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NUTRITIONAL STATUS, ENERGY REQUIREMENTS AND METABOLIC MONITORING IN CRITICALLY ILL CHILDREN: THE NEW PERSPECTIVE OF ARTIFICIAL NEURAL NETWORKS

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Abstract

Nutrition plays a pivotal role in all humans. As early as during foetal life, a correct nutrition has the power to influence the development of organs and tissues, ultimately setting the basis for a healthy life. In critically ill children the risk of malnutrition is of particular importance. Accordingly, an appropriate monitoring of nutritional status and metabolic response, along with the correct assessment of energy requirements and energy balance, is gaining growing clinical relevance as a fundamental prognostic factor and should be considered a specific target in the management of critically ill children. The first step for a tailored nutritional support is the knowledge of patients' resting energy expenditure (REE). Indirect calorimetry (IC) is the gold standard for REE measurement, however, its clinical use is limited across the world for both logistic and technical limitations. Alternatively, REE can be estimated using predictive equations, but this method has been found to be highly inaccurate in pediatric patients. Recent data pointed out that artificial neural networks (ANN) might represent a precise and accurate method to estimate REE in healthy and obese children. However, specific data regarding the applicability of the methodology on critically ill subjects are still missing. This thesis aimed to investigate the potential role of ANN on REE prediction for critically ill children by applying ANN to a dataset containing data on IC performed in our pediatric intensive care unit (PICU). We prospect that data derived from our observations could lead to a more accurate estimation of REE and to a better understanding of the energy requirements of critically ill children.

Riassunto

La nutrizione gioca un ruolo fondamentale in tutti gli esseri umani. Già durante la vita fetale, una corretta alimentazione ha il potere di influenzare lo sviluppo di organi e tessuti, ponendo le basi per una vita sana. Nei bambini gravemente malati il rischio di malnutrizione è di particolare importanza. Di conseguenza, un adeguato monitoraggio dello stato nutrizionale e della risposta metabolica, insieme alla corretta valutazione del fabbisogno energetico e del bilancio energetico, sta acquisendo una crescente rilevanza clinica come fattore prognostico e dovrebbe essere considerato un obiettivo specifico nella gestione dei bambini critici. Il primo passo per un supporto nutrizionale su misura è la conoscenza del dispendio energetico a riposo (REE, *Resting Energy Expenditure*) del paziente. La calorimetria indiretta (IC, *indirect calorimetry*) è il gold standard per la misurazione di REE, tuttavia, il suo uso clinico è limitato dalla scarsa disponibilità della metodica. In alternativa, REE può essere stimato utilizzando equazioni predittive, ma questo metodo è altamente impreciso nei pazienti pediatrici. Dati recenti hanno dimostrato che le reti neurali artificiali (ANN, *Artificial Neural Networks*) possono rappresentare un metodo preciso e accurato per stimare REE nei bambini sani e obesi. Mancano tuttavia dati specifici sull'applicabilità della metodologia nei soggetti critici. Obiettivo di questa tesi è stato indagare il potenziale ruolo di ANN nella predizione di REE dei bambini critici. A questo scopo la tecnologia ANN è stata applicata al set di dati nutrizionali della nostra unità di terapia intensiva pediatrica, comprendente i dati relativi alla calorimetria indiretta. Ci aspettiamo che i risultati emersi dalle nostre osservazioni potrebbero portare a una stima più accurata di REE e a una migliore comprensione del fabbisogno energetico dei bambini critici.

Contents

CHAPTER 1

Introduction

1.1 Nutrition

1.1.1 General aspects

Nutrition is a fundamental component of health and development in all age groups, beginning from pregnancy and early childhood and extending throughout the lifespan [1]. Better quality of diet and balanced nutrition throughout the life-course can improve the quality of life, so that the monitoring of nutritional status and the prevention of malnutrition can be regarded as important actions to prevent illness. Indeed, according to the operative definition of nutritional status, health and nutrition are strictly related to one another (Figure 1).

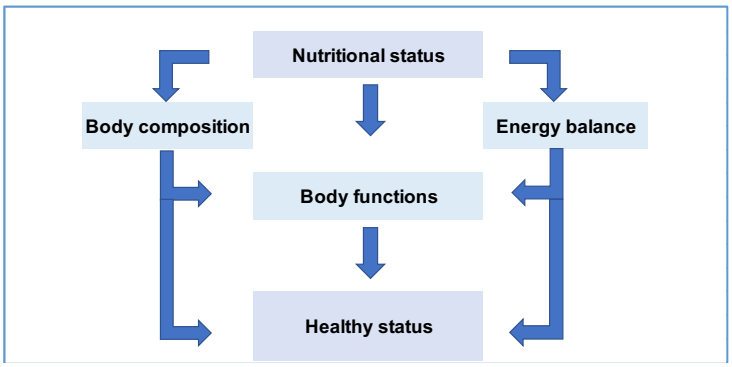

Figure 1. Operative definition of nutritional status (based on concept and findings by Bedogni et al.[2])

As described in Figure 1, nutritional status may be expressed as the resultant between three components: body composition, body functions and energy balance. The interaction of these three entities is fundamental to maintain a balanced life and a healthy status [2]. Therefore, nutritional status and healthy status stand in a two-ways relationship. Optimal nutrition and healthy nutritional status have been linked to stronger immune systems, safer pregnancy and childbirth, lower risk of non-communicable diseases and longevity [3]. On opposite, malnutrition may lead to unhealthiness as much as unhealthiness may lead to malnutrition. For instance, undernutrition is associated with lower life expectancy and morbidity, while overnutrition/obesity is linked to chronic non communicable diseases such as cardiovascular diseases and chronic respiratory diseases. On the other hand, illness has a negative impact on body composition and body functionality, which is frequently mediated by energy imbalance [4]. Indeed, the relationship between nutrition and healthiness has been addressed by the World Health Organisation (WHO) with

the production of specific advice and decision-making tools to diagnose malnutrition and consequently promote corrective actions to restore health and wellbeing of all individuals [5]. Accordingly, it has been proposed that nutritional status and healthiness may be evaluated by analysing body composition, energy balance and body functions of individuals [2].

A correct monitoring of these three functions throughout the life-course and an appropriate intervention to correct eventual unbalances in the diet is a valid preventive strategy to the development of diseases.

1.1.2 Nutritional status assessment

In clinical practice, nutritional status is defined by the analysis of different aspects: clinical appearance, anthropometry and body composition, laboratory (biochemical) values and dietary intake evaluation [6].

1.1.2.1 Clinical appearance

The first step towards the correct assessment of nutritional status in both children and adults is the evaluation of patients' clinical appearance, as well as the identification of specific signs and symptoms that may indicate the presence of nutritional unbalance [7]. The physical appearance of patients alone can give hints of the presence of an underlying nutritional unbalance, such as severe undernutrition or, on the opposite, extreme obesity.

Moreover, clinicians should be able to identify specific signs and symptoms that are linked to a specific nutritional deficit. For instance, the presence of goitre (swallowing of the neck) may suggest a severe deficit of iodine [8]. Although the recognition of such signs and symptom is important for the diagnosis of specific deficiency, it is nowadays very rare to find such striking findings in western hospitals.

In addition to the classic clinical appearance evaluation, for critically ill subject and especially in critically ill children it is also recommended to perform a severity of illness assessment and a risk of malnutrition assessment upon admission. In fact, understanding the severity of illness may be helpful to predict the presence of metabolic changes related to acute inflammation. On the other hand, the assessment of the risk of malnutrition may help clinicians to prevent the occurrence of the condition, reducing the risk of related complications. Many objective illness/malnutrition severity score have been proposed for the pediatric population, two examples are the Pediatric Index of Mortality score 2 (PIM2) and the STRONG kids score for malnutrition [9, 10].

1.1.2.2 Body composition

The assessment of nutritional status based on body composition is widely diffuse in clinical practice. Usually, this can be obtained with the assessment of body composition from basic anthropometric measures [11]. Indeed, classic anthropometry has been regarded as a non-invasive, easy to obtain technique that can be employed by clinicians to define

subjects' body composition and nutritional status. Body weight and body height/length (and head circumference when applicable) are the basic anthropometric measures which allow a simple evaluation of nutritional status by comparing the measures obtained in the single individual to the standard measures of the reference population [2]. However, body weight and height/length taken separately cannot precisely depict the alterations in body composition. In fact, according to the five-components model, body weight derives from the sum of five different components: protein mass, lipid mass, minerals mass, glycogen and total body water. Thus, the variation of any of these components may alter this parameter. On the other hand, length (measured in children under 2 years of age) and height (measured in children equal or over 2 years of age) only provide an insight of the body proportions.

The best way to obtain a valid assessment of nutritional status is therefore to combine body weight and height/length, together or in relation to age, to obtain anthropometric indices, such as height/length for age (HFA/LFA), weight for age (WFA), weight for height/length (WFH/WFL) and the body mass index (BMI), which is the ratio between weight (kg) and the square measure of height (m^2). These indices are important tools to the detect malnutrition (i.e. under- and overnutrition) and classify acute and chronic malnutrition (wasting and stunting respectively) [12].

Besides the classic anthropometry based on the simple

measurement of body weight, height/length and of their related indices, more anthropometric measures can also be employed to define more accurately body lean mass and body fat distribution. For instance, the measurement of body abdominal circumference and mid upper arm circumference (MUAC), as well as the measurement of skinfolds (triceps, biceps, subscapular) can offer a better insight on the actual body content of fat mass. In addition to this, in recent years other more sophisticated methods to assess body composition have been commercialized. Specifically, the bioelectrical impedance analysis (BIA) and the dual-energy x-ray absorptiometry (DEXA) have been progressively more available for clinical practice [13]. However, compared to classic anthropometry, circumferences, skinfolds and new technology for body composition analysis are less used, especially in the critical care setting

1.1.2.3 Laboratory values

Nutritional status assessment can be implemented by the evaluation of some selected biochemical parameters, which may help identify specific deficit before they become clinically manifested [14]. Such parameters can be then monitored throughout clinical recovery from malnutrition to ensure that the eventual deficits are appropriately being corrected.

Protein synthesis and protein turnover is most relevant for the assessment of nutritional status. Blood laboratory values of visceral proteins such as albumin, pre-albumin, retinol

binding protein (RBP) and transferrin are especially useful to detect eventual alterations in the protein pool [15]. In fact, each of the above-mentioned proteins has a different time of turnover and may therefore help outline the different phases of an altered protein status. In particular, deficit in RBP and pre-albumin tend to appear earlier, as they both have a short half-life of respectively 12 hours and 2-3 days. A reduction in the levels of these two proteins may therefore indicate acute nutritional status changes. On opposite, the monitoring of the levels of transferrin and albumin, which have longer half-life (8 and 20 days respectively), may be more helpful in detecting chronic changes in nutritional status. Among the blood values that are less likely to be measured routinely, IGF-1 is considered a useful parameter for early detection of protein-energy imbalance. Additional information on protein synthesis/anabolism and protein turnover/catabolism can be gathered by the monitoring of urine creatinine and urinary nitrogen excretion. Urinary creatinine is especially useful to assess muscle mass and loss. Nitrogen excretion on the other hand is very useful to monitor protein metabolism and to estimate daily protein losses, however it is less easy to obtain and requires 24 hours urine collection.

Other useful biochemical markers for the assessment of nutritional status include the monitoring of haemoglobin (Hb) values, total cholesterol and total proteins, as well as the evaluation of specific micronutrients deficits (iodine, folate, vitamins in general) [16].

The use of laboratory values to assess nutritional status must however be carefully evaluated in hospital setting and especially during acute illness. In fact, the levels of nutritionally relevant proteins may be altered by the active state of inflammation characterising critical patients. Indeed, during acute phase occurring after trauma or sepsis, the production of visceral protein is inhibited, while the production of acute phase protein is favoured. Consequently, the alteration of these proteins may be a reflection of the severity of the illness more than of the acute malnutrition. Therefore, in critical setting it is reasonable to complement the measurement of visceral protein as nutritional status indices with the levels of CRP (C-reactive protein), which is an indicator of the severity of illness and of acute metabolic response [17]. Studies have shown that the levels of CRP are inversely proportional to levels of pre-albumin. Particularly, during acute phase of illness, pre-albumin and visceral proteins are less synthetized, to advantage the production of inflammatory protein such as CRP. On opposite, during the recovery phase, pre-albumin and visceral protein are produced again to allow wound healing and, in case of children, growth, while CRP levels decrease. Finally, nitrogen balance may be of particular use to understand daily protein requirements in critical children. Specifically, the reduction of nitrogen in the urine indicates a return to protein anabolism and, along with reduction of CRP, the resolution of the acute phase [18].

1.1.2.4 Dietary intake evaluation

The definition of nutritional status should ideally also include a complete evaluation of patients' dietary habits, with the help of questionnaire such as the food frequency questionnaire, or the three- or seven-days dietary diary [19]. Such tools allow clinicians to assess patients' daily dietary intakes and to identify potential dietary deficit of macronutrients, as well as micronutrients. Unfortunately, of all the methods available to assess nutritional status, this is the least employed, as it requires time to collect the data and active collaboration from patients or from caregivers.

1.1.3 Nutritional status assessment in children

In children, the classic approach to nutritional status assessment is based on the comparison of individually obtained measures and/or calculated indices with the ideal growth charts/rates from normal healthy infants and children. Therefore, adequate growth monitoring and optimal growth patterns definition are essential to ensure healthy state in children and appropriate intervention to correct growth deviations. Growth curve are graphic representation of the growth pattern of a reference population that consent to compare anthropometric parameters of a single individual with the reference national and/or international standard of the same age/sex population (e.g. percentile tables: Tanner – NCHS – SIEDP). The international WHO growth standards curves, which were

developed using data collected in the WHO Multicentre Growth Reference Study, are the preferred method to assess nutritional status in children internationally [20]. The graphic representation of the growth pattern of a normal children reference population and the definition of precise and statistically defined cut off points to distinguish variations in the growth curve development, allow clinician to identify the intervals in which the nutritional status is considered healthy for the individual, and the values below and above which malnutrition can be diagnosed.

The result of the comparison between the growth curve and the individual measures/indices can be expressed either in percentiles or in Z-score. Reporting the percentile provides a position measure of where the individual stands on the curve, in respect to the reference population[21]. The percentile indicates the percentage of the reference population whose anthropometric parameter is respectively below and above the individual. For instance, if the subjects' BMI is positioned at the 75° percentile, it means that 74.9% of the reference population has a lower BMI than the subject considered, while 24.9% of the reference population has a higher BMI than the subject considered. Children whose percentiles are included in the interval between the third (3°) and ninety-seventh (97°) percentile are considered adequate for age. Instead values lower or higher than these indicate a situation of underweight and overweight respectively. On the other hand, the use of Z-scores is the most appropriate way to

evaluate malnutrition and to monitor growth, since it statistically represents the distance of the measure obtained in the single individual in comparison to the median value for the reference population by expressing it as a standard deviation

1.2 Malnutrition

1.2.1 General aspects

Malnutrition may be defined as the pathological condition that appears when the supply of nutrients and energy does not meet or exceed the demand of the body to guarantee the maintenance of basic and specific functions of the individual and to support growth in children and adolescents [22]. Generally speaking, malnutrition can regard both the amount and the quality of the nutrients introduced with diet or just one of the two components. For this reason, it is crucial to understand that malnutrition does not regard only undernourished subjects, i.e. people who have an insufficient dietary intake in terms of quantity of food and energy intake, but also overnourished subjects, whose diet does not however meet the daily demand for specific nutrients [23].

1.2.1.1 Classification of malnutrition

As already mentioned, the concept of malnutrition comprehends both under and over nutrition. Moreover, depending on its severity, malnutrition can be further

classified in mild, moderate or severe. Normal weight is defined for healthy subjects with a normal nutritional status. On the other hand, overnourished subjects can be either classified as overweight (moderate overnutrition) or obese (severe overnutrition) [24]. On opposite, for subjects that are undernourished and therefore underweight, malnutrition can be further classified in acute or chronic. Acute undernutrition is defined wasting, while chronic malnutrition is defined stunting [25]. It is however important to know that in particularly severe situations, chronic and acute malnutrition can occur at the same time, increasing the risk of short- and long-term consequences for general heath.

1.2.2 Malnutrition in children

The risk of malnutrition should be especially monitored in children and adolescents. Indeed, during early life the energy needed per unit of body mass is higher compared to adults and in addition children require additional energy to support the cost of growth, that is the energy required to support tissues and organs development and the energy needed to maintain these systems. Therefore, nutritional unbalances at young ages have a strong impact on body composition and on growth potential, and may leave consequences long-term, if not accurately addressed. Many studies have reported an association between normal growth deviation and consequent negative outcomes later in life. Early rapid growth has been linked to development of

obesity and cardiovascular diseases in adulthood. On opposite, undernutrition was connected to underdevelopment of cognitive capacity. Moreover, the maximum potential of muscleskeletal health and accretion is achieved in the first two decades of life [26, 27]. Hence, when the time-lapse is passed to achieve the maximum potential accretion, there is no coming back and one could only oppose the natural decline of the body by maintaining the body mass and strength already achieved with correct nutrition and physical exercise. Appropriate growth monitoring during childhood and adolescence is therefore fundamental and growth deviation should be promptly corrected. Of course, malnutrition can have many forms, especially in young patients. The most basic type of general malnutrition in children is protein-energy malnutrition (PEM), which occur when there is a general depletion of both body energy stores (glycogen, adipose tissue…) and tissue proteins. Most frequently, when PEM is present, micronutrients deficiencies are also recognized. PEM is an especially important problem in children from underdeveloped countries, but notably it is frequently recognisable also in children with critical illness [28]. Since PEM is associated with higher risk of mortality and morbidity, when present, the condition should be promptly treated [29].

1.2.2.1 Classification of malnutrition in children

Malnutrition in children is most frequently classified

using anthropometry to define body composition indices, which are then compared with standard growth curve. As already mentioned, internationally the WHO growth charts are the preferred reference method to assess nutritional status for epidemiological studies [30].

Depending on age, the WHO suggests the use of different body composition parameters and Z-score cut off to define presence of malnutrition and its severity. Specifically, in children less than 5 years of age, weight for height (WFH) is the index of current nutritional status. Lower values indicate recent depletion of body mass (wasting), while higher values are diagnostic for overweight or obesity. In older children and adolescents (5 to 18 years), the body mass index (BMI) for age is used instead of WFH. Finally, the presence of long-term growth retardation (stunting) is diagnosed with low height for age (HFA) [31]. The principal cut offs for the diagnosis of malnutrition in children based on WHO Growth Standards are reported in Table 1.

Table 1.

Malnutrition classification in children
Acute undernutrition (wasting)
mild undernutrition -2.0 ≤ z-WFH < -1.0 (0-5 y) or -2.0 ≤ z-BMI for age < -1.0 (5-18 y)
moderate undernutrition -3.0 ≤ z-WFH < -2.0 (0-5 y) or -3.0 ≤ z-BMI for age < -2.0 (5-18 y)
severe undernutrition z-WFH < -3.0 (0-5 y) or z-BMI for age < -3.0 (5-18 y)
Chronic undernutrition (stunting)
z-HFA < -2.0 (0-18 y)
Normal weight
normal weight -1.0 ≤ z-WFH ≤ +2.0 (0-5 y) or -1.0 ≤ z-BMI for age ≤ +1.0 (5-18 y)
Overnutrition
overweight +2.0 < z-WFH ≤ +3.0 (0-5 y) or +1.0 < z-BMI for age ≤ +2.0 (5-18 y)
obesity z-WFH > +3.0 (0-5 y) or z-BMI for age > +2.0 (5-18 y)

Abbreviations: z-WFH= Z-score weight for height; z-BMI= Z-score BMI for age; z-HFA= Z-score height for age

1.2.3 Malnutrition in the hospital setting and in the PICU

The problem of malnutrition in children is even more evident in the hospital setting and especially in the Pediatric Intensive Care Unit (PICU). Indeed, children admitted to hospital facilities have a higher risk of presenting malnutrition upon admission and what is worse, it has been demonstrated that regardless of the condition upon admission, the nutritional status frequently declines during the hospital stay [32, 33]. As a consequence, hospitalized children who develop malnutrition have higher risk of complications (e.g. increased nosocomial infections), greater requirements for high dependency or intensive care, prolonged recovery times and overall a higher risk of morbidity and mortality [29].

1.3 Energy balance and energy requirements

1.3.1 General aspects

The concepts of energy balance and energy requirements are strictly related to one another. In fact, both elements are essential in clinical practice to guarantee optimal nutrition and to prevent malnutrition. Energy balance is by definition the difference between daily energy intake with diet and daily energy expenditure (EE) [34]. When the intake of energy and EE are equivalent, energy balance is obtained. Instead, positive or negative energy balance occur when the energy intake is superior to EE or when the energy intake is

less than the energy consumed, respectively. To maintain energy balance and avoid overnutrition and undernutrition, it is therefore important to correctly define the energy requirements of individual subjects. This is especially important for children and in critically ill pediatric patients.

1.3.2 Energy expenditure

The definition of energy requirements starts with the definition of the EE, that is the energy needed by the organism to fulfil both internal functions (i.e. breathing) and external work (i.e. physical exercise), expressed either in kcal or kJ.

Specifically, EE is the sum of three main components [35]:

- Basal metabolism (60-75%)
- Physical activity (15-30%)
- Diet induced thermogenesis (7-13%)

Theoretically, the evaluation of these three components could provide clinicians with an accurate value of EE. In practice, it is however fundamental to keep in mind that the contribution given by each component may differ from person to person and can vary during the day even in the same individual. Therefore, it is essential to consider the impact of each component separately

1.3.2.1 Basal metabolism

Basal metabolism (BM) is the main component of EE and it represents the energy needed by the organism to

maintain basal physiological conditions and the functionality of cells, tissues, organs and body systems.

Factors that influence basal metabolic rate in humans include basic characteristic, such as age, gender, ethnicity, but also individual factors, such as body composition [36, 37]. For instance, fat free mass (FFM) contained in both organs and tissues requires more energy support compared to fat mass (FM) and is therefore a main predictor of BM, accounting for 73% of its variability.

In healthy individuals, BM is perhaps the EE component that is usually more constant in terms of kJ or kcal needed per day by each individual.

However, the stability of BM energy requirements depends on a precarious equilibrium and therefore it may be subject to abrupt modifications when heathy status is missing.

In clinical practice the concept of BM is frequently assimilated to the concept of resting energy expenditure (REE). Indeed, the conditions required for assessment of BM and of REE are very similar. The BM is measured at rest and in supine position, in subjects that have just awake from their night sleep and fasting from the night before. Similarly, the REE is measured at rest and in supine position, with the subject awake and in post-absorptive state[38]. Overall, the difference between BM and REE in terms of energy consumed is less than 10%.

1.3.2.2 Physical activity

Contrary to BM, the quote of EE that is required for physical activity in healthy subjects may widely vary from day to day and within the same day, in function of the energy cost of the physical activity itself and of course in function of how long the activity lasts. Moreover, it is important to consider that physical activity is not limited to the spontaneous activity that depends on the everyday-life routine (i.e. going to work), but also includes all those activities carried out by individuals as voluntary actions.

1.3.2.3 Thermogenesis

Finally, EE depends also on the thermogenesis induced by the diet, which is determined by the processes of assumption, digestion, absorption, metabolism and deposition of micro- and macronutrients introduced with the diet. As for physical activity, this component of EE can vary more, depending on the quality of the diet introduced by each subject. Specifically, the bigger expense of energy is needed for the utilization of proteins (20-30%), followed by carbohydrates (5-10%) and lipids (0-5%) [38].

1.3.2.4 Additional factors influencing EE

In addition to the three main components discussed above, a minor part of EE may depend on minor thermogenetic components, which, if present, may have an influence on the EE of each individual (*e.g.* presence or

absence of stress, variations in the environmental temperatures, and so on).

1.3.3 Energy expenditure in children

Defining the correct energy needs with EE for each individual becomes more complexed when it comes to measure EE in children. In fact, the assessment of EE in the pediatric age should account for the energy needed for growth, which is the energy required to guarantee the growth of tissues and organs, and the energy needed to sustain the same tissues and organs. Specifically, in the first months of life the cost of growth can take up to the 35% of the energy intake, but it rapidly diminishes during the first year of life to around 3%. Generally during childhood and adolescence the cost of growth is estimated to require to 1-2% of the daily energy intake [39]. Therefore, the role of growth in the evaluation of EE must be carefully assessed by clinicians, especially when treating infants.

1.3.4 Energy expenditure in critically ill child

During critical illness, in both adults and children, the energy requirements can widely vary due to the subjective metabolic response to the illness and general state of inflammation that follows. Indeed, the acute stress that derives from tissue injury, trauma and surgery gives birth to a complex cascade of events that are mediated by cytokines, growth factors and hormones, significantly altering the daily EE [40]. These alterations are proportional to the severity and

duration of the stress and typically result in the catabolism of the endogenous protein, carbohydrates, and fat stores in the attempt to support the metabolic needs. Failure to accurately address these insults can result in significant morbidity and poor patient outcomes.

Normally, the metabolic response to insults can be broken down in three moments. In the first 2 or 3 days, the initial response to body insult is directed towards the conservation of energy, therefore decreasing EE. This initial conservative response is then rapidly followed by an increase of EE and general catabolic response. The duration of this phase depends on the severity of the insult [41]. Finally, when acute stress resolves, EE returns to the physiological values.

The metabolic changes that occur in critical illness are even more variable in pediatric patients, with different studies reporting very different findings on the metabolic state of children treated in the PICU. Some authors have reported normal metabolic state in children admitted in PICU, while some others have reported the presence of hyper- or hypometabolism [18]. This can be explained by the fact that the PICU population is often very heterogeneous, thus, the fact that patients admitted with severe burn are generally hypermetabolic, while patients admitted after elective surgery are not, is not too surprising. Furthermore, energy needs may change over time throughout the course of a child's hospitalization [42]. For example, a febrile child with status epilepticus will have a dramatic raise in metabolic

demand, but if the same child is then mechanically ventilated and sedated, the metabolic state will shift towards hypometabolism and decreased EE. In this scenario, determining the phase of the pediatric stress to appropriate prescription of nutrition therapy during critical illness response is even more essential. Hence, during acute illness the aim should be to provide energy as close as possible to the measured REE in order to avoid energy imbalance and consequent unintended underfeeding or overfeeding.

1.4 Metabolic monitoring: methods to define energy expenditure

1.4.1 General aspects

The assessment of EE has been widely used to characterise alterations in metabolism and to determine daily caloric requirements accompanying a variety of clinical states. In clinical practice, the assessment of EE can be obtained with different approaches and methods, which can be more or less accurate, trustworthy and expensive. Particularly, the methods to assess EE can be divided in two main categories: 1) methods that provide the measurement of EE and 2) methods that provide an estimation of EE.

1.4.2 Methods to measure EE

The methods developed to obtain the actual measurement of EE include direct and indirect calorimetry

(calorimetric techniques), the double-labelled water technique (tracer methodology) and the reverse Fick method [43].

1.4.2.1 Direct calorimetry

Direct calorimetry is based on the concept that EE can be directly assessed by measuring the heat produced by the body. In fact, heat is produced with the synthesis and utilization of ATP and as a consequence its measurement is a signal of the energy consumed during the exam and can therefore be used to assess EE. The principle underneath the methodology is that the energy consumed to produce work is let out in the form of heat. Therefore, EE can be obtained by quantifying the amount of heat produced by metabolism and exchanged with the environment [44].

The big pitfall of direct calorimetry is that the measure requires the confinement of the subject in a controlled environment. Thus, the availability of the technology is limited, as the exam cannot be performed in patients that are in critical conditions since it would require moving the subject to perform the exam in the controlled setting.

1.4.2.2 Indirect calorimetry

Indirect calorimetry is the gold standard method to assess REE in clinical practice [45]. The methodology is based on gas exchange monitoring and uses the Weir equation to combine steady state values of oxygen consumption (VO_2)

and carbon dioxide production (VCO_2), measured by IC, to provide an assessment of REE and respiratory quotient (i.e. the VCO_2/VO_2 ratio), which can provide useful information on the subject's substrate utilisation [43]. Contrary to direct calorimetry, IC application is possible also in critically ill people, who cannot be moved from their beds. In this setting, the exam can be performed both in spontaneously breathing patients and in mechanically ventilated children. However, although IC is the gold standard for REE measurement, the technology is too often unavailable in the PICU. Within a recent survey, only 14% of PICUs have resources to use indirect calorimetry and accordingly, nutritional targets for macronutrients, corrected for age/weight, may vary widely too [46]. Moreover, a limit of the technology in critical care is that the exam cannot be performed in patients with non-invasive ventilation (NIV), which is however commonly employed in the PICU.

Since IC is the preferred method to assess REE in critical setting, more aspects of the methodology will be discussed in paragraph 1.4.3.

1.4.2.3 Double labelled water

Double labelled water is a non-calorimetric method to measure EE. The methodology consists in administrating to the subject drinking water that include a known quantity of two isotopes, deuterium and oxygen-18 [43]. The progressive degradation of these two isotopes over time is than

monitored by the dosage of the levels of each isotope on body liquids, most commonly urine. VCO_2 is then derived from the difference in the velocity reduction of each isotope, the deuterium lost with the urine and the oxygen-18 which is expired with the CO_2 produce by lung gas exchange. From VCO_2, it is then possible to derive the average total energy expenditure (RQ is usually assumed to 0.85), but the results can take up to three weeks.

1.4.2.4 Reverse Fick method

The reverse Fick method is an invasive methodology to measure EE and its application in clinical practice is only limited to selected critically ill patients. The method requires the measurement of the heart ejection volume with a pulmonary catheter using the thermodilution technique. Moreover, the reverse Fick methods demands samples of both arteriosus blood and venous blood, as well as sample of blood in the pulmonary artery to correctly assess VO_2 and VCO_2. One further limit is that RQ is not measured but assumed at a fixed amount.

1.4.3 Indirect calorimetry

Of all the methods developed to measure energy requirements, IC is regarded as the gold standard methodology to assess REE in critically ill pediatric patients [47, 48].

1.4.3.1 Assessment of REE and RQ with IC

The fundamental concept underlying IC is that organism consume oxygen and produce carbon dioxide in order to produce energy. Thus, by measuring VO_2 and VCO_2, we can deduct the energy needed by each patient. Basing on this concept, Weir originally elaborated the Weir equation from the measurement of VO_2, VCO_2 and urinary nitrogen excretion to account for the consumption of protein:

(1) REE (kcal/day) = [3.9 x (VO_2 mL/min) + 1.1 x (VCO_2 mL/min)]x 1.44 -2.17 x UN (urinary nitrogen, g/dL)

However, urinary nitrogen is not readily available in most hospital setting and anyway the production of energy from protein is only limited to 4% of the daily energy produced. Therefore, a modified Weir equation, not accounting for protein consumption is used instead in clinical practice:

(2) REE (kcal/day) = [3.9 x (VO_2 mL/min) + 1.1 x (VCO_2 mL/min)]x 1.44.

The difference between the two equations is however very little and usually less than 2% [49].

In addition to the measurement of REE, IC also provides the respiratory quotient (RQ), which is the ratio between VCO_2 and VO_2 and can provide useful information on substrate utilization in the individual under study.

The physiological values of RQ range between 0.67 and 1.3. Values less than 0.70 may indicate an increased intake of lipids (or ethanol) or increased utilization of lipids due to the lack of carbohydrates substrate. Values superior to 1.0 indicate utilisation of carbohydrates as substrate and are common after meals. Values of 0.84 usually indicate utilisation of both lipids and carbohydrates, denoting a mixed diet [50].

Even though the RQ is a simple and straightforward method to understand substrate utilization, it is important to know that there are many conditions that can influence its value [51, 52]. Specifically, in the critical condition RQ is often considered unreliable. In fact, hypoventilation, acidosis, physical exercise, some drugs, ethanol consumption and overnutrition may increase RQ. On opposite, hyperventilation and alkalosis reduce RQ [52].

Therefore, caution should be used when using RQ as a tool to assess substate utilization in critically ill children.

1.4.3.2 Standard procedure for IC performance

The standard IC should be performed by expert personnel as there are some important conditions that should be met to guarantee the accuracy of the REE measurement. The exam itself consists of a 30 minutes procedure, which allows to obtain measurements of VO_2 and VCO_2 in steady state, that is the fraction of time in which the variation of VO_2 and VCO_2 are minimal and the inspired oxygen fraction

(FiO_2) is stable.

Before starting the exam, it is necessary to calibrate the IC instrument and to complete the basic demographic and anthropometric dedicated section of the machine. Operatively, for an ideal exam the following basic rules for IC should be respected [51]:

- The measurement should be performed in a quiet environment, with the individual in resting condition, preferable from 10-15 minutes before the exam
- The subject should be fasting from at least 5 hours before the exam and should not engage in heavy physical activity in the 5 hours before the exam
- Ideally, the subject should not consume nicotine, caffeine or other stimulating supplements in the 4 hours before the exam
- During the exam the subject should be at rest and not engage in any activity, not even chatting with the operator.

1.4.3.3 IC modalities

The sampling method and the gas analysis is different for the spontaneous breathing patient (canopy mode) compared to the mechanically ventilate (ventilation mode).

In canopy mode, a transparent helmet is placed above the head of the subject. The helmet is connected to the IC through a tube that collects the gas expired by the subject with the aid of an aspiration flow. Inspired O_2 and CO_2 level are measured

at room air. Expired and inspired gases are measured by the IC with specific O_2 and CO_2 cell sensors.

The ventilation mode is a bit more complex since it requires connecting the IC directly to the ventilator flow to sample the inspired gas, and directly to the endotracheal tube to collect he expired gases.

Specifically, there are two ways to measure the gas levels in ventilation mode, breath by breath or mixing chamber. Breath by breath measures gas level in each breath and reports the average value obtained in the final report. Inspired and expired gas must be separated in this methodology and this is very useful for mechanically ventilated patients as it helps reduce the problem of FiO_2 instability. With the mixing chamber methodology, the inspired gas is directed to the chamber and the obtained gases are measured at regular intervals. This system works best if steady state is maintained for most of the exam. Figure 2. shows the different modality of IC sampling and analysis previously discussed [43].

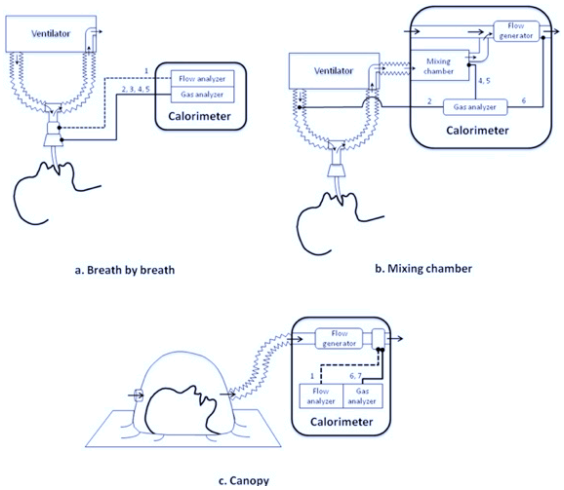

Figure 2. Methods for gas sample collection and analysis (adapted from Oshima et al. [43]).

One final recommendation for the performance of an accurate IC exam regards the prevention of leaks in the IC/canopy or IC/ventilator circuit. In fact, leaking from the circuits may dilute the sample aspired by IC, therefore lowering the accuracy of the analysis. Checking for leaks is especially important when the exam is performed in ventilated patients and should include endotracheal tube, ventilator circuit and tube connection leaks checking. In canopy mode a drape is usually arranged around the perimeter of the helmet, to prevent leaks from the helmet [45].

1.4.4 Mehta equation

One additional method that stands in between from measuring and estimating methods to predict the REE is the Mehta equation, which was recently validated for critically ill children[53]. Briefly, the Mehta equation was derived from the modified Weir equation using VCO_2 data sampled with IC and a fixed RQ of 0.89. The prospective of using VCO_2 data gathered by the ventilator to obtain REE is very appealing for pediatric critical care clinicians. In fact, new generation ventilators that include sensor to monitor VCO_2 are becoming more available in PICUs. Under these premises, the Mehta equation was successively validated by Kerklaan et al. in mechanically ventilated patients with VCO_2 data collected with a ventilator which had an integrated VCO_2 sensor [54].

However, the authors reported that the equation was not sufficiently accurate in children weighting less than 15 kg. Of note, the main reason for the loss of accuracy in the REE prediction in smaller children was the lack of accuracy of the sensor itself, rather than the lack of accuracy of the predictive method.

1.4.5 Methods to estimate EE

Since the current technologies to measure EE are limited by costs, availability and lack of specialized personnel, in clinical practice it is widely accepted that REE can be alternatively assessed using estimation methods instead. Classically, the REE prediction of this formulae/equations is based on parameters that can be easily collected in routine clinical practice and do not require special training of the healthcare personnel. Among all weight, height, age, gender are the most common parameters that are included in the computation of the equations/formulae.

From the beginning of the last century (XX), many predictive equations/formulae have been proposed by researcher and healthcare organisation. Surprisingly the most commonly employed formulae are still the Harris-Benedict equation and the Schofield equation, that have been shared with the public respectively over 100 years ago and over 35 years ago [55, 56]. Other equations/formulae that have been proposed over the years and are employed in pediatric clinical practice, include the FAO/WHO/UNU equation, the Oxford equation and the

Talbot tables [57-59]. In any case, the choice to use prediction equations/formulae to assess REE in children always leaves space to criticism since the equations are notoriously inaccurate, often under- or overestimating energy needs [60]. Indeed, the individuality of each subject is difficult to capture just by relying on basic demographic and anthropometric parameters and this becomes even more evident when these equations are applied for the estimation of REE in critical pediatric patients, whose energy needs are very variable during the course of hospitalization [61].

Over the years, some disease-specific equations have also been developed to estimate REE during critical illness. Yet, these efforts were unsuccessful in capturing the complexity of the metabolic response to acute stress. In a recent study, Chaparro et al. observed that the performance of predictive equations specifically developed for critical illness was below standards in a population of mechanically ventilated children. Interestingly, the authors reported that the estimation of REE using the Talbot tables provides a more accurate estimation of REE in critically ill children, compared to all the other equations considered in the study [62].

In conclusion, the use of predictive equation is not recommendable in critically ill children and the quest to find an alternative methodology for the assessment of REE in critical setting, besides the not often available IC, is still ongoing.

Recently, the use of machine learning has been proposed as

an innovative new way to predict REE. Indeed, the REE prediction based on artificial neural networks (ANN) models has been found to be reliable in healthy children and adults, including obese patients [63, 64]. However, specific data regarding the applicability of this newly proposed methodology on critically ill subjects are still missing. The following paragraph will present an overview on ANN functioning and on its potential application to the REE prediction.

1.4.6 Artificial neural networks functioning and potential role on the assessment of REE

ANN are computerized algorithms that are designed to resemble the interactive processes of the human brain with the purpose to go beyond the traditional 3 or 4 features linear correlation models and allow the study of very complex non-linear phenomena [65]. This fundamental ability of ANN makes them the perfect fit to study the complex kinetic that characterises biological systems. The core elements of ANN models are nodes and connections. Nodes are also called processing elements or artificial neurons. Just as for brain neurons, they receive input from other nodes and from the environment and communicate back their own output to other nodes or to the environment, forming dynamic connections. One fundamental aspect of this learning process is that the connections can change over time, as the models elaborates more input and output. As a result, ANN models

can help with the identification of complex patterns between inputs and outputs, providing an insight of the relationship among the variables in the system. This fundamental characteristic of ANN models makes them a good method to support decision making in the medical field and in many other disciplines. Recent data pointed out that ANN might represent a precise and accurate method to estimate REE in healthy and obese children and adults [63, 64]. However, no study has yet investigated the applicability of ANN for REE prediction in critically ill children.

CHAPTER 2

Aims and hypothesis

The aim of this thesis is to investigate the potential application of ANN algorithms on the topics of nutrition, metabolism and energy requirements of critically ill children.

The main objective is to test the accuracy of ANN for the estimation of REE in a population of critically ill pediatric patients and to compare the accuracy with the other available estimation formulae.

Our hypothesis is that ANN might lead to a more accurate REE estimation compared to the most frequently used estimation formulae and to a better understanding of the energy and metabolic needs of pediatric critical care patients.

CHAPTER 3

Material and methods

3.1 Study population

We enrolled patients consecutively admitted to a 6-bed PICU of a tertiary children's hospital (Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy) in a period of time lasting from 2013 up to 2019. The study was approved by the Ethical Committee of the Policlinico of Milan Hospital and informed consent was obtained.

3.2 Nutritional status and clinical characteristics

A multidisciplinary team completed the nutritional assessment and the anthropometric measurements during the hospital stay. Weight (using a gram scale, accurate to 0.1 kg) and length/height with 417 SECA stadiometer (® SECA Medical Measuring Systems and scales, Birmingham UK) or a flexible but non-stretchable tape measure were recorded.

Body mass index (BMI) was derived (kg/ m^2). Z-scores for weight for age (WFA), BMI for age, Weight for length/height (WFL/WFH) and length/height for age (LFA/HFA) were calculated using the WHO Anthro ® and the WHO Anthro Plus ® software and the WHO reference charts [66, 67]. Stunting (i.e. chronic undernutrition) was diagnosed according to the WHO criteria as HFA < -2. Wasting (i.e. acute undernutrition) was diagnosed according to WHO criteria as WFH z score < -1 (mild), < -2 (moderate) or < -3 (severe) for children aged younger than 5 years and BMI for age z score < -1 (mild), < -2 (moderate) or < -3 (severe) for children older than or equal to 5 years old. Overweight was defined as WFH z score > 2 (for children <5 years), and as BMI for age z score > 1 (for children ≥ 5 years). Obesity was defined as WFH z score > 3 (for children <5 years), and as BMI for age z score > 2 (for children ≥ 5 years). The REE was measured in thermoneutral conditions using an open-circuit IC (Vmax 29®, Sensor Medics, Yorba Linda, CA, USA). VO_2 and VCO_2 were measured in spontaneously breathing (canopy mode) and mechanically ventilated (ventilation mode) children for a period of 30 minutes. Respiratory quotient (RQ) was calculated as VCO_2/VO_2 and REE using the modified Weir formula, not accounting for urinary nitrogen excretion [68]. Steady state conditions were defined as at least 5 minutes with less than 5% variation in RQ, less than 10% variation in VO_2 and in VCO_2 and less than 10% variation in minute ventilation. Data from patients who did not meet steady state

or had a RQ < 0.67 or > 1.3 were excluded. Energy expenditure was estimated using the following predictive equations/formulae: Harris-Benedict, Harris-Benedict for infants, Schofield for weight, Schofield for weight and height, Oxford for weight, Oxford for weight and height, WHO/FAO/UNU, Talbot tables for weight, Talbot tables for height, Mehta equation [55-59].

Clinical characteristics, vital signs (heart rate, blood pressure systolic and diastolic, oxygen saturation-SatO_2 %, respiratory rate and body temperature- °C) and blood values such as haemoglobin (Hb, g/dL), C-reactive protein (CRP, mg/dL), albumin (g/dL), blood glucose (mg/dL) were included in the database. Blood concentrations were measured directly after blood sampling, with methods standardized in the central laboratory of the hospital, when the patient entered the study.

3.3 Modelling of REE with ANN

3.3.1 Data set analysis

A physician expert in ANN analysis conducted the modelling and the data set analysis.

The original data set (Data set 1) consisted of twenty-four variables: Mechanically ventilated, gender (male/female) Ethnic origin (Caucasian, Asian, South American, African), Age, Weight, Height, BMI, BMI Z-score, HFA Z-score, normal weight, overweight, Obese, Wasting (absent, mild, moderate, severe), Stunting, VO_2, VCO_2, RQ. Multivariate analysis was

carried out with supervised ANN, according to the method adopted by Penco et al. [69]. Five different approaches were carried out. First, the analysis was applied to all 24 variables, including the gas values VO_2, VCO_2 and RQ, which are obtained with IC. The reason for developing a model with all variables, gas values included, was purely technical, with the aim of obtaining a "baseline" predictive model developed under the best possible conditions, i.e. with the inclusion of gas exchange monitoring. The system was then tested on four different variants of the data set, the first using a data set with 21 variables, avoiding all gas values (VO_2, VCO_2, RQ), the second including the 21 variables and VO_2, the third including the 21 variables and VCO_2 and the fourth including the 21 variables and RQ. This was done with the purpose to better understand the contribution given by each gas value to the prediction.

In a subgroup of children, it was possible to extend the analysis to include some additional variables. This extended data set (Data set 2) consisted of thirty-two variables, the twenty-four variables mentioned above and eight "functional" inputs: heart rate, blood pressure (systolic and diastolic), $SatO_2$ and body temperature, as well as CRP, Hb and blood glucose. The purpose of including functional inputs was to test the hypothesis that to obtain an accurate estimation of REE during critical state, it may not be sufficient to relay only on basic demographic and anthropometric data. Thus, the inclusion of variables capable of describing

modification in the functional status may help improve the ANN model prediction. As for the original data set, the model was first developed considering all the variables with the scope of obtaining a baseline model. The modelling was then tested on a twenty-nine variables data set, not containing gas values, as well as on a thirty variables data set, including also VCO_2, which can be measured by new generation ventilators or by capnography and therefore may have a clinical relevance.

3.3.2 TWIST (Training with Input Selection and Testing) system

To cut down those variables in the database that are non-relevant for the prediction task, causing the loss of statistical power for inferences, we have employed a special *artificial device* called TWIST, designed ad-hoc to sort out the variables mostly representative for prediction/classification [70]. The TWIST system consists of a combination of other two systems, Training/testing (T&T) and Input Selection (IS), respectively. The T&T system is a robust data re-sampling technique able to rearrange the source sample into further sub-samples, all carrying a similar probability density function. Accordingly, the database is further split into two or more sub-samples with the purpose of checking, testing and validating the ANN models in the most effective way on the basis of available data. The IS system represents an evolutionary 'wrapper' system able to select and minimize

the number of variables while preserving all task-relevant information contained in the data set. The combined action of these two systems enables us to improve the inferential power of the ANN system, while overcoming in parallel a few major technical issues. Both systems are based on an Algorithm, the Genetic Doping Algorithm (GenD) developed at Semeion Research Centre (Rome, Italy) [71].

The TWIST pre-processing may extract the variables mostly meaningful for the prediction/classification task, while simultaneously producing the training and the testing set, respectively, which are extracted from a probability distribution very close to the one proving for the best performance at the task. On these selected variables the functional approximation/prediction task then proceeds by means of a supervised, Multi-Layer Perceptron, with four hidden units. The study sample was then randomly divided into two main sub-samples, one, the training set sub-sample, and, two, the testing sub-sample. After reversing either the training and testing sets a blind prediction was carried out for each record of the data set. The accuracy results were expressed as the average obtained in the two independent testing sets respectively.

3.4 Statistical analysis

The REE value predicted by ANN was compared with the REE measured with IC by univariate linear regression. The mean absolute error (MAE), i.e. the mean of the absolute

difference between the predicted and actual value, and the mean relative error, i.e. the ratio of the MAE of the measurement to the actual measurement, the Pearson coefficient of determination (r^2), and the F-test for two sample analysis of variance, were used to measure the predictive accuracy of ANN, when appropriate. Data are given as mean and standard deviation, absolute or percentile values. Significance was assumed when $p < 0.001$ taking into account the existence of multiple tests. Analyses were performed using SPSS 20.0 (Statistical Package for Social Science. Inc., IL, USA). The same fitting was carried out with all the equations/formulae on study.

CHAPTER 4

Results

4.1 Data set 1

4.1.1 Population characteristics

The whole population of the original data set (Data set 1) consisted of 257 pediatric patients (145 males, 56.4%) of whom 102 (39.5%) were mechanically ventilated. Their characteristics are summarized in Table 2.

Table 2. Anthropometric and metabolic measurements of the study population from Data set 1

	N=257		
Demographic		*Metabolic (indirect calorimetry)*	
Age, years	4.4 (4.8)	VO_2, L/min	0.091 (0.049)
Male, no. (%)	145 (56.4)	VCO_2, L/min	0.071 (0.041)
Anthropometric		RQ	0.77 (0.12)
Weight, kg	15.6 (12.2)	Resting Energy Expenditure, kcal/die	623.3 (325.7)
Height, cm	93.4 (30.5)	*Metabolic (equations/formulae)*	
BMI, kg/m^2	15.9 (3.2)	REE Harris-Benedict equation	824.3 (260.2)
z-score BMI	-0.7 (2.0)	REE Harris-Benedict equation for infants (<12mesi)	964.3 (134.6)
z-score weight for age	-0.9 (1.7)	Schofield (weight) equation	700.9 (347.6)
z-score height for age	-1.2 (1.9)	Schofield (weight and height) equation	703.0 (344.3)
z-score weight for height	-0.6 (2.0)	FAO/WHO/UNU equation	701.4 (353.1)
Outcomes		Oxford (weight) equation	703.1 (335.9)
Mechanically ventilated, no. (%)	102 (39.5)	Oxford (weight and height) equation	705.0 (332.8)
Length of PICU stay, days	10.2 (12.0)	Talbot (weight) equation	650.1 (332.4)
		Talbot (height) equation	675.6 (325.5)
		Mehta equation	564.3 (330.9)
		Mehta equation (ventilated children)	475.6 (257.0)

Data are presented as mean and standard deviation or frequency and percentage.
Abbreviations: BMI= Body Mass Index; PICU= Pediatric Intensive Care

Unit; VO_2= Oxygen Consumption; VCO_2= Carbone Dioxide Production; RQ= Respiratory Quotient; REE= Resting Energy Expenditure; FAO= Food and Agriculture Organization; WHO= World Health Organization; UNU= United Nation University.

4.1.2 Linear correlations

Figure 3 shows the linear correlation values between the study variables and the REE value. As expected, VO_2, VCO_2, height, weight and age were highly correlated with REE. In any case, the absolute value of Pearson R of the other variables is rather low, and this offers a further rationale for the application of ANN especially when avoiding gas values.

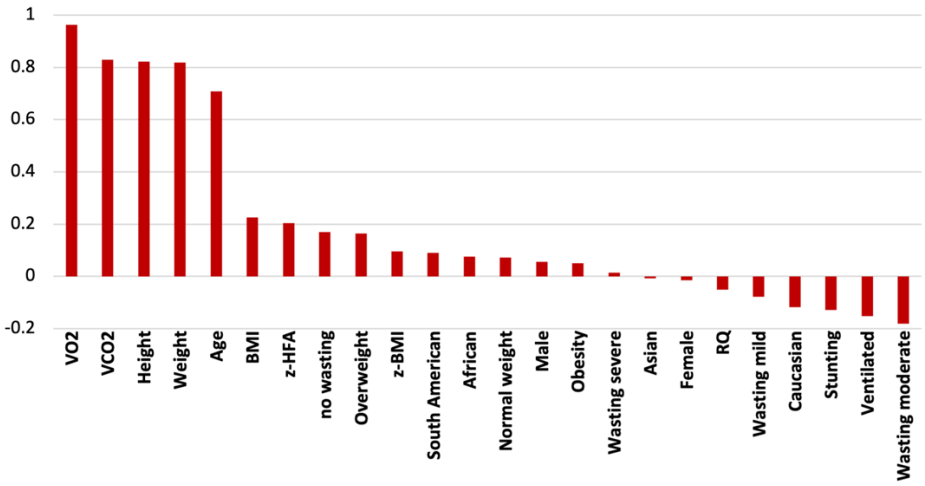

Figure 3. Correlation between the study variables and the REE value (Data set 1).
Abbreviations: VO_2= Oxygen Consumption; VCO_2= Carbone Dioxide Production; RQ= Respiratory Quotient; BMI= Body Mass Index; z-BMI= z-score BMI; z-HFA= z-score Height for Age.

4.1.3 Fitting of REE with the equations

Figure 4 shows the real REE approximation obtained with all the equation/formulae considered in the study. Blue line expresses the true REE values; red line is the corresponding fitting of the method under evaluation and the

dotted line is the tendency line of the method described by polynomial equations (data not shown).

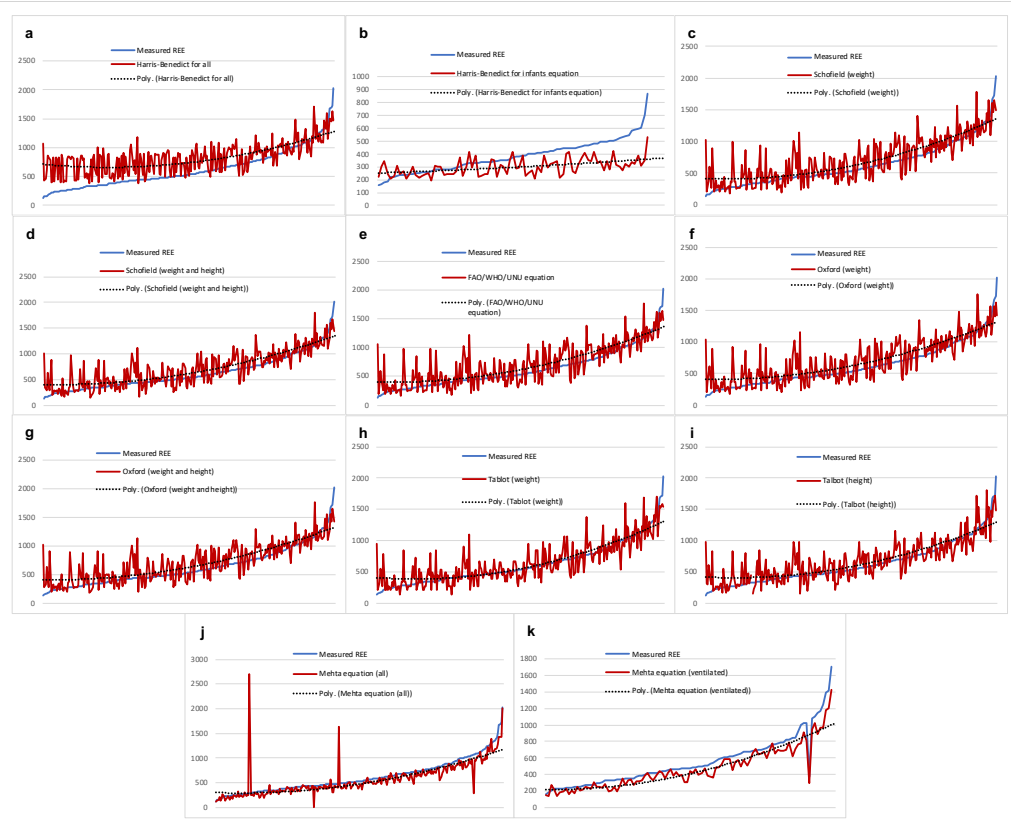

Figure 4. Real REE approximation with predictive equations (Data set 1) Harris-Benedict for all (a), Harris-Benedict for infants (b), Schofield (weight) (c), Schofield (weight and height) (d), WHO/FAO/UNU equation (e), Oxford (weight) (f), Oxford (weight and height) (g), Talbot (weight) (h), Talbot (height) (i), Mehta equation for all subjects (j), Mehta equation in ventilated children (k).

All the equations, except for the Mehta equation appear to systematically overestimate the true REE value and mostly in the left side where true REE reaches the lowest values, especially the Harris Benedict equations (even more with correction <12 months). The contrary is observed at the extreme right of the graphic, where true REE evaluations skip over the estimated values (that in this quite restricted cue result under-estimated).

4.1.4 Fitting of REE with artificial neural networks

4.1.4.1 Baseline analysis (24 variables)

The TWIST® system selected 7 variables carrying the maximal amount of information to build up a predictive model and precisely: height, BMI, gender (male, female), VO_2, VCO_2 and RQ. The final model, based on these seven variables, expressed a functional approximation of the actual REE value within a protocol based on a bipartite division of the data set: training set sub-sample (n= 125) and testing sub-sample (n=132). Training and testing sets were then reversed and consequently for each record of the data set a blind prediction was carried out. Within this approach the neural network tendency line appears to be almost superimposed to the true REE values curve (Figure 5).

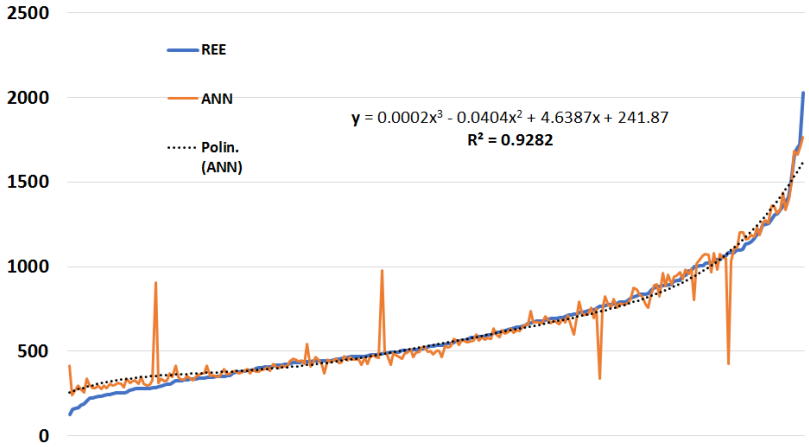

Figure 5. Real REE approximation with neural networks (Data set 1). Blue line expresses the true REE values; orange line is the corresponding fitting of the method under evaluation and the dotted line is the tendency line described by a five-degree polynomial equation.

4.1.5 Comparative statistics between tests on study

The modelling obtained by the ANN reached an average absolute error of 38.06 calories (93.9% accuracy) with a $R^2 = 0.928$. The comparative values obtained with the other equations were worse. The best equation in term of absolute error resulted the Mehta equation (which also requires VCO_2) with an average absolute error of 89.67 calories (84.05% accuracy), followed by the Talbot table for weight with an average absolute error of 141.75 calories (77.17% accuracy). The Harris Benedict equation was the worse option with an average absolute error of 244.16 calorie (60.83% accuracy) (Table 3.)

Table 3. Fitting performances of true REE by methods under study (Data set 1)

Overall group (N=257), measured REE= 623.26 (325.74)									
FITTING METHOD	Predicted REE		Aabsolute error	Accuracy	Relative error	Accuracy	F-Test Two-Sample		
	mean	SD	mean	%	mean	%	F-statistic	P-value (two tails)	Pearson (r^2)
ANN model with gas (baseline)	651.400	329.000	38.060	93.90	0.058	94.16	0.982	0.881	0.928
REE Harris-Benedict equation	824.290	260.227	244.162	60.83	0.611	38.93	1.567	<0.001	0.497
REE Harris-Benedict equation for infants (<12mesi)	299.470	64.510	103.329	72.84	0.254	74.62	3.739	<0.0001	0.288
Schofield (weight) equation	700.868	347.599	164.682	73.58	0.351	64.94	0.878	0.298	0.664
Schofield (weight and height) equation	703.031	344.335	160.821	74.20	0.347	65.25	0.895	0.374	0.671
FAO/WHO/UNU equation	701.444	353.114	168.675	72.94	0.358	64.18	0.851	0.196	0.653
Oxford (weight) equation	703.074	335.851	163.740	73.73	0.352	64.76	0.941	0.624	0.655
Oxford (weight and height) equation	705.000	332.757	158.707	74.54	0.344	65.56	0.958	0.733	0.671
Talbot (weight) equation	650.081	332.385	142.275	77.17	0.300	70.01	0.960	0.746	0.691
Talbot (height) equation	675.642	325.456	147.597	76.32	0.320	67.98	1.002	0.989	0.684
Mehta equation	564.252	330.870	93.280	85.03	0.173	82.71	0.969	0.802	0.685
Mehta equation (ventilated children)	475.560	257.021	89.670	84.05	0.160	84.00	1.380	0.107	0.906

Abbreviations: REE= Resting Energy Expenditure; ANN= Artificial Neural Networks; FAO= Food and Agriculture Organization; WHO= World Health Organization; UNU= United Nation University; SD= standard deviation.

4.1.6 ANN analysis to better appreciate the contribution given by gas values to REE fitting

In this section we show the results obtained with the application of TWIST system to four differential analysis, the first avoiding gas values VO_2, VCO_2, RQ (21 variables), the second avoiding VCO_2 and RQ and maintaining VO_2, the

third avoiding VO_2 and RQ while maintaining VCO_2 and the fourth avoiding VO_2 and VCO_2 while maintaining RQ (22 variables each).

Table 4 shows the summary of the variables selected for the modelling, the predictive results obtained by ANN and the comparison with the results obtained in the original data set.

Table 4. Results obtained in the four data sets relevant to the role of gas values

Data set 1	Original (baseline)	1	2	3	4
Variables number of the data set	24	21	22	22	22
Gas variables	VO_2; VCO_2; RQ	none	VO_2	VCO_2	RQ
Variables selected by TWIST system	Male Female Weight BMI VO_2 VCO_2 RQ	Female Age Weight Height z-score BMI z-score height for age No wasting Wasting mild Wasting moderate Wasting severe Stunting	Weight BMI Obese VO_2	African Weight Height z-score height for age Overweight Wasting severe VCO_2	Mechanically ventilated Male Female Asian Weight Height BMI z-score height for age Normal weight Wasting severe RQ
predictive accuracy	93.90%	75.58%	92.94%	84.44%	77.95%
mean absolute error	38.06	149.1	44.04	96.9	136.8
Person r^2	0.928	0.713	0.914	0.829	0.701

Abbreviation: TWIST system= Training with Input Selection and Testing system; VO_2= Oxygen Consumption; VCO_2= Carbone Dioxide Production; RQ= Respiratory Quotient; BMI= Body Mass Index

4.2 Data set 2

4.2.1 Population characteristics

The purpose of Data set 2 was to provide more functional inputs to the ANN predictive model. Functional parameters were included in the original Data set 1, but not for all subjects. Therefore, the population for Data set 2 was reduced to 199 pediatric patients (112 males, 56.3%) of whom 93 (46.7%) were mechanically ventilated. Patients' characteristics, including vital signs and blood values are

presented in Table 5.

Table 5. Anthropometric, functional and metabolic measurements of the study population from Data set 2

			N=199
Demographic		*Metabolic (indirect calorimetry)*	
Age, years	4.4 (4.7)	VO_2, L/min	0.092 (0.050)
Male, no. (%)	112 (56.3)	VCO_2, L/min	0.069 (0.038)
Anthropometric		RQ	0.75 (0.11)
Weight, kg	16.1 (12.7)	Resting Energy Expenditure, kcal/die	632.3 (339.9)
Height, cm	94.0 (31.1)	*Metabolic (equations/formulae)*	
BMI, kg/m^2	16.1 (3.4)	REE Harris-Benedict equation	833.5 (262.3)
z-score BMI	-0.6 (2.1)	REE Harris-Benedict equation for infants (<12mesi)	718.9 (357.7)
z-score weight for age	-0.9 (1.7)	Schofield (weight) equation	711.5 (353.4)
z-score height for age	-1.1 (1.9)	Schofield (weight and height) equation	712.6 (351.0)
z-score weight for height	-0.6 (2.0)	FAO/WHO/UNU equation	712.4 (358.9)
Outcomes		Oxford (weight) equation	713.1 (340.6)
Mechanically ventilated, no. (%)	93 (46.7)	Oxford (weight and height) equation	714.3 (338.3)
Length of PICU stay, days	10.2 (12.0)	Talbot (weight) equation	661.5 (342.0)
Vital signs		Talbot (height) equation	684.7 (332.8)
Heart rate, bpm	117.6 (30.3)	Mehta equation	552.8 (306.2)
Systolic Blood Pressure, mmHg	103.5 (18.3)	Mehta equation (ventilated children)	463.4 (257.2)
Diastolic Blood Pressure, mmHg	61.0 (14.9)		
Temperature, °C	36.6 (0.7)		
$SatO_2$, %	97.7 (2.7)		
Blood values			
Hemoglobin, mg/dl	9.9 (1.8)		
Blood glucose, mg/dl	106.4 (37.3)		
C-Reactive Protein, mg/dl	5.3 (7.7)		

Data are presented as mean and standard deviation or frequency and percentage.
Abbreviations: BMI= Body Mass Index; PICU= Pediatric Intensive Care Unit; $SatO_2$ %= oxygen saturation; VO_2= Oxygen Consumption; VCO_2= Carbone Dioxide Production; RQ= Respiratory Quotient; REE=Resting Energy Expenditure; FAO= Food and Agriculture Organization; WHO= World Health Organization; UNU= United Nation University.

4.2.2 Linear correlations

The linear correlation values between the study variables and the REE value were very similar to the ones from Data set 1. The gas values (VO_2, VCO_2), height, weight and age were highly correlated with REE. In all other cases, the absolute value of Pearson R of the other variables was low. Figure 6 shows the correlation between the functional

values, which were added in Data set 2 and REE.

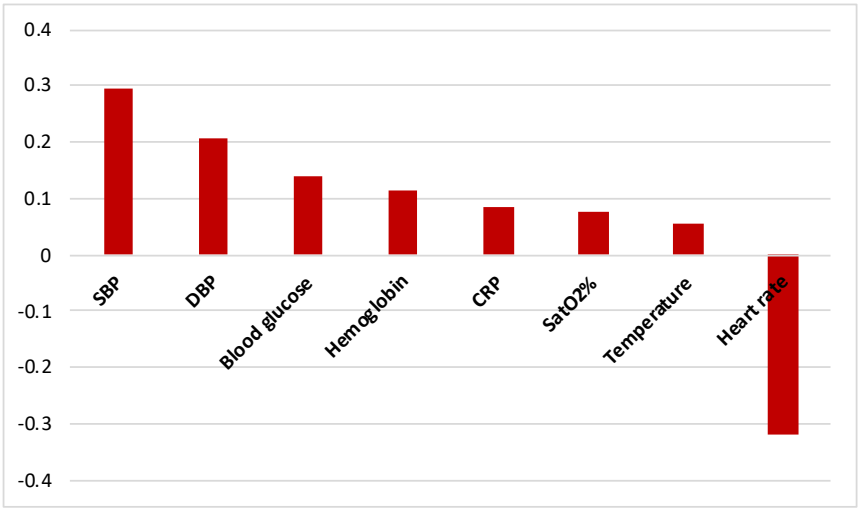

Figure 6. Correlation between the study variables added in Data set 2 and the REE value.
Abbreviations: SBP= Systolic Blood Pressure. DBP= Diastolic Blood Pressure; CRP= C-Reactive Protein; $SatO_2$ %= oxygen saturation.

4.2.3 Fitting of REE with the equations

Figure 7 shows the real REE approximation obtained with all the equation/formulae considered in the study. Blue line expresses the true REE values; red line is the corresponding fitting of the method under evaluation and the dotted line is the tendency line of the method described by polynomial equations (data not shown).

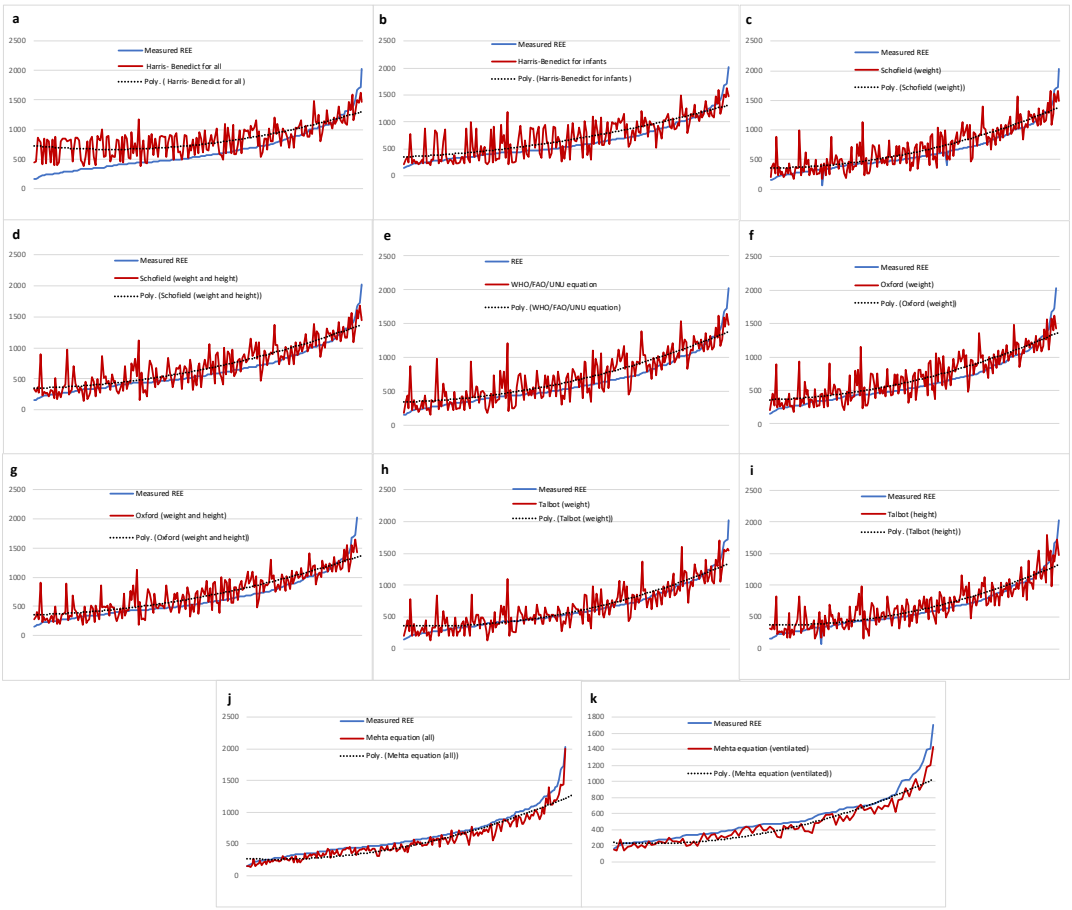

Figure 7. Real REE approximation with predictive equations (Data set 2). Harris-Benedict for all (a), Harris-Benedict for infants (b), Schofield (weight) (c), Schofield (weight and height) (d), WHO/FAO/UNU equation (e), Oxford (weight) (f), Oxford, weight and height) (g), Talbot (weight) (h), Talbot (height) (i), Mehta equation for all subjects (j), Mehta equation in ventilated children (k).

4.2.4 Real REE approximation with artificial neural networks

The inclusion of functional variables in Data set 2 helped improving the prediction of REE by ANN. Figure 8. shows the real REE approximation with ANN from best to worse. The neural network tendency line of the baseline model developed considering also the gas values appears to be almost superimposed to the true REE values curve. The model developed with no gas is the least fitting, while the

VCO_2 models stand somewhere in between the two.

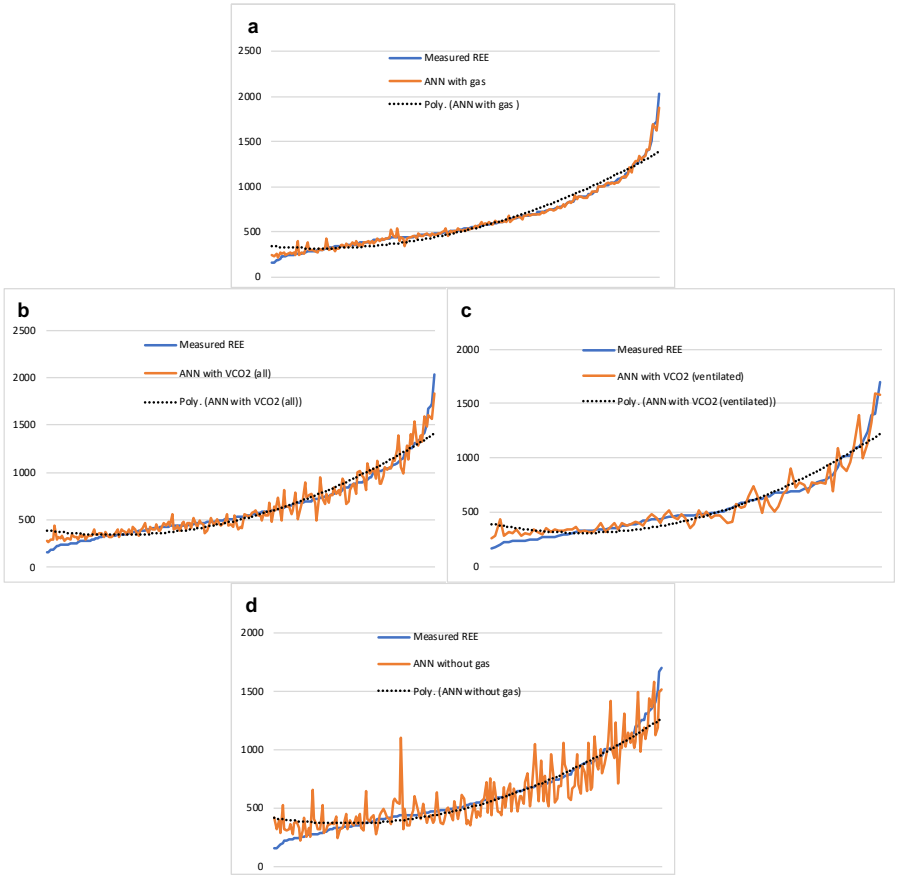

Figure 8. Data set 2 real REE approximation with ANN.
ANN with gas (a), ANN with VCO_2 (all subjects) (b), ANN with VCO_2 (ventilated subjects) (c), ANN without gas (d)

4.2.5 Comparative statistics between all methods on study

All the methods explored for the prediction of REE are displayed in Table 6. As anticipated, the inclusion of functional inputs in Data set 2 provided a clear advantage in terms of performance of the ANN models. The best prediction of REE was obtained with the ANN baseline model (with gas), reaching an average absolute error of 23.29 calories (96.3% accuracy) with a $R^2 = 0.968$. The comparative values obtained with the other fitting methods were worse. The second bests methods for REE estimation in term of

absolute error resulted the ANN model with VCO_2 and the Mehta equation (which also requires VCO_2). The ANN model developed without gas was less fitting, but still better than all the other equations/formulae in the study. The inclusion of functional parameters among the inputs was a decisive improvement for the model. The second next best prediction developed without gas values was confirmed to be the Talbot table for weight, with an average absolute error of 132.74 calories (79.0% accuracy). The Harris Benedict equation was the worse option with an average absolute error of 245.409 calorie (61.2% accuracy).

Table 6. Fitting performances of true REE by methods under study (Data set 2)

Overall group (N=199), measured REE= 632.33 (339.095)									
FITTING METHOD	Predicted REE		Absolute error	Accuracy	Relative error	Accuracy	F-Test Two-Sample		
	mean	SD	mean	%	mean	%	F-statistic	P-value (two tails)	Pearson (r^2)
ANN with gas	630.971	331.334	23.290	96.32	0.050	95.03	1.053	0.718	0.968
ANN with VCO_2	637.302	332.465	65.562	89.63	0.126	87.00	1.046	0.754	0.921
ANN with VCO_2 (ventilated children)	553.119	288.627	66.389	87.96	0.144	85.63	1.101	0.647	0.866
ANN without gas	628.406	312.509	111.743	82.33	0.212	78.76	1.183	0.237	0.808
REE Harris-Benedict equation	833.513	261.628	245.409	61.19	0.603	39.70	1.680	<0.0001	0.529
REE Harris-Benedict equation for infants (<12mesi)	718.888	356.823	182.411	71.15	0.370	62.98	0.903	0.474	0.623
Schofield (weight) equation	711.536	352.519	155.367	75.43	0.310	69.01	0.853	0.265	0.725
Schofield (weight and height) equation	712.630	350.960	151.890	75.98	0.308	69.25	0.938	0.654	0.735
FAO/WHO/UNU equation	712.373	357.990	160.090	74.68	0.317	68.26	0.897	0.446	0.715
Oxford (weight) equation	713.120	339.734	155.013	75.48	0.312	68.75	0.996	0.979	0.722
Oxford (weight and height) equation	714.325	337.474	150.494	76.20	0.306	69.39	1.010	0.946	0.737
Talbot (weight) equation	661.533	341.141	132.739	79.01	0.264	73.59	0.988	0.933	0.751
Talbot (height) equation	681.150	333.430	135.990	78.40	0.274	72.56	0.985	0.913	0.758
Mehta equation	552.822	306.203	83.280	86.83	0.135	86.54	1.233	0.142	0.942
Mehta equation (ventilated children)	463.399	257.225	90.786	83.54	0.165	83.55	1.386	0.647	0.901

Abbreviations: ANN= Artificial Neural Networks; VCO_2= Carbone Dioxide Production; RQ= Respiratory Quotient; REE=Resting Energy Expenditure; FAO= Food and Agriculture Organization; WHO= World Health Organization; UNU= United Nation University.

CHAPTER 5

Discussion

Machine learning may offer a new opportunity to explore the complexity of metabolism and metabolic changes [72]. The use of ANN models to predict REE has been found to be reliable in healthy children and adults, including obese patients [63, 64]. In our research we further investigated the opportunity to employ ANN models to predict REE in critically ill children. We were also interested in understanding what variables our system identifies as important for the REE prediction.

The TWIST system selected VO_2 and VCO_2, RQ, BMI (i.e. the ratio between weight and the square measure of height), weight and gender (female, male) as important variables. The result is consistent with the fact that VO_2 and VCO_2 are the basis to compute the modified Weir equation to obtain REE with IC. Moreover, weight, height and gender are among the variables taken into account to estimate REE by the most commonly employed predictive

equations/formulae, including the Schofield equation, the Harris-Benedict equation, the Talbot tables and many others. The model developed by considering all the gas values (VO_2, VCO_2, RQ) to predict REE was therefore the most accurate and was used as a baseline to understand how accurate ANN can be compared to the modified Weir equation.

To better appreciate the contribution given by each gas value to the REE fitting model we further applied the TWIST system to four variants of the same data set, the first avoiding all gas values VCO_2, VO_2, RQ (21 variables), the second including only VO_2, the third including only VCO_2 and the fourth maintaining only RQ (22 variables each).

When removing VO_2, VCO_2 and RQ altogether, the accuracy of the REE predictive model was similar to most predictive equations/formulae. The Talbot table for weight prediction was more accurate than the model when accuracy was based on the absolute error. The Harris-Benedict equation was the least accurate of all. The accuracy of the model did not considerably improve when RQ was included in the data set analysis.

Instead, the inclusion of either VO_2 or VCO_2 in the data set significantly improved the accuracy of the REE predictive model. In both cases, the accuracy was superior to all the predictive equations/formulae considered for the study.

More interestingly, the model developed including VO_2 was not only superior to the one developed by including VCO_2, but almost as good as the one developed considering all gas

exchange variables (baseline). This finding may be relevant in a more theoretical/physiological perspective, as it may indicate that oxygen consumption is more relevant than carbon dioxide production in defining REE. However, a VO_2 based predictive model would not be useful in clinical practice, since VO_2 is not commonly measured in hospital setting, unless IC is performed.

On the other hand, a VCO_2 based predictive model would be valuable in the critical care setting, as VCO_2 may be monitored in ventilated patients using capnography or even better, using new generation ventilators, which includes VCO_2 monitoring among their functions. The opportunity to use VCO_2 in a predictive algorithm has already been explored by Mehta et al. and Kerklaan et al., who respectively developed and validated a VCO_2 only predictive equation (the Mehta equation) [53, 54]. Of note, in this analysis the accuracy of the VCO_2 predictive model was equal to the accuracy of the Mehta equation.

Taken together, the results discussed so far would not support a call for change in the clinical practice for the assessment of REE in PICU. Evidently, basic demographic and anthropometric parameters alone do not provide sufficient information to allow an accurate prediction of REE with machine learning. Such result is different from what was found for healthy and obese children and proves how far more complex metabolic monitoring is during acute illness, as compared to the physiological status [63].

This final remark leads to the hypothesis that the models developed by ANN would improve if it were possible to include variables capturing the changes from physiology to acute illness. For this reason, in a smaller subset of patients, we were able to include data regarding vital signs and a few blood values in the analysis. As expected, the inclusion of heart rate, blood pressure (systolic and diastolic), $SatO_2$ and body temperature, as well as CRP, Hb and blood glucose, improved the accuracy of the prediction.

Similarly to the original analysis, we applied the TWIST system on different combinations of the same data set, but this time the results were more compelling. All the models developed were superior to the predictive equations/formulae considered in the study. As expected, the model developed including all gas values (baseline model) was the most accurate. The model developed without gas values was less accurate, but still gained a good accuracy for clinical practice. Finally, the most interesting finding was the very good accuracy reached by the VCO_2 model (close to ninety percent). The model was more accurate than the Mehta equation and may represent a refinement of REE prediction based on VCO_2. In any case, this finding needs to be confirmed in clinical practice by testing the model on VCO_2 values actually measured with capnography and/or by ventilators.

Overall, the results of the second analysis confirm the hypothesis that the performance of ANN models on REE

prediction may improve when variables representing changes in body functions are included in the data set.

Our research demonstrates that machine learning overcomes the classic 3 or 4 features linear combination predictive models (on which REE predictive equation/formulae are based) and obtains a more accurate estimation of REE, by improving the number of inputs considered in the model prediction.

Provided that IC remains the preferred and gold standard method to assess REE during critical illness, predicting REE with ANN models represents a better alternative to the common REE estimating equations when IC is not available in the PICU setting.

The current study has some limitations. Since the data were analysed post-hoc, we were unable to recover some variables that could have added useful information to our model. For instance, we did not have a recorded severity of illness score (*e.g.* Pediatric Risk of mortality Index II, PIM2). Moreover, we had insufficient data to assess the effects of sedation, analgesia, vasoactive drugs or other pharmacological therapies on patients. Finally, even though blood test values and vital signs were collected in the database, many data were missing. Therefore, when we decided which variables to add for the second analysis, we chose to include all vital signs except for respiratory rate and only CRP, Hb and blood glucose, among the blood values, because this combination was the best compromise between the wish to add more

"functional" inputs to the model and the need to maintain a reasonable number of subjects for the analysis.

Despite these limitations, this is the first study to develop a REE predictive model with ANN for critically ill children and to offer an insight on the potentials of machine learning to provide a valid and accurate REE prediction to clinicians.

CHAPTER 6

Conclusions

The delivery of optimal nutrition to critically ill children is very important, yet difficult to obtain. Assessing patients' energy requirements represents the starting point to prevent risks of undernutrition or overnutrition, which are associated with negative outcomes (i.e. longer duration of PICU recovery). Machine learning represents an easy to obtain, cost effective solution to predict REE with good accuracy.

Prospectively, the application of the VCO_2 model could be tested on patients ventilated with new generation ventilators, which include VCO_2 monitoring, and also in patients monitored with capnography. However, we also believe that more research should be done on the topic of machine learning, potentially exploring the role of more variables, as they may add relevant information and lead to the development of an even better model for REE prediction.

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