classes of integral membrane proteins known as transporters that move molecules and ions across biomembranes. Uniporters are also transporters but move only one type of molecule down its concentration gradient and are not classified as cotransporters.
- **Clinical features:** Patients with heart failure present with a variety of symptoms, most of which are non-specific. The common symptoms of congestive heart failure include fatigue, dyspnoea, swollen ankles, and exercise intolerance, or symptoms that relate to the underlying cause.
- **Clinodactyly:** is a medical term describing the curvature of a digit (a finger or toe) in the plane of the palm, most commonly the fifth finger (the "little finger") towards the adjacent fourth finger (the "ring finger"). It is a fairly common isolated anomaly which

often goes unnoticed, but also occurs in combination with other abnormalities in many genetic syndromes. Clinodactyly is an autosomal dominant trait that has variable expressiveness and incomplete penetrance. When identified prenatally, for example during obstetric ultrasonography, it may be an indication for intrauterine sampling for foetal chromosome analysis as it is statistically correlated with increased risk of chromosome aberration in the foetus.

- **Comorbidity:** is the presence of one or more additional diseases or disorders co-occurring with (that is, concomitant or concurrent with) a primary disease or disorder; in the countable sense of the term, a comorbidity (plural comorbidities) is each additional disorder or disease. The additional disorder may be a behavioural or mental disorder. The term can indicate either a condition existing simultaneously but independently with another condition or a related medical condition. The latter sense of the term causes some overlap with the concept of complications. For example, in longstanding diabetes mellitus, the extent to which coronary artery disease is an independent comorbidity versus a diabetic complication is not easy to measure, because both diseases are quite multivariate and there are likely aspects of both simultaneity and consequence. The same is true of intercurrent diseases in pregnancy. In other examples, the true independence or relation is not ascertainable because syndromes and associations are often identified long before pathogenetic commonalities are confirmed (and, in some examples, before they are even hypothesized). In psychiatric diagnoses it has been argued in part that this "'use of imprecise language may lead to correspondingly imprecise thinking', [and] this usage of the term 'comorbidity' should probably be avoided." However, in many medical examples, such as comorbid diabetes mellitus and coronary artery disease, it makes little difference which word is used, as long as the medical complexity is duly recognised and addressed.

- **Congenital heart defect:** is a problem with the structure of the heart. It is present at birth. Congenital heart defects are the most common type of birth defect. The defects can involve the walls of the heart, the valves of the heart, and the arteries and veins near the heart.
- **Cognitive deficit:** is an inclusive term used to describe impairment in an individual's mental processes that lead to the acquisition of information and knowledge, and drive how an individual understands and acts in the world. The following areas constitute domains of cognitive functioning: Attention.
- **Cognitive functions:** can be defined as cerebral activities that lead to knowledge, including all means and mechanisms of acquiring information. Cognitive functions encompass reasoning, memory, attention, and language and lead directly to the attainment of information and, thus, knowledge.

- **Cranial nerve XII:** or hypoglossal nerve is the twelfth cranial nerve, and innervates all the extrinsic and intrinsic muscles of the tongue, except for the palatoglossus which is innervated by the vagus nerve. It is a nerve with solely a motor function. The nerve arises from the hypoglossal nucleus in the brain stem as a number of small rootlets, passes through the hypoglossal canal and down through the neck, and eventually passes up again over the tongue muscles it supplies into the tongue. There are two hypoglossal nerves in the body: one on the left, and one on the right. The nerve is involved in controlling tongue movements required for speech and swallowing, including sticking out the tongue and moving it from side to side. Damage to the nerve or the neural pathways which control it can affect the ability of the tongue to move and its appearance, with the most common sources of damage being traumatic injury, surgery in the local area, and motor neuron disease. The first recorded description of the nerve is by Herophilos in the third century BC. The name hypoglossus springs from the fact that its passage is below the tongue.

- **C-reactive protein (CRP):** is a substance produced by the liver that increases in the presence of inflammation in the body. An elevated C-reactive protein level is identified with blood tests and is considered a non-specific "marker" for disease.
- **Creatinine (CR):** is a waste product produced by muscles from the breakdown of a compound called creatine. Almost all creatinine is filtered from the blood by the kidneys and released into the urine, so blood levels are usually a good indicator of how well the kidneys are working.
- **Creatinine blood test:** is used to assess kidney function. It is frequently ordered along with a BUN (blood urea nitrogen) test or as part of a basic or comprehensive metabolic panel (BMP or CMP), groups of tests that are performed to evaluate the function of the body's major organs. BMP or CMP tests are used to screen healthy people during routine physical exams and to help evaluate acutely or chronically ill people in the emergency room and/or hospital. Sometimes, creatinine may be performed as part of a renal panel to evaluate kidney function.
- **C-terminal telopeptide (CTx):** can be used as a biomarker in the serum to measure the rate of bone turnover. It can be useful in assisting clinicians to determine a patient's nonsurgical treatment response as well as evaluate a patient's risk of developing complications during healing following surgical intervention. The test used to detect the CTX marker is called the Serum CrossLaps, and it is more specific to bone resorption than any other test currently available.
- **Cytocentrifuge:** A centrifuge used for depositing cells suspended in a liquid on a slide for microscopic examination.
- **Cytoplasmic:** also called protoplasmic streaming, the movement of the fluid substance (cytoplasm) within a plant or animal cell. The motion transports nutrients, proteins, and organelles within cells.

- **2,6 Dimethyl-heptanoyl carnitine:** is an acylcarnitine. Numerous disorders have been described that lead to disturbances in energy production and in intermediary metabolism in the organism which are characterised by the production and excretion of unusual acylcarnitines.
- **Duodenal atresia:** also known as duodenojejunal atresia, is the congenital absence or complete closure of a portion of the lumen of the duodenum. It causes increased levels of amniotic fluid during pregnancy (polyhydramnios) and intestinal obstruction in newborn babies.
- **Electrographic seizure activity:** One of the hallmarks of clinical electroencephalography is the identification of patients with epileptic seizures, where electrographic seizure activity remains one of the most specific and sensitive findings in electroencephalogram (EEG) recordings.
- **Encoding genes:** Genes that encode proteins are composed of a series of three-nucleotide sequences called codons, which serve as the "words" in the genetic "language". The genetic code specifies the correspondence during protein translation between codons and amino acids.
- **Epicanthal folds:** is a skin fold of the upper eyelid covering the inner corner of the eye. It is often seen as a normal finding in very young children and is also common in people of Asiatic decent.
- **Epileptic seizures:** also known as an epileptic fit, seizure or fit, is a brief episode of signs or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. The outward effect can vary from uncontrolled jerking movement (tonic-clonic seizure) to as subtle as a momentary loss of awareness (absence seizure). Diseases of the brain characterised by an enduring predisposition to generate epileptic seizures are collectively called epilepsy. However, seizures can also occur in people who do not have

epilepsy for various reasons including brain trauma, drug use, elevated body temperature, low blood sugar and low levels of oxygen. Additionally, there are a number of conditions that look like epileptic seizures but are not. A first seizure generally does not require long term treatment with anti-seizure medications unless there is a specific problem on either electroencephalogram or brain imaging. 5–10% of people who live to 80 years old have at least one epileptic seizure and the chance of experiencing a second seizure is between 40% and 50%. About 50% of patients with an unprovoked apparent "first seizure" have had other minor seizures, so their diagnosis is epilepsy. Epilepsy affects about 1% of the population currently and affected about 4% of the population at some point in time. Most of those affected—nearly 80%—live in developing countries.

- **Epileptic spasms:** is a sudden flexion, extension or mixed flexion-extension of proximal and truncal muscles, lasting 1-2 seconds i.e. longer than a myoclonic jerk (which lasts milliseconds) but not as long as a tonic seizure (which lasts > 2 seconds). Spasms typically occur in a series, usually on wakening.
- **Fine motor skills:** or dexterity is the coordination of small muscles, in movements—usually involving the synchronisation of hands and fingers—with the eyes. The complex levels of manual dexterity that humans exhibit can be attributed to and demonstrated in tasks controlled by the nervous system. Fine motor skills aid in the growth of intelligence and develop continuously throughout the stages of human development.
- **Functional capacity evaluation (FCE):** is set of tests, practices and observations that are combined to determine the ability of the evaluated to function in a variety of circumstances, most often employment, in an objective manner. Physicians change diagnoses based on FCEs.
- **Gastroesophageal reflux:** is a digestive disorder that affects the lower oesophageal sphincter (LES), the ring of muscle between the oesophagus and stomach. Many people,

including pregnant women, suffer from heartburn or acid indigestion caused by GERD. Doctors believe that some people suffer from GERD due to a condition called hiatal hernia. In most cases, GERD can be relieved through diet and lifestyle changes; however, some people may require medication or surgery.

- **Glucocorticoids (GCs):** lie in a class of corticosteroids, which are a class of steroid hormones. Glucocorticoids are corticosteroids that bind to the glucocorticoid receptor (GR) that is present in almost every vertebrate animal cell. The name glucocorticoid (glucose + cortex + steroid) is composed from its role in regulation of glucose metabolism, synthesis in the adrenal cortex, and its steroidal structure. A less common synonym is glucocorticosteroid. GCs are part of the feedback mechanism in the immune system which reduces certain aspects of immune function, such as reduction of inflammation. They are therefore used in medicine to treat diseases caused by an overactive immune system, such as allergies, asthma, autoimmune diseases, and sepsis. GCs have many diverse (pleiotropic) effects, including potentially harmful side effects, and as a result are rarely sold over the counter. They also interfere with some of the abnormal mechanisms in cancer cells, so they are used in high doses to treat cancer. This includes inhibitory effects on lymphocyte proliferation, as in the treatment of lymphomas and leukaemia, and the mitigation of side effects of anticancer drugs. GCs affect cells by binding to the glucocorticoid receptor (GR). The activated GR complex, in turn, up-regulates the expression of anti-inflammatory proteins in the nucleus (a process known as transactivation) and represses the expression of proinflammatory proteins in the cytosol by preventing the translocation of other transcription factors from the cytosol into the nucleus (transrepression). Glucocorticoids are distinguished from mineralocorticoids and sex steroids by their specific receptors, target cells, and effects. In technical terms, "corticosteroid" refers to both glucocorticoids and

mineralocorticoids (as both are mimics of hormones produced by the adrenal cortex), but is often used as a synonym for "glucocorticoid." Glucocorticoids are chiefly produced in the zona fasciculate of the adrenal cortex, whereas mineralocorticoids are synthesized in the zona glomerulosa. Cortisol (or hydrocortisone) is the most important human glucocorticoid. It is essential for life, and it regulates or supports a variety of important cardiovascular, metabolic, immunologic, and homeostatic functions. Various synthetic glucocorticoids are available; these are widely utilised in general medical practice and numerous specialties either as replacement therapy in glucocorticoid deficiency or to suppress the immune system.

- **Glutamate:** In neuroscience, glutamate generally refers to the anion of glutamic acid in its role as a neurotransmitter: a chemical that nerve cells use to send signals to other cells. It is by a wide margin the most abundant neurotransmitter in the vertebrate nervous system. It is used by every major excitatory function in the vertebrate brain, accounting in total for well over 90% of the synaptic connections in the human brain. Chemical receptors for glutamate fall into three major classes, known as AMPA receptors, NMDA receptors, and metabotropic glutamate receptors. Many synapses use multiple types of glutamate receptors. AMPA receptors are ionotropic receptors specialised for fast excitation: in many synapses they produce excitatory electrical responses in their targets a fraction of a millisecond after being stimulated. NMDA receptors are also ionotropic, but they differ from AMPA receptors in being permeable, when activated, to calcium. Their properties make them particularly important for learning and memory. Metabotropic receptors act through second messenger systems to create slow, sustained effects on their targets. A fourth class, known as kainite receptors, is similar in many respects to AMPA receptors, but much less abundant. Because of its role in synaptic plasticity, glutamate is involved in cognitive functions such as learning and memory in the brain. The form of

plasticity known as long-term potentiation takes place at glutamatergic synapses in the hippocampus, neocortex, and other parts of the brain. Glutamate works not only as a point-to-point transmitter, but also through spill-over synaptic crosstalk between synapses in which summation of glutamate released from a neighbouring synapse creates extrasynaptic signalling/volume transmission. In addition, glutamate plays important roles in the regulation of growth cones and synaptogenesis during brain development as originally described by Mark Mattson.

- **Glutamine:** is an α-amino acid that is used in the biosynthesis of proteins. Its side chain is similar to that of glutamic acid, except the carboxylic acid group is replaced by an amide. It is classified as a charge-neutral, polar amino acid.
- **Gross motor function measure (GMFM):** is a clinical tool designed to evaluate change in gross motor function in children with cerebral palsy.
- **Health-related quality of life (HRQoL):** is a multidimensional concept that includes domains related to physical, mental, emotional, and social functioning. It goes beyond direct measures of population health, life expectancy, causes of death, and focuses on the impact health status has on quality of life.

- **Haematocrit (Ht or HCT):** It is also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF). It's the volume percentage (%) of red blood cells in blood and stands for the degree of blood viscosity. The haematocrit blood test determines the percentage of red blood cells (RBC's) in the blood. Blood is composed mainly of red blood cells and white blood cells suspended in an almost clear fluid called serum. The haematocrit test indicates the percentage of blood by volume that is composed of red blood cells. The condition called "anaemia" results from having too few red blood cells. Anaemia causes a variety of symptoms. The haematocrit is a basic test that can tell a physician a lot about a person's health.

- **Haemoglobin (Hb or Hgb):** is a protein in red blood cells that carries oxygen throughout the body. In many cases, a low haemoglobin count is only slightly lower than normal and doesn't affect how you feel. If it gets more severe and causes symptoms, your low haemoglobin count may indicate you have anaemia.
- **High-density lipoprotein cholesterol (HDL-C):** is commonly measured to assess the risk of heart disease. There is an inverse relationship between HDL-C and the risk of heart disease. It is believed that HDL's act as scavengers, picking up excess cholesterol in the blood and transporting it to the liver where it's broken down.

- **Hippocampus:** is a major component of the brains of humans and other vertebrates. Humans and other mammals have two hippocampi, one in each side of the brain. The hippocampus belongs to the limbic system and plays important roles in the consolidation of information from short-term memory to long-term memory, and in spatial memory that enables navigation. The hippocampus is located under the cerebral cortex (allocortical) and in primates in the medial temporal lobe. It contains two main interlocking parts: the hippocampus proper (also called Ammon's horn) and the dentate gyrus.
  In Alzheimer's disease (and other forms of dementia), the hippocampus is one of the first regions of the brain to suffer damage; short-term memory loss and disorientation are included among the early symptoms. Damage to the hippocampus can also result from oxygen starvation (hypoxia), encephalitis, or medial temporal lobe epilepsy. People with extensive, bilateral hippocampal damage may experience anterograde amnesia (the inability to form and retain new memories).

- **Hirschsprung's (HIRSH-sproongz) disease:** is a condition that affects the large intestine (colon) and causes problems with passing stool. The condition is present at birth (congenital) as a result of missing nerve cells in the muscles of the baby's colon.

- **Homeostasis:** the tendency toward a relatively stable equilibrium between interdependent elements, especially as maintained by physiological processes.

- **Homocysteine:** is a non-protein α-amino acid. It is a homologue of the amino acid cysteine, differing by an additional methylene bridge (-CH_2-). It is biosynthesised from methionine by the removal of its terminal C^{ε} methyl group. Homocysteine can be recycled into methionine or converted into cysteine with the aid of certain B-vitamins. A high level of homocysteine in the blood (hyperhomocysteinemia) makes a person more prone to endothelial cell injury, which leads to inflammation in the blood vessels, which in turn may lead to atherogenesis, which can result in ischemic injury. Hyperhomocysteinemia is therefore a possible risk factor for coronary artery disease. Coronary artery disease occurs when an atherosclerotic plaque blocks blood flow to the coronary arteries, which supply the heart with oxygenated blood. Hyperhomocysteinemia has been correlated with the occurrence of blood clots, heart attacks and strokes, though it is unclear whether hyperhomocysteinemia is an independent risk factor for these conditions. Hyperhomoscyteinemia has also been associated with early pregnancy loss and with neural tube defects

- **Human chrorionic gonadotropin (hCG) blood:** The test is done to check for the hormone hCG in blood or urine. Some hCG tests measure the exact amount. Some just check to see if the hormone is present. HCG is made by the placenta during pregnancy. The test can be used to see if a woman is pregnant. Or it can be done as part of a screening test for birth defects. HCG may also be made by certain tumours, especially those that come from an egg or sperm. (These are called germ cell tumours.) HCG levels are often tested in a woman who may have tissue that is not normal growing in her uterus. The test also may be done to look for molar pregnancy or a cancer inside the uterus. Several hCG tests may be

done after a miscarriage to be sure a molar pregnancy is not present. In a man, hCG levels may be measured to help see if he has cancer of the testicles.

- **4-Hydroxyphenyl acetate:** also known as hydroquinone monoacetate or 4-acetoxyphenol, belongs to the class of organic compounds known as phenol esters. These are aromatic compounds containing a benzene ring substituted by a hydroxyl group and an ester group. 4-Hydroxyphenyl acetate is an extremely weak basic (essentially neutral) compound (based on its pKa). 4-Hydroxyphenyl acetate exists in all living organisms, ranging from bacteria to humans. These are aromatic compounds containing a benzene ring substituted by hydroxyl group and an ester group.
- **Hypogonadism:** Male hypogonadism is a condition in which the body does not produce enough testosterone — the hormone that plays a key role in masculine growth and development during puberty — or has an impaired ability to produce sperm or both. It also means diminished functional activity of the gonads—the testes in males or the ovaries in females—that may result in diminished sex hormone biosynthesis. In layman's terms, it is sometimes called *interrupted stage 1 puberty*. Low androgen (e.g., testosterone) levels are referred to as hypoandrogenism and low oestrogen (e.g., estradiol) as hypoestrogenism, and may occur as symptoms of hypogonadism in both sexes, but are generally only diagnosed in males and females respectively. Other hormones produced by the gonads that hypogonadism can decrease include progesterone, dehydroepiandrosterone sulphate (DHEA-S), anti-Müllerian hormone, activin, and inhibin. Spermatogenesis in males, and ovulation in females, may be impaired by hypogonadism, which, depending on the degree of severity, may result in partial or complete infertility.
- **Hypothyroidism:** also called underactive thyroid or low thyroid, is a common disorder of the endocrine system in which the thyroid gland does not produce enough thyroid

hormone. It can cause a number of symptoms, such as poor ability to tolerate cold, a feeling of tiredness, constipation, depression, and weight gain. Occasionally, there may be swelling of the front part of the neck due to goitre. Untreated hypothyroidism during pregnancy can lead to delays in growth and intellectual development in the baby, which is called cretinism. Worldwide, too little iodine in the diet is the most common cause of hypothyroidism. In countries with enough iodine in the diet, the most common cause of hypothyroidism is the autoimmune condition Hashimoto's thyroiditis. Less common causes include: previous treatment with radioactive iodine, injury to the hypothalamus or the anterior pituitary gland, certain medications, a lack of a functioning thyroid at birth, or previous thyroid surgery. The diagnosis of hypothyroidism, when suspected, can be confirmed with blood tests measuring TSH and thyroxine levels. Prevention at the population level has been with the universal salt iodisation. Hypothyroidism can be treated with levothyroxine. The dose is adjusted according to symptoms and normalisation of the thyroxine and TSH levels. Thyroid medication is safe in pregnancy. While a certain amount of dietary iodine is important, excessive amounts can worsen certain types of hypothyroidism. Worldwide about one billion people are estimated to be iodine deficient; however, it is unknown how often this results in hypothyroidism. In the United States, hypothyroidism occurs in 0.3–0.4% of people. Subclinical hypothyroidism, a milder form of hypothyroidism characterized by normal thyroxine levels and an elevated TSH level, is thought to occur in 4.3–8.5% of people in the United States. Hypothyroidism is more common in women than men. People over the age of 60 are more commonly affected. Dogs are also known to develop hypothyroidism and in rare cases cats and horses can also have the disorder.

- **Hypotonia:** commonly known as floppy baby syndrome, is a state of low muscle tone (the amount of tension or resistance to stretch in a muscle), often involving reduced

muscle strength. Hypotonia is not a specific medical disorder, but a potential manifestation of many different diseases and disorders that affect motor nerve control by the brain or muscle strength. Hypotonia is resistance to passive movement, whereas muscle weakness results in impaired active movement. Central hypotonia originates from the CNS, while peripheral hypotonia is related to problems within the spinal cord, peripheral nerves and/or skeletal muscles. Recognising hypotonia, even in early infancy, is usually relatively straightforward, but diagnosing the underlying cause can be difficult and often unsuccessful. The long-term effects of hypotonia on a child's development and later life depend primarily on the severity of the muscle weakness and the nature of the cause. Some disorders have a specific treatment but the principal treatment for most hypotonia of idiopathic or neurologic cause is physical therapy, occupational therapy for remediation, and/or music therapy. Hypotonia is thought to be associated with the disruption of afferent input from stretch receptors and/or lack of the cerebellum's facilitatory efferent influence on the fusimotor system, the system that innervates intrafusal muscle fibres thereby controlling muscle spindle sensitivity. On examination a diminished resistance to passive movement will be noted and muscles may feel abnormally soft and limp on palpation. Diminished deep tendon reflexes also may be noted. Hypotonia is a condition that can be helped with early intervention.

- **Infantile spasms:** also known epileptic spasms, juvenile spasms or West syndrome is an uncommon-to-rare epileptic disorder in infants, children and adults. It is named after the English physician, William James West (1793–1848), who first described it in an article published in The Lancet in 1841. The original case actually described his son, James Edwin West (1840–1860). Other names for it are "Generalised Flexion Epilepsy", "Infantile Epileptic Encephalopathy", "Infantile Myoclonic Encephalopathy", "jackknife

convulsions", "Massive Myoclonia" and "Salaam spasms". The term "infantile spasms" can be used to describe the specific seizure manifestation in the syndrome, but is also used as a synonym for the syndrome itself. West syndrome in modern usage is the triad of infantile spasms, a pathognomonic EEG pattern (called hypsarrhythmia), and developmental regression - although the international definition requires only two out of these three elements. The syndrome is age-related, generally occurring between the third and the twelfth month, generally manifesting around the fifth month. There are various causes. The syndrome is often caused by an organic brain dysfunction whose origins may be prenatal, perinatal (caused during birth) or postnatal.

- **Insulin:** is a peptide hormone produced by beta cells of the pancreatic islets. It regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of, especially, glucose from the blood into fat, liver and skeletal muscle cells. In these tissues the absorbed glucose is converted into either glycogen via glycogenesis or fats (triglycerides) via lipogenesis, or, in the case of the liver, into both. Glucose production (and excretion into the blood) by the liver is strongly inhibited by high concentrations of insulin in the blood. Circulating insulin also affects the synthesis of proteins in a wide variety of tissues. It is therefore an anabolic hormone, promoting the conversion of small molecules in the blood into large molecules inside the cells. Low insulin levels in the blood have the opposite effect by promoting widespread catabolism. Pancreatic beta cells (β cells) are known to be sensitive to glucose concentrations in the blood. When glucose concentrations in the blood are high, the pancreatic β cells secrete insulin into the blood; when glucose levels are low, secretion of insulin is inhibited. Their neighbouring alpha cells, by taking their cues from the beta cells, secrete glucagon into the blood in the opposite manner: increased secretion when blood glucose is low and decreased secretion when glucose

concentrations are high. Glucagon, through stimulating the liver to release glucose by glycogenolysis and gluconeogenesis, has the opposite effect of insulin. The secretion of insulin and glucagon into the blood in response to the blood glucose concentration is the primary mechanism responsible for keeping the glucose levels in the extracellular fluids within very narrow limits at rest, after meals, and during exercise and starvation. If pancreatic beta cells are destroyed by an autoimmune reaction, insulin can no longer be synthesised or be secreted into the blood. This results in type 1 diabetes mellitus, which is characterised by abnormally high blood glucose concentrations, and generalised body wasting. In type 2 diabetes mellitus the destruction of beta cells is less pronounced than in type 1 diabetes, and is not due to an autoimmune process. Instead there is an accumulation of amyloid in the pancreatic islets, which disrupts their anatomy and physiology. Type 2 diabetes is characterised by high rates of glucagon secretion into the blood which are unaffected by, and unresponsive to the concentration of glucose in the blood glucose. Insulin is still secreted into the blood in response to the blood glucose. As a result, the insulin levels, even when the blood sugar level is normal, are much higher than they are in healthy persons. There are a variety of treatment regimens, none of which is entirely satisfactory. When the pancreas's capacity to secrete insulin can no longer keep the blood sugar level within normal bounds, insulin injections are given. The human insulin protein is composed of 51 amino acids, and has a molecular mass of 5808 Da. It is a dimer of an A-chain and a B-chain, which are linked together by disulphide bonds. Insulin's structure varies slightly between species of animals. Insulin from animal sources differs somewhat in effectiveness (in carbohydrate metabolism effects) from human insulin because of these variations. Porcine insulin is especially close to the human version, and was widely used to treat type 1 diabetics before human insulin could be produced in large quantities by recombinant DNA technologies. The crystal

structure of insulin in the solid state was determined by Dorothy Hodgkin. It is on the WHO Model List of Essential Medicines, the most important medications needed in a basic health system.

- **Iris Brushfield spots:** are small, white or greyish/brown spots on the periphery of the iris in the human eye due to aggregation of connective tissue, a normal iris element. The spots are named after the physician Thomas Brushfield, who first described them in his 1924 M.D. thesis. They are focal areas of stromal hyperplasia, surrounded by relative hypoplasia, and are more common in patients with lightly pigmented irises.

- **Leukaemia:** a malignant progressive disease in which the bone marrow and other blood-forming organs produce increased numbers of immature or abnormal leukocytes. These suppress the production of normal blood cells, leading to anaemia and other symptoms.

- **Ligamentous laxity:** or *ligament laxity*, is a term given to describe "loose ligaments". Ligamentous laxity is a cause of chronic body pain characterised by loose ligaments. When this condition affects joints in the entire body, it is called *generalised joint hypermobility*, which occurs in about five percent of the population, and may be genetic. Loose ligaments can appear in a variety of ways and levels of severity. It also does not always affect the entire body. One could have loose ligaments of the feet, but not of the arms. Someone with ligamentous laxity, by definition, has loose ligaments. Unlike other, more pervasive diseases, the diagnosis does not require the presence of loose tendons, muscles or blood vessels, hyperlax skin or other connective tissue problems. In heritable connective tissue disorders associated with joint hyper-mobility (such as Marfan syndrome and Ehlers–Danlos syndrome types I–III, VII, and XI), the joint laxity usually is apparent before adulthood. However, age of onset and extent of joint laxity are variable in Marfan syndrome, and joint laxity may be confined to

the hands alone, as in Ehlers–Danlos syndrome type IV. In most people, ligaments (which are the tissues that connect bones to each other) are naturally tight in such a way that the joints are restricted to 'normal' ranges of motion. This creates normal joint stability. If muscular control does not compensate for ligamentous laxity, joint instability may result. The trait is almost certainly hereditary, and is usually something the affected person would just be aware of, rather than a serious medical condition. However, if there is widespread laxity of other connective tissue, then this may be a sign of Ehlers-Danlos syndrome. Ligamentous laxity may also result from injury, such as from a vehicle accident. It can result from whiplash and be overlooked for years by doctors who are not looking for it, despite the chronic pain that accompanies the resultant spinal instability. Ligamentous laxity will show up on an upright magnetic resonance imaging (MRI), the only kind of MRI that will show soft tissue damage. It can be seen in standing stress radiographs in flexion, extension, and neutral views as well, and also digital motion X-ray, or DMX. An advantage to having lax ligaments and joints is the ability to withstand pain from hyperextension; however, this is also a disadvantage as a lack of perceived pain can prevent a person from removing the ligament from insult, leading to ligament damage. People with hypermobile joints (or "double-jointed" people), almost by definition, have lax ligaments.

- **Low-density lipoprotein cholesterol (LDL-C):** LDL is one of the body's lipoproteins and is an important carrier of cholesterol. Blood levels of low-density lipoprotein cholesterol (LDL-C) are often assessed when evaluating the risk of future heart disease.
- **Lumbar vertebrae:** are, in human anatomy, the five vertebrae between the rib cage and the pelvis. They are the largest segments of the vertebral column and are characterised by the absence of the foramen transversarium within the transverse process (as it is only found in the cervical region), and by the absence of facets on the sides of the body (as

only found in the thoracic region). They are designated L1 to L5, starting at the top. The lumbar vertebrae help supporting the weight of the body, and permit movement.

- **Macrophages:** are a type of white blood cell that engulfs and digests cellular debris, foreign substances, microbes, cancer cells, and anything else that does not have the types of proteins specific to healthy body cells on its surface in a process called phagocytosis. These large phagocytes are found in essentially all tissues, where they patrol for potential pathogens by amoeboid movement. They take various forms (with various names) throughout the body (e.g., histiocytes, Kupffer cells, alveolar macrophages, microglia, and others), but all are part of the mononuclear phagocyte system. Besides phagocytosis, they play a critical role in nonspecific defence (innate immunity) and also help initiate specific defence mechanisms (adaptive immunity) by recruiting other immune cells such as lymphocytes. For example, they are important as antigen presenters to T cells. In humans, dysfunctional macrophages cause severe diseases such as chronic granulomatous disease that result in frequent infections. Beyond increasing inflammation and stimulating the immune system, macrophages also play an important anti-inflammatory role and can decrease immune reactions through the release of cytokines. Macrophages that encourage inflammation are called M1 macrophages, whereas those that decrease inflammation and encourage tissue repair are called M2 macrophages. This difference is reflected in their metabolism; M1 macrophages have the unique ability to metabolise arginine to the "killer" molecule nitric oxide, whereas rodent M2 macrophages have the unique ability to metabolise arginine to the "repair" molecule ornithine. Human macrophages are about 21 micrometres (0.00083 in) in diameter and are produced by the differentiation of monocytes in tissues. They can be identified using flow cytometry or immunohistochemical staining by their specific expression of proteins such

as CD14, CD40, CD11b, CD64, F4/80 (mice)/EMR1 (human), lysozyme M, MAC-1/MAC-3 and CD68. Macrophages were first discovered by Élie Metchnikoff, a Russian zoologist, in 1884.

- **Maladaptive behaviour:** Generally, adaptive behaviour is the collection of skills that people learn and employ to be able to function in everyday life. These skills range from talking to getting dressed to going to work. They allow us to adapt to the demands of life and fulfil our needs. This behaviour refers to the type of behaviour that inhibits a person's ability to adjust to certain situations. We inevitably face challenges and conflicts in daily life and must adapt our behaviour to face them. Sometimes, however, people can develop a tendency to escape these challenges rather than with them. There are several types of maladaptive behaviour, ranging from relatively minor impairments (such as nail-biting and separation difficulties), to more severe impairments (such as self-harm).
- **3-Methylxanthine:** is a caffeine and a theophylline metabolite.
- **7-methylxanthine:** is a purine base found in most human body tissues and fluids and in other organisms and an oxopurine that is xanthine in which the hydrogen attached to the nitrogen at position 7 is replaced by a methyl group. It is an intermediate metabolite in the synthesis of caffeine. It has a role as a plant metabolite, a human xenobiotic metabolite and a mouse metabolite.
- **Missense mutation:** in genetics, a missense mutation is a point mutation in which a single nucleotide change results in a codon that codes for a different amino acid. It is a type of nonsynonymous substitution.
- **Mitochondrial acylpyruvase**: an enzyme is able to hydrolyze acetylpyruvate and fumarylpyruvate in vitro.

- **Mitochondrial disease:** is a group of disorders caused by dysfunctional mitochondria, the organelles that generate energy for the cell. Mitochondria are found in every cell of the human body except red blood cells, and convert the energy of food molecules into the ATP that powers most cell functions. Mitochondrial diseases are sometimes (about 15% of the time) caused by mutations in the mitochondrial DNA that affect mitochondrial function. Other causes of mitochondrial disease are mutations in genes of the nuclear DNA, whose gene products are imported into the mitochondria (mitochondrial proteins) as well as acquired mitochondrial conditions. Mitochondrial diseases take on unique characteristics both because of the way the diseases are often inherited and because mitochondria are so critical to cell function. The subclass of these diseases that have neuromuscular disease symptoms are often called a mitochondrial myopathy.

- **Monocytes:** are type of white blood cell, or *leukocyte*. They are the largest type of leukocyte and can differentiate into macrophages and myeloid lineage dendritic cells. As a part of the vertebrate innate immune system monocytes also influence the process of adaptive immunity. There are at least three subclasses of monocytes in human blood based on their phenotypic receptors.

- **Muscular strength:** is the ability to exert a maximal amount of force for a short period of time. In the gym, that may be bench pressing heavy barbell 5-8 repetitions. Think about lifting that heavy box when moving – that requires strength. Muscular strength is much different from muscular endurance. Strength is a measure of how much force the muscles can exert, while endurance is the measure of how many times the muscles can repeat a specific exertion of force. Unlike muscular endurance which is controlled by slow twitch fibres, strength is determined by fast twitch fibres which focus more on quick bursts of energy rather than long, drawn out ones. In addition to understanding the definition of muscular strength, it's also important to understand the benefits of strong muscles.

Building muscle strength helps with body alignment, makes performing everyday actions easier, increases metabolism, and relieves stress. It is also a much different procedure when it comes to improving strength. The most widely used method is lifting a weight that is 70% of your maximum 10-12 times. You repeat this three times with 1-5 minute breaks in between sets. Another thing to remember is that you should never repeat strength training on a specific muscle more than once every 48 hours. If you do, you can cause numerous injuries and basically wear the muscle out so you receive no improvement at all.

- **Myoclonic encephalopathy (EME):** is a rare epilepsy syndrome seen in neonates and infants. It is also known as neonatal myoclonic encephalopathy. It is usually diagnosed before 3 months of age. In retrospect, the first seizure could be felt towards the last trimester (when the baby is in the mother's womb) or is seen during the first 10 days of life. Motor and cognitive problems can be seen in a baby that can get progressively worse. EME can affect both boys and girls equally.

- **Nephelometry:** is a method to detect particles in liquid samples. The principle is to measure forwarded scattered light when a laser beam passes through a sample and the light is deflected by the particles. The technique used in immunology to determine the levels of several blood plasma proteins. For example, the total levels of antibodies isotypes or classes: Immunoglobulin M, Immunoglobulin G, and Immunoglobulin A. It is important in quantification of free light chains in diseases such as multiple myeloma. Quantification is important for disease classification and for disease monitoring once a patient has been treated (increased skewing of the ratio between kappa and lambda light chains after a patient has been treated is an indication of disease recurrence). It is performed by measuring the turbidity in a water sample by passing light through the sample being measured. In nephelometry, the measurement is

made by measuring the light passed through a sample at an angle. This technique is widely used in clinical laboratories because it is relatively easily automated. It is based on the principle that a dilute suspension of small particles will scatter light (usually a laser) passed through it rather than simply absorbing it. The amount of scatter is determined by collecting the light at an angle (usually at 30 and 90 degrees). Antibody and the antigen are mixed in concentrations such that only small aggregates are formed that do not quickly settle to the bottom. The amount of light scatter is measured and compared to the amount of scatter from known mixtures. The amount of the unknown is determined from a standard curve. Nephelometry can be used to detect either antigen or antibody, but it is usually run with antibody as the reagent and the patient antigen as the unknown. In the Immunology Medical Laboratory, two types of tests can be run: "end point nephelometry" and "kinetic (rate) nephelometry". End point nephelometry tests are run by allowing the antibody/antigen reaction to run through to completion (until all of the present reagent antibodies and the present patient sample antigens that can aggregate have done so and no more complexes can form). However, the large particles will fall out of the solution and cause a false scatter reading, thus kinetic nephelometry was devised. In kinetic nephelometry, the rate of scatter is measured right after the reagent is added. As long as the reagent is constant the rate of change can be seen as directly related to the amount of antigen present.

- **Neurophysiology:** is a branch of physiology and neuroscience that is concerned with the study of the functioning of the nervous system. The primary tools of basic neurophysiological research include electrophysiological recordings, such as patch clamp, voltage clamp, extracellular single-unit recording and recording of local field potentials, as well as some of the methods of calcium imaging, optogenetics, and molecular biology. Neurophysiology is related

to electrophysiology, neurobiology, psychology, neurology, clinical neurophysiology, neuroanatomy, cognitive science, biophysics, mathematical biology, and other sciences concerning the brain.

- **Neurotransmitters:** also known as chemical messengers, are endogenous chemicals that enable neurotransmission. They transmit signals across a chemical synapse, such as a neuromuscular junction, from one neuron (nerve cell) to another "target" neuron, muscle cell, or gland cell. Neurotransmitters are released from synaptic vesicles in synapses into the synaptic cleft, where they are received by receptors on the target cells. Many neurotransmitters are synthesised from simple and plentiful precursors such as amino acids, which are readily available from the diet and only require a small number of biosynthetic steps for conversion. Neurotransmitters play a major role in shaping everyday life and functions. Their exact numbers are unknown, but more than 100 chemical messengers have been uniquely identified.
- **Perceptual motor:** Perception is the ability to collect and process information from the environment around you. For example, a newborn might wake up suddenly and begin crying when a toy crashes to the ground. They heard (meaning they perceived) the noise stimulus and responded to it. Over time, these two skills overlap and become linked, meaning physical movements are matched appropriately in response to an environmental cue or stimulus that was perceived. Motor behaviour development refers to all of the movements the body is capable of making, including those of the eyes and head. For example, when the baby begins crying in the scenario above, he may also start waving his arms and legs trying to signal his mother to pick him up. Perceptual motor development occurs in infants, toddlers, and young children, as they learn to match their physical responses to stimuli they perceive from their surrounding environment.

- **Phenylalanine** (Phe) is an amino acid found in many **foods** and **used** by the body to produce proteins and other important molecules. High **phenylalanine foods** include beef, chicken, pork, tofu, fish, beans, milk, nuts, seeds, pasta, whole grains, and vegetables like sweet potatoes. The recommended daily intake of **phenylalanine** and tyrosine is 25mg per kilogram of body weight or 11mg per pound. It has been studied for its effects on depression, pain and skin disorders. However, **phenylalanine** can cause intellectual disabilities, brain damage, seizures and other problems in people with genetic disorder phenylketonuria (PKU).
- **Phonological awareness:** Phonemic awareness refers to the specific ability to focus on and manipulate individual sounds (phonemes) in spoken words. Phonemes are the smallest units comprising spoken language. Phonemes combine to form syllables and words.
- **Physical activity (PhA):** is defined as any bodily movement produced by skeletal muscles that require energy expenditure. Physical inactivity (lack of physical activity) has been identified as the fourth leading risk factor for global mortality (6% of deaths globally). Moreover, physical inactivity is estimated to be the main cause for approximately 21–25% of breast and colon cancers, 27% of diabetes and approximately 30% of ischaemic heart disease burden.
- **Pocketing food:** means getting food stuck in cheek.
- **Potassium/sodium hyperpolarisation-activated cyclic nucleotide-gated channel 3:** this gene encodes (HCN3 in humans) a multi-pass membrane protein that functions as a voltage gated cation channel. The encoded protein is a member of a family of closely related cyclic adenosine monophosphate-binding channel proteins.
- **Procollagen type 1 N-terminal propeptide (P1NP):** is a peptide formed during type 1 collagen synthesis, and its plasma concentration is an index of the rate of bone turnover.

Plasma P1NP increases in states of high bone turnover such as normal growth, healing fractures, Paget's disease, osteoporosis, hyperparathyroidism, and hyperthyroidism. It can be used to monitor therapy in Paget's disease and osteoporosis. Its use as a predictor of future bone loss in osteoporosis is not yet well established. The assay measures total P1NP (intact P1NP plus a smaller peptide), which is partially cleared by the kidney; hence P1NP levels increase in renal failure. However, P1NP is not a marker of liver fibrosis.

- **Protein phosphatase 1 regulatory subunit 1A** (PPP1R1A): a potent protein phosphatase 1 (PP1) inhibitor; may be important in hormonal control of glycogen metabolism. However, its role in tumour development is largely undefined.
- **Proteome:** is the entire set of proteins that are produced or modified by an organism or system. This varies with time and distinct requirements, or stresses, that a cell or organism undergoes.
- **Proteomic:** is the large-scale study of proteins which was coined in 1997 in analogy with genomics, the study of the genome. Thereon, it has benefited greatly from the genetic information of the Human Genome Project; and is also emerging scientific research and exploration of proteomes from the overall level of intracellular protein composition, structure, and its own unique activity patterns. Proteomic is often specifically used for protein purification and mass spectrometry.
- **Psychomotor development:** refers to changes in a child's cognitive, emotional, motor, and social capacities from the beginning of life throughout foetal and neonatal periods, infancy, childhood, and adolescence. It occurs in a variety of domains and a wide range of theories makes understanding children's development a challenging undertaking. Different models have tried to interpret the origins of human behaviour, the pattern of developmental changes over time, and the individual and contextual factors that could

direct child development. No single theory has been able to account for all aspects of child development, but each of them may contribute an important piece to the child development puzzle.

- **Questionnaire for Children's Health Related Quality of Life (QCHRQoL):** is set up to provide a systematic, valuable and reliable description of health-related quality of life when we care about children of age 6 to 15 years.
- **Reflex epilepsies:** are a group of epilepsy syndromes in which a certain stimulus brings on seizures. The stimulus can be something simple in the environment or something more complex like reading, writing, doing arithmetic, or even thinking about specific topics. The types of seizures that may occur are varied, but 85% are generalised tonic-clonic (grand mal) seizures. Other seizure types include absence seizures (staring) and myoclonic seizures (jerking of the eyes, head, or arms). The most common form of reflex epilepsy is photosensitive epilepsy, in which flashing lights trigger seizures. These seizures are usually found to be primary generalised seizures. Occasionally, partial seizures (arising from a small portion of the brain) may also present as a reflex epilepsy.
- **Renal physiology:** this encompasses all functions of the kidney, including maintenance of acid-base balance; regulation of fluid balance; regulation of sodium, potassium, and other electrolytes; clearance of toxins; absorption of glucose, amino acids, and other small molecules; regulation of blood pressure; production of various hormones, such as erythropoietin; and activation of vitamin D.
- **Salivary buffering capacity (SBC):** is the ability of saliva in keeping stable oral pH, within the limits of normality, that is, it is the capacity of saliva in neutralising the acids and/or bases within oral cavity, contributing to oral health. Therefore, saliva has a buffer capacity which neutralises acids in the mouth. This capacity is based on several systems such as the phosphate system and the carbonic acid/bicarbonate system. In unstimulated

saliva, the concentration of inorganic phosphate is rather high while the concentration of carbonic acid/bicarbonate system is low. The carbonic acid/bicarbonate system is the most important buffer in stimulated saliva due to its higher concentration.

- **Seizure:** occurs when there's abnormal electrical activity in the brain. Seizures may go virtually unnoticed. Or, in severe cases, they may produce a change or loss of consciousness and involuntary muscle spasms called convulsions. Seizures usually come on suddenly and vary in duration and severity. A seizure may be a one-time event, or you may have seizures repeatedly. Recurrent seizures are called epilepsy, or a seizure disorder. Less than one in 10 people who has a seizure develops epilepsy. Experts classify seizures into two general categories and many subtypes based on the pattern of the attack. Generalised seizures involve both sides of the brain from the start of the attack. Common subtypes include tonic-clonic (grand mal) and absence seizures (petit mal). Febrile and infantile spasms are two types of generalized seizures that occur almost exclusively in young children. Partial (or focal) seizures are the second major seizure type. These begin in a specific area of the brain and may be contained there. Or they may spread to the entire brain.

- **Sense of coherence (SOC):** was put forward by Aaron Antonovsky in 1979 to explain why some people becomes ill under stress and others stay healthy. It arose from the salutogenic approach, that is, the search for the origins of health rather than the causes of disease. The SOC gained widespread attention and has since been linked to health outcomes in many studies. Hence, the SOC is defined as: "The extent to which one has a pervasive, enduring though dynamic, feeling of confidence that one's environment is predictable and those things will work out as well as can reasonably be expected." In other words, it is a mixture of optimism and control.
  It has three components – comprehensibility, manageability, and meaningfulness. Comprehensibility is the extent to which events are perceived as making logical sense,

that they are ordered, consistent, and structured. Manageability is the extent to which a person feels they can cope. Meaningfulness is how much one feels that life makes sense, and challenges are worthy of commitment. Professor Antonovsky believed that, in general, a person with a strong SOC is more likely to feel less stress and tension, and to believe that he or she can meet demands. The SOC was developed to apply across cultures, and versions of the questionnaire have been used. The concept interacts with a person's natural coping style, upbringing, financial assets, and social support – the extent to which these are available is a major determinant in the development of a strong or weak SOC.

- **Single transverse palmar crease (simian crease or simian line):** is a single crease that extends across the palm of the hand, formed by the fusion of the two palmar creases (known in palmistry as the heart line and the head line).
- **Social functioning:** The ability of the individual to interact in the normal or usual way in society can be used as a measure of quality of care.
- **Somatisation:** is a tendency to experience and communicate psychological distress in the form of somatic symptoms and to seek medical help for them. Furthermore, it is the generation of physical symptoms of a psychiatric condition such as anxiety.
- **Superoxide dismutase** [Cu–Zn] (SOD, EC 1.15.1.1): an enzyme that alternately catalyses the dissimulation (or partitioning) of superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Hydrogen peroxide is also damaging and is degraded by other enzymes such as catalase. Thus, SOD is an important antioxidant defence in nearly all living cells exposed to oxygen. One exception is *lactobacillus plantarum* and related lactobacilli, which use a different mechanism to prevent damage from reactive (O_2^-).

- **Thrombotic thrombocytopenic purpura (TTP):** is a rare blood disorder characterised by clotting in small blood vessels of the body (thromboses), resulting in a low platelet count. In its full-blown form, the disease consists of the pentad of microangiopathic haemolytic anaemia, thrombocytopenic purpura, neurologic abnormalities, fever, and renal disease.
- **Upslanting palpebral fissures:** the eye opening appears slanted upwards from the inner corner of the eye to the outer corner of the eye.
- **Uridine:** is a glycosylated pyrimidine-analogue containing uracil attached to a ribose ring (or more specifically, a ribofuranose) via a β-N_1-glycosidic bond. It is one of the five standard nucleosides which make up nucleic acids, the others being adenosine, thymidine, cytidine and guanosine.
- **Verbal short-term memory (STM):** measures and typically developing individuals' ability to learn the phonological *form* of novel words but not their ability to learn the physical *referent* of new words.
- **Vigabatrin:** brand name Sabril, is an antiepileptic drug that inhibits the breakdown of γ-aminobutyric acid (GABA) by acting as a suicide inhibitor of the enzyme GABA transaminase (GABA-T). It is also known as γ-vinyl-GABA, and is a structural analogue of GABA, but does not bind to GABA receptors.

- **Visuospatial short-term memory (VSTM):** is typically construed as a set of basic processing mechanisms for the maintenance and manipulation of visuospatial information that underpins a range of cognitive functions, such as orientation and navigation in the environment and mental imagery. Generally, VSTM is associated with maintaining and processing visual and spatial information when that information is no longer present in the immediate surroundings, so it serves a critical function in the construction and maintenance of an integrated representation of the visual world.
- **White blood cells or leukocytes (WBCs):** also called leukocytes or leucocytes, are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All white blood cells are produced and derived from multipotent cells in the bone marrow known as haematopoietic stem cells. Leukocytes are found throughout the body, including the blood and lymphatic system. All white blood cells have nuclei, which distinguishes them from the other blood cells, the enucleated red blood cells (RBCs) and platelets. Types of white blood cells can be classified in standard ways. Two pairs of broadest categories classify them either by structure (granulocytes or agranulocytes) or by cell division lineage (myeloid cells or lymphoid cells). These broadest categories can be further divided into the five main types: neutrophils, eosinophils, basophils, lymphocytes, and monocytes. These types are distinguished by their physical and functional characteristics. Monocytes and neutrophils are phagocytic. Further subtypes can be classified; for example, among lymphocytes, there are B cells, T cells, and NK cells. The number of leukocytes in the blood is often an indicator of disease, and thus the WBC count is an important subset of the complete blood count. The normal white cell count is usually between 4×10^9/L and 11×10^9/L. In the US this is usually expressed as 4,000 to 11,000 white blood cells per microliter of blood. They make up approximately 1% of the total blood volume in a healthy

adult, making them substantially less numerous than the RBCs at 40% to 45%. However, this 1% of the blood makes a large difference to health, because immunity depends on it. An increase in the number of leukocytes over the upper limits is called leukocytosis. It is normal when it is part of healthy immune responses, which happen frequently. It is occasionally abnormal, when it is neoplastic or autoimmune in origin. A decrease below the lower limit is called leukopenia. It weakens the immune system.

- **Xaa-Pro aminopeptidase 1 (XPNPEP1):** an enzyme contributes to the degradation of bradykinin. Catalyses the removal of a penultimate prolyl residue from the N-termini of peptides, such as Arg-Pro-Pro.

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