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Department of Clinical Sciences and Community Health

Laboratory of Medical Statistics, Biometry and Epidemiology "G.A. Maccacaro"

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NUTRITION IN FRAGILE LIFE PHASES:

FERTILITY, PREGNANCY AND HOSPITALIZED CHILDREN

PhD Student: Valentina De Cosmi

Matr n: R11246

PhD Tutor: Prof. Fabio **Parazzini**

PhD Coordinator: Prof. Carlo **La Vecchia**

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ABSTRACT

This thesis deals with nutrition and diet, during the most fragile phases of life: fertility, pregnancy and hospitalized children. The duplex aim of this thesis is to investigate the dietary habits of subfertile couples attempting a pregnancy and the effects of bad nutrition on growth and metabolism in hospitalized children. It is divided into theoretical and experimental parts. The theoretical part studies firstly, the determinants of reproductive factors that influence male and female fertility and, secondly, the importance of maintaining a good nutritional status during hospital recovery in a pediatric population. The topic approached in this thesis belongs to a public health field since delivering and guarantee a good nutritional state in all ages of life is known to be crucial in the prevention of both under and overnutrition, which have both negative effects on long-term health.

In the first experimental part, three studies that investigate data from a cohort of subfertile couples, presenting to the Infertility Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, are described. The aim is to analyze dietary and lifestyle habits of both males and females that may affect fertility. Dietary habits have been collected with the use of a previously validated Food Frequency Questionnaire. The results showed that a moderate alcohol intake appears associated with better semen quality in the male sample. While there is evidence to support that alcohol does have an impact on fertility, it is also difficult to establish a definitive link as there is no standard "drink" or comparative way to measure alcohol consumption. We could not analyze the role of heavy or binge drinking, which are consistently associated to detrimental effects on semen quality. Regarding female fertility, in literature there is evidence of a lower risk of in vitro fertilization failure in women reporting higher adherence to Mediterranean Diet. The analysis here described does not show a statistically significant effect of Mediterranean Diet on oocyte quality and success rate after assisted reproductive techniques.

In the second experimental part, The Italian Pediatric Nutrition Survey which analyzed data from many Italian pediatric hospitals is presented, and metabolic data of children recovered in the Pediatric Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan are discussed. The aim of this this second part is to show how much pediatric malnutrition is still unrecognized in hospital setting, where, an accurate nutritional and metabolic evaluation should be recommended. Strong evidences suggest that nutrition modulates the capacity to exit from the state of stress and disease, with possible repercussions on growth and development and nutrition imbalances may affect the prognosis during hospital stay. In the national survey a high prevalence of both acute and chronic malnutrition among hospitalized pediatric patients in Italy emerges, especially in infants and young children and nutritional support is only given to a small number of the malnourished children. In disease condition, the metabolic response to stress is highly variable and cannot be easily predicted, consequently it is difficult to predict the right amount of calories and nutrients that a child needs starting from the calculation of the resting energy expenditure. In this thesis was demonstrated that the commonly employed equations, WHO, Harris-Benedict, Schofield, and Oxford formulae should not be used to estimate metabolism in hospitalized children. Feeding strategies based on these equations might result in unintended underfeeding or overfeeding. The development and validation of more accurate equations will be an initial step in precipitating a culture shift which places greater emphasis on the importance of nutritional delivery as a therapeutic intervention, rather than supportive care.

RIASSUNTO

La mia tesi si occupa della nutrizione durante le fasi più delicate e fragili della vita: il mantenimento della fertilità, la gravidanza e l'età pediatrica. Il lavoro ha un duplice scopo: lo studio delle abitudini alimentari di coppie infertili e degli effetti di una nutrizione scorretta sulla crescita e sul metabolismo del bambino ospedalizzato e si articola in due sezioni: teorica e sperimentale. La parte teorica studia, in primo luogo, i fattori che influenzano la fertilità maschile e femminile e, in secondo luogo, l'importanza di mantenere un buon stato nutrizionale durante il ricovero ospedaliero in una popolazione di età pediatrica. Gli argomenti affrontati in questa tesi ricoprono un ruolo importante di salute pubblica, poiché fornire e garantire un buono stato nutrizionale in tutte le epoche della vita, a partire dalla nascita, è noto per essere cruciale nella prevenzione degli effetti negativi sulla salute a lungo termine a cui sia l'alimentazione per difetto, che per eccesso, conducono.

Nella prima parte sperimentale sono descritti i risultati di tre studi che analizzano i dati di una coorte di coppie subfertili afferenti all'Ambulatorio di Procreazione Medicalmente Assistita della Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico di Milano, con lo scopo di valutarne le abitudini alimentari e lo stile di vita. I risultati hanno mostrato che un moderato consumo di alcol sembra essere associato a una migliore qualità del liquido seminale nel campione maschile. Sebbene vi siano prove a supporto del fatto che l'alcol abbia un impatto sulla fertilità, è molto difficile stabilire un legame definitivo, soprattutto con un consumo moderato. Per quanto riguarda la fertilità femminile, in letteratura vi è evidenza di un minor rischio di fallimento della fecondazione in vitro nelle donne che riferiscono una maggiore aderenza alla dieta mediterranea. L'analisi qui descritta non mostra un effetto statisticamente significativo della dieta mediterranea sulla qualità degli ovociti e sulla percentuale di successo dopo le tecniche di fecondazione assistita.

La seconda parte del lavoro è dedicata allo studio dello stato nutrizionale di bambini

ospedalizzati, del loro metabolismo basale e delle equazioni esistenti per predirlo. Lo scopo è mettere in luce come la malnutrizione ospedaliera sia ancora un problema non riconosciuto e come un'accurata valutazione dello stato metabolico e nutrizionale possa essere d'aiuto nel migliorare la prognosi delle malattie. Nell'indagine "Italian Pediatric Nutrition Survey" a cui la Clinica Pediatrica De Marchi della Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano ha partecipato, il team multidisciplinare medico e nutrizionale di ciascuna struttura coinvolta ha valutato lo stato di nutrizione di ciascun paziente, ed è emerso come vi sia un'elevata prevalenza di malnutrizione sia acuta che cronica. Nelle condizioni di malattia, la risposta metabolica allo stress è altamente variabile e non può essere facilmente prevista, di conseguenza una corretta valutazione dei fabbisogni è complessa ed è difficile prevedere la giusta quantità di calorie e nutrienti di cui un bambino ha bisogno a partire dal calcolo del dispendio energetico a riposo. Il mio lavoro dimostra che le equazioni comunemente impiegate per la stima del dispendio energetico a riposo (le formule WHO, Harris-Benedict, Schofield e Oxford) non dovrebbero essere utilizzate per stimare il metabolismo nei bambini ospedalizzati poiché sovra o sottostimano il reale valore del dispendio energetico a riposo. Definire l'intervento nutrizionale più adeguato per il paziente, permette di gestire al meglio il ricovero e di evitare eventuali ricadute della patologia. Saranno necessari nuovi e ulteriori studi per testare se delle equazioni popolazione-specifiche saranno in grado di stimare in maniera più accurata il dispendio energetico di base. I risultati saranno orientati al miglioramento della gestione nutrizionale del paziente pediatrico nel contesto ospedaliero e potranno essere un punto di partenza per la creazione di nuovi standard di assunzione di energia nella popolazione pediatrica.

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CHAPTER 1

Introduction and Rationale

The overarching aim of this thesis is to provide a critical overview of the role of nutrition in different phases of life. On one side, nutrition during pregnancy and on the other side nutrition in children, in hospital and community setting. Nutrition during pregnancy and in early ages of life have the primary role of planning the future individual, the secondary, later on, to maintain an optimal health status and of modulating the capacity to exit from the state of stress and disease, with possible repercussions on growth and development. The role of optimizing nutritional management in health condition and disease is well known and ask for a multidisciplinary team able to take into account all the factors that influence it. Good nutrition in hospital setting helps to improve the outcomes both of children and adults.

The first theme of my work is the role of nutrition in periconceptional period and during pregnancy, where infertility is taken as a model. Unbalanced diets affect adult fertility, in particular some groups of nutrients influence it. I describe an epidemiological investigation of infertility, including analytical research of the prevalence and determinants of it. The work concentrates on the role of alcohol consumption on male fertility and on the adherence to Mediterranean Diet on female fertility. Maintenance of normal body mass may be effective in the prevention of infertility resulting from ovulatory disorders. Underweight and, to a larger degree, overweight and/or obesity, are related to the enhanced risk of infertility. Insulin resistance is an important pathogenic mechanism that may impair ovulation. Adequate intake of monounsaturated fatty acids, derived mainly from vegetable fats, as well as avoidance of trans isomers of unsaturated fatty acids which are present in industrially products, like sweets, crisps, fast-foods, powdered soups and margarines, may be effective in the prevention of infertility in females. In males, higher consumption of fish, chicken,

fruit, vegetables, legumes and whole grains is significantly and positively associated with progressive sperm motility and other semen quality parameters.

The second theme on which the thesis focuses, is the pediatric malnutrition in hospital and Resting Energy Expenditure (REE) as one of the main actor involved in the regulation of the response to stress. There are lots of equations to predict REE in children, but many of them are not accurate to predict metabolism in disease condition. The work will concentrate on the reliability of these equations and on the nutritional status of the populations studied. There is evidence for the need to measure energy expenditure accurately, in order to track the dynamic energy needs of the ill patients, instead of prescribing nutritional needs according to static predictive equations. In children, few studies have investigated the validity of predictive equations versus indirect calorimetry and all concluded that the equations are less reliable. Recently, questioning whether indirect calorimetry is a necessity or a luxury in a Pediatric Intensive Care Unit (PICU) led to the conclusion that more than 72% of patients would derive benefit from the measurement.

Nutrition, from preconception to adulthood, encompasses all of these factors and has the potential to positively or negatively shape the individual or population health trajectories and their intergenerational differences.

CHAPTER 2

Aims and Objectives

The two aims of the thesis are as follows:

- To review the literature surrounding the definition and determinants of infertility, with particular focus on reproductive risk factors. To study the dietary factors that influence male and female fertility in a cohort of subfertile couples.
- To investigate the prevalence of malnutrition in pediatric hospital setting and to evaluate the accuracy of commonly employed REE prediction formulae versus indirect calorimetry.

Aim 1: Specific Objectives

- To study the dietary factors that influence male fertility in a cohort of subfertile couples, presenting for evaluation to the Infertility Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan.
- To study the relation between alcohol intake and semen variables in male subject of this cohort.
- To study the dietary factors, like coffee intake, alcohol consumption, and lifestyle habits that influence female fertility.

Aim 2: Specific Objectives

- To investigate the prevalence of malnutrition and related nutritional support among hospitalized children in Italy.
- To study the role and the accuracy of prediction formulae for the assessment of resting energy expenditure in hospitalized children with acute and chronic disease.

- To study the child’s metabolic needs during acute illness

CHAPTER 3

Literature review on diet and pregnancy

3.1 Investigation of early life and reproductive risk factors

Infertility can be considered a characteristic of a couple, with female or male factors implicated, or in some cases, both. Infertility is considered a major public health problem, since it is associated with significant medical, social, economic and demographic consequences. The economic costs of infertility are characterized by the financial costs to individuals and the health services in terms of medical investigations and treatment, and also the costs of complications resulting from such treatment and the resulting births. There are also considerable psychological and social implications of both infertility and infertility-related services and treatment. Infertility remains an issue of great interest, with constant media reports alerting the public about rising infertility. Multiple factors affect estimates of prevalence, including methodological issues such as definitions used, trends towards delayed child bearing, differing patterns of help-seeking behavior, and the increased use of medical treatment to aid fertility. Despite the relatively high proportion of couples who experience fertility problems, estimates suggest that true unresolved infertility (sterility) is a rare outcome. In Europe, the prevalence of infertility has been estimated at around 14% [1].

Some risk factors for infertility such as age and lifestyle factors have been the topic of considerable epidemiological research. However, other risk factors have received little investigation. For example, little is known about the role of early life factors, an association which deserves more research given the epidemiological popularity of the `fetal origins hypothesis' linking early life factors, particularly markers of in utero growth, to various health outcomes in adulthood.

3.2 Impacts of diet on reproductive health

Diet has been recognized as an important factor influencing fetal and maternal health. Reproductive health is influenced by certain vitamins and food groups rather than others. Adequate levels of substances such as homocysteine, folate and vitamin B_{12} have been associated with a higher rate of success in infertility treatments. Few data, however, are available on the average levels of micronutrients in the blood of reproductive-aged women [2].

Aspects of a male's diet may have an impact on his fertility. Consuming a diet rich in carbohydrates, fiber, folate, and lycopene [3], fruit and vegetables [4] correlates with improved semen quality. Consuming lower amounts of both proteins and fats were more beneficial for fertility [3]. Antioxidants are another potential benefit since they scavenge reactive oxygen species (ROS). Oxidative stress can result both in sperm protein, lipid and DNA damage and sperm dysfunction [5]. A high amount of antioxidants has been demonstrated to increase semen quality, compared to low or moderate amounts [6]. Vitamin E and selenium decreased levels of malondialdehyde (MDA), a marker for damage done by ROS [7]. This result was confirmed by another study that report that Vitamin E decreased MDA levels, increased spermatozoa motility, and led to 21% couples conceiving over a 2.5 year period versus no conceptions in men who took a placebo (n = 52) [8]. Antioxidants were also associated with a significant increase in pregnancy rate (Odds Ratio-OR 4.18; Confidence Interval-CI 2.65-6.59; P <0.00001; n = 964) [9].

Moreover, a woman's diet may affect fertility too, in particular ovulation. Replacing carbohydrates with animal protein was demonstrated to be detrimental to ovulatory fertility (OR 1.18) [22]. On the contrary, when carbohydrates are replaced with vegetable protein there is a protective effect (OR 0.5) [10]. Trans fats in the diet drastically increase the risk of ovulatory infertility (Relative Risk-RR 2.31) [11]. The use of multivitamins also has an effect: women who take multivitamins may be less likely to experience ovulatory infertility [12]. Chavarro et al. found that women with

high “fertility diet” scores, who followed a diet with a higher mono-unsaturated to trans-fat ratio, major consumption of vegetable over animal protein, high-fat over low-fat dairy, a decreased glycemic load, and an increased intake of iron and multivitamins had lower rates of infertility due to ovulation disorders ($p < 0.001$) [13].

3.2.1 Obesity

An individual’s weight is considered a marker of a person’s eating habits and amount of activity. Body weight can have significant outcomes on global health, including cardiovascular disease, diabetes and male and female infertility [14]. The obesity epidemic has recently become is a serious issue, particularly in industrialized nations where adult obesity increased to 35.7% in 2010 [15]. The reasons of this increasing may be due to energy-rich diets and insufficient physical exercise [16]. Obese men are three times more likely to be infertile than men of a normal weight [17]. Many studies demonstrated that an increase in Body Mass Index (BMI) is correlated with a decrease in sperm concentration [18, 19], a decrease in motility [20] and increased DNA damage in sperm [21,22].

A relationship also exists between obesity and erectile dysfunction (ED). Corona et al. reported that 96.5% of men with metabolic syndrome presented with ED [23]. The enzyme aromatase is responsible for the conversion of androgens to estradiol and it is found primarily in adipose tissue [24]. ED may be the consequence of this conversion: as the amount of adipose tissue increases, there is more aromatase available to convert androgens, and serum estradiol levels increase [21,24]. Inhibin B and leptin may also be affected by obesity. Inhibin B levels have been reported to decrease with increasing weight, which results in decreased Sertoli cells and consequently sperm production [25]. Leptin is a hormone involved in numerous functions, like appetite control, inflammation, and insulin secretion [26]. Leptin resistance could play a role in male infertility [26].

A study conducted in mice demonstrated that leptin was nearly five times higher in obese mice than lean mice, and that the higher leptin levels corresponded to five times lower fertility in the obese mice [26]. In a systematic review, Boots & Stephenson reported a miscarriage rate of 10.7% in women with a normal BMI, versus 13.6% in obese women (OR: 1.31; 95% CI 1.18-1.46) [27]. Bellver et al. found a negative correlation between increasing BMI and implantation [28]. A decreased ongoing pregnancy rate of 38.3% per cycle was also found in women who were overweight in comparison to the 45.5% in non-overweight women (n = 2656) [28]. There is speculation that these negative outcomes may be related to follicular environment, which differs in women who are obese. The negative effects of obesity on fertility in women may be reversible and after losing an average of 10.2 kg, 90% of obese previously anovulatory women began ovulating [29].

3.2.2 Underweight

Women and men who are underweight are also at risk of infertility. Underweight men tend to have lower sperm concentrations than those who are at a normal BMI [21]. The majority of the available studies focus on the impact of obesity. For this reason, more research is needed to investigate the effects that being underweight may have on male fertility. For women, having extremely low amounts of body fat are associated with ovarian dysfunction and infertility [30]. The risk of ovulatory infertility increases in women with a BMI below 17 (RR 1.6) [31]. A meta-analysis of 78 studies, which included 1025794 women, found that underweight women had an increased risk of pre-term birth (RR 1.29) [32]. Eating disorders can negatively affect menstruation, fertility, and maternal and fetal well-being [33]. It was found that among infertile women suffering from amenorrhea or oligomenorrhea due to eating disorders, 58% had menstrual irregularities [33]. Freizinger et al. reported 20.7% of infertile women seeking intra uterine insemination had been diagnosed with an eating disorder, suggesting that women with history of eating disorders may be at

a higher risk for infertility [34].

3.3 Impact of exercise on reproductive health

A healthy amount of exercise both in men and women can be beneficial for fertility. Men who exercised at least three times a week for one hour typically scored higher in almost all sperm parameters in comparison to men who participated in more frequent and rigorous exercise [35]. Studies in obese male rats showed that diet combined with exercise increase sperm motility (1.2 times) and sperm morphology (1.1 times), and decrease sperm DNA damage (1.5 times) and ROS (1.1 times) [36].

In obese women moderate physical activity, combined with weight loss, confer a protective effect on fertility [29]. Excessive exercise, when energy demand exceeds dietary energy intake, negatively alter energy balance and may result in hypothalamic dysfunction and alterations in gonadotropin-releasing hormone (GnRH) pulsatility, leading to menstrual abnormalities, negatively affecting the reproductive system [37]. An OR of 3.5 for infertility was found in women who exercised every day (n = 24,837) [38]. A study examining 2232 women undergoing in vitro fertilization (IVF) found that women who engaged in cardiovascular exercise for 4 hours or more per week for as little as one year prior to the treatment had a 40% decrease in live birth rate (OR .6; 95% CI .4-.8), as well as higher risks of cycle cancellation (OR 2.8; 95% CI 1.5-5.3) and implantation failure (OR 2.0; 95% CI 1.4-3.1) [39].

3.4 Recreational activities

3.4.1 Cigarette smoking

35% of reproductive-aged males and 30% of women smoke [40]. While it is well documented that cigarette smoke is associated with a number of potential health complications such as

cardiovascular disease, more research is needed to establish a link to infertility. Reduced total sperm count, density [41], motility [42,43], altered morphology [41,43], semen volume [41], and fertilizing capacity [44] are reported in men who smoke. Men who smoke before or during attempts to conceive, decrease their fertility (OR 1.6) in comparison to non-smokers [45]. Calogero et al. concluded from their study that smoking could reduce the mitochondrial activity in spermatozoa, and lead to a decreased fertilization capacity [42]. Smoking can increase DNA damage, too [42,49-52]. Anomal endocrine function have been reported in smokers, with an increase in serum levels of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and decreases in testosterone [29]. The same happens in smokers-women. The reductions in fertility among them may be due to decreases in ovarian function and a reduced ovarian reserve. Sharara et al. found that the incidence of reduced ovarian reserve was significantly higher in women who smoked than in age-matched non-smokers (12.31% and 4.83%, respectively), and that these women had similar fertilization and pregnancy rates [53].

3.4.2 Alcohol

Many studies have been conducted on the effects of alcohol and aspects of health, including fertility. However, evidence is not always consistent. A negative association between alcohol intake and semen quality has been suggested by some authors [54, 55], although other studies did not confirm this finding [56, 57]. According to a recent meta-analysis of 15 cross-sectional studies, occasional consumption does not adversely affect semen variables, whereas a negative association with semen volume and normal morphology emerged for daily consumption [58]. However, these findings could not be controlled for confounders such as smoking and age. Women who drink large amounts of alcohol have a higher chance of experiencing an infertility examination than moderate drinkers (RR = 1.59, CI 1.09 –2.31) in comparison to those who consumed low amounts, who had a decreased chance of experiencing an infertility examination (RR 0.64; CI 0.46-0.90) (n = 7393)

[60]. A common result of drinking is a hangover. Women who experienced hangovers were more likely to be infertile than women who did not experience hangovers [61], suggesting that the amount of alcohol consumed does matter. While it is clear alcohol can have an impact, the amount it takes to negatively influence reproductive function is not clear as there is no standard "drink". Amounts of alcohol ranging from one drink a week to 5 units a day can have various effects including increasing the time to pregnancy (P = 0.04; 95% CI .85-1.10), decreasing probability of conception rate by over 50% [62] and decreasing implantation rate, increasing both the risk of spontaneous abortion (OR 4.84) [63] and of fetal death [64], and causing anovulation, luteal phase dysfunction, and abnormal blastocyst development [65]. Researchers believe that these effects may be due to hormonal fluctuations including increases in estrogen levels, which reduce FSH and suppress both folliculogenesis and ovulation [60,65], but many mechanisms are still unknown.

3.4.3 Caffeine

Caffeine has been reported to have negative effects on female fertility. The effects that are emphasized in recent research are miscarriage, spontaneous abortion, fetal death and still birth. Women who consumed more than 100 mg of caffeine a day were more likely to experience a miscarriage. There may be a narrow window for caffeine to impact fertility: consuming more than 375 mg of caffeine a day rise the OR for spontaneous abortion versus a consumption of 200 mg a day [63].

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CHAPTER 4

Literature review on malnutrition

4.1 General definition

Malnutrition is the imbalance between the supply of nutrients and energy and the body's demand for these to ensure growth, maintenance and specific functions. Malnutrition means "poor nutrition" and it occurs when person's diet does not contain the right amount and the right quality of nutrients. It is a continuum that starts with a nutrient intake inadequate to meet physiological requirements, followed by metabolic alterations and consequently by impairment of body composition [1]. The term "malnutrition" is commonly used as an alternative to undernutrition but technically it also refers to overnutrition. Undernutrition is an imbalance between nutrient requirement and intake, resulting in deficits of energy, proteins or micronutrients; on the contrary in an overnutrition condition the intake of nutrients is oversupplied. These conditions may occur both in healthy and hospitalized people, and in this case, malnutrition affects also the length of stay and the recovery.

4.1.1 Causes of malnutrition

The causes of malnutrition are complex and multifactorial. Malnutrition is frequently caused by medical as well as non-medical social factors as social isolation, inability to gain and/or prepare adequate food, as well as poverty. Some causes of malnutrition are associated with environmental, economic, and sociopolitical factor. Food insecurity, defective maternal and child caring practices, unsafe water, poor sanitation, and inadequate health services are main causes in low income Countries, directly influenced by causes such as limited education, poverty and marginalization.

Food security is related to a complex interaction of factors that include agricultural and food production policies, regulation of food marketing and advertisement, and food subsidies.

In developed countries, the main cause of malnutrition is disease: response to trauma, infection or inflammation may alter metabolism, appetite, absorption, or assimilation of nutrients. In addition, underlying acute disease (e.g., sepsis, trauma, burn and cancer), chronic illness (e.g., inflammatory bowel disease, congenital heart disease, cystic fibrosis and severe neuromuscular impairment) or medications, may increase the imbalance between substrate supply and demands. Mechanical obstructions in the gastrointestinal tract may lead to reduced food intake by causing nausea or vomiting, pain or discomfort induced by the passage of food. Drug-related side effects: (e.g. chemotherapy, morphine derivatives, antibiotics, sedatives, neuroleptics, digoxin, anti-histamines, captopril, etc.) can cause anorexia or interfere with the ingestion of food [2]. The reasons for developing malnutrition in sickness are multifactorial, but decreased nutritional intake, increased energy and protein requirements, increased losses together with inflammation probably play the central role. It is demonstrated that in inhospitable patients undiagnosed malnutrition (both over and under-nutrition) prolong hospital stay, and worse comorbidities, leading to functional impairment and frailty, finally affecting quality of life and survival [3,4]. A number of screening tests have been developed for the assessment of undernutrition (e.g., the Mini-Nutritional Assessment (MNA) [5], the Malnutrition Universal Screening Tool (MUST) [6], the Nutritional Risk Screening (NRS) [7], the Subjective Global Assessment (SGA) [8].

4.2 Malnutrition in infancy and childhood

Children are more vulnerable to malnutrition due to the higher amount of energy required for growth and development, and their limited energy reserves. Growth depends on a permanent increase in fat and lean body mass, which requires positive energy and nitrogen balance. In infancy

malnutrition is associated with reduced or delayed mental and psychomotor development [9]. Prolonged nutritional imbalance induces growth retardation. Illness-related malnutrition in children may be attributed to nutrient loss, increased energy expenditure, decreased nutrient intake, or altered nutrient utilization. These factors are seen frequently in relation to acute illnesses as well as chronic diseases such as cystic fibrosis, chronic kidney disease, malignancies, congenital heart disease (CHD), gastrointestinal (GI) diseases, and neuromuscular diseases. In addition to the anthropometric changes in acute malnutrition, chronic malnutrition may be characterized by stunting (decreased height velocity). Although several studies have reported a prevalence of illness-related malnutrition of 6%–51% in hospitalized children, this condition is probably under recognized [10,11]. Lack of uniform definitions, heterogeneous nutrition screening practices, and failure to prioritize nutrition as part of patient care are some of the factors responsible for under recognition of the prevalence of malnutrition and its impact on clinical outcomes [12].

4.3 Hospital malnutrition

Malnutrition is common in the hospital setting and can adversely affect clinical outcomes and costs. Addressing hospital malnutrition has the potential to improve quality of patient care and clinical outcomes and reduce costs. Today it is estimated that at least one third of patients arrive at the hospital malnourished [13] and, if left untreated, many of these patients will continue to decline nutritionally, which may adversely impact their recovery and increase their risk of complications and readmission. Effective management of malnutrition requires collaboration among multiple clinical disciplines. All members of the clinical team must be involved, including nurses who perform initial nutrition screening and develop innovative strategies to facilitate patient compliance; dietitians who complete nutrition assessment/ diagnosis and develop evidence-based interventions; pharmacists who evaluate drug-nutrient interactions; and physicians who oversee the overall care

plan and documentation. Nutrition intervention for malnourished patients is a low-risk, cost-effective strategy to improve quality of hospital care. Unfortunately, despite the availability of validated screening tools, malnutrition continues to be under-recognized [14,15]. Moreover, among patients who are not malnourished upon admission, approximately one third may become malnourished while in the hospital [16]. Many of the adverse outcomes influenced by malnutrition are potentially preventable. Nosocomial infections are a prime example.

4.3.1 Pediatric hospital setting

The prevalence of acute malnutrition among hospitalized children in Europe is generally high although it can vary considerably (between 6.1% and 31.8%) due to the applied methodology and the analysis population [17]. In particular, the prevalence of acute and chronic malnutrition on hospital admission varies from 6.1% to 19% and 8.7% to 12.8%, respectively [18]. In the last 10 years, its prevalence has not decreased [19]. There is a further negative impact on children's growth and development if undernutrition is prolonged [20]. The nutritional status of children often declined after admission to the hospital and this condition may influence both short and long term health outcomes as children should be in a permanent positive energy balance (anabolic state) to maintain optimal growth and development [21]. Malnutrition has been reported to be highly prevalent in children with an underlying disease. In countries like Brazil and Turkey, the prevalence of acute hospital malnutrition on admission reach alarming figures, ranging from 33.8% to 52.4% [22-24]. Most of the studies use BMI or weight for height greater than two Standard Deviations (SD) to define acute malnutrition and height for age less than 2 SD to determine chronic malnutrition [25]. Some researchers use as the criterion hospital-acquired malnutrition the quantification of any weight loss [19], others define as a loss of weight greater than 2% [18] or a decrease in BMI greater than 0.25 SD [26]. In France Sermet-Gaudelus et al. reported that 191

(65%) of the 296 children admitted to the pediatric or pediatric surgery units lost weight during hospital stay; 44.5% of children had lost 2-5% of their body weight, and 25.6% children had lost more than 5% of their body weight on discharge [18]. An Italian study found that the BMI of 19.5% children on discharge had decreased by more than 0.25 standard deviations [26].

The latest studies show that the prevalence of hospital-acquired malnutrition has not changed [23]. In a study conducted in 2013 in a tertiary hospital in Belgium, 109 (31.8%) of the 343 children who completed the study had lost weight [27]. More recently [19], a multicentric study of 2567 patients aged one month to 18 years from 14 centers in 12 European Countries found that 217 (23%) of the 938 patients with hospital stays longer than four days lost weight. On the other hand, there is an epidemic of childhood obesity over recent years: the prevalence of obesity has been reported to be even higher in hospitalized children than in children seen in community settings [28]. Some studies highlight the importance of nutritional counseling to obese children's parents, who are not completely aware of the problem and its implication [29].

4.3.2 Impact of nutrition intervention on key outcomes

The benefits of nutrition intervention in terms of improving key clinical outcomes are well documented. Numerous studies, have shown the potential of specific nutrition interventions to substantially reduce complication rates, length of hospital stay, readmission rates, cost of care, and, in some studies, mortality [30, 31]. Nutrition intervention strategies represent a broad spectrum of options that can be organized into four categories: (1) food and/or nutrient delivery; (2) nutrition education; (3) nutrition counseling, and (4) coordination of nutrition care. Food and/or nutrient delivery requires an individualized approach that includes energy- and nutrient-dense food, complete oral nutrition supplements (ONS) that provide macronutrients (from carbohydrate, fat, and protein sources) combined with micronutrients (mixtures of complete vitamins, minerals, and trace

elements); enteral nutrition (EN); and/or parenteral nutrition (PN). The value of EN and PN is well established in selected patient populations. In addition, numerous studies have shown improved body weight, lean body mass (LBM), and grip strength with dietary counseling and with ONS [32].

Length of Stay

In a prospective study conducted at the Johns Hopkins Hospital, an early nutrition screening involving a team and earlier intervention to address malnutrition, reduced the length of hospital stay by an average of 3.2 days in severely malnourished patients and this translated into substantial cost savings of $1514 per patient [30]. Two meta-analyses have shown significantly reduced length of hospital stay in patients receiving ONS compared with control patients. One analysis demonstrated a reduced average length of hospital stay ranging from 2 days for surgical patients to 33 days for orthopedic patients ($P<0.004$) [33]. Likewise, in a recent meta-analysis of nine randomized trials, high-protein ONS significantly reduced length of stay by an average of 3.8 days compared with routine care (N=1227) [34].

Readmissions

Hospital readmission rate is another outcome that can be improved through nutrition intervention. Thirty-day readmission rates decreased from 16.5% to 7.1% in a community hospital that implemented a comprehensive malnutrition clinical pathway program focused on identification of at-risk patients, nutrition care decisions, inpatient care, and discharge planning [35].

Mortality

Several meta-analyses have also demonstrated reduced mortality in patients receiving optimized nutrient care. An analysis of 11 studies (N=1965) found significantly lower mortality rates among hospitalized patients receiving ONS (19%) compared with control patients (25%; $P<0.001$) [33]. This represented a 24% overall reduction in mortality, and patients with lower average BMI (<20)

receiving ONS had a greater reduction in mortality. Among elderly patients, hospitalized for hip fracture, significantly fewer patients had an unfavorable combined outcome (mortality or medical complication) if they received ONS vs routine care (RR=0.52; 95% CI 0.32 to 0.84) [36]. Collectively, all these data provide solid evidence that nutrition intervention significantly contributes to improved clinical outcomes and reduced cost of care [37, 38].

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CHAPTER 5

Literature review on Energy Metabolism

5.1 Energy expenditure and energy requirements

Human beings need energy to perform and regulate all biochemical processes that maintain the structural and biochemical integrity of the body; to perform internal work of circulation, respiration, and muscle contraction; and to perform external work [1]. All energy used for body maintenance, activity and growth is derived from chemical free energy of food provided by carbohydrates, fats, proteins and alcohol. Our ability to use the chemical free energy of diet results from the development of the biochemical, structural and physiologic apparatus that permits the transformation of chemical free energy into other energy forms essential for life.

Part of the energy from food, on the order of 5%, is thermodynamically obligated for conversion to heat because the entropy of the metabolic end products is greater than the initial substances. Conversion of food energy into high-energy biochemical compounds is an inefficient process, with approximately 50% lost as heat. Through biochemical transformations, approximately 45% of the energy of food is available to the body, primarily as adenosine triphosphate (ATP). Eventually, all the energy of food is lost from the body in the form of heat or external work. The potential energy contribution of food is determined experimentally by measuring the heat evolved in a bomb calorimeter when foodstuffs are completely combusted to carbon dioxide (CO_2) and water [2]. The actual amount of heat evolved per gram of foodstuff varies according to its chemical composition. Average values are 4.1 kcal/g of carbohydrate, 9.3 kcal/g of fat, and 5.4 kcal/g of protein. The body cannot oxidize nitrogen, and therefore energy resulting from the oxidation of the nitrogenous

component of protein is unavailable to the body. Consequently, only 4.2 kcal/g protein is potentially available to the body. The physiologic fuel value is compromised further by the apparent digestibility of various foodstuffs that vary among food sources. These factors result in physiologic fuel values of 4 kcal/g for carbohydrate, 9 kcal/g for fat, and 4 kcal/g for protein, also known as the Atwater factors. The physiologic fuel value for alcohol is 7 kcal/g. Protein oxidation is largely determined by protein intake, whereas the relative contributions of glucose or free fatty acids (FFAs) to the fuel mix are more variable. Glucose oxidation is adjusted to carbohydrate intake to maintain stable glycogen stores. Fat intake, in contrast, does not promote its own oxidation, and under conditions of positive energy balance, some fat will be deposited. Most cells can use the metabolic intermediates of carbohydrates, fats, and proteins interchangeably to regenerate ATP, with a few exceptions. The brain preferentially uses glucose and is able to use ketone bodies after adaptation to starvation, but it does not use FFAs. Red blood cells also depend on glucose. At rest, the brain (20%), internal organs (25% to 30%), and skeletal muscle (20%) account for the majority of energy turnover. During vigorous activity, skeletal muscle overwhelms the utilization of other tissues. In the postabsorptive state, FFAs are mainly oxidized by muscle, whereas during exertion, muscle's own glycogen reserve is used, with a subsequent shift toward use of FFAs mobilized from muscle fat stores and adipose tissue. When alcohol is consumed, it promptly appears in the circulation and is oxidized at a rate determined largely by its concentration and by the activity of liver alcohol dehydrogenase. Oxidation of alcohol rapidly reduces the oxidation of the other substrates used for ATP regeneration. Ethanol oxidation proceeds in large part through conversion to acetate and oxidative phosphorylation. Approximately 80% of the energy liberated by ethanol oxidation is used to drive ATP regeneration, and approximately 20% is released as heat.

An evaluation of individual energy expenditure is important to deliver adequate nutrition in particular in a hospital setting. Total energy expenditure (TEE) is most commonly calculated from

measured (mREE) or estimated resting energy expenditure (eREE) using a constant correction for the thermic effect of food and a variable correction for physical activity [3].

5.2 Components of total energy expenditure

TEE expended over 24 hours is the sum of basal energy expenditure (BEE), the energy expenditure of physical activity (EEPA), the thermic effect of food (TEF) and, in less frequent situations, cold-induced thermogenesis.

Basal energy expenditure

BEE is the energy needed to maintain all vital body functions:
- at the cellular level: the pumping of ions across membranes to maintain normal chemical gradients, the turnover of proteins and other cellular constituents;
- at the organ level: e.g. the contraction of cardiac and respiratory muscles.

BEE is defined as the energy used to maintain the basic physiological functions of the body at rest under strictly defined conditions: after an overnight fast corresponding to 12-14 hours of food deprivation, awake, supine, resting comfortably, motionless, no strenuous exercise in the preceding day, being in a state of "mental relaxation" and in a thermoneutral environment. BEE is the main component (45-70 %) of TEE [4].

Factors that influence basal metabolic rate in humans are:

- Body surface and body mass: they are positively correlated with basal metabolic rate but negatively correlated to basal metabolic rate per m^2 body surface or kg of body mass.
- Fat free mass (FFM): muscular tissue and especially organs expend more energy compared to fat tissue. FFM contains the metabolically active compartments of the body and therefore is the

major predictor of basal metabolism. FFM was the single best predictor of REE, and it accounts for 73% of its variability, instead fat mass (FM) accounted for only an additional 2%. Together, the brain, liver, heart, and kidneys account for approximately 60% to 70% of REE in adults, but they represent less than 6% of body weight. Skeletal muscle accounts for only 20% to 30% of REE and comprises 40% to 50% of body weight. The lower body fat percentage in males is one reason why men generally have a 10- 15% higher basal metabolic rate than women.

- Age: after the age of 20 years, basal metabolic rate declines at a rate of approximately 1% to 2% per decade in weight-constant persons. This decline is attributable to loss of FFM and gain of fat associated with aging.

- Diet: starvation can reduce basal metabolic rate by 30%.

- Body temperature: for each centigrade raise in body temperature, basal metabolic rate increases by approximately 10%.

- Ethnicity: the BEE, expressed per kilogram of body weight or per kilogram of FFM, is on the order of 5% to 10% lower in African-Americans compared with whites. Differences in relative contributions of organs and tissues to FFM may explain the differences in BEE among ethnic groups [5].

- Thyroid, adrenal and sympatic activity influence basal metabolic rate.

Resting energy expenditure

REE is the amount of calories required by the body at rest during a 24-h period and represents 70% to 80% of the calories used by the body. It is the resting metabolic rate that defines the energy released to maintain normal basal physiological functioning, when the body is at rest and no extra energy is used for muscular effort. In many studies, for practical reasons since conditions for

measuring BEE are more stringent, REE instead of BEE is measured. REE is measured in conditions less stringent than the ones for measurement of BEE (i.e. 3- to 4-hour fasting period is required and the time of day and prior physical activity are not controlled), so that REE is usually slightly higher than BEE (approximately up to 10%-20%).

Thermogenesis

Thermogenesis increases basal metabolism in response to food ingestion. It has two components: obligatory and facultative [6,7]. Obligatory thermogenesis depends on the energy cost of digesting, absorbing and processing or storing nutrients. The magnitude of this component is determined by the metabolic fate of the ingested substrate. Obligatory thermogenesis may be potentiated by a frequent meal pattern and increased meal size. Facultative or regulatory thermogenesis represents the additional energy expenditure not accounted for the known energy costs of obligatory thermogenesis. The sympathetic nervous system plays a role in modulating facultative thermogenesis. When the ingestion of a meal is the stimulus, the process can be referred to as postprandial thermogenesis, thermic effect of a meal or heat increment. These metabolic processes increase REE, and their energy expenditure is known as the TEF. The increments in EE above BEE, divided by the energy content of the food consumed, vary from 5% to 10% for carbohydrate, 0% to 5% for fat, and 20% to 30% for protein. A mixed meal elicits an increase in EE equivalent to approximately 10% of the calories consumed.

A change in environmental temperature (cold temperature) induces the production of heat in response to temperatures below thermoneutrality. Cold-induced thermogenesis can be divided into two types: shivering thermogenesis and non-shivering thermogenesis. The thermoneutral zone (or the critical temperature) is the environmental temperature at which oxygen consumption and metabolic rate are lowest [8]. The relative contribution of cold-induced thermogenesis to TEE has decreased in recent decades due to the increase in time spent in enclosed and heated environments.

Physical activity

EE for physical activity represents the most variable component of TEE, both within and between subjects, ranging from 15 % of TEE in very sedentary individuals to 50 % or more of TEE in highly active individuals. Physical activity level (PAL) is defined as the ratio of TEE/BEE and is commonly used to describe typical activity levels.

Moderate levels of exercise do not appear to increase subsequent EE markedly. Substrate utilization during exercise depends mainly on relative intensity. Fat is the main energy source in muscle and at the whole-body level during rest and mild exercise [9]. As exercise intensity increases, a shift from the predominant use of fat to carbohydrate occurs. Other factors such as exercise duration, gender, training status, and dietary history play secondary roles [10]. The peak rate of fat oxidation is achieved at approximately 45% of oxygen consumption (VO_2) max, and for exercises at greater than 50% of VO_2 max, the oxidation of FFAs declines in muscle, both as a percentage of total energy and on an absolute basis. The main carbohydrate energy source is muscle glycogen, supplemented by blood glucose and lactate. If exercise persists beyond 60 to 90 minutes, fat oxidation will rise as carbohydrate fuel sources become depleted. In this case, the intensity of exercise must drop because of depletion of muscle glycogen, decreased blood glucose, and fatigue [11].

Growth

The increase in EE induced by growth results from the expenditure for protein and lipid synthesis and their deposition in newly-formed tissue. The energy requirement for growth relative to maintenance is low, except for the first months of life. As a percentage of total energy requirements, the energy cost of growth decreases from 35% at 1 month to 3% at 12 months of age, and it remains low until puberty, at which time it increases to 4% [12]. During childhood, girls grow slightly more

slowly than boys, and girls have slightly more body fat. During adolescence, the gender differences in body composition are accentuated [13]. Adolescence in boys is characterized by rapid acquisition of FFM, a modest increase in FM in early puberty, followed by a decline. Adolescence in girls is characterized by a modest increase in FFM and continual FM accumulation.

Pregnancy

The additional energy requirements of pregnancy include increased basal metabolism and energy cost of physical activity and energy deposition in maternal and fetal tissues. The BEE increases as a result of the metabolic contribution of the uterus and fetus and the increased internal work of the heart and lungs [14]. In late pregnancy, the fetus accounts for approximately 50% of the increment in BEE. The energy cost of weight-bearing activities was increased by 19% after 25 weeks of gestation. The gross energy cost of non-weight-bearing activities increased on the order of 10% and the net cost on the order of 6% in late pregnancy [15]. The energy cost of tissue deposition can be calculated from the amount of protein and fat deposited in the fetus, placenta, amniotic fluid, uterus, breasts, blood, extracellular fluid, and adipose tissue.

Lactation

Consistent with the additional energy cost of milk synthesis, basal metabolism of lactating women increased on the order of 4% to 5% [16]. Although TEE may be slightly lower in the first months postpartum, TEE does not appear to differ from nonpregnant, nonlactating values thereafter [17]. Energy cost of lactation is estimated from milk production rates and the energy density of human milk. Milk production rates averaged 0.78 L/day from 0 to 6 months postpartum [18] and 0.6 L/day from 6 to 12 months postpartum [19]. Energy density measured by bomb calorimetry or proximate macronutrient analysis averaged at 0.67 (range, 0.64 to 0.74) kcal/g [20]. Energy mobilized from

maternal tissue stores can subsidize the energy cost of lactation. Gradual weight loss averaging -0.8 kg/month in the first 6 months postpartum is typical in well-nourished lactating women [17].

5.3 Measuring energy expenditure

Methods used to measure EE in humans include direct calorimetry, indirect calorimetry, and noncalorimetric methods [21]. Direct calorimetry is the measurement of the heat emitted from the body over a given period. A direct calorimeter chamber measures heat loss by radiation, convection, conduction, and latent heat arising from vaporization of water. Heat sink calorimeters capture the heat produced by liquid-cooled heat exchangers. Gradient layer calorimeters measure heat loss by a network of thermocouples in series surrounding the insulated chamber. Indirect calorimetry estimates heat production indirectly by measuring VO_2, VCO_2 and the respiratory quotient (RQ). Another approach to measure TEE is the doubly labelled water technique (DLW). This method is based on the differences in turnover rates of ^2H_2O and $H_2{}^{18}O$ in body water. After equilibration both ^2H and ^{18}O are lost as water whereas only ^{18}O is lost by respiration as CO_2. The difference in the rate of turnover of the two isotopes can be used to calculate the VCO_2. The DLW technique is validated against indirect calorimetry and is now considered to be the gold standard for measurements of TEE under free-living conditions. The advantage of this technique is the noninvasive, nonintrusive manner in which it measures TEE. The main disadvantages of the method are the high cost of isotopes and expensive, sophisticated mass spectrometric equipment and the expertise required to measure ^{18}O and ^2H.

Indirect Calorimetry

The measurement of EE is the most accurate method to assess energy needs. REE can be measured indirectly with a metabolic cart, using the analysis of expired gases to derive the volume of air that

passes through the lungs, the amount of oxygen extracted from it and the amount of carbon dioxide that is expelled into the atmosphere as a byproduct of metabolism. This technique arose from the observations of Lavoisier and Laplace that heat production of animals as measured by calorimetry was equal to that released when organic substances are burned, and that the same quantities of oxygen were consumed by the two processes. The ultimate goal of nutrient metabolism is to produce energy. The most common way of extracting the chemical energy of a substrate is to completely oxidize it to carbon dioxide and water. The heat generated by biologic combustions is utilized to maintain body temperature. Because of its isothermia, however, the body cannot use heat to perform work. The chemical energy of oxidizable substrates is therefore transferred on to some all-purpose carriers, which bring the free energy to where it is needed. Chemical (biosynthesis), osmotic (active transports), and mechanical (muscular contraction) work is thus made possible.

This technique has become the most commonly used to measure the rate of energy production and substrate oxidation in patients, both in clinical practice and in research studies. All indirect calorimeters (IC) monitors use inspired and expired gas volumes and concentrations to calculate VO_2 and VCO_2. Metabolic monitors are now available as portable bedside modules enabling the accurate estimation of patient metabolic demands and most can measure VO_2 with an accuracy of more than 95%. The closed-circuit ICs that are used most frequently collect expired air via a face mask or canopy or directly from the ventilator's exit port. The inspired air source is room air or oxygen from inside the calorimeter. Open-circuit ICs are those in which inspired gas source is room air or from the ventilator [22]. The principle of IC is derived from the fact that total average daily energy expenditure in kcal can be calculated by measuring the amount of oxygen used, and carbon dioxide released. In this model, all the oxygen that is consumed is completely used and the CO_2 that is expired is derived from complete oxidation of fuels. The formulae used to calculate REE are shown below. The equations are based on the classic work of Weir, first published in 1949 and later modified [23].

Energy Expenditure (Kcal/d) = 3.941 x VO_2 (L/min) + 1.106 x VCO_2 (L/min) - 2.17 x UrN (g/dl)

Weir demonstrated that the error in neglecting the effect of protein metabolism on the caloric equivalent of oxygen is 1% for each 12.3% of the total calories that arise from protein. Therefore, the foregoing equation can be reduced to the following:

Energy Expenditure (Kcal/d) = 3.9 x VO_2 (L/min) + 1.1 x VCO_2 (L/min)

The respiratory quotient

RQ is defined as the ratio VCO_2/VO_2 and reflects substrate utilization. The complete oxidation of glucose results in an RQ equal to 1.0. The complete oxidation of fat and proteins results in an RQ averaging about 0.71 and 0.84, respectively, depending on the chemical structure of the foodstuff. Based on several physiologic studies, a very specific well-documented physiologic range for the overall RQ exists between 0.67 to 1.3 [10]. With these limits of physiologic capability, values for RQ measured at the time of IC that are outside this range may be interpreted as nonphysiologic and presumably generated by some error in calibration, or leak in the system. In this manner, the overall RQ is a clinically useful parameter to help substantiate test validity for IC measurements [24].

The measured RQ of a subject on a typical Western diet should theoretically fall in a range between 0.85 and 0.90. If the subject is overfed and lipogenesis occurs, the RQ may be displaced upward above 1.0. Alternatively, if the patient is underfed and begins to use endogenous fat stores to meet caloric requirements, the measured RQ may be displaced downwards, below 0.85. Used in this manner, the RQ in theory becomes a valuable tool for nutrition assessment, identifying the metabolic consequences of over- and underfeeding [25]. This principle has been supported in the past by studies that suggested that high carbohydrate feedings, especially when given in excess of

caloric requirements, lead to net lipogenesis, increased VCO_2, and a resultant rise in RQ [26]. Many factors (e.g. underlying chronic disease, acid/base disturbances, hyper or hypoventilation, pharmacological agents, the stress response caused by acute disease process) may displace the measured RQ in a manner that is difficult to identify clinically. These results suggest that while IC provides sufficient accuracy in estimating total 24-hour energy expenditure from the short-term measured REE, considerable uncertainty exists in using this method to assess carbohydrate and fat use. Therefore, one of the major clinical benefits of the measured RQ is to validate an IC study confirming that the measured values for RQ fall in the physiologic range [24].

Timing and duration of IC measurements

There is no consensus regarding the optimal timing or duration of IC measurements. No major differences in REE were found when comparing prolonged measurements (24 h) to a much shorter duration (30 min) [27]. Longer periods of measurements have been recommended for metabolically unstable patients. In metabolically stable patients a 5-min steady-state test may give precise estimation of the 24-h REE. Some have suggested the need for repeated measurements, as the metabolic course of critically ill patients is dynamic and changes through hospitalization [28].

A prospective study examined factors associated with successful IC testing using the standard 5-min protocol in mechanically ventilated children [29]. The study examined the agreement between the REE obtained using the standard 5-min protocol and two abbreviated 4-min and 3-min protocols as well as the Schofield prediction equation. REE during a shortened period was optimally correlated with 24-h REE measurements only if steady-state criteria were met, that is, when minute-to-minute VO_2 and VCO_2 varied by not more than 10% consecutively for 5 min. However, some patients may fail to achieve a steady state by these criteria and a reliable measurement of REE may not be obtained. Indeed, the number of patients who were able to reach steady state was nearly doubled when using the 3-min protocol. The authors concluded that the abbreviated protocols

allowed REE measurements to be obtained in most patients with reasonable accuracy and may decrease the need to rely on inaccurate equations when assessing energy expenditure in children who fail IC testing by standard steady-state criteria. The use of IC should be recommended to individualize nutrition, and that follow-up be extended beyond the doors of the hospital [17]. At present, there are limited data regarding the use of IC in children. Indeed, there are many challenges associated with its use, including the use of specialized equipment, technical support, high settings on conventional ventilation and it may be difficult to obtain a steady state during the IC measurement due to the presence of fever, feeding regimens, uncontrolled movements, degree of sedation, and environmental noise. The standard use of IC is limited due to equipment availability, staffing and cost. The recent American Society for Parenteral and Enteral Nutrition (ASPEN) clinical guidelines for nutrition support of the critically ill child, suggested that IC measurements should be obtained when possible in pediatric patients with suspected metabolic alterations or malnutrition [31]. A recent prospective chart review, attempted to determine how many PICU patients would be candidates for IC measurements during their first week of stay, based on the current ASPEN recommendations [32]. The review hypothesized that > 50% of patients admitted to the PICU would meet these criteria and would benefit from IC measurements. This prospective chart review included 150 consecutive patients admitted to PICU during a 7-week period. The review found that IC was indicated in 72% of patients, with the most frequent indications for IC being overweight/obesity (32.4%), hypermetabolism (26.4%), not meeting nutrition goals (13.7%), and mechanical ventilation (11.5%). Patients with neurologic/seizure and respiratory disorders were responsible for 66% of the suggested indications for IC. The review concluded that in addition to the ASPEN criteria, further prioritization for IC measurements should be given to patients ages < 2 years who are at risk for developing nutrition deficiencies given their high basal metabolic rate, weight (underweight or overweight) or PICU length of stay (> 5 days).

Limitations of IC

There are many technical issues and methodological problems which affect the correct measurement of EE and the interpretation of the results. The first problem is the collection of expired gases: all the situations which prevent a complete collection make impossible to perform the IC. Examples include air leaks from the ventilator circuit and around endotracheal tubes or through chest drains while CO_2 removal across hemodialysis membranes is also not taken into account by IC. FiO_2 above 0.6 or high-applied positive end-expiratory pressure (PPEP) in ventilatory mode, or when the metabolic monitor is connected to ventilators with a large bias flow, IC cannot separate inspired and expired gases related to the bias flow. All these variables make the use of IC. Trained operators should actively look for correct calibration, equipment instabilities and adequate length of measurement to ensure valid results.

5.4 Metabolic needs during acute illness

Acute illness as sepsis, burns, inflammation, surgery or other severe infections, are the major causes of admission into PICUs. In this condition, the metabolic response to stress varies and it is influenced by many factors. It has precise pathophysiological mechanisms, clinical consequences, and therapeutic implications. It is divided into three phases—the acute phase, the stable phase, and the recovery phase—all characterized by specific neuroendocrine, metabolic, and immunologic modifications. Predominantly includes catabolic processes that, in many circumstances, may increase physiological instability and resource wasting. All these conditions in addition to therapeutic interventions, such as mechanical ventilation and the administration of vasoactive or sedative agents change energy and nutrients requirements. It has been suggested that growth ceases during the metabolic response to illness or injury in children [33]. In its original description, the response was characterized as biphasic, with brief ebb phase followed by a hypermetabolic flow phase [34]. This hypermetabolic phase is catabolic in nature. It is driven initially by a cytokine

surge and increased counter regulatory hormones with insulin and growth hormone resistance. The result is breakdown of endogenous body stores, in particular muscle mass, to provide free amino acids that are used for the inflammatory response, tissue repair, and wound healing [35]. This is an adaptive phenomenon where autocannibalism sustains the individual during periods of low nutrient availability after the insult or injury. Recent accounts of measured energy expenditure have shown a muted hypermetabolic response after major illness, injury, or surgery. One exception is burn injury, which is characterized by a profound hypermetabolic response that may be sustained for several weeks [36]. By contrast, metabolic measurements in most other illnesses reveal a muted and brief hypermetabolic response. So, metabolism and energy needs of the critically ill child seem to fluctuate from a hypermetabolic to hypo- metabolic state through the continuum of the PICU stay. During this phase is important to prevent both overfeeding and underfeeding and to avoid waste of LBM as well as the worsening of any existing nutrient deficiencies [34]. Overfeeding leads to increased CO_2 production, respiratory failure, hyperglycemia, and fat deposits in the liver, on the contrary underfeeding may lead to malnutrition, muscle weakness, and impaired immunity. Hence, a careful approach to accurately determine energy needs during illnesses is essential. Both underestimation and overestimation of energy needs may impact the ability to match the requirements with intake, and result in unintended energy imbalance [37].

5.5 The prediction of energy expenditure

The determination of energy expenditure, as seen above, IC allows accurate assessment of REE but it requires specialized equipment and trained personnel, factors that limit its availability and it is used only in a minority of centers worldwide [39]. Suboptimal levels of feeding are associated with poor clinical outcomes including poor wound healing, higher complication rates and increased mortality [40-41]. Conversely, excess energy intake is associated with longer duration of mechanical ventilation, prolonged ICU admission and increased overall length of stay [42].

Equations for predicting REE are historically based on easily measurable parameters such as body mass, height, sex, age and ethnicity. These equations are derived by regression analysis of the data from a group of subjects whose REE is measured by direct or indirect calorimetry. The first set of equations was proposed in 1919 by Harris and Benedict, and has been one of the most used set of equations [42]. The database included a small number of subjects (136 men and 103 women), with no children or adolescents below the age of 15 years, and a significant number of measurements were obtained by the use of closed-circuit IC. In 1985, Schofield synthesized the results of 114 different studies and included a total of 7173 BEE measurements [43]. These data were subsequently used for the development of the Food and Agriculture Organization (FAO)/World Health Organization (WHO)/ United Nations University (UNU) equations, as well as the Schofield equations (called Schofield [weight] and Schofield [weight and height]).

The FAO/WHO/UNU equations were developed based on 7500 measurements from children and adolescents 3–18 years of age from both underdeveloped and developed countries [44]. Other two equations are: Mifflin-St. Jeor equation that incorporate patient's height, age and gender and Ireton-Jones equations, for obese individuals [45,46]. Likewise, the Penn State and Swinamer equations have been used specifically in critically ill patients, and include factors such as ventilation rate and core temperature which may further influence energy expenditure. However, caution regarding the use of these prediction equations is warranted due to underrepresentation in most validation studies of adult and children with diseases all of whom may differ physiologically due to acute illness or chronic organ insufficiency [47]. Underestimations in energy expenditure for these equations ranged from 18-27%, while overestimations ranged from 5-12%. The Mifflin-St Jeor equation, alternately, had the strongest performance in healthy, non-obese adults, but underestimated energy intake in obese adults [47]. Henry in 2005 also developed a new database including 10552 basal metabolic rate values collected from 166 previous investigations, from a wide range of researchers [48]. These algorithms included a larger number of people from tropical areas and excluded the

Italian subjects from the Schofield/FAO/WHO/UNU database, as it was believed that these subjects may have been responsible for the overestimation of predicted REE.

Fig 1. Most common equations used to predict EE in children.

Harris-Benedict equation (kcal/d)
Boys: 66.4730 + (5.0033 × height) + (13.7516 × weight) − (6.7550 × age)
Girls: 655.0955 + (1.8496 × height) + (9.5634 × weight) − (4.6756 × age)

Schofield-W 3–10 y Girls: 22.5 × weight + 99
Boys: 22.7 × weight + 495
11–18 y Girls: 17.5 × weight + 651
Males: 12.5 × weight + 746

Schofield-HW 3–10 y Girls: 16.97 × weight + 1.618 × height + 371.2
Boys: 19.6 × weight + 1.033 × height + 414.9
11–18 y Girls: 8.365 × weight + 4.65 × height + 200
Boys: 16.25 × weight + 1.372 × height + 515.5

Schofield equations (kj/d) (1 kcal = 4.186 kj)
< 3 y Boys: (0.0007 × weight) + (6.349 × height) − 2.584
Girls: (0.068 × weight) + (4.281 × height) − 1.730
3–10 y Boys: (0.082 × weight) + (0.545 × height) + 1.736
Girls: (0.071 × weight) + (0.677 × height) + 1.553
10–18 y Boys: (0.068 × weight) + (0.574 × height) + 2.157
Girls: (0.035 × weight) + (1.948 × height) + 0.837

White (kJ/d) 17 × age [mo] + (48 × weight [kg]) + (292 × body temp °C) − 9677

FAO/WHO/UNU equations
< 3 y Boys: (kcal/d): (60.9 × weight) − 54
Girls: (kcal/d): (61 × weight) − 51
3–10 y old (1 kcal = 4.186 kJ)
Boys: (kJ/g): (95 × weight) + 2071
Girls: (kJ/d): (94 × weight) + 2088
10–18 y Boys: (kcal/d): (16.6 × weight) + (77 × height) + 572
Girls (kcal/d): (7.4 × weight) + (482 × height) + 217

Maffeis equations (kJ/d) (1 kcal = 4.186 kJ)
Boys: (28.6 × weight) + (23.6 × height) − (69.1 × age) + 1287
Girls: (35.8 × weight) + (15.6 × height) − (36.3 × age) + 1552

Fleisch equation (kcal/d)
Boys: 1–12 y: 24 × BSA × (54−0.885 × age)
13–19 y: 24 × BSA × (42.5 − [0.64 × {age − 13}])
Girls: 1–10 y: 24 × BSA × (54−1.045 × age)
11–19 y: 24 × BSA × (42.5 − [0.778 × {age − 11}])

5.5.1 Validity of predicted versus measured REE

As seen above, many equations exist for predicting REE, but their accuracy in estimating energy requirements for critically ill patients and children in particular, is not clear. As a result, energy requirements of ill infants and children are difficult to predict accurately. Most predictive equations are typically derived from studies of healthy non-hospitalized individuals and few have been validated in special population, as mechanically ventilated patients. One of the problems with developing an accurate predictive equation for critically ill children in the PICU is the large heterogeneity regarding age, weight, muscle mass, level of growth and maturity, diagnosis, and

severity of illness. Ideally, predictive equations should provide results within 10% of measured energy expenditure [49]. Vasquez-Martinez et al. performed a prospective study of 43 ventilated critically ill children during the first 6 h post-injury, in which they compared MREE by continuous IC with predictive energy expenditure calculated using the Harris-Benedict, Caldwell-Kennedy, Schofield, FAO/WHO/UNU, Maffies, Fleisch, Kleiber, Dreyer, and Hunter equations [50]. Most of the predictive equations overestimated measured energy expenditure, and MREE and predictive energy equations differed significantly except for the Fleisch and Caldwell- Kennedy equations, which were found to be the best predictors of energy expenditure. Bott and colleagues compared measured versus predictive resting energy expenditure in 52 children with bronchopulmonary dysplasia (BPD) and in 30 healthy children, using four predictive equations, namely, Schofield-Weight (-W), Schofield-Height&Weight (-HW), Harris-Benedict and FAO equations [51]. They concluded that the Harris-Benedict equation best predicted REE in children with BPD while the Schofield-W was best in healthy children. In a study of 91 severely burned children (> 40% body surface area), Suman and colleagues compared the REE measured by IC with predictive equations in this very hypermetabolic population [52]. Good agreement was obtained between FAO/WHO/UNU, Schofield-HW, and Harris-Benedict equations. However, the predicted REEs were significantly lower than the measured REEs. In a prospective observational study, Hardy et al. [53] examined whether a similar hypermetabolic response to that observed in adults exists in children and compared a newly derived predictive equation specific for the PICU (the White equation) with measured REE and with the age appropriate Schofield-predictive equation [54]. The White equation was accurate only in 30% of measurements [8]. In another prospective study, predictive equations including the Schofield, the White, and the WHO equations were compared with IC in mechanically ventilated children who underwent surgery for congenital heart disease [55]. They also compared REE with the Schofield equation using a stress correction factor, which is widely used to estimate energy expenditure in critically ill children. They found poor correlation

between MREE and predicted energy requirements, with none of the predictive equations predicting requirements within 10% of the REE. The Schofield equation with added stress factor had the lowest percentage difference [55]. In general, most of these studies came to the same conclusion: current predictive equations do not accurately predict required energy needs in the ill children and need to be reevaluated in the context of the variability of the metabolic state in various conditions such as surgery, cardio-pulmonary bypass, and postoperative organ dysfunction. Additionally, the dynamic alterations in energy metabolism that characterize illness, particularly if critical, can only accurately be assessed with repeated IC, which remains the gold standard [55].

5.6 References

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CHAPTER 6

Experimental Section: Study of infertile couples referring to the Infertility Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan

This chapter details the design, methods and analysis for the research conducted using data from a study on the impact of lifestyle habits and diet on Assisted Reproductive Techniques (ARTs) in Italian infertile couples, focusing on the relation between diet's variables and IVF outcomes.

6.1 Population

From September 2014 to December 2016, in randomly selected days, subfertile couples, presenting for evaluation to the Infertility Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, and eligible for ART, were invited to participate into an ongoing prospective cohort study on the role of lifestyle habits and diet on ART outcome. The study protocol was approved by the local Institutional Review Board. All procedures were in accord with the Helsinki Declaration and all participants provided written informed consent.

6.2 Data collection and Methods

Study participation was proposed during the diagnostic phase. Couples were interviewed on the day of oocyte retrieval. On the same day, a semen sample was collected and analyzed prior to proceed IVF or intra-cytoplasmic sperm injection (ICSI). The time interval between the proposal of the study and the interview was generally less than one month.

Both partners of couples who agreed to participate were interviewed by centrally trained personnel, using a standard questionnaire to obtain information on general socio-demographic characteristics,

personal and health history and habits (including smoking, physical activity, alcohol intake and methylxanthine-containing beverages consumption). Couples who do not speak fluent Italian were excluded. Information on diet was obtained using a previously validated food frequency questionnaire (FFQ) [1,2,3]. Patients were asked to report about their usual weekly food consumption in the last year. The FFQ includes the average weekly consumption of 78 food items or food groups (such as the major sources of animal fats – i.e. red meat, milk, cheese, ham, salami – folates, vitamins – vegetables and fruit – pasta and bread consumption, cake, sweets and chocolate, fish) and beverages. Intakes lower than once per week, but at least once per month, was coded 0.5 per week. Seasonal consumption was also considered (weekly consumption of vegetables/fruits available in limited periods during the year, weighted for months of consumption). Energy and mineral, macro- and micronutrient intake was estimated using the most recent update of an Italian food consumption database [4]. BMI was classified according to WHO indications [5]. Smoking habits were categorized as never, former or current, and number of cigarettes smoked daily and duration of smoking were recorded. Caffeine intake from coffee (60 mg per cup), cappuccino (75 mg per cup), tea (45 per cup), decaffeinated coffee (4 per cup) and chocolate (6mg/10g) was calculated [6].

Occupational physical activity (PA) was described as heavy (or very heavy), light/moderate, mainly standing or mainly sitting. Leisure PA was recorded in term of hours/week: <2, 2 to 4, ≥5. No information was collected about intensity or type of leisure PA. Calories intake was calculated by the FFQ [1,2,3]. Information on alcohol intake was collected as usual weekly consumption (1 unit=125 mL wine or 330 mL beer or 30 mL spirits, all containing approximately 12.5 gr of ethanol). An intake lower than one unit per week were codified as 0.5.

6.3 First Objective

To analyze the relation between alcohol intake and semen variables.

6.3.1 Subjects

From the above mentioned database, we analyzed data regarding the male partner. The overall participation rate was close to 95%. This high participation rate was mainly due to the fact that couples were interviewed during the period spent waiting for the different diagnostic and therapeutic phases. Considering this time off and the non-sensitive character questions, couples did not generally refuse to participate. Men were considered as having risk factors for impaired fertility, if they had a history of previous chemio- or radiotherapy, as well as previous reproductive organ diseases, such as orchiectomy, cryptorchidism and varicocele. These data were retrieved from clinical records.

Sperm analysis

Men were instructed to abstain from ejaculation for 2 to 5 days before semen analysis and to report the specific time of abstinence. Semen samples were obtained by masturbation and collected into a sterile plastic container provided and labeled with the date and time of collection. All seminal fluid examinations were carried out by the laboratory of the Unit, where samples were maintained at room temperature until complete liquefaction. Duration of complete liquefaction (<1 h) was documented, until 1 h was reached. Semen analysis was performed with standardized methods according to the WHO guidelines [7]. The following variables were taken into consideration: volume (mL), sperm concentration (spermatozoa N/mL) and motility (%). Sperm motility was classified into total (progressive + non-progressive motility) and progressive. Total sperm count was calculated as volume × sperm concentration. As semen samples were collected specifically to

carry out ART procedures, sperm morphology was evaluated only in partners of those couples undergoing IVF and after semen capacitation (and not on rough samples).

The laboratory personnel was trained using the European Society of Human Reproduction and Embryology (ESHRE) Special Interest Group in Andrology Basic Semen Analysis Course [8].

6.3.2 Statistical analysis

Categorical or ordinal variables were described as frequency (percentage, %), continuous variables as mean (±SD) if normally distributed and medians (interquartile range, IQR) if not. Four domains of semen quality were assessed: volume, concentration, total count and motility. At the univariate analysis, groups were compared by means of Kruskal-Wallis test, even if they were normally distributed. In order to perform a multivariate analysis including potential confounders, non-normal (skewed) distributions of semen parameters were square-root transformed and included in a general linear model. Adjusted medians and 95% CI were calculated back-transforming the adjusted means and their 95% CIs. In the model, we included as potential confounders variables associated to alcohol intake or semen quality at univariate analysis. Given that the relation between alcohol intake and sperm parameters was potentially different in men with or without risk factors for impaired fertility, we planned to perform an analysis in strata for this variable. The multivariate analysis also showed a significant relation between sperm motility and leisure PA, therefore we also performed a further analysis in strata for PA. All reported P-values are based on two-sided tests and considered statistically significant if below 5%.

6.3.3 Results

From September 2014 to December 2016, 327 men were enrolled, aged 39.2 years on average (SD 5.2, range 27-60). Among them, four did not provide complete information about lifestyle and were excluded. The final analyses were conducted in a sample of 323 men, aged 39.3 years on average

(SD 5.3, range 27-60). The median daily alcohol intake was 8.30 g (IQR 2.72-15.95). Excluding 31 men who did not drink at all, we determined tertiles of daily alcohol intake: 0.01-5.44, 5.45-14.20 and ≥14.21 g per day. Tertiles corresponded to a weekly consumption of <1-3, 4-7, and ≥ 8 alcohol units, respectively. In the last category, the highest value of consumption was 108.13 g/day (60 units per week), while the median alcohol consumption was 21.21 g/day (about 12 units per week).

Patients' characteristics according to alcohol intake are described in Table 1: alcohol intake was inversely associated with age, and positively with caffeine consumption and calories intake, although the highest caloric intake was observed in abstainers. Never smokers were less frequently alcohol drinkers than both former and current smokers.

Table 2 shows the median values of semen variables according to demographic characteristics and lifestyle patterns. Alcohol intake was associated to semen volume, sperm concentration and total sperm count, with no dose-effect relation. Men drinking 4-7 alcohol units per week had the highest semen volume. The highest median concentrations were observed in abstainers and in men drinking ≥8 units/week; total count was also associated to alcohol intake, but did not show a dose-dependent relation, although a significant rank correlation was observed between these two variables (Spearman rho=0.12, p=0.038). Days of abstinence were positively correlated to semen volume (Spearman rho=0.14, p=0.01) and inversely to sperm motility (Spearman rho=-0.11), with borderline significance (p=0.07). We accounted for the observed difference among men in groups of alcohol intake using a general linear model equation, that included age (associated to alcohol intake and semen variables), days of abstinence, leisure PA, and risk factors for impaired male fertility (associated with at least 1 semen variable), smoking status, caffeine consumption, calories intake (associated with alcohol intake). Previous ART cycles did not relate to alcohol intake nor to semen quality: therefore, this variable was not included in the final model. However, we also rerun the model including this information, without significant modifications in the results.

In the multivariate analysis, we still found a relation between alcohol intake and semen volume, concentration and total count (Table 3). Back-transforming semen volume, and using men with <1-3 units per week of alcohol intake as the reference group, we observed that men drinking 4-7 units/week had a significantly higher median semen volume, that both men in 4-7 and ≥8 units/week group had significantly higher sperm concentration (p=0.047 and p=0.004, respectively) and that abstainers had higher median concentration as well (p=0.017). Total count was also associated to alcohol intake: men drinking 4-7 and ≥8 units/week had higher total count than men drinking <1-3 units/week (p=0.006 and p=0.009, respectively) but without dose-dependent relation. No association emerged with sperm motility.

In the multivariate model, the presence of risk factors for impaired male fertility was significantly associated to worse sperm concentration (19.4 vs 40.9 mil/mL, p<0.0001), total count (48.8 vs 100.2 mil, p=0.0002) and motility (29.3% vs 39.6%, p<0.0001). Leisure PA was related to sperm motility, with the lowest motility in the intermediate level of PA: 31.3% in men with 2-4 hours per week vs 37.0% in those with ≥5 hours per week (p=0.012) of leisure PA. A further analysis was performed, aiming at better understanding the role of alcohol intake in strata of impaired male fertility and physical activity: medians and 95% CIs of sperm variables are shown in Table 3, according to alcohol intake. Considering an alcohol intake of <1-3 units/week as the reference category, we found that semen volume was significantly lower in abstainers with low level of leisure PA; a trend of increasing volume with increasing alcohol intake, with a maximum at 4-7 units/week was consistently found in all strata. Concentration and total sperm count increased with higher level of alcohol intake in men without risk factors for impaired fertility, and was significant both in those drinking 4-7 and ≥8 units per week. As regards leisure PA, no significant relation was observed in men with ≥5 hours/week, whereas in subject with <2 and 2-4 hours/week concentration and total count were positively related to alcohol intake, although no dose-relation was seen. As in the overall analysis, alcohol intake was not associated with sperm motility in any subgroups. We

checked terms for interactions between alcohol and, in turn, smoking, PA, risk factors for male impaired fertility, age class, and caffeine intake. None of them was significant (data not shown). Lastly, we estimated the association between high alcohol intake and semen quality, comparing 39 men who drank ≥14 units/week: in a model including the before mentioned variables, no statistically or clinically significant association was observed, both including and excluding non-drinkers from the analysis.

Table 1. Demographic characteristics and lifestyle patterns according to tertiles of alcohol intake.

	Overall N=323 (%)		**Alcohol intake (units/week)**			**P***
		Abstainers N=31 (9.6%)	**<1-3 N=97 (30.0%)**	**4-7 N=98 (30.3%)**	**≥8 N=97 (30.0%)**	
Alcohol intake (gr, median, range)	8.3 (0-108.13)	0 (0-0)	2.63 (0.85-5.44)	9.58 (5.70-14.20)	21.21 (14.27-108.13)	-
Age						
<35	60 (18.6)	3 (9.7)	13 (13.4)	19 (19.4)	25 (25.8)	
35-39	126 (39.0)	7 (22.6)	39 (40.2)	42 (42.9)	38 (39.2)	
≥40	137 (42.4)	21 (67.7)	45 (46.4)	37 (37.8)	34 (35.0)	0.0007
Mean ± SD	39.3 ± 5.2	41.4 ± 5.0	40.1 ± 5.4	38.8 ± 4.8	38.4 ± 5.4	0.01[#]
College degree	131 (40.6)	9 (29.0)	40 (41.2)	44 (44.9)	38 (39.2)	0.55
Risk factor for impaired male fertility	66 (20.4)	3 (9.7)	20 (20.6)	22 (22.4)	21 (21.6)	0.28
Previous ART cycles	184 (57.0)	19 (61.3)	54 (55.7)	57 (58.2)	54 (55.7)	0.75
Days of abstinence mean ± SD	3.9 ± 1.9	4.0 ± 2.5	3.9 ± 1.9	4.0 ± 2.2	3.8 ± 1.5	0.85[#]
BMI						
<25.0	146 (45.3)	16 (51.6)	41 (42.3)	54 (55.1)	35 (36.5)	
25.0-29.9	148 (46.0)	13 (41.9)	46 (47.4)	33 (33.7)	56 (58.3)	
≥30.0	28 (8.7)	2 (6.4)	10 (10.3)	11 (11.2)	5 (5.2)	0.54
Mean ± SD	25.3 ± 3.0	24.6 ± 2.8	25.5 ± 3.1	25.0 ± 3.2	25.5 ± 2.8	0.25[#]
Smoking						
Never	129 (39.4)	17 (54.8)	50 (51.6)	37 (37.8)	25 (25.8)	
Former	93 (28.9)	6 (19.4)	22 (22.7)	32 (32.6)	33 (34.0)	
Current	101 (31.7)	8 (25.8)	25 (25.8)	29 (29.6)	39 (40.2)	0.004

0-9 cig/die	46 (14.1)	2 (6.4)	13 (13.4)	18 (18.4)	14 (14.4)	
≥10 cig/die	15 (13.9)	6 (19.4)	12 (12.4)	11 (11.2)	25 (25.8)	0.001
Caffeine intake (mg/day)						
0-127	110 (34.1)	15 (48.4)	38 (39.2)	30 (30.6)	27 (27.8)	
128-214	105 (32.5)	9 (29.0)	29 (29.9)	41 (41.8)	26 (26.8)	
≥215	108 (33.4)	7 (22.6)	30 (30.9)	27 (27.6)	44 (45.4)	0.005
Mean ± SD	175 ± 99	152 ± 108	168 ± 100	177 ± 89	189 ± 105	0.24#
Occupational physical activity						
Heavy	67 (20.8)	12 (38.7)	17 (17.5)	19 (19.4)	19 (19.8)	
Light/moderate	68 (21.1)	9 (29.0)	20 (20.6)	21 (21.4)	18 (18.8)	
Mainly standing	47 (16.6)	4 (12.9)	17 (17.5)	10 (10.2)	16 (16.7)	
Mainly sitting	140 (43.5)	6 (19.4)	43 (44.3)	48 (49.0)	43 (44.8)	0.07
Leisure physical activity						
<2 h/wk	133 (41.7)	19 (63.3)	47 (49.0)	29 (29.6)	38 (40.0)	
2-4 h/wk	112 (35.1)	6 (20.0)	29 (30.2)	41 (41.8)	36 (37.9)	
≥5 h/wk	74 (22.1)	5 (16.7)	20 (20.8)	28 (28.6)	21 (22.1)	0.11
Calories intake (Kcal/ day) median (IQR)	1899 (1623-2290)	2110 (1795-2377)	1740 (1429-2145)	1858 (1720-2257)	2028 (1683-2404)	0.0002§

* Cochran-Mantel-Hanszel chi-square test, if not otherwise indicated
# analysis of variance
§ Kruskal-Wallis test

Table 2. Median sperm parameters (IQR) according to demographic characteristics and lifestyle patterns.

Characteristics	N	Volume (mL)			Concentration (mil/mL)			Total count (mil)			Motility (A+B) %		
		median	(Q1	Q3)	median	(Q1	Q3)	median	(Q1	Q3)	median	(Q1	Q3)
Overall	323	2.6	(1.7	3.7)	32.0	(9.7	67.0)	76.0	(27.4	155.7)	41.0	(30.0	49.0)
Age													
<35	60	**2.8**	**(2.0**	**3.5)**	45.0	(15.9	72.0)	**116.8**	**(35.7**	**213.0)**	44.0	(31.0	54.0)
35 -39	126	**2.7**	**(1.8**	**4.0)**	30.0	(8.7	57.0)	**70.0**	**(21.4**	**144.0)**	40.0	(29.0	50.0)
≥40	137	**2.3**	**(1.4**	**3.5)**	30.0	(10.0	70.0)	**75.4**	**(21.6**	**138.0)**	40.0	(31.0	46.0)
College degree													
No	192	2.6	(1.8	3.7)	30.5	(9.7	68.0)	75.2	(29.9	156.0)	42.0	(31.0	50.0)
Yes	131	2.7	(1.5	3.7)	32.0	(9.8	57.0)	78.9	(24.0	144.0)	40.0	(28.0	48.0)
Risk factor for impaired male fertility													
No	257	2.5	(1.5	3.5)	**37.0**	**(13.7**	**72.0)**	**88.8**	**(34.5**	**166.5)**	**42.0**	**(32.5**	**50.0)**
Yes	66	2.9	(1.9	4.5)	**13.7**	**(4.7**	**34.0)**	**36.2**	**(15.2**	**72.0)**	**33.0**	**(21.0**	**45.0)**
Previous ART cycle													
No	139	2.3	(1.5-	3.8)	28.0	(10.0-	65.0)	70.0	(25.4-	147.7)	39.0	(26.0-	48.0)
Yes	184	2.7	(1.8-	3.7)	33.0	(9.7-	68.0)	84.0	(29.0-	167.5)	41.0	(33.0-	50.0)
BMI													
<25.0	148	2.8	(1.8	3.9)	33.0	(10.4	63.0)	84.7	(28.1	150.0)	40.0	(31.0	48.0)
25.0 -29.9	150	2.4	(1.5	3.3)	30.0	(9.6	67.5)	70.0	(24.0	151.9)	42.0	(28.0	50.0)
≥30.0	28	2.7	(2.0	4.3)	33.0	(8.0	66.0)	82.5	(28.1	175.5)	41.0	(31.0	51.0)
Smoking													
Never	129	2.6	(1.8	4.0)	30.0	(9.7	58.0)	75.7	(21.6	140.0)	38.0	(29.0	46.0)
	93												

Former	101	2.7	(1.7 -	3.7)	30.5	(10.0 -	65.5)	69.8	(25.5 -	165.0)	44.0	(34.0 -	51.0)
Current	46	2.6	(1.7 -	3.5)	34.5	(9.9 -	70.0)	83.0	(34.5 -	153.0)	43.0	(32.0 -	49.0)
0 -9 cig/die	15	2.7	(1.7 -	3.5)	35.0	(11.0 -	75.0)	87.3	(37.5 -	142.5)	41.0	(27.0 -	50.0)
≥10 cig/die		2.5	(1.7 -	3.3)	30.0	(8.7 -	69.0)	80.1	(21.4 -	168.0)	44.0	(36.0 -	49.0)
Daily alcohol intake (units/wk)													
Abstainer	31	**1.8**	**(1.2 -**	**2.5)**	**42.0**	**(18.0 -**	**75.0)**	**85.4**	**(37.8 -**	**151.9)**	41.5	(32.0 -	47.5)
<1-3	97	**2.4**	**(1.7 -**	**3.5)**	**24.5**	**(5.9 -**	**50.0)**	**51.5**	**(15.2 -**	**114.7)**	38.0	(29.5 -	46.0)
4-7	98	**3.0**	**(2.0 -**	**4.0)**	**31.0**	**(8.7 -**	**71.0)**	**87.9**	**(20.2 -**	**182.1)**	42.0	(32.0 -	50.0)
≥8	97	**2.6**	**(1.5 -**	**4.0)**	**39.0**	**(16.0 -**	**72.0)**	**84.0**	**(37.4 -**	**156.4)**	42.0	(28.0 -	50.0)
Caffeine intake (mg/day)													
0 -127	110	2.8	(1.8 -	4.0)	32.5	(10.0 -	61.5)	86.5	(23.4 -	151.9)	39.0	(30.5 -	49.5)
128 -214	108	2.5	(1.7 -	3.7)	31.0	(8.7 -	70.0)	70.2	(24.0 -	156.4)	41.0	(31.0 -	48.0)
≥215	109	2.5	(1.5 -	3.3)	30.0	(10.1 -	63.5)	79.6	(32.7 -	149.0)	42.0	(28.0 -	49.5)
Occupational physical activity													
Heavy	67	2.7	(1.4 -	3.7)	29.0	(6.6 -	65.0)	54.0	(17.6 -	150.0)	41.0	(36.0 -	50.0)
Light/moderate	66	2.6	(1.8 -	4.0)	30.5	(12.6 -	63.5)	76.0	(35.7 -	156.0)	43.5	(32.0 -	48.5)
Mainly standing	48	2.3	(1.7 -	3.9)	40.0	(15.9 -	82.0)	100.1	(37.5 -	180.0)	41.0	(23.0 -	46.0)
Mainly sitting	145	2.7	(1.8 -	3.6)	31.5	(9.7 -	64.0)	79.9	(21.6 -	148.0)	40.0	(28.5 -	50.0)
Leisure physical activity													
<2 h/wk	137	**2.5**	**(1.6 -**	**3.1)**	33.0	(8.7 -	66.0)	70.2	(17.5 -	156.4)	**43.0**	**(33.0 -**	**51.0)**
2 -4 h/wk	112	**2.6**	**(1.7 -**	**3.9)**	31.5	(9.9 -	70.0)	75.4	(31.8 -	156.2)	**37.5**	**(25.5 -**	**45.5)**
≥5 h/wk	74	**3.0**	**(2.0 -**	**4.0)**	32.5	(11.3 -	57.0)	85.9	(36.1 -	147.4)	**42.0**	**(30.0 -**	**49.0)**

Bold: $p<0.05$; Kruskal-Wallis test

Table 3. Adjusted median sperm parameters (95% CI) according to alcohol intake, in strata of risk factor for impaired male fertility and leisure physical activity.

Alcohol intake (units/wk)	Overall[a] N=323	Risk factor for impaired male fertility[b]		Leisure physical activity (hours/wk)[c]		
		No N=257	Yes N=66	<2 N=133	2-4 N=112	≥5 N=74
Volume (mL)						
Abstainers	**2.1 (1.6- 2.6)**	1.9 (1.5- 2.4)	1.8 (0.7- 3.5)	**1.7 (1.2- 2.3)**	2.1 (1.2- 3.3)	3.1 (1.8- 4.7)
<1-3	2.7 (2.4- 3.0)	2.4 (2.1- 2.8)	3.3 (2.6- 4.1)	2.6 (2.2- 3.1)	2.4 (1.9- 3.0)	3.1 (2.4- 3.9)
4-7	**3.1 (2.8- 3.5)**	2.8 (2.5- 3.2)	3.7 (3.0- 4.5)	2.5 (2.0- 3.1)	**3.2 (2.6- 3.8)**	3.7 (3.0- 4.4)
≥8	2.7 (2.4- 3.1)	2.7 (2.4- 3.0)	2.4 (1.8- 3.0)	2.5 (2.1- 3.0)	2.3 (1.8- 2.9)	3.7 (2.9- 4.5)
Concentration (mil/mL)						
Abstainers	**36.4 (22.8- 53.1)**	45.0 (28.8- 64.8)	66.4 (22.8- 132.7)	26.9 (12.2- 47.2)	**59.8 (24.4- 110.7)**	29.5 (7.6- 65.7)
<1-3	19.3 (13.2- 26.4)	27.5 (19.6- 36.7)	14.1 (5.6- 26.4)	19.5 (10.4- 31.5)	20.9 (10.4- 35.0)	18.6 (8.2- 33.0)
4-7	**28.6 (21.4- 36.8)**	**42.7 (33.2- 53.4)**	12.3 (4.5- 23.9)	23.9 (12.8- 38.6)	33.8 (21.0- 49.6)	32.2 (20.1- 47.0)
≥8	**34.0 (26.0- 43.1)**	**47.6 (37.1- 59.3)**	22.4 (12.1- 35.8)	**36.2 (23.0- 52.4)**	**40.1 (25.4- 58.1)**	28.7 (15.5- 46.0)
Total sperm count (million)						
Abstainers	74.6 (42.6- 115.4)	93.9 (55.5- 142.2)	127.7 (33.4- 283.1)	46.3 (16.8- 90.6)	128.5 (42.2- 261.7)	78.3 (20.6- 173.2)
<1-3	48.5 (32.3- 68.0)	64.9 (44.7- 88.9)	48.8 (21.9- 86.4)	41.2 (20.3- 69.3)	52.1 (23.0- 92.9)	56.2 (26.8- 96.2)
4-7	**85.1 (64.4- 108.7)**	**117.7 (91.3- 147.3)**	55.5 (26.9- 94.2)	53.8 (27.2- 89.4)	**105.0 (65.5- 153.7)**	**105.1 (69.6- 147.8)**
≥8	**84.1 (63.0- 108.2)**	**121.6 (93.8- 153.0)**	43.0 (20.3- 74.0)	**80.9 (49.5- 120.0)**	86.8 (49.9- 133.9)	92.3 (53.5- 141.5)
Motility (%)						
Abstainers	33.4 (27.2- 40.2)	37.9 (31.8- 44.7)	37.3 (12.8- 74.6)	35.9 (27.8- 45.0)	30.3 (17.4- 46.7)	41.2 (26.3- 59.3)
<1-3	33.4 (29.6- 37.5)	38.7 (34.9- 42.8)	28.9 (18.1- 42.3)	36.8 (30.8- 43.2)	33.6 (26.1- 42.0)	32.5 (25.2- 40.8)
4-7	35.3 (31.6- 39.3)	40.0 (36.4- 43.8)	33.0 (21.6- 46.8)	40.9 (34.1- 48.4)	29.7 (23.4- 36.6)	35.1 (28.6- 42.3)
≥8	34.9 (31.1- 38.9)	41.3 (37.4- 45.4)	28.1 (19.0- 39.0)	41.4 (35.1- 48.2)	28.8 (22.4- 35.9)	34.5 (26.9- 43.1)

bold: p<0.05 as compared to <1-3 units/week of alcohol intake

Adjusted medians were calculated back-transforming adjusted means of square-rooted variables and their corresponding 95% CI.

a Adjusted for age (<35, 35-39, ≥40 years), risk factor for impaired male fertility (no/yes), caffeine (tertiles of daily intake), smoking (never, former, current), leisure physical activity (<2, 2-4, ≥5 hours/week), days of abstinence and daily calories intake(as continuous variables)

b Adjusted for age (<35, 35-39, ≥40 years), caffeine (tertiles of daily intake), smoking (never, former, current), leisure physical activity (<2, 2-4, ≥5 hours/week), days of abstinence and daily calories intake(as continuous variables)

c Adjusted for age (<35, 35-39, ≥40 years), risk factor for impaired male fertility (no/yes), caffeine (tertiles of daily intake), smoking (never, former, current), days of abstinence and daily calories intake(as continuous variables)

6.3.4 Discussion

In this study, moderate alcohol intake appeared associated with increased semen volume, sperm concentration and total sperm count in the whole sample. A similar pattern was observed in subgroups of leisure PA and risk factors for impaired male fertility, although these estimates often lacked statistical significance. Considering that in our study both semen volume and sperm concentration were positively associated to alcohol consumption, total sperm count was positively related to alcohol intake as well. In this analysis, moderate alcohol intake relation with sperm concentration and total count was significant in the entire cohort, in men without risk factors for impaired fertility and in those with low and intermediate level of leisure PA. Actually, this trend was observed in all subgroups considered in our analyses: in some cases, differences were not significant, probably because of the small sample size of each group. A study on 1221 young Danish men [9] found that sperm concentration and total sperm count were negatively associated with increasing habitual alcohol intake. A case-control study [10] concluded that semen volume and sperm concentration were lower in alcoholics compared with abstainers. However, in other studies alcohol did not seem to play any role. Considering the peculiar group of men enrolled from Fertility Clinics, Martini et al. [11] and López Teijón et al. [12] found no association, whereas Goverde et al. (1995) reported that alcohol did not seem associated with poor semen quality, although excessive alcohol consumption may affect an already suboptimal sperm morphology. The inconsistency between our findings and previous studies may be due to the different way of categorization of alcohol consumption and to the different drinking habits of the populations studied. For example, in Martini et al.'s study [11], the comparison was performed between patients who drank any quantity of alcohol and those who did not drink at all in the past six months, therefore the effect of low and high alcohol intake (about 25% of drinkers included in the study consumed more than 28 units/week) could not be discerned. Men included in the study of Jensen et al. [9] also had higher levels of alcohol intake than subjects in our sample: although the negative effects of alcohol intake

were consistently found at high doses (in men who drank more than 25 units/week), sperm parameters of men with 0 and 1-5 units per week were largely similar, if not better in the latter. In our sample, a relatively low alcohol intake was frequent: although 90% of men reported some alcohol consumption, about one third drank no more than 3 units per week and one third no more than 7 units/week. Therefore, the majority had levels of alcohol intake similar to the lowest consumption category of Jensen et al.'s study [9].

A relation between alcohol drinking and semen parameter is biologically plausible. It is known that beer and wine contain polyphenols such as resveratrol or xanthohuminol, which were demonstrated to have a strong therapeutic and cell protective potential [14,15]. Accordingly, it can be suggested that these compounds might stand behind the observed beneficial effects found in this study. On the other hand, different studies experimentally proved that alcohol has a detrimental effect at all levels of the male reproductive system: it interferes with the function of the hypothalamic-pituitary-testicular axis, impairing gonadotropin secretion with consequent decreasing of testosterone levels [10,16]. Likewise, the ratio between free estradiol and free testosterone is modified by alcohol consumption [17]. Studies on heavy alcohol intake [10, 18] related the low semen volume to the testosterone reduction due to alcohol abuse, causing damage to Leydig cells or impairment of hypothalamic-pituitary-gonadal axis. Conversely, Jensen et al. [9] found increasing testosterone levels (total and free) with increasing alcohol intake in young Danish men. With few exceptions, patients in our cohort had a moderate alcohol intake and the detrimental effects, at these levels of consumption, might be balanced by increasing testosterone levels and cell protective potential of resveratrol or xanthohuminol. However, the mechanisms underlying the positive association between moderate alcohol intake and semen parameters, if true, are not easily comprehensible and need to be further investigated. Some limitations and strengths of our study deserve to be commented.

A first important limitation is that our findings should be referred only to patients of infertile couples. The information regarding alcohol use was self-reported, thus some misclassification may have occurred. However, studies investigating reproducibility and validity of self-reported alcohol drinking [19,20], in different populations, found satisfactory correlation coefficients (at least 0.61). Further, in Italy alcohol consumption is socially accepted and recommendations to avoid alcohol for fertility preservation are not routinely advocated during assisted reproduction procedures. On the contrary, underreporting of cigarette consumption was possible, due to a lower social acceptability of smoking [21]. However, an underreporting should tend to reduce the estimated association between alcohol and semen parameters. Among the strengths of this study, we mention the relatively large sample size, which is even more significant as this is a single institution study. Men were interviewed in the same Institution by the same personnel, and participation was practically complete. Moreover, we analyzed the role of alcohol in men with or without other conditions associated with infertility. We also accounted for potential biases, such as age, smoking, BMI, calories intake, days of abstinence, that have been reported to be associated with semen quality [22]. In conclusion, in this cohort of male partners of subfertile couples undergoing ART cycles, alcohol intake was not negatively associated with semen quality. In particular, higher semen volume was observed in men with 4-7 units/week of alcohol intake, and ≥8 units/week were not negatively associated with other seminal variables. Patients drinking 4-7 units per week also showed a higher total sperm count in the subgroup of men with no risk factors for impaired fertility, and in those with 2-4 and ≥5 hours/week of leisure physical activity.

6.3.5 Conclusion

Considering the high proportion of moderate drinkers included in our population, we could not analyze the role of heavy or binge drinking, which are consistently associated to detrimental effects on semen quality. Considering that reassuring results of our study were related to moderate intake, all men undergoing assisted

reproduction should be advised to limit alcohol consumption. As this study has not addressed all concerns regarding the effect of male drinking on reproduction and fertility, other domains of reproductive outcomes need further investigation.

6.4 Second Objective

To analyze the role of Mediterranean Diet in females and IVF outcomes.

6.4.1 Subjects

From the database presented in paragraph 6.1 we analyzed the data obtained from the female partner. The overall participation rate was close to 95%. A woman was considered a smoker if she had smoked more than one cigarette/day for at least one year; an ex-smoker if she had smoked more than one cigarette/day for at least one year, but had stopped more than one year before the interview, and a non-smoker if she had never smoked more than one cigarette/day.

Adherence to Mediterranean Diet

The adherence to the Mediterranean Diet was assessed through an a priori score (Mediterranean Diet Score, MDS), developed by Trichopoulou and colleagues [23], modified for cross-sectional design. It included nine components of diet: cereals (pasta, bread and potatoes), legumes, vegetables, fruits, fish, monounsaturated fatty acids (MUFA)/saturated fatty acids (SFA) ratio, meat (and meat products), dairy products and alcohol intake. For each score component and for each study subject, a value of 0 or 1 was attributed: for components that are frequently consumed in the traditional Mediterranean Diet (i.e., cereals, legumes, vegetables, fruit, fish and high MUFA/SFA ratio), subjects were assigned a value of 1 if they had a consumption above or equal to the study-specific median among the whole sample, and 0 otherwise; for components less frequently consumed (i.e., meat and dairy products), participants with a consumption lower than the study-specific median were assigned a value of 1 and 0 otherwise. For alcohol intake, women consuming 10 g to less than 50 g of ethanol per day were attributed 1 point, and 0 otherwise. The MDS was calculated by adding up the points for each of the nine individual components; thus, it varied between 0 and 9, the higher the score the stronger the adherence to Mediterranean Diet.

Protocol of stimulation

Patients were managed according to a standardized clinical protocol as reported in details elsewhere [24]. Briefly, protocol of stimulation and drugs dosages were decided based on clinical characteristics and biomarkers of ovarian reserve. In case of hypo-response or abnormal follicular growth, the cycle could be canceled before ovum pick up. A freeze all strategy was conversely preferred in case of hyper-response. Oocyte retrieval was performed 36 h after ovulation triggering and embryo transfer was generally performed two to five days after oocyte insemination according to embryo quantity and quality. However, embryo transfer was postponed through embryo vitrification in the following conditions: 1) if the number of retrieved oocytes exceeded 15 or if serum estradiol level exceeded 4000 pg/ml in order to reduce the incidence of Ovarian Hyper-stimulation Syndrome (OHSS); 2) if serum progesterone exceeded1500 pg/ml at the time of ovulation triggering. Viable non-replaced embryos were vitrified mostly at the blastocyst stage. Women with frozen embryos were scheduled for natural cycle embryo transfer if they referred regular menstrual cycles and a mean cycle length between 24 and 35 days. Embryo transfer was performed 4–6 days after LH surge (detected with the use of urinary sticks) according to the embryo age. No luteal phase support was given. Hormone replacement treatment was prescribed if women had irregular menstrual cycles or if the monitoring of the natural cycle failed. Couples underwent ART with conventional IVF or ICSI as clinically indicated.

Serum Human chorionic gonadotropin (hCG) assessment to detect pregnancy was performed +14/16 days after ovulation triggering or LH surge. Women with positive hCG values underwent a transvaginal sonography three weeks later. Clinical pregnancy was defined as the presence of at least one intrauterine gestational sac. All clinical information (including infertility diagnoses) was collected from medical records.

6.4.2 Statistical analysis

Clinical pregnancy was considered the main objective of the study. Considering a 30% of pregnancy rate per cycle, as usual in our Fertility Centre, this study was powered to detect a 1.5 increase of risk in the highest tertile of intake as compared to the lowest (α=0.05, β=0.80).

Multiple outcomes were considered in this analysis: 1. Number of retrieved good quality oocytes; 2. Embryo transfer; 3. Clinical pregnancy; 4: Live birth. Categorical variables were described as frequency (N) and percentage (%) and compared using the Pearson or Mantel-Haenzsel (MH) chi-square, as appropriate. Continuous variables were described as mean and standard deviation (SD) if normally distributed, or median and interquartile range (IQR) if not normally distributed, and analyzed using analysis of variance and Kruskal-Wallis test respectively. In order to perform a multivariate analysis including potential confounders, non-normal (skewed) distributed numbers of good quality oocytes were square-root transformed and included in a general linear model. Adjusted medians and 95% confidence interval (CI) were calculated back-transforming the adjusted means and their 95% CIs. In the model, we included as potential confounders variables associated to MDS or number of good quality oocytes at univariate analysis.

We estimated relative risks (RRs) of each outcome and corresponding 95% CIs in categories of MDS (approximate tertiles) in the year before the interview. To account for potential confounders, we included terms for variables that were associated with MDS and/or with at least one IVF outcome, in the multiple log-binomial regression model (as indicated in table footnotes).

All the analyses were performed using the SAS software, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

6.4.3 Results

During the study period, out of 501 eligible women, 27 (5.4%) did not provide complete information about their diet and were excluded. Comparing women included and excluded from the

present analysis, we did not observe any significant difference in term of socio-demographic characteristics and ART outcomes. In the remaining 474 included women, mean age was 36.6 years (standard deviation, SD, 3.6, range 27-45) and mean body mass index (BMI) was 22.3 kg/m^2 (SD 4.0, range 17.0-42.0). Twenty-six women were obese (BMI > 30 kg/m^2).

Table 4 shows the characteristics of considered women according to adherence to MDS. No significant association was observed between MDS and age, BMI, education, occupational physical activity, cause of infertility, and previous ART cycles. MDS was related to daily calories intake and leisure physical activity.

Of the 474 cycles, 414 (87.3%) resulted in embryo transfer, 150 (31.6%) in clinical pregnancies, 117 (24.7%) in live births. Out of 33 clinical pregnancies not resulting in live birth, 32 ended in miscarriage and one in induced abortion. Age was the main risk factor for IVF failure: as compared to women aged less than 35, RR for not achieving embryo transfer was 2.09 (95% CI 1.03-4.22) for women aged 35-39 and 2.40 (95% CI 1.12-5.13) for those aged ≥40 years. The corresponding figures were 1.19 (95% CI 1.00-1.40) and 1.41 (95% CI 1.18-1.67) for clinical pregnancy; 1.20 (95% CI 1.03-1.39) and 1.37 (95% CI 1.18-1.59) for live birth. After adjusting for age, previous IVF cycles were associated to higher risk of unavailability of embryos for transfer (RR 1.79, 95% CI 1.05-3.03) and exercising for ≥5 hour per week to higher risk of non-achieving clinical pregnancy (RR 1.25, 95% CI 1.05-1.49) as compared to <2 hours per week. Table 5 shows the relation between MDS level and clinical results. Numbers of good quality oocytes were not significantly different among classes of MDS.

Adjusted RRs for IVF failure at each step (embryo transfer, clinical pregnancy, live birth) were calculated including age, leisure PA, daily calories intake, and previous IVF cycles. Analyses were also performed in strata of age (Table 5), previous ART cycles, and cause for infertility (data not shown): findings consistently showed that MDS was not significantly associated with IVF outcomes, although a slightly lower risk of adverse outcome was consistently observed in the MDS

4-5 group. The only exception was a significant higher number of good quality oocytes (4.9 vs 3.8 in MDS 0-3, p=0.024) and a lower risk of not achieving clinical pregnancy in women with an intermediate level of MDS (Adjusted RR 0.83, 95% CI 0.70-0.99, p=0.04). Finally, we analyzed the association between food groups included in the MDS and ART outcomes: we did not find any significant association with components of MDS (data not shown).

Table 4. Demographic characteristics of 474 women, according to Mediterranean Diet Score (MDS).

	MDS								P*
	All		0-3		4-5		6-9		
	N	%	N=132	27.8%	N=200	42.2%	N=142	30.0%	
Age (years)									
<35	132	27.8	40	30.3	62	31.0	30	21.1	
35-39	232	49.0	58	43.9	103	51.5	71	50.0	0.14
≥40	110	23.2	34	25.8	35	17.5	41	28.9	
College degree	245	51.7	60	45.4	111	55.5	74	52.1	0.26
Cause of infertility									
Male factor only	124	26.2	36	27.3	57	28.5	31	21.8	
Endometriosis	101	21.3	32	24.2	33	16.5	36	25.4	
Tubal	52	11.0	19	14.4	21	10.5	12	8.4	
Low ovarian reserve	91	19.2	20	15.2	37	18.5	34	24.0	0.32
Ovulatory	20	4.2	5	3.8	8	4.0	7	4.9	
Unexplained	86	18.1	20	15.2	44	22.0	22	15.5	
BMI									
≤24.9	380	80.2	106	80.3	156	78.0	118	83.1	
25.0-29.9	62	13.1	16	12.1	30	15.0	16	11.3	0.32
≥30.0	29	6.1	10	7.6	13	6.5	6	4.2	
Occupational physical activity									
Heavy/moderate	131	27.6	40	30.3	56	28.0	35	24.6	
Mainly standing									

Mainly sitting	104 237	21.9 50.0	23 68	17.4 51.5	43 100	21.5 50.0	38 69	26.8 48.6	0.51
Leisure physical activity <2 h/wk 2-4 ≥5	 255 169 49	 53.8 35.6 10.3	 79 41 12	 59.8 31.1 9.1	 110 73 16	 55.0 36.5 8.0	 66 55 21	 46.7 38.7 14.8	0.02
Previous ART cycle	275	58.0	75	56.8	111	55.5	89	62.7	0.28
Mean calories (Kcal/day), median (IQR)	1749	446	1624	358	1752	480	1860	441	<0.0001
FSH, median (IQR)	7.3	5.8-8.9	7.2	5.7-8.7	7.3	5.7-8.6	7.3	5.9-9.6	0.65
AMH, median (IQR)	1.6	0.8-3.2	1.7	0.8-3.0	1.9	0.9-3.5	1.4	0.8-2.8	0.14
Outcomes									
Oocytes, median (IQR)	5.0	3.0-8.0	4.0	3.0-7.0	6.0	3.0-8.5	5.0	3.0-8.0	0.09
Implantation	414	87.3	114	86.4	178	89.0	122	85.9	0.89
Clinical pregnancy	151	31.9	37	28.0	74	37.0	40	28.4	0.98
Live birth	117	24.7	33	25.0	51	25.5	33	23.4	0.73

Sometimes the sums do not add up to the total because of missing values

* Cochrane-Mantel-Haenszel chi-square

BMI: body mass index; ART: assisted re production techniques; IQR: interquartile range; FSH: follicle-stimulating hormone; AMH: anti-mullerian hormone

Table 5. Relative risks for failure in clinical outcomes of ART, in 474 women according to Mediterranean Diet Score.

	N	Number of high quality oocytes		Implantation		ARR (95% CI)	Clinical pregnancy		ARR (95% CI)	Live birth		ARR (95% CI)
MDS		median	95% CI	Yes	%		Yes	%		Yes	%	
Overall												
1-3	132	4.4	3.7-5.0	114	86.4	1	37	28.0	1	33	25.0	1
4-5	200	5.1	4.5-5.7	178	89.0	0.81 (0.45-1.46)	74	37.0	0.94 (0.84-1.04)	51	25.5	1.00 (0.90-1.10)
6-9	142	4.8	4.2-5.4	122	85.9	0.87 (0.48-1.57)	40	28.2	0.97 (0.86-1.09)	33	23.2	0.99 (0.88-1.10)
Trend						χ^2=0.19, p=0.66			χ^2=0.24, p=0.63			χ^2=0.06, p=0.81
≤35 years old												
1-3	51	5.7	4.7-6.8	48	94.1	n.e.	20	39.2	1	19	37.2	1
4-5	79	6.0	5.1-6.9	72	91.1	n.e.	34	43.0	0.96 (0.79-1.16)	27	34.2	1.01 (0.82-1.25)
6-9	44	5.5	4.5-6.7	40	90.9	n.e.	17	38.6	0.98 (0.79-1.22)	15	34.1	1.00 (0.79-1.28)
Trend									χ^2=0.03, p=0.86			χ^2=0.00, p=0.97
>35 years old												
1-3	81	3.8	3.0-4.6	66	81.5	1	17	21.0	1	14	17.3	1
4-5	121	4.9	4.2-5.7	106	87.6	0.66 (0.33-1.28)	40	33.1	0.83 (0.70-0.99)	24	19.8	0.96 (0.84-1.10)
6-9	98	4.5	3.7-5.3	82	83.7	0.78 (0.41-1.50)	23	23.5	0.93 (0.78-1.10)	18	18.4	0.97 (0.84-1.12)
Trend						χ^2=0.44, p=0.51			χ^2=0.46, p=0.50			χ^2=0.12, p=0.73

ARR: adjusted relative risk; CI: confidence interval

The final model included age class (<35, 35-39, ≥40, when appropriate), previous ART cycles (no, yes), leisure physical activity (<2, 2-4, ≥5 hours/week), daily calories intake (Kcal, continuous variable). n.e.: not evaluable

6.4.4 Discussion

In our sample of 474 women, adherence to Mediterranean diet, estimated as Mediterranean Diet Score, was not associated to successful IVF outcomes. Moreover, no significant relation was observed between food groups and number of good quality oocytes, availability of embryos for transfer, clinical pregnancy and live birth. We only found a significant slight positive effect of intermediate MDS on good quality oocytes number and achieving clinical pregnancy: however, this did not result in a higher proportion of live birth.

Most published studies show a positive relation between healthy diet and successful ART cycles.

In 161 couples undergoing ART treatment in a Fertility Clinic in Rotterdam (The Netherlands) between 2004 and 2007, Vujkovic et al. [25] used a 104-items questionnaire to identify two dietary patterns: a "health-conscious-low processed" pattern, characterized by high intakes of fruits, vegetables, fish, and whole grains and low intakes of processed products (snacks, meats, and mayonnaise), and a Mediterranean one, with high intakes of vegetable oils, vegetables, fish, and legumes and low intakes of snacks. Both patterns were associated with red blood cell folates, but only the Mediterranean diet increased the probability of pregnancy, with an odds ratio of 1.4 (95% CI 1.0-1.9).

In a later study, 199 couples were enrolled between 2007 and 2010 in Rotterdam (The Netherlands), if candidate for their first ART cycle [26]. They were asked about their dietary habits using six questions regarding the intake of six main food groups, that is, fruits, vegetables, meat, fish, whole wheat products and fats. Based on this information, an estimate of nutritional habits was calculated. A higher Preconception Dietary Risk score (PDR) indicated a better dietary quality. Clinical pregnancy, that represented the primary outcome, was achieved in 26% of couples. After adjusting for woman's age and smoking habits, partner's PDR, BMI of the couple, and treatment indication, Twigt et al. found that PDR of the woman and the chance of ongoing pregnancy were positively associated (odds ratio 1.6, 95% CI 1.1–2.2).

More recently (2013-2016), in a sample of 244 non-obese women undergoing their first IVF treatment, Karayiannis et al. [27] calculated a Mediterranean diet Score (MedDietScore) ranging between 0 and 55, with higher score indicating greater adherence: in tertiles of MedDietScore, they did not observe a relation with intermediate ART outcomes (oocytes yield, fertilitzation rate and measures of embryo quality). On the contrary, clinical outcomes such as pregnancy (50% vs 29%) and live birth (49% vs 27%) were significantly higher in the third tertile, as compared to the lower. The association was still present after accounting for age, ovarian hyper-stimulation protocol, BMI, physical activity, anxiety levels, infertility diagnosis, caloric intake and supplements use.

Our study only observed a protective effect of intermediate adherence score to Mediterranean diet on oocyte number and clinical pregnancy, but no effect on live birth.

It has been hypothesized that the beneficial effects of a Mediterranean diet are mainly due to the high intake of vegetable oil [28], containing nutrients such as linoleic acid, and of fruits and vegetables in general, although this association was not observed in a "health-conscious" diet [25] not including the consumption of vegetable oil. On the other hand, it has also been suggested that the intake of high-residue pesticide vegetables may contrast the positive effect of an otherwise healthy diet. It has to be noted, however, that this confounder is unlikely in our study: the 2017 report of Italian Health Ministry [29] on control of pesticides residues found that less than 1% of horticultural products contained residues over the regulatory limits, whereas 54% did not contain residues at all and 45% was under the regulatory limits.

The inconsistence of our findings with those of Karayiannis et al. [27], despite similarities between considered food groups, may also be due to the fact that our questionnaire did not specify whether cereals (pasta, bread, rice) were consumed as whole or refined. Given that the beneficial effect seemed limited to whole grains intake [30], it is possible that cereals considered in MDS were at least partially refined, thus contributing to increase the glycemic load, associated with higher risk of

ovulatory infertility [28]. Indeed, the whole cereals intake in Italy is among the lowest in Europe [31].

Potential limitations should be considered. All information was self-reported by the woman, so some misclassification could have occurred. However, in Italy, dietary counseling is not routinely advocated by gynecologists before IVF (including in the Center where the study was run), so underreporting of unhealthy diet should be unlikely. Other sources of bias, including selection or confounding factors, are also unlikely to have produced marked effects, especially considering that cases and controls were interviewed in the same institution and that participation was practically complete. With regard to other biases, we analyzed information on nutritional status, and their inclusion into the model did not change the estimated RR. Further, the questionnaire was satisfactorily reproducible [32]. Another potential limitation is study power. For example, with our data, comparison between the lowest and the highest tertile could identify a risk of pregnancy loss of about 1.8.

6.4.5 Conclusion

Our study does not show any clear association between adherence to a Mediterranean diet and oocyte quality or successful IVF. However, at the light of previous research and of general benefits due to a healthy diet (including those on obstetrics outcome), women candidates to ART should be counseled about their dietary habits.

6.5 Third Objective

To analyze the relationship between women lifestyle habits, modern alcohol and coffee consumption and smoking and outcomes of assisted reproduction.

6.5.1 Subjects

From the database presented in paragraph 6.1 we analyzed the data obtained from the female partner. Patients were managed according to a standardized clinical protocol as reported in paragraph 6.5.1.

6.5.2 Statistical analysis

Clinical pregnancy was considered the main objective of the study. Considering a 30% of pregnancy rate per cycle, as usual in our Fertility Centre, this study was powered to detect a 1.5 increase of risk in the highest tertile of intake as compared to the lowest (α=0.05, β=0.80).

Multiple outcomes were considered in this analysis: 1. Number of retrieved good quality oocytes; 2. Implantation; 3. Clinical pregnancy; 4: Live birth. Categorical variables were described as frequency (N) and percentage (%) and compared using the Pearson or Mantel-Haenzsel (MH) chi-square, as appropriate. Continuous variables were described as mean and standard deviation (SD) if normally distributed, or median and interquartile range (IQR) if not normally distributed, and analyzed using analysis of variance and Kruskal-Wallis test respectively. In order to perform a multivariate analysis including potential confounders, non-normal (skewed) distributed numbers of good quality oocytes were square-root transformed and included in a general linear model. Adjusted medians and 95% confidence interval (CI) were calculated back-transforming the adjusted means and their 95% CIs. In the model, we included as potential confounders variables associated to alcohol intake, current smoking, leisure PA or number of good quality oocytes at univariate analysis.

We estimated relative risks (RRs) of each outcome and corresponding 95% confidence intervals (CIs) in categories of alcohol intake (approximate tertiles), current smoking (no, ≤ 5, >5 cigarettes/day) and leisure PA (<2, 2-4, ≥5 hours/week) in the year before the interview. To account for potential confounders, we included terms for variables, that were associated with these modifiable lifestyles, and/or with at least one ART outcome, in the multiple log-binomial regression model (as indicated in table footnotes). Terms for interaction were tested: alcohol x leisure PA, alcohol x smoking, leisure PA x smoking, alcohol x smoking x leisure PA.

All the analyses were performed using the SAS software, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

6.5.3 Results

From September 2014 to December 2016, out of 501 women undergoing ART cycle, 9 (1.8%) did not provide complete information about their lifestyle, or were lost to follow-up, and were excluded from this analysis. Analysis was then performed on 492 ART cycle outcomes from 492 women.

Mean age was 36.6 years (standard deviation, SD, 3.6 range 27-45) and mean body mass index (BMI) was 22.3 kg/m^2 (SD 3.9, range 16.4-41.7). Thirty women (6.1%) were obese (BMI ≥ 30.0 kg/m^2).

The characteristics of women according to alcohol, smoking habits and PA are shown in Table 6.

The median (range) pre-treatment alcohol intake was 1.9 (0.0–27.3) g/day: 140 (28.5%) women did not drink at all, 117 (23.8%) were in the first (0.01-2.27 g/day), 122 (24.8%) in the second (2.28-5.74 g/day) and 113 (23.0%) in the third tertile of alcohol consumption (≥ 5.75 g/ day); 28 (5.7%) women drank at least one unit per day (highest intake 27.35 g/day).

Higher pre-treatment alcohol intake was associated with age (MH chi-square 4.99, p=0.026), college degree (MH chi-square 20.89, p<0.0001), lower BMI (MH chi-square 16.99, p<0.0001), and occupational PA (MH chi-square4.52, p=0.033).

Currently smoking was inversely associated with college degree (MH chi-square 13.33, p=0.0003) and previous ART cycles (MH chi-square 6.75, p=0.009).

Only 16 women (3.2%) exercised more than 7 hours per week, so we merged the two categories 5-7 and >7 hours/week. Leisure PA was associated with college degree (MH chi-square 4.84, p=0.028) and inversely with daily calories intake (MH chi-square 11.91, p=0.0006).

Of the 492 initiated cycles, 427 (86.8%) resulted in embryo transfer, 157 (31.9%) in clinical pregnancy, 121 (24.6%) in live births. Out of 36 clinical pregnancies not resulting in live birth, 34 ended with miscarriage, one with an induced abortion, and one was extrauterine.

Age was the main risk factor for ART failure. The median of good quality oocytes was 6 (IQR 4-9) in women <35 years old, 5 (IQR 3-8) in 35-39, 3 (IQR 2-6) in women aged ≥ 40 years (p<0.0001). As expected, women with diagnosis of low ovarian reserve had significantly lower number of good oocytes (3, IQR 2-5 vs 6, IQR 3-9, p<0.0001). No association was observed at univariate analysis with alcohol intake, current smoking or leisure PA.

As compared to women aged < 35, RR for not achieving embryo transfer was 2.01 (95% CI 1.03-3.93) for women aged 35-39 and 2.29 (95% CI 1.11-4.72) for those aged ≥40 years. The corresponding figures were 1.16 (95% CI 0.98-1.37) and 1.40 (95% CI 1.18-1.66) for clinical pregnancy; 1.19 (95% CI 1.03-1.37) and 1.37 (95% CI 1.19-1.78) for live birth.

At univariate analysis, leisure PA ≥ 5 hours/week was significantly associated with higher risk of not achieving clinical pregnancy (RR 1.25, 95% CI 1.08-1.45), whereas no relationship was observed with implantation and live birth. Alcohol intake and current smoking were not significantly associated with implantation, clinical pregnancy, and live birth.

Table 7 shows the relation between alcohol intake, current smoking, leisure PA and clinical results, accounting for potential confounders. Adjusted medians of good quality oocytes were not significantly different among classes of alcohol intake, current smoking, and leisure PA. Adjusted RRs (ARR) for ART failure at each step (implantation, clinical pregnancy, live birth) were

calculated including in the model age, college degree, BMI, occupational PA, previous ART cycles and calories intake. No significant association was observed between smoking, alcohol intake and leisure PA, thus they were not mutually adjusted. Terms for interaction between smoking, alcohol intake and leisure PA did not show any significance and were excluded from the final models.

High intakes were also investigated. In this sample, 28 (5.7%) women drank at least 1 alcohol unit per day. Although the RRs for implantation failure was higher, it was not significant as compared to abstainers, and no effect was observed on clinical pregnancy or live birth.

Six women smoked at least 20 cigarettes per day during the year before ART procedure: number of retrieved good quality ooctyes was lower (borderline significance) and ARR for implantation was significantly higher. Lastly, we performed an analysis of caffeine intake in relation with ART outcomes, confirming the previous finding observed in a sample from this population (24): caffeine intake by women in the year prior to the ART procedure was not associated with negative ART outcomes (data not shown).

Table 6. Demographic characteristics of 492 women, according to alcohol intake, caffeine intake and smoking habits.

	Alcohol intake (g/day)								**Current smoking**						**Leisure Physical Activity**					
	Abstainers 0		**1st tertile 0.01-2.27**		**2nd tertile 2.28-5.74**		**3rd tertile ≥5.75**		**No**		**≤5 cig/day**		**>5 cig/day**		**< 2 hours/week**		**2-4 hours/week**		**≥5 hours/week**	
	N=140	**28.5%**	**N=117**	**23.8%**	**N=122**	**24.8%**	**N=113**	**23.0%**	**N=272**	**55.2%**	**N=91**	**18.5%**	**N=130**	**26.4%**	**N=256**	**54.1%**	**N=175**	**35.6%**	**N=51**	**10.4%**
Age (years)																				
<35	**46**	**32.9**	**38**	**32.5**	**25**	**20.5**	**27**	**23.9**	108	28.9	12	29.6	16	33.3	78	29.3	47	26.9	11	21.6
35-39	**70**	**50.0**	**50**	**42.7**	**64**	**52.5**	**59**	**52.2**	197	49.0	22	52.4	24	50.0	123	46.2	89	50.9	31	60.8
≥40	**24**	**17.1**	**29**	**24.8**	**33**	**27.0**	**27**	**23.9**	97	24.1	8	19.0	8	16.7	65	24.4	39	22.3	9	17.6
College degree	**49**	**35.0**	**64**	**54.7**	**70**	**57.4**	**72**	**63.7**	**220**	**54.7**	**24**	**57.1**	**11**	**22.9**	**120**	**45.1**	**110**	**62.9**	**25**	**49.0**
Cause of infertility																				
Male factor	37	26.4	35	29.9	33	27.0	23	20.4	106	26.4	11	26.2	11	22.9	68	25.6	47	26.9	13	25.5
only	26	18.6	25	21.4	24	19.7	22	19.5	77	19.2	8	19.0	12	25.0	51	19.2	36	20.6	10	19.6
Low ovarian	27	19.3	21	18.0	28	23.0	27	23.9	84	20.9	7	16.7	12	25.0	64	24.1	29	16.6	10	19.6
reserve	10	7.1	5	4.3	4	3.3	0	0	19	4.7	0	0	0	0	12	4.5	5	2.9	2	3.9
Endometriosis	11	7.9	17	14.5	11	9.0	15	13.3	38	9.4	8	19.0	8	16.7	26	9.8	21	12.0	7	13.7
Ovulatory	29	20.7	14	12.0	22	18.0	26	23.0	78	19.4	8	19.0	5	10.4	45	16.9	37	21.1	9	17.6
Tubal																				
Unexplained																				
BMI (Kg/m^{-2})																				
<18.5	**12**	**8.6**	**11**	**9.4**	**12**	**9.8**	**12**	**10.6**	39	9.7	6	14.3	2	4.2	28	10.5	15	8.6	4	7.8
18.5-24.9	**98**	**70.0**	**80**	**68.4**	**97**	**79.5**	**94**	**83.2**	302	75.1	30	71.4	37	77.1	190	71.4	137	78.3	42	82.4
25.0-29.9																				

≥30.0	**16**	**11.4**	**15**	**12.8**	**8**	**6.6**	**7**	**6.2**	39	9.7	3	7.1	4	8.3	29	10.9	13	7.4	4	7.8
	14	**10.0**	**11**	**9.4**	**5**	**4.1**	**0**	**0**	22	5.5	3	7.1	5	10.4	19	7.1	10	5.7	1	2.0
Occupational PA																				
Heavy	**50**	**35.7**	**27**	**23.3**	**33**	**27.1**	**28**	**24.8**	112	28.0	12	28.6	14	29.2	83	31.3	40	22.9	15	30.0
Mainly standing	**32**	**22.9**	**30**	**25.9**	**25**	**20.5**	**19**	**16.8**	84	21.0	7	16.7	15	31.2	60	22.6	32	18.3	14	28.0
Mainly sitting	**58**	**41.4**	**59**	**50.9**	**63**	**51.6**	**66**	**58.4**	104	51.0	23	54.8	19	39.6	122	46.1	103	58.9	21	42.0
Previous ART cycle	84	60.0	67	57.3	69	56.6	65	57.5	**243**	**60.4**	**22**	**52.4**	**20**	**41.7**	155	58.3	98	56.0	32	62.8
Mean calories (Kcal/day), mean (SD)	1711	(458**)**	1705	(401**)**	1782	(415**)**	1812	(493**)**	1747	(444)	1748	(343)	1779	(526)	**1788**	**(444)**	**1739**	**(425)**	**1597**	**(481)**

Bold: p<0.05; sometimes the sums do not add up to the total because of missing values

ART: assisted reproduction technique; PA: physical activity; SD: standard deviation

Table 7. Relative risks for failure in clinical outcomes of ART, in 492 women according to alcohol, current smoking and leisure physical activity

	N	Number of high quality oocytes (adjusted median, 95% CI)	Implantation Yes		ARR (95% CI)	Clinical pregnancy Yes		ARR (95% CI)	Live birth Yes		ARR (95% CI)
Alcohol intake			N	%		N	%		N	%	
Abstainers	140	4.1 (3.5-4.8)	16	11.4	1	92	65.7	1	102	72.9	1
1st tertile	117	3.9 (3.2-4.5)	14	12.0	1.08 (0.55-2.12)	72	61.5	0.95 (0.80-1.12)	84	71.8	1.00 (0.89-1.13)
2nd tertile	122	3.9 (3.2-4.5)	16	13.1	1.02 (0.53-1.96)	91	74.6	1.10 (0.93-1.29)	98	80.3	1.07 (0.95-1.21)
3rd tertile	113	4.1 (3.5-4.8)	19	16.8	1.32 (0.71-2.47)	80	70.8	1.05 (0.89-1.23)	87	77.0	1.05 (0.92-1.19)
≥1 drink/day	28	4.9 (3.6-6.5)	5	17.8	1.34 (0.53-3.40)	19	67.9	1.05 (0.78-1.41)	20	71.4	1.01 (0.76-1.34)
Current smoking											
No	402	4.0 (3.6-8.3)	52	12.9	1	271	67.4	1	303	75.4	1
≤ 5 cig/day	42	3.6 (2.7-8.8)	8	19.0	1.64 (0.83-1.22)	31	73.8	1.12 (0.88-1.41)	33	78.6	1.06 (0.89-1.27)
> 5 cig/day	48	3.8 (2.9-8.9)	5	10.4	1.04 (0.43-2.51)	33	68.8	1.06 (0.86-1.30)	35	72.9	0.99 (0.85-1.16)
≥20 cig/day	6	2.3 (0.7-9.2)*	2	33.3	5.58 (1.65-19.0)	5	83.3	1.10 (0.74-1.64)	5	83.3	1.09 (0.68-1.76)
Leisure PA											
<2 h/wk	266	4.1 (3.6-8.4)	35	13.2	1	179	67.3	1	199	74.8	1
2-4	175	3.9 (3.4-8.3)	20	11.4	0.85 (0.50-1.42)	113	64.6	0.99 (0.92-1.08)	128	73.1	0.99 (0.91-1.08)
≥5	51	3.8 (2.9-9.0)	10	19.6	1.55 (0.81-2.45)	43	84.3	1.10 (0.97-1.25)	44	86.3	1.09 (0.95-1.25)

ARR: adjusted relative risk; CI: confidence interval; PA: physical activity; the final model included age class, college degree, BMI class (<25.0, ≥25.0), occupational PA, previous ART cycles, calories intake

* p=0.057 as compared to non current smokers

6.5.4 Discussion

Potential limitations should be considered. All information on smoking and drinking was self-reported by women, so some underestimates could have occurred. However, in Italy, alcohol consumption is socially accepted and recommendations to avoid alcohol to protect fertility have not received widespread attention and are not routinely advocated by gynecologists before IVF.

Other sources of bias, including selection or confounding factors, are also unlikely to have produced marked effects, especially considering that all women were interviewed in the same institution and that participation was practically complete. Moreover, we analyzed information on nutritional status, and their inclusion into the model did not change the estimated RRs. The questionnaire was satisfactorily reproducible [32]. Another potential limitation is study power. Comparing the clinical pregnancy percentage in women who did not drink at all and those in the 3rd tertile of intake, the power of detecting a significant difference was about 13%. Using our data, with 30% prevalence of abstainers, we could identify a RR of not achieving clinical pregnancy of 1.8 for drinkers.

Lastly, this study only included women presenting for ART, thus the findings are not generalizable to the wider population.

Alcohol

The role of alcohol intake on spontaneous fertility has been analyzed during the last 4-5 decades and it has been associated with decreased fertility and decreased chance of conception [33,34]. A recent meta-analysis of observational studies (cohort and case-control studies) that investigated the relation between female alcohol consumption and fecundity, including about 100,000 women, suggested that female alcohol consumption was associated with reduced fecundability [35]. In biological terms, alcohol may lower fertility affecting endogenous hormone levels [36] and embryo quality [37]. In principle, to evaluate the association between alcohol intake and fertility, ART procedures may represent a more easily evaluable model. The role of alcohol intake on ART

success rate has been analyzed in some studies showing inconsistent results [38,39]. Part of these differences may be due to the high heterogeneity between studies, in particular in terms of prevalence of intake. For example, in Boston, US, 30% of women reported >12 g/day of alcohol intake, while in the present study this proportion was 5.7%; consequently, the Authors could find an increased risk of adverse outcome, whereas they also found no association between low to moderate alcohol consumption [38]. Along this line, we did not find any association between alcohol intake and ART outcomes, but we were not able to analyze the effect of high alcohol intake. Taken together, data derived from literature suggest a possible but still extremely controversial relationship between alcohol consumption and IVF results, maybe with differences in effects according to the different pattern of exposure.

Smoking

We did not find any association between smoking and clinical pregnancy or live birth.

Since it appears to have a detrimental effect on spontaneous fertility [40], it has been suggested that cigarette smoking may affect IVF outcomes as well.

However, individual studies available on this issue do not always support a significant association between smoking and IVF success or oocyte quality. For example, in a study conducted in Australia [41] the mean number of oocytes retrieved from women undergoing IVF treatment did not significantly differ between regular smokers (11.1 oocytes retrieved, SD 6.5), ex-smokers (11.8, SD 10.1) or non-smokers (11.2, SD 7.6) which indicated that smoking might not influence oocytes production. Interestingly, the same analysis also showed that fertilization rates were not influenced by current female smoking status. However, fertilization rate did decrease as female years of smoking increased ($p < 0.001$). The same evidence emerged from a large Dutch nationwide retrospective analysis conducted on 8457 patients [42]: no significant difference was observed in

the mean number of oocytes retrieved between non-smokers and smoking women, yet there were significantly lower clinical pregnancy and live birth rates for smoking patients.

These findings regarding clinical pregnancies and live birth rate were further confirmed by more recent meta-analysis and reviews [43,44].

In our sample, good quality oocytes number was significantly lower in women who had smoked 20 or more cigarettes per day in the year before ART procedure, and risk of implantation failure significantly higher than in non-smokers. However, only six women had such a high level of smoking, thus our estimates should be considered with caution. No significant effect was observed on clinical pregnancy and live birth. The campaign against smoking before and during pregnancy has had generally success worldwide, thus heavy smoking is very uncommon not only among pregnant patients but also among women seeking pregnancy.

Leisure Physical Activity

At univariate analysis, an increased risk of implantation failure was suggested in women with ≥5 hours/week of leisure PA, but this result was not confirmed in the multivariable analysis.

Findings from literature are inconsistent. Among 2,232 patients prospectively enrolled before their first IVF cycle, Morris et al. [45] found that women who exercised 4 or more hours per week, for 1-9 years before ART cycle, were more likely to experience an implantation failure (OR 2.0, 95% CI 1.4-3.1) or pregnancy loss (OR 2.0, CI 1.2-3.4) than women who did not report exercise.

On the contrary, in a group of 131 women [46] those who were physically more active during the ART procedure were more likely to have an increased implantation rate and a live birth; none of these women met the criteria for high PA, so that the comparison was done between low and moderate PA. On the same line, an observational study [47] found that a self-reported active lifestyle in the preceding year affected favorably the ART outcome in 121 women, with clinical pregnancy more likely in women with a level above median for each kind of activity: active living

(OR 1.96, 95% CI 1.09-3.50), sports/exercise (OR 1.48, 95% CI 1.02–2.15), and total activity (OR 1.52, 95% CI 1.15–2.01).

Recently, findings from the EARTH Study [48] suggested that time spent in moderate-to-vigorous physical activities before IVF was not associated with probability of implantation, clinical pregnancy or live birth, in 273 women who underwent 427 IVF cycles.

Two studies focused on the effects of PA in obese women. In 2011, Moran et al. reported a small study comparing two groups of obese women, randomized to a lifestyle intervention (n=21), including PA) or standard treatment (N=25), to be ceased before embryo transfer; no difference was observed [49]. A cohort study [50] suggested that in obese infertile patients with regular PA, the chances to obtain a pregnancy and a live birth were higher (RR 3.22, 95% CI 1.53-6.78 and 3.71, 95% CI 1.51-9.11, respectively), in comparison with those without regular PA. In our sample of mostly normal weight women, leisure PA did not significantly affect ART outcomes, although an increased risk of adverse outcomes was suggested in women with the highest level of activity.

6.5.5 Conclusion

Our study did not show an effect of alcohol consumption, current smoking and physical activity level on number of good quality oocytes and success rate after ART procedures.

Considering that reassuring results of our study were related to moderate alcohol intake and cigarette smoking, conservatively, all women seeking pregnancy should be advised to limit or avoid alcohol drinking and, of course, smoking. Moderate physical activity as a part of a healthy lifestyle is also advisable, although current knowledge does not support convincing evidence of a direct beneficial effect.

6.6 References

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CHAPTER 7

Experimental Section: Hospital malnutrition and Resting Energy Expenditure in children

7.1 First Objective: Italian Pediatric Nutrition Survey

The study was designed and coordinated by the Italian Society of Pediatric Gastroenterology and Nutrition (SIGENP). Our center, Pediatric Department, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, participated in the process of data collection and analysis.

7.1.1 Aim

To investigate the prevalence of malnutrition and related nutritional support among hospitalized children in Italy.

7.1.2 Data collection and Methods

The study was designed and coordinated by SIGENP in a crowd-sourcing manner. An open access website (http://nday.biomedia. net) was specifically constructed to collect information on hospital characteristics (institution type, i.e. university or general hospital), location, number of beds; participants were recruited from general pediatrics and all medical pediatric specialties, pediatric surgery and pediatric onco-hematology wards. The Nutrition Survey of 16 April 2015 was advertised through the website. An e-mail was sent to all members of SIGENP and the Italian Society of Pediatrics (SIP), in which participating centers were asked to join for the study and login with given credentials. Starting on 16 April and for the next 48 h the website was open to receive patient data.

Anonymous (each center supplied its own patient data without sharing identity with the coordinating authors) information was gathered on patient's sex, age, weight and length/height, admission diagnosis (Fig. 2); with the adjunction of premature and small for gestational age children), existence of chronic diseases (none or one of 16 choices) and use of nutritional support: ONS, EN or PN. In particular, the participating centers were asked a closed question (Yes or No) for every type of nutritional support. For the positive answer, they were further asked to specify which of the reported choices applied. The latter are: modular (one or two nutrients) or complete ONS, nasogastric, gastrostomy or jejunostomy tube feeding and parenteral nutrition (more than two nutrients given intravenously). Patients may receive more than one type of nutritional support. After excluding 141 patients whose only weight was available (mainly due to inaccurate height/length due to spastic posture), 1790 complete records were obtained for hospitalized patients aged 0-20 years old. Z-scores of anthropometric measurements were calculated with Epi Info 7.1.5 and defined nutritional status using the ASPEN criteria [1]: wasting or acute malnutrition, intended as a low weight-for-height [19], was identified by BMI and Weight-for-Length Z-score (<-1 mild, <-2 moderate, <-3 severe), stunting or chronic malnutrition, defined as low stature for age [19], by Height-for-Age Z-score <-2. Overweight is defined by BMI or Weight-for-Length Z-score > 2. WHO 2006 and CDC (Center for Disease Control) 2000 growth charts were used respectively for children younger and older than two years old [1].

7.1.3 Statistical Analysis

The distribution of data was checked using the Shapiro-Wilkinson test. Given the skewed distribution, continuous data were expressed as median value and IQR while categorical variables were expressed as proportions (percentages). Differences in proportions were tested by chi-squared test. The risk of acute and chronic malnutrition in chronically ill patients was expressed by the risk

ratio. Significance level was fixed at $p<0.05$. Statistical analysis was performed using STATA 11, StataCorp 4905 Lakeway Drive, College Station, Texas 77845 USA. Given the anonymous collection of data, the Ethical Committee of the coordinating center advised that ethical approval was not necessary.

7.1.4 Results

The website registered 2003 accesses of which 1994 were valid records e patients aged more than 20 years and those without available anthropometric measurements were excluded. The data were collected from 14 out of the 20 Italian regions, 73 hospitals (9 children's hospitals, 18 university hospitals and 46 general hospitals) and 101 wards: 84.6% pediatrics, 6.7% pediatric surgery and 8.7% pediatric onco-hematology. 1071 (59.8%) patients come from third level pediatric hospitals and 719 (40.2%) from general hospitals, median age 5.5 years (IQR 10.3), 53.3% males. More than 50% of children (52.9%) were aged 0-6 years, 58.8% of children suffered from chronic diseases. The characteristics of study population are reported in Table 8.

Table 8. Characteristics of study population

Patients (N)	1790
Sex (M/F)	954/836
Age in years (median, IQ range)	5.5 (10.3)
Age groups: N (/%)	
0–2 yrs	505 (28.2%)
2–6 yrs	442 (24.6%)
6–10 yrs	262 (14.6%)
10–14 yrs	311 (17.4%)
14–20 yrs	270 (15.1%)
Chronic disease: N (%)	1052 (58.8%)

Wasting or acute malnutrition identified by BMI or Weight for Length (WfL) was detected in 28.7% of the patients in the following forms: 15.5% of patients were classified as mild or at risk of malnutrition (Z-score between - 1 and - 2), 6.5% as moderate malnutrition (z-score between - 2 and

- 3) and 6.7% as severe malnutrition (Z-score < - 3). Stunting or chronic malnutrition, identified by Height or Length Z-score, was detected in 17.3% of patients of whom 9.1% showed moderate malnutrition (Z-score between - 2 and - 3) and 8.2% severe malnutrition (z-score < - 3). 111 out of 309 (36%) patients with stunting showed coexisting wasting (BMI or weight for length Z-score < - 2). Both wasting and stunting were more frequent in the youngest patients of study population: 146/514 (46%) and 195/309 (63%) respectively in acute and chronic malnourished patients were aged 0-6 years. For detailed information about patients' age and malnutrition rate see Table 9.

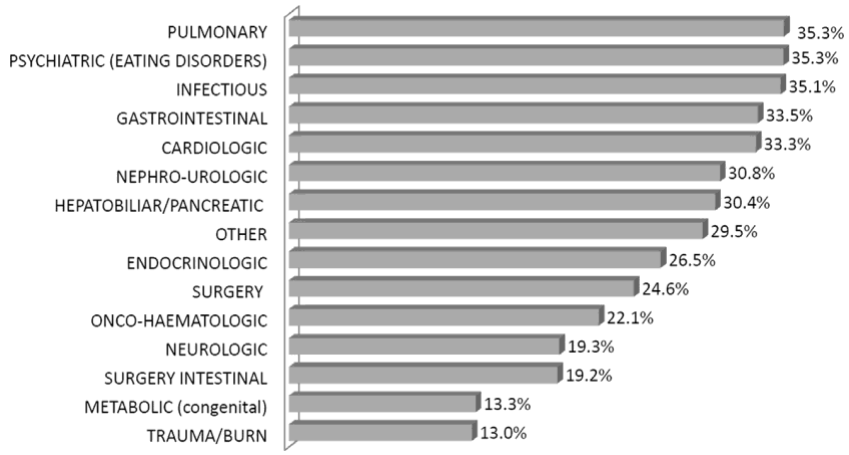
Table 9. Age related nutritional status of pediatric hospitalized patients based on Z-scores of anthropometric measurements.

Nutritional status by:	0–2 years n = 505 (28.2%)	2–6 years n = 442 (24.7%)	6–10 years n = 262 (14.6%)	10–14 years n = 311 (17.4%)	14–20 years n = 270 (15.1%)	Total n = 1790 (100%)
Weight-for-length or BMI z-score (% of 1790 patients)						
Normal	17.4	14.6	10.5	12.5	10.3	65.3
Overweight	1.8	1.6	0.6	1.4	0.6	6
Wasting (total)	9	8.5	3.6*^	3.5*^	4.1	28.7
Mild malnutrition	5	4.4	2.5	2.1	1.6	15.5
Moderate malnutrition	2	1.9	0.6	0.8	1.3	6.5
Severe malnutrition	2.1	2.2	0.6	0.6	1.2	6.7
Height-for-Age z-score (% of 1790 patients)						
Normal	20.6	21.4	12.9	15.2	12.6	82.7
Stunting (total)	7.6	3.3*	1.7*	2.2*	2.5*	17.3
Moderate malnutrition	3.5	2	1.2	1.2	1.3	9.1
Severe malnutrition	4.1	1.3	0.5	1.0	1.2	8.2

*Statistically significant difference vs. 0–2 years group; ^statistically significant difference vs. 2–6 years group.

Regarding the admission diagnosis, patients with psychiatric (including eating disorders), infectious, pulmonary, gastrointestinal and cardiological pathologies had an elevated rate of wasting with more than 30% of them being malnourished (Fig. 2).

Fig 2. Acute malnutrition rate by admission diagnosis



The prevalence of wasting and stunting was higher amongst children with chronic diseases. Wasting or acute malnutrition prevalence was 34.1% vs. 27.1% ($p = 0.002$) and stunting or chronic malnutrition prevalence was 24.5% vs. 8.3% ($p \ll 0.001$) in patients with and without chronic diseases respectively. Table 10 shows the rate of chronic disease and acute or chronic malnutrition. Chronically ill children had significantly ($p < 0.005$) higher risk of malnutrition, either wasting or stunting. The risk ratio for acute malnutrition or wasting among chronically ill patients is 1.1 (CI 95% 0.11; 0.25) whilst for stunting or chronic malnutrition was 3 (CI 95% 1.06; 1.13).

Table 10. Prevalence of acute and chronic malnutrition in patients with or without chronic disease.

*Malnutrition chronic disease compared to no chronic disease patients ($p < 0.005$).

Diseases	Patients n (%)							
		Acute Malnutrition				Chronic malnutrition		
	Total	Mild	Moderate	Severe	Total	Moderate	Severe	Total
Neurologic	178 (9.9)	23 (8.3)	13 (11.1)	16 (13.3)	52 (10.1)	30 (18.6)	22 (15.0)	52 (16.8)
Gastrointestinal	82 (4.6)	20 (7.2)	4 (3.4)	5 (4.2)	29 (5.6)	11 (6.8)	8 (5.4)	19 (6.1)
Hepatobiliar/pancreatic	24 (1.3)	3 (1.1)	2 (1.7)	3 (2.5)	8 (1.6)	1 (0.6)	4 (2.7)	5 (1.6)
Surgery (intestinal)	20 (1.1)	4 (1.4)	1 (0.9)	1 (0.8)	6 (1.2)	–	4 (2.7)	4 (1.3)
Surgery (other)	46 (2.6)	10 (3.6)	2 (1.7)	5 (4.2)	17 (3.3)	2 (1.2)	5 (3.4)	7 (2.3)
Onco-hematologic	195 (10.9)	24 (8.7)	12 (10.3)	10 (8.3)	46 (8.9)	17 (10.5)	12 (8.2)	29 (9.4)
Nephro-urologic	85 (4.7)	10 (3.6)	6 (5.1)	7 (5.9)	23 (4.5)	12 (7.4)	13 (8.8)	25 (8.1)
Cardiologic	68 (3.8)	11 (4.0)	8 (6.8)	6 (5.0)	25 (4.9)	10 (6.2)	14 (9.5)	24 (7.8)
Pulmonary	57 (3.3)	8 (2.9)	6 (5.1)	10 (8.3)	24 (4.7)	5 (3.1)	6 (4.1)	11 (3.6)
Metabolic (congenital)	25 (1.4)	1 (0.4)	1 (0.9)	3 (2.5)	5 (1.0)	3 (1.9)	7 (4.8)	10 (3.2)
Endocrinologic	47 (2.6)	9 (3.2)	1 (0.9)	1 (0.8)	11 (2.1)	7 (4.3)	5 (3.4)	12 (3.9)
Infectious	6 (0.3)	2 (0.7)	1 (0.9)	–	3 (0.6)	–	1 (0.7)	1 (0.3)
Psychiatric (Eating Disorders)	55 (3.1)	5 (1.8)	11 (9.4)	3 (2.5)	19 (3.7)	2 (1.2)	1 (0.7)	3 (1.0)
Ex preterm	28 (1.6)	8 (2.9)	2 (1.7)	2 (1.7)	12 (2.3)	3 (1.9)	14 (9.5)	17 (5.5)
Ex small for gestational age	7 (0.4)	2 (0.7)	–	2 (1.7)	4 (0.8)	–	1 (0.7)	1 (0.3)
Other	129 (7.2)	12 (4.3)	9 (7.7)	9 (7.5)	30 (5.8)	16 (9.9)	12 (8.2)	28 (9.1)
Total chronic disease patients	1052 (58.8)	**152 (54.8)***	**79 (67.6)***	**83 (69.2)***	**314 (61.1)***	**119 (73.5)***	**129 (87.8)***	**248 (80.3)***
No chronic disease patients	738 (41.2)	**125 (45.2)**	**38 (32.4)**	**37 (30.8)**	**200 (38.9)**	**43 (26.5)**	**18 (12.2)**	**61 (19.7)**
Total	1790 (100)	277 (100)	117 (100)	120 (100)	514 (100)	162 (100)	147 (100)	309 (100)

Bold text stands for the principal categories of patients, all the upper categories are undergroups of the total chronic disease patients.

As expected, the rate of acute and chronic malnutrition was higher in pediatric hospitals than in pediatric wards of general hospitals. Respectively in general and pediatric hospitals the prevalence of acute malnutrition or wasting was 5.3% vs. 7.4% ($p = 0.37$) for moderate malnutrition and 4.5% vs. 8.2% ($p \ll 0.001$) for severe malnutrition, whilst the prevalence of stunting was 7.4% vs. 10.2% ($p = 0.001$) for the moderate malnutrition and 5.3% vs. 10.2% ($p \ll 0.001$) for the severe malnutrition. The prevalence of overweight did not differ between the inpatients in the two types of hospitals (5.6% vs. 6.2%; $p = 0.15$). Nutritional support (of any kind) was given to only 23.5% of

children affected by acute malnutrition (Fig. 2: 17%, 25.5% and 36.7%, respectively for mild, moderate and severe malnutrition). Furthermore 10.7% of non-malnourished and 11.2% of overweight patients received some nutritional support. Nutritional support was more frequent among stunted than in wasted patients (31.1% vs. 23.5%, $p \ll 0.001$) (Table 11). ONS (modular or complete) were used in 11.7% of both wasted and stunted patients, EN in 11.5% and 19.4% (via naso-gastric tube or gastrostomy), PN in 6.8% and 8.4% respectively in wasted or stunted patients; some patients received a combination of two.

Fig 3. Nutritional support in Italian hospitalized children

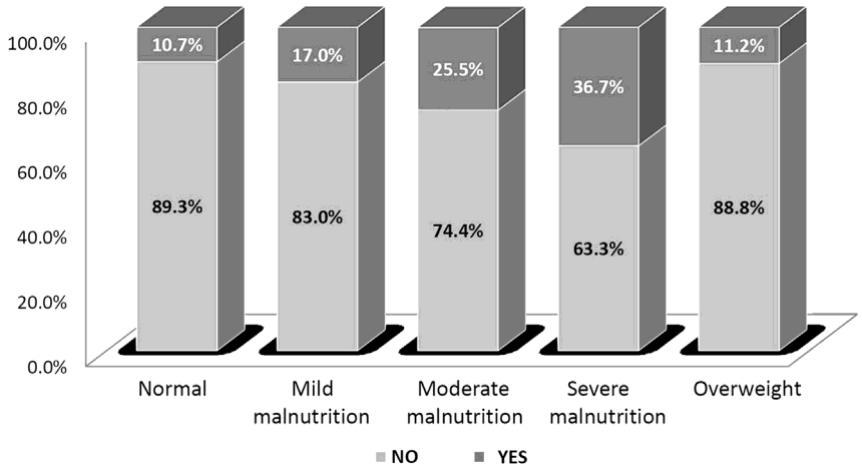


Table 11. Types of nutritional support provided to malnourished patients. Some patients received more than one type of nutritional support

Nutritional support	Wasted patients	Stunted patients
	n (%)	n (%)
Malnourished patients	514	309
Nutritional support (any type)	121 (23.5)	96 (31.1)
ONS complete	33 (6.4)	12 (3.9)
ONS modular	27 (5.3)	24 (7.8)
Enteral nutrition – nasogastric tube feeding	33 (6.4)	30 (9.7)
Enteral nutrition – gastrostomy tube feeding	26 (5.1)	30 (9.7)
Enteral nutrition – jejunostomy tube feeding	0	0
Parenteral nutrition	34 (6.6)	26 (8.4)

ONS: Oral Nutritional Support.

7.1.5 Discussion

The data reported here well represent the Italian population of hospitalized children as they come from a nationwide selection of Italian hospitals. Italian Pediatric Nutrition Survey involved third level referral centers and general pediatric departments and all types of hospitals, permitting appreciation of the real figure of malnutrition prevalence in hospitalized children in Italy. Most studies have reported hospital malnutrition in children's hospital, which are more likely to admit children with severe diseases and extreme malnourished inpatients and our study confirm these data. We purposely also included patients in small pediatric wards to obtain an unbiased figure of nutrition and the malnutrition rate in both contexts is far from negligible. Our study is the first to report nation-wide epidemiological data. The studies published previously involved single hospital samples from Europe, USA and New Zealand and one 1-day cross-sectional survey. Our data confirm the high prevalence of malnutrition among hospitalized children [2]. The acute malnutrition rate observed in our mixed pediatric population falls within the range of reported rates from specialized and general hospitals [5-11]. However, the results of various studies are difficult to compare because of the heterogeneity of methods used to define malnutrition and the population studied. The highest rate of acute malnutrition in our study was observed in the younger age groups in agreement with other published studies [2,5]. This could be due to two reasons: first - the population of hospitalized children mainly consists mainly of infants and young children up to 6 years old; second the younger the age the higher the susceptibility to energy deficit and to metabolic effects of inflammation and hormonal alterations induced by acute and chronic pathologies on nutrition status and growth. In our study, in contrast with Pawellek et al. [7], this range extended up to 6 years of age both for acute and chronic malnutrition (Table 9). These findings underline the need to make a correct and early malnutrition diagnosis and to provide adequate nutritional therapy in order to prevent its harmful short and long term impact in infants and young children. Stunting is reported by a few studies although it is widely accepted as a parameter of chronic malnutrition

parameter. Chronic nutrition deficiency that causes long-term faltering growth is the suggested mechanism [12], confirmed recently by the new definition of malnutrition by the Academy of Nutrition and Dietetics and the ASPEN [1]. Stunting rate in hospitalized children is highly variable between studies but heavily influenced by the presence of an underlying chronic disease. Some of the conditions with the highest malnutrition rates are cardiac and renal chronic diseases, malignancies, cerebral palsy, etc. [13-17]. Our data showed stunting, in decreasing ranking, neurologic, oncologic, cardiologic and nephrologic malnourished patients. No studies consider the coexisting wasting in stunting patients. Our data showed 1/3 of stunted patients had BMI or weight for length Z-score < - 2 identifying a slice of the population critically malnourished. Considering that short stature may be the result of factors intrinsic to chronic pathologies and not by nutritional deficit, we need to identify chronic malnourished patients that require nutritional therapy. The design of our study (1-day survey) did not allow us to establish the extent that the short stature is affected by energy deficit to requirements or other factors (hormonal, inflammatory…); it is however likely that only few children with non-nutritional causes of stunting have entered this study.

The percentage of patients affected by an underlying chronic disease was higher in our population (>50% vs. 33%) than that reported by other authors [6]. The predominance of pediatric third level hospital patients on those of pediatric wards of general hospitals in our population may be an explanation. The presence of a chronic disease increased the risk of both acute and chronic malnutrition, and the reported high percentage of chronically ill among the hospitalized children emphasizes the need for nutritional surveillance as the malnutrition rate is reported to increase during hospitalization even in patients affected by mild clinical conditions [3].

To our knowledge, no studies have reported epidemiological data on the type of nutritional support utilized in malnourished patients. The present study strengthens evidence of the under- recognition

of the prevalence of malnutrition in hospitalized children given the low percentage of malnourished patients receiving any kind of nutritional therapy. We think that the application of the nutritional therapy by the clinicians is based on clinical reasons and not on recognizing malnutrition or its severity. This can be explained by the delivery of nutritional support to normal and overweight/obese patients as well as the high percentage of untreated malnourished children (Fig. 3). Data did not show any preference of the clinicians for the type of nutritional support but it can be noted that parenteral nutrition is chosen equally with enteral nutrition (oral or tube feeding) either in acute or chronic malnourished patients. Considering the fact that the use of parenteral nutrition in hospitalized children is far from evidence-based, the ESPGHAN (European Society for Pediatric Gastroenterology Hepatology and Nutrition) guidelines on parenteral nutrition recommend its employment only when adequate oral or enteral feeding is precluded [18].

In chronically malnourished (stunted) children there was a slight preponderance of tube feeding (SNG or gastrostomy) and a lower preference for complete ONS. This may be related to the lower availability of pediatric oral supplements than in adults as well as to the long-term need for nutritional support in those patients. The modular ONS added to natural foods to provide more energy or nutrients is probably better known to pediatricians who are familiar with supplementing of human milk.

Limitations of the study: pediatric patients were screened on a single day which did not necessarily correspond to admission day. This may reduce the sensitivity and precision of the study in the estimation of the prevalence and entity of wasting, as hydration or other therapies, especially the intravenous ones may result in an increased weight and the borderline wasting patients may not be detected. Furthermore, we did not ask about the duration of malnutrition in order to comply with the ASPEN definition of chronicity of malnutrition. Another limitation is the absence of other data,

outside of the admission diagnosis or the presence of a chronic disease that would explain causes of stunting other than malnutrition.

7.1.6 Conclusion

There is a high prevalence of both acute and chronic malnutrition among hospitalized pediatric patients in Italy, especially in infants and young children; it is likely to be under-recognized as nutritional support is only given to a small number of the malnourished children.

Given its adverse effects on short and long-term clinical outcomes and healthcare costs, malnutrition should be promptly recognized and treated properly. The univocal diagnostic method offered by Z-score of anthropometric measurements and the related classification has proved more effective for the identification of malnutrition than the different standard methods used in most Italian pediatric hospitals. Its implementation in everyday clinical practice would help to improve overall nutrition assessment and consistency.

7.1.7 References

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7.2 Second objective: Accuracy of prediction formulae for the assessment of resting energy expenditure in hospitalized children

7.2.1 Aim

To evaluate the accuracy of commonly employed REE prediction formulae versus indirect calorimetry in hospitalized children.

7.2.2 Data Collection and methods

We performed a cross-sectional study of 236 infants, children, and adolescents consecutively admitted to the Intermediate Care, Nephrology, Intensive Care, Emergency, and Cystic Fibrosis Units of the De Marchi Pediatric Hospital (Milan, Italy) between September 2013 and March 2015. Patients from all Units were excluded from the study in the presence of RQ <0.67 or >1.3; need of supplemental oxygen; and inability to maintain the fasting state for at least 4 hours. Values of RQ between 0.67 and 1.3 were used as marker of validity of the IC measurements following McClave et al [1]. Patients from the Nephrology Unit were excluded from the study in the presence of nephrotic syndrome; treatment with intravenous methylprednisolone; kidney transplantation with circulating antidonor antibodies; and hemodialysis or peritoneal dialysis with acute disease, for example, influenza. Patients from the Emergency Unit were excluded from the study in the presence of gas leaking >10% and FIO_2 >40%. Patients with FIO_2 > 40% were excluded from the study because, even if the inspired oxygen will contribute to the measured REE, such contribution is expected to substantially distort the measure at values of $FIO_2 \geq 60\%$ [2]. The study was approved by the Ethical Committee of the De Marchi Pediatric Hospital and the parents of the children gave their written informed consent.

Anthropometry

Weight, length (age <2 years), or height (age ≥ 2 years), arm circumference, and triceps skinfold were measured following international guidelines [3]. BMI was calculated as weight(kg)/length or height $(m)^2$. Standard deviation scores (SDS) of weight, length, height, weight-for-length, weight-for-height, BMI, arm circumference, and triceps skinfold were calculated using the WHO reference data [4,5]. WHO SDS could be calculated for the following intervals of age and anthropometric dimensions: weight-for-age from age 0 to 10 years; length-for-age from age 0 to 2 years; height-for-age from age 2 to 18 years; weight-for-length for length 45 to 110 cm; weight-for-height for height 65 to 120 cm; arm circumference-for-age from age 0.25 to 5 years; and triceps skinfold-for-age from age 0.25 to 5 years.

Measurement of Resting Energy Expenditure

REE was measured in thermoneutral conditions using an open-circuit IC (Vmax 29, Sensor Medics, Yorba Linda, CA). An 8-hour fasting period was recommended for all patients, but a fasting period of at least 4 hours was acceptable for patients ages 2 years or younger. In spontaneously breathing patients, a canopy was positioned around the patient's head and the expired air was drawn from the hood at a fixed rate [6]. In patients requiring mechanical ventilation, the calorimeter was connected to the ventilator (Babylog VN500, Dräger, Andover, MA). No changes in the ventilator settings were done for at least 1 hour before the REE measurement. Steady state was defined as at least 5 minutes with <5% variation in RQ, <10% variation in oxygen consumption, and <10% variation in minute ventilation [1]. After the steady state was reached, the REE measurement was performed for at least 30 minutes. REE was obtained from oxygen uptake and carbon dioxide output using Weir's equation [7].

Estimation of Resting Energy Expenditure

REE was estimated using the 5 most commonly employed formulae: the WHO formula [8], the

Harris-Benedict formula [8], the Schofield-W formula [8], the Schofield-WH formula [8], and the Oxford formula [9].

7.2.3 Statistical Analysis

Most variables were not Gaussian distributed and all are reported as 25th, 50th, and 75th percentiles. Bland-Altman plots of the bias (EEE - mREE) versus the average [(EEE + mREE)/2] and of the percent bias [(EEE - mREE)/mREE] versus the average were used to evaluate the presence of proportional bias [10,11]. The association between the bias and the average was evaluated using the Pearson product-moment correlation coefficient. Because proportional bias was detected in all cases, the Bland-Altman limits of agreement were not calculated [12]. The absolute bias was Gaussian distributed, as determined by using kernel density plots and the Shapiro-Wilk test. The comparison of the measured and estimated values of REE was performed using Student t test for paired data. The percent bias was not Gaussian distributed. We evaluated the association of the percent bias of the Schofield-W formula with sex, age, weight, and respiratory insufficiency (RI) using multivariable median regression [13]. The response variable was percent bias (continuous, %) and the predictors were sex (discrete, 0 = female; 1 = male), age (continuous, years), weight (continuous, kg), and RI (discrete, 0 = no; 1 = yes). The continuous predictors were in linear relation with the outcome, as detected by using multivariable fractional polynomials [14]. Statistical analysis was performed using Stata 14.1 (Stata Corporation, College Station, TX).

7.2.4 Results

Clinical, Anthropometric and Metabolic Features of the Patients

A number of 236 consecutive patients (200 Caucasians 85% and 123 boys 52%) aged 0.04 to 17.7 years were studied. Among them, 210 (89%) were spontaneously breathing. The reasons for hospitalization were (in order of frequency) the following: respiratory insufficiency (RI) (n = 81); kidney disease (n = 51); rheumatic disease (n = 32); cystic fibrosis (n = 18); blood disease (n = 17);

gastrointestinal disease (n = 16); neurological disease (n = 12); infectious disease (n = 6); and slow growth (n = 3). The anthropometric and metabolic measurements of the patients are given in Table 12. The measurements of weight and length or height were available in all 236 patients, those of arm circumference in 223 (95%), and those of triceps skinfold in 219 (93%). The median SDS of weight-for-age, length-for-age, height-for-age, weight-for-length, weight-for-height, and BMI-for-age were negative, signaling values always below the 50th percentile. In detail, the median BMI-for-age was -0.33 SDS, corresponding to the 37th percentile and 28 children (12%) had a BMI-for-age <2 SDS. The median (IQR) REE was 895 (419–1315) kcal/day.

Table 12. Anthropometric and metabolic measurements of the study population

	N	P_{50}	P_{25}	P_{75}
Age (years)	236	6.6	0.8	11.4
Weight (kg)	236	19.7	7.9	36.7
Weight-for-age (SDS WHO*)	152	−0.74	−1.67	0.16
Length (cm)	79	62.0	57.0	72.0
Length-for-age (SDS WHO*)	79	−0.55	−1.70	0.41
Height (cm)	157	135.0	116.0	152.0
Height-for-age (SDS WHO*)	157	−0.72	−1.49	0.12
Weight-for-length (SDS WHO*)	79	−0.43	−1.56	0.80
Weight-for-height (SDS WHO*)	49	−0.16	−1.06	0.34
Body mass index (kg/m^2)	236	15.9	14.7	18.5
Body mass index-for-age (SDS WHO*)	236	−0.33	−1.20	0.69
Arm circumference (cm)	223	17.0	14.0	21.0
Arm circumference-for-age (SDS WHO*)	72	−0.34	−1.62	0.68
Triceps skinfold (mm)	219	9.6	7.7	12.7
Triceps skinfold-for-age (SDS WHO*)	71	−0.01	−0.42	1.19
Resting energy expenditure (kcal/day)	236	895	419	1315
Resting energy expenditure (kcal/kg weight)	236	40	32	53

P_x = Xth percentile; SDS = standard deviations scores; WHO = World Health Organization.

*WHO SDS could be calculated for the following intervals of age and anthropometric dimensions: (1) weight-for-age from age 0 to 10 years; (2) length-for-age from age 0 to 2 years; (3) height-for-age from age 2 to 18 years; (4) weight-for-length for length 45 to 110 cm; (5) weight-for-height for height 65 to 120 cm; (6) arm circumference-for-age from age 0.25 to 5 years; (7) triceps skinfold-for-age from age 0.25 to 5 years.

Accuracy of the Prediction Formulae

Table 13 gives the absolute and percent bias of the WHO, Harris-Benedict, Schofield, and Oxford formulae. Figure 4 shows the presence of negative proportional bias for all formulae, especially for the Harris-Benedict formula. Because proportional bias was detected also for percent bias (not shown), limits of agreement were not calculated. Because IC is a reference method, the values reported in Table 13, however, do accurately quantify the prediction error and its interindividual variability. The estimated REE was <80% and >120% of measured REE in 16% and 28% of patients using the WHO formula; 10% and 41% using the Harris-Benedict formula; 15% and 27% using the Schofield-W formula; 14% and 26% using the Schofield-HW formula; and 14 and 28% using the Oxford formula. This offers a rough but clinically useful measure of how many children would be underfed or overfed using these formulae [15]. Using a stricter criterion [16], the estimated REE was <90% and >110% in 35% and 36% of patients using the WHO formula; 22% and 46% using the Harris-Benedict formula; 35% and 35% using the Schofield-W formula; 35% and 33% using the Schofield-HW formula; and 33% and 33% using the Oxford formula. Figure 5 plots the joint contribution of sex, age, weight, and RI to the percent bias of the Schofield formula (multivariable median regression). Sex ($P = 0.9$), age ($P = 0.9$), and weight ($P = 0.8$) were not associated with the percent bias but RI was (35%, 95% CI 23–46, $P < 0.001$).

Table 13. Absolute and percent bias associated with the estimation of resting energy expenditure from the WHO, Harris-Benedict, Schofield, and Oxford formulae.

	n	Mean	SD	P_{50}	P_{25}	P_{75}
Bias-WHO (kcal)[†]	236	−1	234	−11	−134	117
Bias-WHO (%)[‡]	236	—	—	−2	−16	25
Bias-Harris-Benedict (kcal)[†]	236	82[*]	286	76	−103	270
Bias-Harris-Benedict (%)[‡]	236	—	—	8	−9	65
Bias-Schofield weight (kcal)[†]	236	2	215	−11	−129	125
Bias-Schofield weight (%)[‡]	236	—	—	−1	−14	25
Bias-Schofield weight & height (kcal)[†]	236	−2	214	−18	−134	120
Bias-Schofield weight & height (%)[‡]	236	—	—	−2	−14	22
Bias-Oxford (kcal)[†]	236	−5	221	−14	−130	121
Bias-Oxford (%)[‡]	236	—	—	−2	−14	24

P_x = Xth percentile; SD = standard deviation; WHO = World Health Organization.

[*] $P < 0.001$ (Student t test for paired data).

[†] Absolute bias was calculated as (estimated resting energy expenditure − measured resting energy expenditure). Absolute bias was Gaussian-distributed and Student t test for paired data was used to compare estimated and measured values.

[‡] Percent bias was calculated as [(estimated resting energy expenditure − measured resting energy expenditure)/measured energy expenditure)]. Percent bias was not Gaussian-distributed.

Figure 4. Bland-Altman plots of the bias versus the average for the WHO, Harris-Benedict, Schofield, and Oxford formulae.

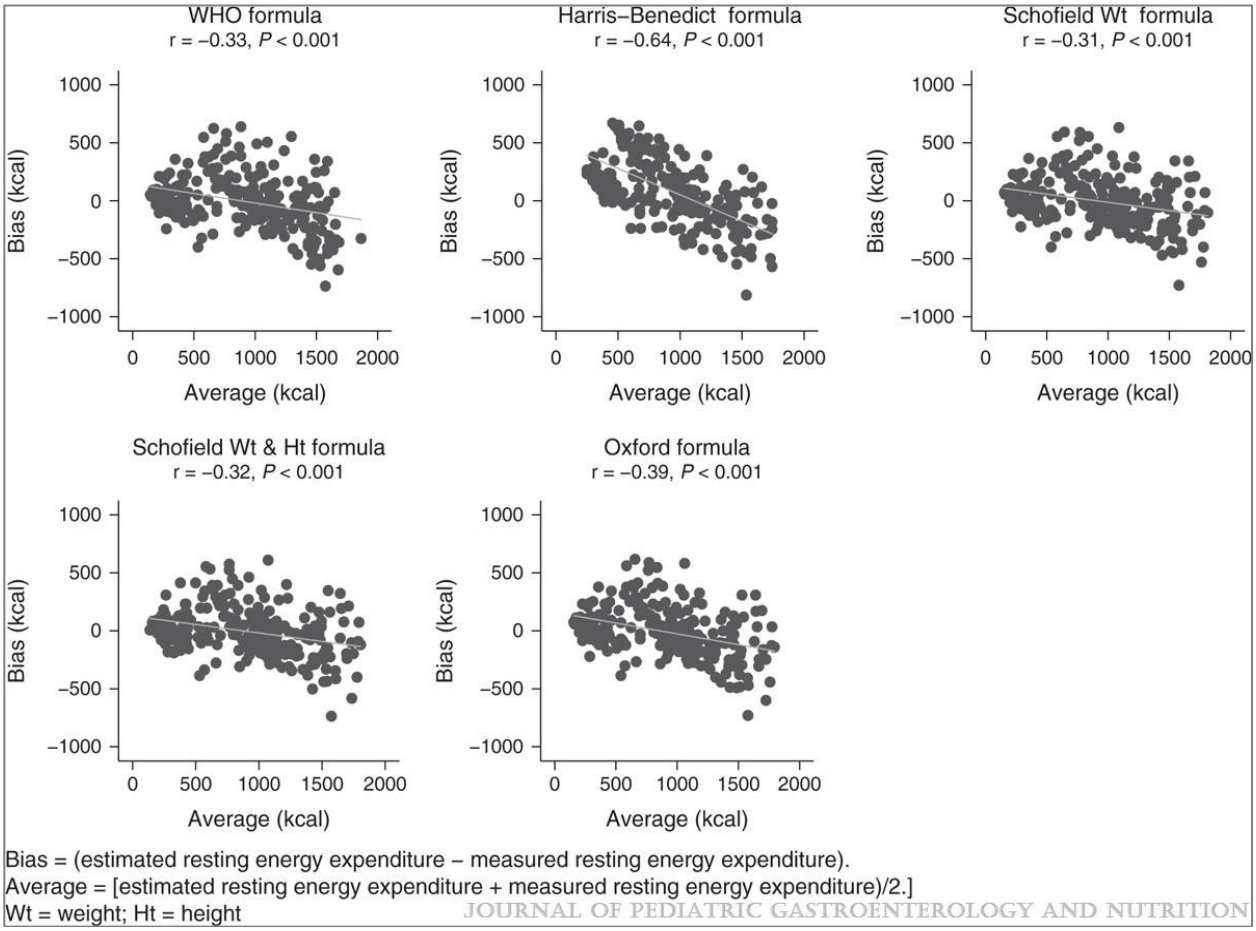
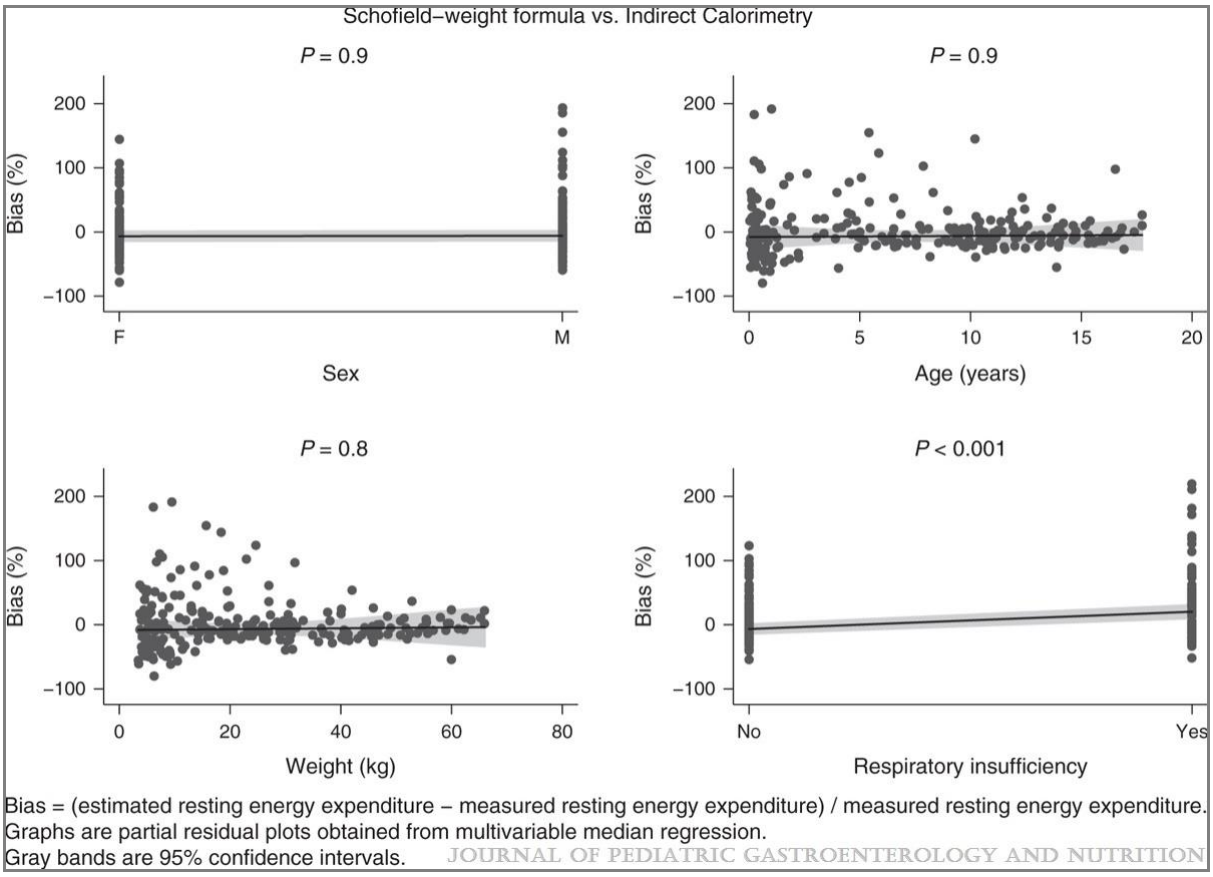


Figure 5. Association of the percent bias of the Schofield-weight formula with age, weight, and respiratory insufficiency (multivariable median regression).



7.2.5 Discussion

Most of the available clinical validation studies of REE formulae have been performed in mechanically ventilated children [18,19]. In the present study, we evaluated the accuracy of the most commonly employed REE prediction formulae [8,9] in a large sample of hospitalized children.

All formulae except the Harris-Benedict formula gave accurate estimates of REE at the population level (small mean bias) but were not accurate enough to be employed at the individual level (large SD of the bias). This finding has important implications for the treatment and prevention of hospital malnutrition [19]. Our results highlight the risk of underfeeding or overfeeding in hospitalized

children whose energy prescription is based on REE estimated from commonly used prediction formulae. In order to evaluate how REE formulae perform in a “mixed” pediatric hospital setting, we chose to study a heterogeneous population of hospitalized children. An obvious limitation of this approach is that, for most of the diseases that we studied, we do not reach a sufficient number of children to test whether purposely developed REE population-specific formulae perform better than traditional REE formulae. Further studies should be performed to test whether population-specific formulae can improve the accuracy of REE estimation in the pediatric hospital setting. Moreover, in the present study, we did not evaluate the contribution of nutritional rehabilitation [20] and of the ebb and flow phases of trauma [21] to the bias of estimated REE. These are clinically important modifiers of REE worthy of further clinical investigation.

The mean bias of the WHO (-1 kcal), Schofield-W (2 kcal), Schofield-HW (-2 kcal), and Oxford (-5 kcal) formulae was much lower than the mean bias of the Harris-Benedict (82 kcal) formula. If one considers the large SD of the bias shown by all formulae, it is, however, clear that none of these formulae can be applied satisfactorily at the individual level. The greater absolute and proportional bias associated with the Harris-Benedict formula was not unexpected, owing to the fact that its development sample included only adults. The similar accuracy of the Schofield and Oxford formulae was also not unexpected because most of their development sets consisted of common subjects. Our findings about the accuracy of the Schofield formulae agree with those of other researchers who studied mechanically ventilated children or children recovered in PICU [22,23]. It is also worth noting that, in a clinical population made mostly of children with failure to thrive [24], the Schofield-W formula proved slightly better than the Harris-Benedict formula.

Interestingly, despite the highly variable age and weight of our children, we found that these factors were not associated with the percent bias of the Schofield-W formula. The contribution of RI to the bias of the Schofield-W formula was, however, clinically relevant and shows that the presence of RI

should be always taken into account by studies investigating the accuracy of estimated REE in the clinical setting. Our conclusion that REE formulae should not be used in hospitalized children, be they under mechanical ventilation or not, is the same offered by most studies of mechanically ventilated children [22,25,26].

7.2.6 Conclusion

The WHO, Harris-Benedict, Schofield, and Oxford formulae should not be used to estimate REE in hospitalized children. Further studies are needed to test whether population-specific formulae can improve the accuracy of REE estimation in the hospital setting and to test whether factors such as nutritional rehabilitation can affect the ability of IC to estimate REE in hospitalized children.

7.2.7 References

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7.3 Third Objective: Nutritional status, metabolic state and nutrient intake in children with bronchiolitis

7.3.1 Aim

To describe the nutritional status, metabolic state and the accuracy of equations used to estimate REE in infants hospitalized with bronchiolitis.

7.3.2 Data collection and Methods

In this prospective observational study, we enrolled infants less than 2 years of age with a clinical diagnosis of acute viral bronchiolitis [1] admitted to the Pediatric Department between October 2014 and March 2015. Patients needing supplemental oxygen were not eligible for the study, because of technical difficulties in performing indirect calorimetry. The study was approved by the local Ethics Committee and informed consent was obtained. Assuming a mean REE (±SD) of 346±85kcal/die, the estimated minimum sample size was 19 per group to achieve a α=0.05 and ß=80%. A multidisciplinary team completed the nutritional assessment and the anthropometric measurements on admission and prior to hospital discharge. Weight (using a gram scale, accurate to 0.1kg) and length (using the 417 SECA stadiometer VR SECA Medical Measuring Systems and scales, Birmingham, UK, or a flexible but non-stretchable tape measure) were measured [2,3]. Z-scores for weight for age (WFA), BMI, weight for length (WFL) and length for age (LFA) were calculated using the WHO Anthro Plus software, and the WHO reference charts [4,5], Malnutrition was diagnosed according to WHO criteria if WFL z-score was < 2. REE was measured in thermoneutral conditions after 4-h fasting, using an open-circuit indirect calorimeter (Vmax 29VR , Sensor Medics, Yorba Linda, CA) and a transparent canopy. Minute-to-minute VO_2 and VCO_2 were measured during steady state and were reported in ml per kg of body weight [6]. Data from patients who did not meet steady state or had a respiratory quotient <0.67 or >1.3 were excluded.

Energy expenditure was estimated using the Harris–Benedict, the Schofield and the FAO-WHO equations [7,8]. Based on the ratio of MREE and the EEE by Schofield equation, individual patients were classified as, (a) normometabolic (MREE:EEE = 0.9–1.1), (b) hypermetabolic (MREE:EEE >1.1) and (c) hypo-metabolic (MREE:EEE<0.9) [9,10].

A 24-h food recall was used to determine daily nutrient intake. We classified the feeding status of patients based on the ratio of actual energy intake (AEI) and MREE, as underfed (AEI:MREE <0.8), overfed (AEI:MREE >1.2) or normally fed (AEI:MREE=0.8–1.2). Biochemical characteristics, including albumin and prealbumin, were measured on admission, with methods standardized in the central laboratory of the hospital.

7.3.3 Statistical analysis

Data are presented as median ± IQR. We used the Bland–Altman analysis to determine mean bias and limits (95% CIs) of agreement between MREE and each of the EEE. Differences between groups were assessed with Wilcoxon signed rank test and analysis of variance (ANOVA), as appropriate. Statistical significance was defined at $p < 0.05$.

7.3.4 Results

We enrolled 35 consecutive eligible patients (27 Caucasians, four Africans, three Hispanics, one Asiatic and 19 boys, 54%) aged 2.7–11.7 months, during the study period. Table 14 shows the demographic, anthropometric and clinical characteristics of children on admission. Globally, 45.7% of children were malnourished, overweight (25.7%) or undernourished (20%). Gas exchange, metabolic status and macronutrient intake both at admission and at discharge are reported in Table 15. One IC measurement did not reach the steady state and was excluded from the analysis. At admission, 63% of the patients were either hypermetabolic (25.8%) or hypometabolic (37.1%). There were no significant differences in the WFL-z scores between admission and discharge.

Bland–Altman plots showing the agreement between MREE by IC and EEE by three different predictive equations are depicted in Figure 6. The mean bias (limits of agreement) for Harris–Benedict was 61.0 (41 to 163%); for FAO-WHO was 9.9 (74.4 to 94.2%) and for Schofield was 8.9 (73.9 to 91.8%). The median daily protein and actual energy intake for the cohort was 3g/kg and 94.1/kg, respectively, and 80% of the cohort in which we performed the 24-h recall was overfed (AEI:MREE>120%) and none of the children were underfed.

Table 14. Demographic, anthropometric data and nutritional status on admission in children admitted to the hospital with bronchiolitis.

	Median (IQR 1,3)
Age, months	5.4 (2.7–11.7)
Weight, kg	6.9 (4.8–8.5)
Length, m	0.6 (0.6–0.7)
BMI, kg/m^2	15.4 (14.6–16.6)
Weight for length *z* score	−0.5 (−0.9–0.8)
Undernourished	7.0 (20.0)
Overweight/obese	9.0 (25.7)
BMI, *z* score	−0.5 (−1.6–0.6)
Weight for age *z* score	−0.4 (−1.9–0.09)
Length for age *z* score	−1.0 (−1.8–0.5)
Respiratory rate breaths/min	50.0 (28.0–80.0)
Cardiac rate bpm	140.0 (98.0–180.0)
O_2 saturation %	95.0 (85.0–100.0)
Temperature °C	36.2 (35.5–38.0)
Albumin[a], g/L	0.043 (0.041–0.045)
Prealbumin[b], g/L	0.120 (0.092–0.177)
Etiological agent bronchiolitis	%
RSV	51
Rhinovirus	14
hMPV	6
Coronavirus	3
Mixed	26

Data are presented as median (1–3 quartile) and N and % when appropriate. a Data available for only 34 patents. b Data available for only 26 patients.

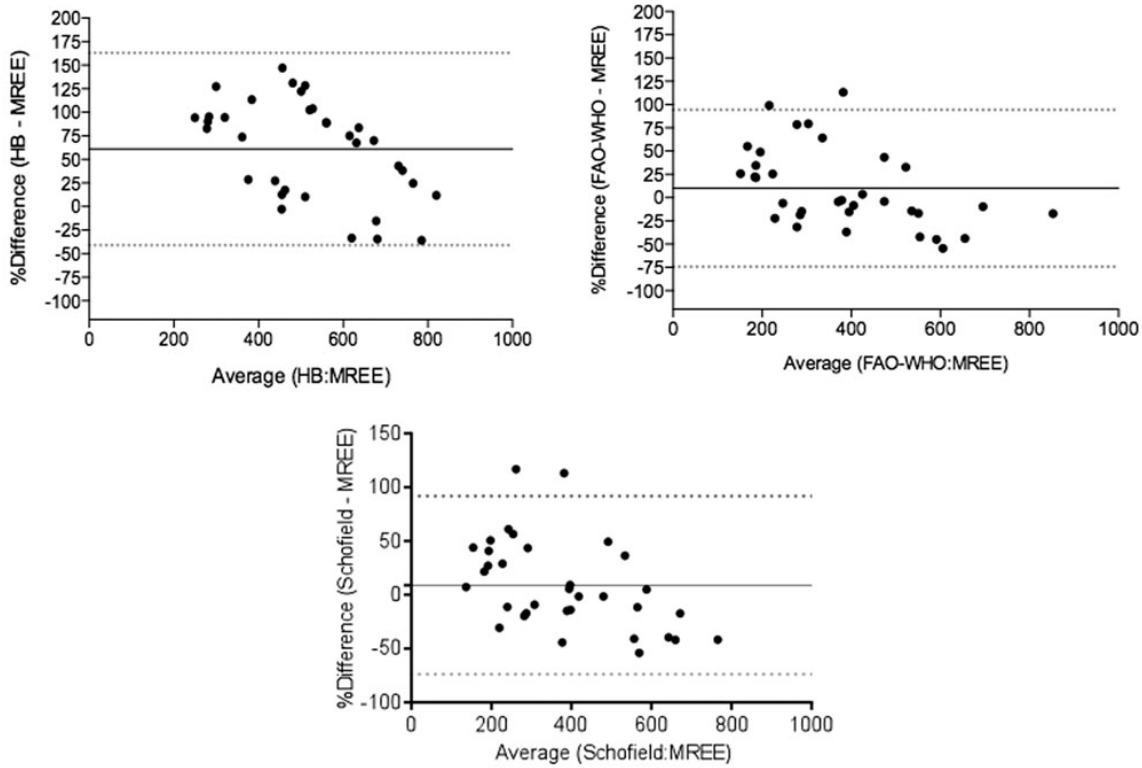
Table 15. Gas exchange, metabolic status and macronutrient intake at admission and at discharge.

	Admission		Discharge		
	N	Median (IQR 1,3)	*N*	Median (IQR 1,3)	*p* Value
VO2 mL/kg/min	34	12.2 (10.0–17.6)	21	9.1 (5.5–11.1)	NS
VCO2 mL/kg/min	34	10.4 (8.5–14.9)	21	7.3 (4.8–9.3)	NS
RQ	34	0.8 (0.8–0.9)	21	0.8 (0.8–0.9)	NS
MREE, kcal/d	34	346.5 (172.5–478.3)	21	424 (288.0–617.0)	NS
EEE by Harris–Benedict equation, kcal/d	35	626.1 (487.2–824.3)	21	789.1 (475.2–848.8)	NS
EEE by Schofield equation, kcal/d	35	360.0 (248.0–515.6)	21	366.0 (251.2–564.6)	NS
EEE by FAO-WHO equation, kcal/d	35	372.9 (241.9–462.1)	21	421.7 (251.1–516.3)	<.05
MREE/HB	34	0.5 (0.4–0.7)	21	0.6 (0.5–0.9)	NS
MREE/Schofield	34	1.1 (0.7–1.4)	21	1.3 (0.8–1.5)	NS
MREE/FAO-WHO	34	1.0 (0.7–1.2)	21	1.2 (0.8–1.4)	NS
Protein intake, g/kg	10	2.9	7	2.4	NS
Protein intake, %	10	9.7 (7.5–14.9)	7	7.4 (7.1–17.0)	NS
Lipid intake, g	10	30.2 (26.9–32.1)	7	47.9 (36.1–47.3)	NS
Lipid intake, %	10	47.9 (36.9–49.9)	7	45.1 (39.1–50.7)	NS
CHO intake, g	10	67.3 (62.1–88.5)	7	91.6 (76.6–110.0)	NS
CHO intake, %	10	43.3 (42.6–48.2)	7	42.5 (41.7–49.7)	NS
Metabolic status (MREE:EEE Schofield)					
Normometabolic, *N* and %	34	13 (37.1)	21	4.0 (19.0)	
Hypermetabolic, *N* and %	34	8 (25.8)	21	10.0 (48.0)	
Hypometabolic, *N* and %	34	13 (37.1)	21	7.0 (33.0)	
Feeding status					
Daily actual energy intake, kcal/kg	10	94.1 (74.9–127.6)	7	116.8 (91.2–150.9)	
Actual energy intake/REE	10	1.83 (1.5–2.0)	7	2.6 (1.5–3.5)	
Normal fed, *N* and %		2.0 (20.0)		1.0 (14.3)	
Underfed, *N* and %		0		1.0 (14.3)	
Overfed, *N* and %		8.0 (80.0)		5.0 (71.4)	

Data are presented as median (1–3 quartile) and N and % when appropriate. N=number of patients where the stated characteristic was assessed. Respiratory quotient (RQ); Measured resting energy expenditure (MREE); Estimated energy expenditure(EEE).

Fig. 6. REE in children with bronchiolitis on hospital admission.

Agreement between indirect calorimetry and three different predictive equations. The x-axis shows the average measured energy expenditure by the two methods (kcal/d). The y-axis shows the % difference in measured energy expenditure between the two methods (kcal/d). If the two methods of measurement had good agreement, the points should be centered on the "0" y-axis, regardless of the average measured resting energy expenditure. In the population study, the higher the average MREE, the more likely the predictive equation is to underestimate MREE (negative difference), and the lower the average MREE, the more likely the predictive equation is to overestimate the MREE (positive difference on y-axis).



7.3.5 Discussion

Our results provide some insights into the nutrient needs and challenges to nutrient delivery in children hospitalized with bronchiolitis. Malnutrition was recorded in a majority of patients on admission. In particular, this cohort had a high prevalence of overweight/obesity. The hospital stay was short, average 7 days, and both nutritional and metabolic states did not change significantly during this period. Accurate data related to daily caloric intake were available in a small subgroup of patients. In this subgroup, we identified eight children who were overfed and none was underfed. We found only 13 children who were normometabolic, while the majority of them were either hypometabolic or hypermetabolic.

Other studies show that patients did not change their weight during hospitalization in agreement with our data [11,12] but high percentages of undernutrition and risk of malnutrition are noteworthy, 21.7% and 17.5%, respectively [13]. While a preexisting malnutrition status is a strong predictor of mortality from acute lower respiratory tract infection in preschool-aged children [12, 14] and a marker of further nutritional and clinical deterioration, attention should be paid to the prevalence of obesity, too. We decided to use WHO growth charts, because only 51% of children in the study population were Italian, the others were Caucasians-not Italian and other ethnicities and the obesity prevalence was comparable to that described in northern Italian children [15].

Our results do not support the notion that low caloric intake is common among infants with bronchiolitis. In previous report, suboptimal nutrient delivery in children hospitalized with bronchiolitis has been associated with prolonged length of stay, low early hospital caloric intake and a slow rate of improvement [16]. In our subgroup, descriptive statistics revealed that the median caloric intake of infants at admission was 94 kcal/kg, while median protein intake was 2.9g/kg, a value higher than recommended intakes [17]. Providing both protein and energy intakes above-recommended intakes [18] may promote protein anabolism in critically ill infants [9].

Many equations have been developed as surrogate methods to predict REE, but they are not always accurate to carefully monitor the energy needed to support growth and metabolic requests in disease conditions and they assume a lack of heterogeneity among individuals through ages in both genders [19]. In the current study, MREE and energy expenditure assessments with equations were not in agreement. The limits of agreement were wide and individual values of estimated energy expenditure must be interpreted with caution for these patients. Furthermore, looking at the slope of the three Bland–Altman Plots, in younger infants with lower mean energy expenditure (kcal/kg) the estimated energy expenditure overestimated MREE; on the contrary in older patients, the equations underpredicted the measured energy expenditure.

Limitations

There are a number of limitations of the current study, beyond those already mentioned. Few patients were enrolled during the epidemic season and some data at discharge were lost (40%). Dietary intake data compared to resting energy expenditure were available in a very limited number of patients at admission and discharge, respectively, 10 versus 7. Nevertheless, the results suggest that an individualized metabolic and nutritional assessment in patients with bronchiolitis might be useful. This should be further elucidated in larger studies. Since our study was performed in a single Italian center, multicentric studies could more easily respond to open questions with a more meaningful statistical power. Accurate measurement of energy expenditure requires the achievement of oxygen and carbon dioxide exchange at a steady state. Certain procedures in the inpatient setting may affect the oxygen dynamics and accuracy of the examination; for this reason, we excluded one measure. Finally, the role of the different viruses in establishing different metabolic expenses, and therefore, needs, in bronchiolitis, may be matter of further research.

For this aim, the current sample size was insufficient. Beyond RSV and rhinovirus, also the metabolic challenges in coinfection (ranging widely among studies from 6% to more than 30%) should be evaluated [20]. In children in whom we performed the 24-h recall, we identified eight who were overfed and none was underfed. These results represent the comparison between MREE and intake and do not take into account the energy burden from diet induced thermogenesis, activity and growth. Nevertheless, hospitalized infants are generally quite sick and spend most of their time in bed. Furthermore, they are spending most of their energy to fight the acute infection, so that the energy spent for growth is likely negligible during this short period of time.

7.3.6 Conclusion

Infants with acute bronchiolitis present with a high prevalence of malnutrition and altered metabolic state, which is not accurately estimated by standard equations. Heterogeneity in metabolic state suggests the need for an individualized approach to nutrition [21]. It would be desirable to focus on providing optimal nutrition and perform targeted indirect calorimetry on high-risk patients, to prevent cumulative excesses, deficits in energy balance and reduce length of stay [22]. A suboptimal nutritional status may persist also after the acute illness, so that a global health follow-up may be indicated to evaluate the growth of the child after the episode.

7.3.7 References

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7.4 Fourth Objective: The Metabolic Response to Stress and Infection in Critically Ill Children, The Opportunity of an Individualized Approach

7.4.1 Aim

The following narrative review aims to discuss the metabolic changes in critically-ill children and the potential of developing personalized nutritional interventions. Through a literature search strategy, I have investigated the importance of blood glucose levels, the nutritional aspects of the different phases of acute stress response, and the reliability of the available tools to assess the energy expenditure.

7.4.2 Methods

Electronic databases (Pubmed, Medline, Embase, Google Scholar, Knowledge Finder) were used to locate and appraise relevant studies. We carried out the search to identify the articles published in English on (i) glycemic control, (ii) nutritional interventions in the different acute stress phases, (iii) the role of the parenteral nutrition and of the immunity-enhancers nutrients and, finally, (iv) the usefulness of predictive equations and indirect calorimetry use in critically ill children. Relevant articles published from January 2007 to March 2017 were identified using the following groups of key terms: “metabolic response” AND “critical illness” AND “infections”; “hyperglycemia” AND “critically ill children” OR “nutritional status” OR “nutrition” OR “nutritional sciences” AND “critical care”; OR “hospitalized children” OR “child, hospitalized” “insulin therapy” AND “critically ill children”; “energy expenditure” AND “critically ill children”; “nutrition” AND “critically ill children” OR “PICU”. Randomized Control Trials (RCTs) and largest non-RCTs studies were considered. In the eligible studies, we focused on data on infective events (or inflammation status), need of mechanical ventilation, length of PICU stay, and mortality rate (number of deaths) for the different nutritional approaches.

7.4.3 Results

Hyperglycemia and Glycemic Control in Stress Conditions

Stress hyperglycemia remains an unsolved medical condition. It is usually defined as blood glucose level >11.1 mmol/L [1]. Its incidence is very high, ranging between 56% and 86% among patients requiring intensive care [1]. Mechanical ventilation, vasopressor/inotropic infusion, continuous renal replacement therapy, infections, and long lengths of stay are the main risk factors of hyperglycemia that are commonly associated with worse outcomes [2].

Several trials have investigated the consequences of glycemic alterations and of their correction, providing inconclusive results. The effect of targeting age-adjusted normoglycemia with insulin infusion was investigated in a large prospective, randomized, controlled study including 700 critically-ill pediatric patients. Subjects were randomly assigned to intensive insulin treatment (to target blood glucose concentrations of 2.8–4.4 mmol/L in infants and 3.9–5.6 mmol/L in children) or conventional insulin infusion to prevent blood glucose from exceeding 11.9 mmol/L. A significantly shorter PICU stay was found in the intensively treated group (5.5 days vs. 6.2 days, $p = 0.017$) [3]. However, among several neurocognitive outcomes analyzed in a four-year follow-up of these children, only motor coordination ($p \leq 0.03$) and cognitive flexibility ($p = 0.02$) were worse in conventional insulin infusion group [4]. Another study on 97 patients with a median age of two years demonstrated that hyperglycemia was associated with higher morbidity (e.g., need of mechanical ventilation at 30 days) in meningococcal sepsis [5]. Other investigators found significantly reduced number of complications (e.g., incidence of sepsis) with lower (6.7–7.2 mmol/L) compared to higher (8.3–8.9 mmol/L) glucose targets in pediatric patients with burns [6]. On the contrary, a large multicenter trial, including more than 1300 critically-ill children randomly assigned to conventional glycemic control (glucose below 12 mmol/L) or tight glycemic control (4–7 mmol/L), found that the tight glycemic control had no significant effect on need of mechanical

ventilation and mortality [7]. In this study, however, the upper limit of tight glycemic control was higher than in previous RCT, reducing the number of patients undergoing insulin treatment. In "The Heart and Lung Failure–Pediatric Insulin Titration (HALF-PINT)" trial (Clinical Trials ID: NCT01565941), glycemic control targeted to blood levels between 2.8–4.4 mmol/L in infants and 3.3–5.6 mmol/L in children was neither associated with lower length of PICU stay nor with mortality, as compared to levels between 8.3 and 10 mmol/L [8].

It may also be considered that tight glycemic control is difficult to achieve and, very often, intensive insulin therapy inevitably increases the risk of hypoglycemic episodes [9]. In turn, if severe and prolonged, low glucose levels may also cause major adverse events. A study investigating the effects of hypoglycemia found that critically-ill infants spending more than 50% of the time with lower (4.4–6.1 mmol/L) glucose levels had more frequent complications (e.g., renal failure) than those with higher (>11.1 mml/L) glucose levels [10]. However, in the Leuven pediatric study, although hypoglycemia was more common in children on intensive insulin therapy and patients developing hypoglycemia had a higher risk of death, this association was not significant and it could be explained by duration of PICU stay [3,4]. Other similar studies showed contrasting results on the association between hypoglycemia episodes and neurological consequences [11,12].

Randomized clinical trials on glycemic control and nutrition in critically-ill children are reported in Table 16. As a result of this section, the targets of glycemic control are still debated. Since normoglycemia is mostly associated with favorable outcomes, in terms of hospital stay and mortality, both hyperglycemia and hypoglycemia should be adequately prevented or promptly managed. However, the adoption of a tight glucose range for critically-ill patients is debated [13]. Indeed, currently, there is no recommendation available on hyperglycemia management provided from pediatric international societies. A "common sense approach" suggests keeping blood glucose between 7.8 and 10.0 mmol/L [14].

The Acute Phase: Metabolic Steps and Nutritional Implications

In acute stress conditions, circulating glucose and glycogen stores are rapidly depleted. Hence, hepatic gluconeogenesis, fatty acid beta-oxidation, and ketogenesis become the primary source of energy. At a further stage, the energy necessary for the increased gluconeogenesis is provided from either lactate or proteins and Aminoacids (AAs) [15]. These physiologic mechanisms are mediated by the insulin release switch-off followed by a production of glucagon, cortisol, and epinephrine, and activation of the sympathetic activity. They aim at providing sufficient energy for body metabolism and, among them, ketone bodies mainly supply central nervous energy requirements. During severe infections increased levels of inflammatory cytokines [e.g., Interleukin 1 (IL1) and Tumor Necrosis Factor (TNF)], Adreno-Cortico-Tropic Hormone (ACTH), and growth hormone tend to amplify these pathways and enhance protein breakdown [16–18]. Although these mechanisms are well known, management of ketosis in acute stress conditions is still challenging. On the one hand, ketone bodies are organic acids and, therefore, consume bicarbonates leading to blood acidosis and may cause malaise, nausea, and vomiting [16,19]. On the other hand, the amount of fatty acids resulting from lipolysis may even exceed energy requirements and glucose supplementation may rapidly lead to hyperglycemia and hepatic steatosis [15,16]. Yet, no randomized control trial has been conducted so far on different strategies to manage ketosis in critically-ill children.

Failing to provide adequate amounts of nutrients in the acute phase of stress response also results in exacerbation of existing nutritional problems in children. Malnutrition and infection may indeed interact, reinforcing each other even in milder stage of disease [20-23]. Furthermore, restricted nutritional support may stimulate autophagy, a survival mechanism by which cells break down their own damaged components to recycle intracellular nutrients and generate energy during starvation [23–25]. However, the acute stress response is a complex condition where hypercatabolism and

muscular tissue consumption often cannot be reversed even with increased provision of nutrients ("futile cycle of nutrients") [26]. On the other hand, overfeeding, i.e., a caloric intake/REE ratio >110% or >120% [27], is associated with increased morbidity (e.g., delayed ventilator weaning), prolonged hospitalization, and a higher mortality [28]. Overfeeding may also inhibit autophagy, leading to an increased risk of cell death and organ dysfunction [26]. Being the lower and upper limits of individual energy requirements unknown in critically-ill children and may largely vary in the acute phase of stress conditions, a characteristic paradigm of under- and overfeeding is the unpredictable combination of metabolic and feeding patterns [29].

Stable and Recovery Phases

During the stable phase, both an early normalization of the catabolic counter-regulatory hormone levels and an increased effect of anabolic hormones occur, however, proteins continue to be wasted while fat stores remain relatively intact [30]. During this phase, the risk of muscle atrophy remains high, especially if this condition lasts several weeks. On the contrary, in the recovery phase, protein synthesis exceeds protein break down. Nutrition in both these phases should slowly increase to allow recovery and growth [31]. A recent systematic review and a single-center study in mechanically-ventilated children calculated a minimum intake of, respectively, 57 and 58 kcal/kg/day to achieve a positive nitrogen balance [32,33]. In both studies, a protein intake of 1.5 g/kg/day was suggested to attain nitrogen balance. In these studies, however, no difference was made between the stable and recovery phases.

Nutrition: Method of Administration and Immunity-Enhancers Nutrients

It is generally accepted that enteral nutrition is advised in the stable phase and mostly in the recovery phase. In the acute phase, it may also be of benefit, but its composition and timing of administration should be cautiously considered [34].

A large RCT, including 1440 patients from three different PICUs, found that early (within 24 h) parenteral supplementation of AAs was associated with a higher rate of infections and longer PICU stay [35]. Yet, the heterogeneity of the population and the different glycemic control strategies were likely to be biased across the participating centers [34,35]. On the contrary, in a retrospective study of more than 5100 critically-ill children, early enteral nutrition, over the first 48 h of admission, was associated with a lower mortality rate in those with a PICU length of stay at least 96 h [36].

Many strategies to optimize enteral nutrition have been developed recently. It has been suggested that an immune-enhancing formula (i.e., giving patients perioperative nutritional supplements with immunonutritional additives) might improve the general and metabolic conditions in adults with infection [37]. In particular, the importance of AAs, dietary nucleotides, and lipids in modulating immune function has been recognized. For instance, the arginine plasma concentrations are strongly related to the severity of systemic inflammation, being especially low during the acute phase of critical illness [38]. Dietary supplementation with arginine might have positive effects on immune function and reparative collagen synthesis [39]. The role of glutamine supplementation is controversial: experimental work proposed various mechanisms of action, but none of the randomized studies in early life showed any effect on mortality and only a few showed some effect on inflammatory response, organ function, and a trend for infection control [39,40]. Briassoulis et al., in a blinded, randomized, controlled trial, compared nitrogen balance, biochemical indices, antioxidant catalysts, and clinical outcomes in critically-ill children given an immune-enhancing formula or conventional early enteral nutrition [41]. In their cohort, immunonutrition had a favorable effect on some biochemical indices (e.g., natremia) and antioxidant catalysts. However, the mortality rate did not differ between the two groups [41]. A further single-center, randomized, blinded controlled trial in 38 children with septic shock, performed by the same group, compared the effect of early enteral feeding using immune-enhancing with non-immune-enhancing formulas on cytokines. The study showed that immune-enhancing nutrition can interfere with the production

of interleukin-6, but no evidence was found regarding the impact on the short-term outcome [42]. Finally, no clinical effect was provided by the immune-enhancing diet in a study including 40 ventilated children with severe head injury [43]. Overall, the present section suggests that, besides the role of the appropriate timing of the parenteral and enteral nutrition, some specific nutrients, particularly AAs, may contribute to an improvement of the immune response. Newer formulations of enteral or parenteral mixtures of AAs meeting the individual needs of different critically-ill populations should be tested [13].

Energy Expenditure Assessment

In critically-ill children, measured or calculated REE has been proposed to estimate energy intake requirements in children [44]. The five most commonly-used REE prediction formulas are: the WHO formula, the Harris–Benedict formula, the Schofield-W formula, the Schofield-HW formula, and the Oxford formula [16]. These formulas have been validated in healthy children. However, they were found inaccurate in critically-ill children [29,31,45]. In a prospective cohort study, performed in a PICU setting, standard equations overestimated the energy expenditure and an 83% incidence of overfeeding with cumulative energy excess of up to 8000 kcal/week was observed [46]. Another prospective study found that MREE during critical illness was much lower than the energy expenditure predicted by formulas [47]. Further studies came to similar conclusions [48,49].

IC has been proposed as a reference method to measure REE. This technique is used to measure the rate of energy production and substrate oxidation in children, both in clinical and research settings. The recent clinical guidelines of the ASPEN for nutritional support of the critically-ill child, suggest that IC measurements should be obtained in patients with suspected metabolic alterations or malnutrition [50]. A study including 150 patients found that 72% of PICU patients were candidates for IC accordingly to the ASPEN guidelines [51]. Particularly, authors suggested prioritizing performing IC in patients <2 years of age, malnourished (underweight/overweight) on admission, or

with a PICU stay of >5 days [43]. In recent years, the reliability of ventilator-derived VCO_2 equations was investigated. These simplified methods measure the VCO_2 derived from measurements of exhaled gas volume and CO_2 concentrations and seem to be a promising tool when IC is not available or applicable [52]. Recent studies have demonstrated that this technique is a promising option for the determination of energy requirements in children on mechanical ventilation [21]. However, since the variability of RQ influences the accuracy of the $EEVCO_2$ calculation (EE from CO_2 measurements), and many of these approaches assume that the RQ value is a fixed value, the validity of this technique as an alternative to IC is questionable in some circumstances [52]. Strides have been made to build new, compact metabolic monitors to measure REE in PICU and to validate them. However, again, there is a wide range of conditions that may compromise their accuracy. For instance, metabolic monitors' errors were shown to be significantly affected by oxygen concentration and minute ventilation and when used during inhaled anesthesia [53].

7.4.4 Conclusion

For critically-ill children, the role of nutrition is evolving from a simple supportive function to the possibility of an effective co-adjuvant therapy. However, the variability of metabolic responses to stress requires testing the hypothesis of an individualized approach to nutrition in critically-ill children, since available data are mainly derived from healthy individuals [54]. Additionally, the possible "intermediary" role of the microbiome should be investigated in future studies in PICUs [54]. Unfortunately, as previously mentioned, in stress conditions, the futile cycle of nutrients may make most interventions that are effective in healthy subjects useless. Indeed, inconclusive data are available in most important issues of critically-ill child nutrition (such as the glycemic control) and no international recommendation exists for the management of many common problems in their

day-to-day care [13]. The determination of individual macronutrients needed in the various phases of stress are still more hypothetical than evidence-based. Finally, problems with the current predictive equations and lack of availability of IC are likely to result in continued under- or overfeeding in many critically-ill children, with the associated morbidity [55]. New efforts are urgently needed to develop individualized nutrition strategies and evaluate their effect on relevant health outcomes.

Table 16. RCTs on glycemic control and nutrition in critically-ill children.

Authors, years	Country	Groups	Primary Outcomes	Results
Vlasselaers et al.	Belgium	Tight glycemic control (interventional group) n = 700, conventional glycemic control (control group) n = 351, age = 0–16 years. Statistical power = 80%	Effect of tight glycemic control on duration of PICU stay and inflammation	PICU stay was shorter (5.5 vs. 6.2 days, p = 0.017) and C-reactive protein change after 5 days lower (–9.8 mg/L vs. 9.0 mg/L, p = 0.007) in interventional vs. control group
Mesotten et al.	Belgium	Tight glycemic control (interventional group) n = 222, conventional glycemic control (control group) n = 234, age $\leq$ 16 years. Statistical power = 80%	Effect of tight glycemic control on long-term follow-up of neuro-cognitive-outcomes	No significant difference between the two groups was observed
Jeschke et al.	USA	Tight glycemic control (interventional group) n = 60, conventional glycemic control (control group) n = 159, age = 0–16 years. The study was underpowered	Effect of tight glycemic control on infectious events	Sepsis was less frequent (p < 0.05) in interventional than in control group (8.2% and 22.6% of patients, respectively)
Macrae et al.	England	Tight glycemic control (interventional group) n = 694, conventional glycemic control (control group) n = 675, age = 0–16 years. Statistical power = 80%	Effect of tight glycemic control on days alive and free from and free from mechanical ventilation at 30 days after enrollment	No significant difference between the two groups was observed
Agus et al.	USA	Tight glycemic control (interventional group) n = 349, conventional glycemic control (control group) n = 360, age = 2 weeks–17 years. Statistical power = 80%	Effect of tight glycemic control on length of PICU stay	No significant difference between the two groups was observed
Agus et al.	USA	Tight glycemic control (interventional group) n = 490, conventional glycemic control (control group) n = 490, age = 0–36 months. Statistical power = 80%	Effect of tight glycemic control on mortality, length of PICU stay, and infectious events	No significant difference between the two groups was observed
Sadhwani et al.	USA	Tight glycemic control (interventional group) n = 121, conventional glycemic control (control group) n = 116, age = 0–36 months. Statistical power not reported	Effect of tight glycemic control on neurodevelopment follow-up	No significant difference between the two groups was observed
Vanhorebee et al.	Belgium	Tight glycemic control (interventional group) n = 349, conventional glycemic control (control group) n = 351, age = 0–16 years. Statistical power = 80%	Effect of tight glycemic control on neurological injury biomarkers	No significant difference between the two groups was observed
Vanhorebee et al.	Belgium, Netherlands, Canada	Early parenteral nutrition (interventional group) n = 723, late parenteral nutrition (control group) n = 717, age = 0–17 years. Statistical power = 70%	Effect of macronutrients supplementation timing on infections, need of mechanical ventilation, and length of PICU stay	The early provision of amino-acids, and not glucose or lipids, was associated with worse outcomes
Briassoulis et al.	Greece	Immunonutrition (interventional group), n = 25. Conventional enteral nutrition (control group) n = 25, age = 8–9.2 years. Statistical power not reported	Effect of immunonutrition on biochemical nutritional markers and hard outcomes (mortality, length of PICU stay, and need of mechanical ventilation)	Immunonutrition had a favorable effect on few nutritional biochemical markers, but not on hard outcomes

Briassoulis et al.	Greece	Immunonutrition (interventional group), $n = 15$, conventional enteral nutrition (control group) $n = 15$, age = 6.5–7.9 years. Statistical power not reported	Effect of immunonutrition on interleukins in septic children	IL-6 levels were lower (11.8 vs. 38.3 pg/mL, $p < 0.001$) and IL-8 higher (65.4 vs. 21 pg/mL, $p < 0.03$) in interventional group compared with control group
Briassoulis et al.	Greece	Immunonutrition (interventional group), $n = 20$, conventional enteral nutrition (control group) $n = 20$, age = 6–10.5 years. Statistical power not reported	Effect of immunonutrition on biochemical nutritional markers and hard outcomes (mortality, length of PICU stay, and need of mechanical ventilation) in severe head injury patients	Except for IL-8 levels and nitrogen balance, no difference was observed between the two groups

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CHAPTER 8: Conclusions

8.1 Summary of key findings

Lifestyle factors, including age when starting a family, nutrition, weight management, exercise, psychological stress, cigarette smoking, alcohol and caffeine consumption, are modifiable behaviors that may impact fertility. The main evidence suggests that age play a large role in determining fertility. When both partners consider their ages together they may be able to increase their odds of having a successful pregnancy. Other evidences regard the role of proper nutrition, weight, and exercise. In this work, the role of diet has been taken into account and emerges that choosing proper nutrition and following a healthy lifestyle, before and during attempts to conceive, may be crucial for improving fertility for both men and women. In this thesis data from a study on the impact of lifestyle habits and diet on ARTs in Italian infertile couples have been analyzed. Moderate alcohol intake appears associated with increased semen volume, sperm concentration and total sperm count in the whole sample. While there is evidence to support that alcohol does have an impact on fertility, it is also difficult to establish a definitive link as there is no standard "drink" or comparative way to measure alcohol consumption. We could not analyze the role of heavy or binge drinking, which are consistently associated to detrimental effects on semen quality. Regarding female fertility, in literature there is evidence of a lower risk of IVF failure in women reporting higher MDS scores. The analysis here described does not show a statistically significant effect neither of Mediterranean Diet nor of moderate alcohol, moderate coffee intake and smoking on oocyte quality and success rate after ART procedures. It has to be considered that the sample analyzed had a moderate consumption of alcohol, coffee and cigarettes, so that it was impossible to evaluate the effect of heavy drinking or smoking on IVF outcomes. As general recommendation, all couples who are attempting to achieve a pregnancy should be advised to limit or avoid alcohol

drinking and smoking, while maintaining caffeine intakes within suggested limits for consistence. It is important to understand the ways in which lifestyle behaviors may benefit or harm fertility in order to minimize complications and to maximize fertility outcomes. By understanding the impact of lifestyle on reproductive health, and by actively modifying lifestyle behaviors, men and women are capable of controlling their own fertility potential.

Moreover, nutrition has the role of modulating the capacity to exit from the state of stress and disease, with possible repercussions on growth and development. Nutrition imbalances may affect also the prognosis during hospital stay, both in adult and in children, so that hospital-acquired malnutrition, is very frequent. Its genesis is multifactorial, some factors are inherent to the patient, and others, to the stay, and its management requires the healthcare team to consider nutrition a part of inpatient care. In this work, a high prevalence of both acute and chronic malnutrition among hospitalized pediatric patients in Italy emerges, especially in infants and young children and nutritional support is only given to a small number of the malnourished children. One of the strategies to improve the nutritional care is the accurate count of energy intakes, starting from the metabolism's measure or estimate. In the last part of the thesis was demonstrated that the commonly employed equations, WHO, Harris-Benedict, Schofield, and Oxford formulae should not be used to estimate REE in hospitalized children. In particular, infants with acute bronchiolitis present with a high prevalence of malnutrition and altered metabolic state, which was not accurately estimated. Feeding strategies based on these equations might result in unintended underfeeding or overfeeding. Metabolic response to stress is variable and cannot be easily predicted, suggesting the need for an individualized approach to nutrition. Cumulative effects of energy imbalance can negatively impact patient outcomes and must be prevented. The role of nutrition is evolving from a simple supportive function to the possibility of an effective co-adjuvant therapy. An individualized approach to nutrient delivery, with attention to delivery of prescribed nutrients, and increased awareness of energy needs, has the potential for improving clinical outcomes.

8.2 Future Research

Further research is needed to individuate a dietary pattern able to preserve fertility both in males and in females, taking into account the whole complexity of the relations between nutrients, in order to make specific recommendations. Eliminating every exposure is unrealistic; however, identifying, eliminating, or minimizing even one factor may have significant positive effects on fertility for both men and women. Interventions may consider not only nutritional patterns, but also behavioral modifiers and other socioeconomic and built environment factors that affect lifestyle and adherence. Regarding nutrition in early life and the role of metabolism, few studies have been conducted to investigate the role of predictive equations in healthy children and results are controversial. Some agree in considering these equations accurate to measure REE in health conditions even if they have been created in early ‘90s and even if they involve extrapolation from an adult-age population. Other studies disagree in considering these equations accurate. A major step forward to the comprehension of malnutrition related mechanism may be the accurate development of new predictive equations not only in ill children, but also in healthy ones.

CHAPTER 9: Annex

9.1 List of published papers

- Agostoni C, Edefonti A, Calderini E, Fossali E, Colombo C, Battezzati A, Bertoli S, Milani G, Bisogno A, Perrone M, Bettocchi S, **De Cosmi V**, Mazzocchi A, Bedogni G. **Accuracy of prediction formulae for the assessment of resting energy expenditure in hospitalized children**. J Pediatr Gastroenterol Nutr. 2016 Dec;63(6):708-712.

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- **De Cosmi V**, Mehta NM, Boccazzi A, Milani GP, Esposito S, Bedogni G, Agostoni C. **Nutritional status, metabolic state, nutrient intake in children with bronchiolitis.** Int J Food Sci Nutr. 2017 May;68(3):378-383.

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- **De Cosmi V**, Milani GP, Mazzocchi A, D'Oria V, Silano M, Agostoni C. **The metabolic response to stress and infection in critically ill children: the opportunity of an individualized approach.** Nutrients. 2017 Sep 18;9(9).

- Mazzocchi A, D'Oria V, **De Cosmi V**, Bettocchi S, Milani GP, Silano M, Agostoni C. **The Role of Lipids in Human Milk and Infant Formulae.** Nutrients. 2018 May 4;10(5).

- Scaglioni S, **De Cosmi V**, Ciappolino V, Parazzini F, Brambilla P, Agostoni C. **Factors influencing children's eating beahviours.** Nutrients. 2018 May 31;10(6).

- Ricci E, Noli S, Ferrari S, La Vecchia I, Cipriani S, **De Cosmi V**, Somigliana E, Parazzini F. **Alcohol intake and semen variables: cross-sectional analysis of a prospective cohort study of men referring to an Italian Fertility Clinic.** Andrology. 2018 Jul 18.

9.2 List of under-submission papers

- The role of genetic predisposition, programming during fetal life and family conditions in the development of pediatric NASH.

- Adherence to the Mediterranean diet and outcomes of assisted reproduction: results from an Italian study.

- Pre-treatment maternal lifestyle and outcomes of assisted reproduction: an Italian cohort study.

- Micronutrients intake and the risk of poor semen quality: cross-sectional analysis of men referring to an Italian Fertility Clinic.