Advances in Health and Disease

Advances in Health and Disease

Volume 33



Lowell T. Duncan
Editor

Nova Biomedical

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ADVANCES IN HEALTH AND DISEASE

VOLUME 33

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ADVANCES IN HEALTH AND DISEASE

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VOLUME 33

LOWELL T. DUNCAN
EDITOR

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PREFACE

This volume comprises 9 chapters, each concerning a recent advancement in health and disease. Chapter 1 critically appraises the current literature related to pediatric obesity and asthma, summarizes the evidence on the impact of excess body fat on the pulmonary system in pediatric asthma patients, and discusses possible mechanisms as well as effective public health strategies for reducing the increasing obesity trend and mitigating asthma risk. Chapter 2 details the nutritional and bioactive composition of lactuca sativa, commonly called lettuce, including its antioxidant, cholesterol-lowering, cardioprotective, neuroprotective, anti-diabetic, anti-inflammatory, anti-proliferative, and anti-cancer properties. Chapter 3 attempts to shed light into the pathogenetic mechanisms connecting metabolic syndrome and nonalcoholic fatty liver disease (NAFLD) to the emergence and progression of hepatocellular carcinoma, emphasizing the evolving epidemiological profile of this type of cancer. Chapter 4 is also about lettuce and provides another perspective on the nutritional composition and health benefits among different varieties of the vegetable. Chapter 5 deals with the health promoting effects of onions (allium cepa l.), including their insulin secretagogue effect, hypoglycemic influence, hypolipidemic action, antioxidant effect, cardioprotective effect, gallstone prevention, cancer preventive potential, and digestive stimulant action, all of which are scientifically validated. Chapter 6 remarks on the

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difficulties associated with diagnosing rare diseases and describes several systems-level interventions that can be expected to limit the diagnostic delays experienced by patients with rare diseases and the frustration associated with years or decades of untreated symptoms. Chapter 7 describes the prevalence and effects of iron deficiency and anemia as well as treatment options for these conditions. Chapter 8 once again focuses on lettuce, this time exploring the nutritional and anti-cancer effects of carotenoids from different varieties of lettuce. Finally, Chapter 9 describes an application for the diagnosis of hypothermia in forensic practice wherein the color difference between blood in the left cardiac chamber and blood in the right cardiac chamber can be examined using a CO-oximeter.

Chapter 1 - The global surge in pediatric obesity and asthma are two disorders requiring urgent attention in public health and account for significant patient, familial and societal economic burden including school and employment absentees, hospital care and pharmaceutical expenditures. Overweight and obesity as measured by body mass index (BMI) is a risk factor for many conditions including pediatric asthma, that has immediate and long-term negative impact on children's health physically, psychologically, socially as well as on academic performance. The obese-asthma phenotype in children is more difficult to treat, is related to increased and more severe exacerbations, need for hospitalization, days absent from school, impaired lung function, reduced medication response, poor asthma control and quality of life compared to normo-weight peers. Despite the growing evidence on the obese-asthmatic phenotype, little is known about its specific characteristics in children. The purpose of this chapter is to critically appraise the current literature and summarize the evidence on the impact of excess body fat on the pulmonary system in pediatric asthma patients. Possible mechanisms are also discussed. In conclusion, paediatric obesity remains a growing global issue with significant detrimental health impact for paediatric respiratory health. Effective public health strategies focusing on reducing the increasing obesity trend, have the potential to reduce asthma risk, patient burden and overall cost. The findings of this review highlight the complexity and heterogeneity of asthma in children and imply that deficits observed in lung function of pediatric asthma patients

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might be due to obesity-related systemic inflammation, mechanical overload, metabolic dysfunction, oxidative stress and hormonal effects of excess body fat including disproportionate lung growth. Childhood may represent a critical window of opportunity to intervene and prevent long term consequences. Phenotyping and lifestyle interventions promoting a healthy diet, daily physical exercise and outdoor play combined with asthma therapy should be considered key points in the management of obesity in pediatric asthma patients. Future longitudinal studies are recommended to determine the mechanisms underlying the obese-asthma phenotype in children.

Chapter 2 - *Lactuca sativa*, commonly called as lettuce, is a well-known leafy vegetable consumed worldwide that possessing high nutritional value. The nutritional and bioactive composition of lettuce differs depending on the origin and the type of lettuce. Lettuce is low in calories and own numerous health benefits mainly due to its high availability of vitamins, phytochemicals (phenolics and carotenoids), macro and micronutrients. Several *in vitro* and *in vivo* studies have demonstrated the antioxidant, cholesterol-lowering, cardioprotective, neuroprotective, anti-diabetic, anti-inflammatory, anti-proliferative, anti-cancer activities of the lettuce bioactives. In addition, clinical studies have shown lettuce consumption markedly increased the bioavailability of phenolics and carotenoids in the plasma, improved glucose metabolism and reduced total lipids in the liver. However, the explication of molecular and metabolic mechanisms of lettuce derived bioactives need to be studied further for the health benefits and safety and regular consumption.

Chapter 3 - During the past decades, the globally increasing incidence of metabolic syndrome and obesity has attained the character of an epidemic. Additionally, Non Alcoholic Fatty Liver Disease (NAFLD), strongly associated with type 2 diabetes mellitus and metabolic syndrome, has emerged as a major contributor to chronic liver disease and cirrhosis, with a global prevalence of about 25,2%. In the meantime, despite the development of anti-viral therapies and organized vaccination programs, emerging data demonstrate that the incidence of hepatocellular carcinoma (HCC) is continuously rising. However, the current epidemiological landscape of HCC is believed to be quite different than that of the past century, with

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obesity, metabolic syndrome, type 2 diabetes mellitus and NAFLD acting as the leading modifiers in the development of HCC. This review attempts to shed light into the pathogenetic mechanisms connecting metabolic syndrome and NAFLD to the emergence and progression of hepatocellular carcinoma, emphasizing on the evolving epidemiological profile of this type of cancer.

Chapter 4 - *Lactuca sativa* (lettuce) is one of the major leafy vegetable widely grown across the world. There are numerous varieties of lettuce and it remains to be the most widely consumed vegetable, mainly in the form of salads, as it is rich in nutrition. Lettuce is commonly eaten in raw form without cooking or processing; hence the quality and quantity of the bioactives in lettuce are not compromised. Bioactives of lettuce includes carotenoids, phenolics, fibers, vitamins and minerals that generally differ among the lettuce varieties. Green lettuce is rich in Vitamin C, whereas red lettuce is rich in phenolic content. Lactucaxanthin, a xanthophyll carotenoid, also the isomer of lutein, is a novel bioactive carotenoid abundantly present in lettuce. Studies have shown that the lettuce bioactives exhibited antioxidant, anti-diabetic, cholesterol lowering, anti-inflammatory and anticancer activities. In this book chapter, lettuce's nutritional composition and health benefits among the different varieties of lettuce are summarized.

Chapter 5 - Several medicinal properties of onion have been recognized since ancient times and used in traditional systems of medicine for treating diseases. Bioactive phytochemicals present in this spicy vegetable have now been understood to promote human health in many ways. The mechanism of such an action has also been elucidated by researchers in clinical studies and experimental models. Insulin secretagogue effect, hypoglycemic influence, hypolipidemic action, antioxidant effect, cardioprotective effect, gallstone prevention, cancer preventive potential, and digestive stimulant action are among the scientifically validated health effects of onion consumption. Its ability to suppress oxidative stress and the hypolipidemic potential have far-reaching implications in the prevention of several degenerative diseases. Generally, the antidiabetic influence of dietary onion was higher when consumed along with dietary fiber-rich fenugreek seeds, which also exerts

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an antidiabetic effect by an independent mechanism. Onion also enhances the bioavailability of micronutrients from plant foods.

Chapter 6 - Occam's razor proffers guidance in the diagnosis of rare diseases, suggesting that the most likely diagnosis is that which can explain every symptom. This logic should lead a clinician who is attuned to their patient to arrive at a short list of the compatible causes which can explain all of—even the slightest—findings of their patient's physical exam. In conflict with this enduring philosophical principle, however, Hickam's dictum observes that it is not always so straightforward: one patient can experience multiple conditions simultaneously. With respect to the very rarest of diseases, statistics appear to support Hickam's dictum in that two relatively common ailments may in fact be a more likely explanation for a patient's constellation of symptoms than a singular and remarkably atypical disease. Consequently, the reality of rare disease diagnosis is defined by a process commonly referred to as a "diagnostic odyssey": a journey fraught with diagnostic delays and misdiagnoses, which a patient is forced to negotiate even while experiencing the symptoms of their illness. Intuitively, medical training and clinical practice afford greater exposure to more common diseases, necessarily resulting in many uncommon and unusually maladies—simply by virtue of their rarity—resisting easy diagnosis. Despite the obstacles therefore inherent in rare disease diagnosis, several systems-level interventions can be expected to limit the diagnostic delays experienced by patients with rare diseases, and the frustration associated with years or decades of untreated symptoms. These interventions include increased integration at the levels of medical training and practice, the utilization of emerging digital resources designed to assist with rare disease diagnosis, and the adoption of patient navigators to assist and advocate for patients during their diagnostic odyssey. While many of the challenges intrinsic to diagnosing rare diseases are therefore ineradicable, interventions designed to ameliorate the diagnostic odyssey can nonetheless be meaningful for patients.

Chapter 7 - Recently, iron deficiency and anemia due to iron deficiency is one of the most common medical conditions in clinical practice worldwide. Since in emerging countries mostly malnutrition, insufficient

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uptake of iron, and parasitic infestations are main cause, in developed countries iron deficiency is still not rare due to vegaterian diet and malignancies. Iron deficiency is usually asymptomatic but also can be seen with widespread clinical features, such as; weakness, fatigue, exortional dyspnea, restless leg syndrome. To diagnose iron deficiency, traditional laboratory tests are complete blood count and peripheral blood smear tests. Transferrin saturation and ferritin tests are the most commonly used in diagnosis. Rarely, bone marrow biopsy is needed. Oral iron administration is first line treatment for iron deficiency but intravenous administration of iron can be preferred in conditions such as: drug intolerance, deep anemia, chronic renal failure. Reticulocyte count and complete blood count can be used for evaluating therapy response. Iron deficiency anemia is one of the most common medical condition and important cause of mortality and morbidity so that diagnosis, treatment and follow up is important.

Chapter 8 - *Lactuca sativa* (Lettuce) is one of the most common green leafy vegetable cultivated across the world. Lettuce is mostly consumed in raw form and used to prepare curries, salads and soups. Lettuce is less in calories and is a good source of iron, folate, vitamin C and fiber. Wide varieties of lettuce include romaine, loose-leaf, iceberg, butterhead-leaf (green/red) and stem lettuce. Depending on the variety, the composition of lettuce also changes. Among all the varieties, romaine lettuce is rich in dietary fiber while the butterhead is rich in iron and folate content. Green leaf and baby romaine are rich in Vitamin C levels while β-carotene and lutein are rich in butterhead and romaine lettuce. Phenolic compounds are typically high in red romaine and red leaf varieties. Lutein, β-carotene, violaxanthin, neoxanthin, zeaxanthin and lactucaxanthin are the major carotenoids of lettuce. Lettuce is well documented for its numerous biological activities such as antioxidant, anti-diabetic, cholesterol-lowering, anti-inflammatory, anti-cancer, etc.

Chapter 9 - Hypothermia is one of the external causes of death due to a cold environment. There are few or no specific autopsy findings in cases of fatal hypothermia. However, it has been reported that, in cases of hypothermic death, blood in the left cardiac chamber is bright red compared to that in the right cardiac chamber, which might be a characteristic autopsy

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sign of hypothermic death. This color difference is due to the difference in oxygen content in blood in the two chambers. Examination of this difference using a CO-oximeter would provide valuable information in cases of hypothermia. In the present paper, the authors discuss application of the CO-oximeter for the forensic diagnosis of hypothermia.

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Chapter 1

IMPACT OF PEDIATRIC OBESITY ON LUNG PHYSIOLOGY

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ABSTRACT

The global surge in pediatric obesity and asthma are two disorders requiring urgent attention in public health and account for significant patient, familial and societal economic burden including school and

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employment absentees, hospital care and pharmaceutical expenditures. Overweight and obesity as measured by body mass index (BMI) is a risk factor for many conditions including pediatric asthma, that has immediate and long-term negative impact on children's health physically, psychologically, socially as well as on academic performance. The obese-asthma phenotype in children is more difficult to treat, is related to increased and more severe exacerbations, need for hospitalization, days absent from school, impaired lung function, reduced medication response, poor asthma control and quality of life compared to normo-weight peers. Despite the growing evidence on the obese-asthmatic phenotype, little is known about its specific characteristics in children. The purpose of this chapter is to critically appraise the current literature and summarize the evidence on the impact of excess body fat on the pulmonary system in pediatric asthma patients. Possible mechanisms are also discussed.

In conclusion, paediatric obesity remains a growing global issue with significant detrimental health impact for paediatric respiratory health. Effective public health strategies focusing on reducing the increasing obesity trend, have the potential to reduce asthma risk, patient burden and overall cost. The findings of this review highlight the complexity and heterogeneity of asthma in children and imply that deficits observed in lung function of pediatric asthma patients might be due to obesity-related systemic inflammation, mechanical overload, metabolic dysfunction, oxidative stress and hormonal effects of excess body fat including disproportionate lung growth. Childhood may represent a critical window of opportunity to intervene and prevent long term consequences. Phenotyping and lifestyle interventions promoting a healthy diet, daily physical exercise and outdoor play combined with asthma therapy should be considered key points in the management of obesity in pediatric asthma patients. Future longitudinal studies are recommended to determine the mechanisms underlying the obese-asthma phenotype in children.

INTRODUCTION

The global surge in pediatric obesity and asthma are two disorders requiring urgent attention in public health and account for significant patient, familial, societal and economic burden including school and employment absentees, hospital care and pharmaceutical expenditures [1, 2]. Both conditions share common genetic, environmental and lifestyle factors (such as hereditary, unhealthy diet and physical inactivity) affecting the child patient physically, psychologically, socially, along with sleep patterns and

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low academic performance [1, 3-5]. Asthma and obesity have their roots in childhood and can persist into adulthood causing substantial disability and poor quality of life [1, 3].

The Obesity Pandemic in Children

Overweight and obesity are defined as excessive accumulation of body fat that is detrimental to health and fundamentally caused by an energy imbalance between calories consumed and expended [6]. Alarmingly, the global prevalence of obesity has tripled over the last 30 years, irrespective of socioeconomic status, gender and age [6]. According to World Health Organization statistics, in the mid childhood age group (5-19 years), more than 340 million children and adolescents, worldwide, were overweight or obese in 2016, documenting a radical increase from 4% in the seventies to approximately 18% in 2016 (% Overweight (2016): 18% girls, 19% boys) [6]. During the same time period, obesity rates have increased from just 1% to 14% (% Obesity (2016): 6% girls, 8% boys), that is 124 million in the 5-19 years age group. Over 60% of children who are overweight before puberty will be overweight in early adulthood [6]. Modifications in today's dietary patterns consisting of an increased intake of energy-dense foods (such as fast food, sweets, sugary drinks) that are rich in fat and sugars and of low nutritional value, as well as large portion sizes combined with decreased physical activity and increased sedentariness (more hours spent television viewing, videos and computer games) [3], have contributed to the epidemic rise of this disease in the pediatric population. In terms of public health, this is significant because childhood obesity is the principal risk factor responsible for earlier onset of various comorbidities related to the cardiovascular, metabolic, endocrine, gastrointestinal and respiratory systems including asthma as well as a higher risk of premature death and disability in adulthood [3, 7]. These serious health complications are attributed to either the increased fat mass or release of peptides from enlarged fat cells [3, 7].

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Asthma Definition and Pathophysiology

There is general consensus that asthma is defined as a chronic heterogeneous inflammatory disease of the upper airways that is associated with airway inflammation, edema, hypersecretion of mucus and hyperresponsiveness [8] that are presented clinically by symptoms of wheeze, cough, dyspnea and chest tightness, especially at night and early morning [9]. Furthermore, this process of chronic airway inflammation leads to structural changes in airways (remodeling) that involve thickening of the basal membrane and hyperplasia of smooth muscle and goblet cells [10]. It is believed that asthma is initiated by the complex interaction among genes, environmental and lifestyle determinants [9]. Exposure to allergens activates an immune response in airways that include infiltration of T cells, eosinophils and basophils, consequently leading to onset of symptoms [11]. There are multiple triggers, including house dust mites, mold, pollen, cigarette smoke, air pollution, respiratory infections, pet hair, high fat diet, sedentary lifestyle and co-morbidities such as obesity (high BMI) [12]. A comprehensive list of risk factors is presented in Table 1.

Table 1. Risk factors for asthma development

Direction of association between risk factor and childhood asthma	
Positive	Negative
Age	High altitude
Male gender	Day care center attendance
Hereditary	Family size
Maternal asthma	Living in rural areas
Asthma in siblings	Farm animals
Birth rank-first born	Cats*
High-income lifestyle	Dogs*
Fast food	Fruit and vegetables
Trans fat	Mediterranean Diet
Salt	Probiotics
Atopic sensitization	Breastfeeding
Premature birth	Raw milk
Low birth weight	Fish oils (Ω3)
Fast infant weight gain	Selenium
Caesarean delivery	Vitamin A

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Direction of association between risk factor and childhood asthma	
Positive	Negative
Rhinitis	Vitamin D
Stress	Vitamin E
High BMI	Magnesium
Physical inactivity	
Sedentary behavior (TV viewing, computer time, video games)	
Respiratory infections	
Physical exertion	
High-intensity exercise	
Medication	
Paracetamol	
Beta-agonists	
Antibiotics	
Molds	
Inhaled Exposures	
Maternal/Paternal Smoking	
Child Smoking	
Indoor air pollution	
House dust mites	
Outdoor air pollution	
Weather changes	
Humidity	
Thunderstorms	

*Inconsistent evidence

In italics are shown risk factors shared by obesity and asthma.

References: Beasley et al., 2015. Risk factors for asthma: is prevention possible? *Lancet* 386 (999):1075-1085.

Sahoo et al., 2015. Childhood obesity: causes and consequences. *J Family Med Prim Care 4* (2), 187–192.

Clinical diagnosis is established based on the presence of symptoms and documented variability in expiratory airflow limitation as measured by the gold standard of pulmonary function tests, spirometry [13].

Pediatric Asthma Prevalence

In children (5-14 years), asthma is the most common chronic disease, ranking among the top 10 conditions for disability-adjusted life years worldwide [14]. Latest data collected from the largest multi-national

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epidemiological study, the International Study of Asthma and Allergies in Childhood (ISAAC) Phase 3, that included 280 centers in 106 countries involving 1, 187 496 children up to 14 years old, documented that the global asthma prevalence was 11.6% in children aged 6–7 years and 13.7% in 13–14 years [15, 16]. Wide variations exist among countries up to 13-fold with prevalence of wheezing symptoms in children ≤ 14 years ranging from approximately 1% to 38%, with over 20% in Anglo-Saxon countries [15, 16]. The highest prevalence of severe asthma (~ 50%), measured as wheezing symptoms was observed in Eastern Mediterranean, Africa, India, Latin America and Anglo Saxon countries [16].

Although asthma incidence and prevalence are higher in children, morbidity, and mortality predominate in adults [17]. One third of children experience wheeze in the first three years of life, a common feature of respiratory infection rather than 'true' airway disease and most will lose symptoms by the age of six years [18]. Nevertheless, 40% of cases will continue to wheeze and develop asthma [19], being more common in boys' pre-puberty, after which it predominates in girls [20] suggesting that sex hormones may play a role in asthma etiology [21]. In addition, early menarche before 11 years increased asthma incidence in girls. Animal studies have shown that estrogen increased and testosterone decreased Th2-mediated airway inflammation [21]. Children with persistent and late onset wheeze (after 3 years old) have greater risk of having persistent childhood asthma [18]. In the long-term many children with early onset of persistent asthma or wheeze may have reduced airway and lung development with sub-optimal lung function potential [22]. These lung deficits can track into adulthood and juvenile asthma can contribute to increased risk of *Chronic Obstructive Pulmonary Disease* (COPD) in later life [22, 23].

Compared to adult patients, there is substantial diversity in pediatric asthma severity, pathophysiology, aetiology, clinical presentation (phenotype) and response to therapy, rendering asthma treatment to be a challenge, despite advances in knowledge of this complex condition and in drug therapy. In the real-life clinical setting, current asthma management guidelines are based on adult recommendations which do not always adequately address pediatric patients. Therefore, diagnosing asthma and

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establishing good asthma control as early as possible in childhood would reduce patient burden, prevent progression of asthma into adulthood, improve quality of life and facilitate significant long term cost savings for the patient and the healthcare system.

Asthma Heterogeneity

Asthma is not a single disease entity but is considered to be an umbrella term for a collection of several diseases with distinct molecular pathways (endotypes) and varying clinical presentations (phenotypes) that share common clinical features of wheeze, dyspnea, chest tightness and cough of varying intensity and degree of airway limitation [9]. This heterogeneity of traits renders asthma diagnosis and treatment to be difficult for the pediatrician.

Classification of childhood asthma phenotypes is based on patient's age during onset of symptoms, wheezing patterns (early, transient, persistent, late-onset), type of airway inflammation (eosinophilic, neutrophilic, paucicellular), disease severity and response to medication therapy [24]. Discrete phenotypes identified during early childhood (wheezing onset ≤ 3 years) include early, transient, persistent late-onset [25]; in mid-childhood (≥ 5 years) atopic, [26, 27], non-atopic [28], obese [29], severe asthma [28, 30] and steroid-resistant asthma [30, 31]. Hence, stratification of patients according to phenotype would improve accuracy of diagnosis, allow better asthma management by being able to predict the development and progression of disease and most importantly to tailor pharmacotherapy individually.

Obese-Asthma Origins in Utero

Obesity represents a serious risk factor throughout the lifespan. Whether surplus body weight precedes asthma, asthma predisposes to obesity or whether both conditions simply co-exist is a matter of scientific controversy.

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According to the developmental origins of health and disease hypothesis, exposure to certain environmental factors during early life can have significant consequences on health in offspring during childhood and adult life [32]. In fact, obesity induced asthma has been linked in utero. It has been shown that maternal obesity and excessive maternal weight gain during gestation were correlated with high risk of childhood asthma [33]. Recently, Liu et al. (2020) in a comprehensive meta-analysis of 22 observational studies involving 145, 574 mother-child pairs with age of follow-up from 14 months to 16 years, documented that maternal overweight or obesity pre-pregnancy significantly increased the risk of wheeze and asthma in progeny from 13% to 40% [34]. For every increment in pre-pregnancy maternal BMI, a 3% increased risk of childhood asthma and wheeze was observed. Furthermore, the degree of gestational weight gain (very low, moderate, high) was also found to be positively associated with elevated asthma risk and wheeze in childhood at 26%, 12% and 24% respectively. Forno et al. (2014), in an earlier meta-analysis, published that maternal obesity in pregnancy was associated with 30% higher odds of asthma or wheeze ever (OR: 1.31, 95% CI: 1.16-1.49) and 20% increase in current asthma in offspring (OR: 1.21, 95% CI: 1.07-1.37) [33]. A one unit (1-kg/m^2) increase in maternal BMI was associated with a 2% to 3% increase in the odds of childhood asthma. Additionally, high gestational weight gain was associated with 16% higher odds of asthma or wheeze ever (OR: 1.16; 95% CI: 1.001-1.34).

Interestingly, data from 12, 050 subjects participating in eight (8) European birth cohort studies reported that in children with rapid weight gain trajectory (as assessed by BMI) during the first 2 years of life had higher incidence of asthma risk up to age of 6 years than those with less pronounced weight gain in early childhood [35]. The high asthma risk in offspring conferred by maternal obesity or gestational weight gain could be modulated by alterations in immunity (eosinophils and monocytes), levels of inflammatory cytokines (IL-6, TNF-α, INF-α) [36], lipid profiles in cord blood and early post-natal life [37], metabolic and oxidation changes in the placenta and fetus [38] as well as elevated leptin levels and insulin resistance in neonates [39].

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Obese-Asthma Link in Juveniles

Obesity and asthma form a harmful synergy during the mid-childhood period. Observational studies have demonstrated that obese-asthmatic children experience higher frequency of severe exacerbations [40, 41], more night symptoms [42], days wheezing [43], emergency visits [42-44], hospitalization [43, 45] and length of stay [40, 41], mechanical ventilation [40, 41], medication use [42, 44], missed school days [42, 44] and reduced quality of life [43] than normo-weight counterparts.

Several studies suggest that the marked increase in obesity observed during the past 20 years may partly contribute to the increase in asthma prevalence that has concomitantly been observed. In a cross-sectional study, Forno et al. (2014) reported that BMI, per cent body fat and waist circumference were all associated with increased asthma morbidity severity, poor lung function and asthma control as measured by decrements in FEV_1/FVC in Puerto Rican children 6-14 years [44]. Furthermore, Chen et al. (2014) found that central obesity as a measure of adiposity predicted asthma risk and poor lung function in Taiwanese children 9-12 years of age [46]. Concrete evidence from systematic reviews and meta-analyses advocate that surplus body fat in children increased asthma prevalence [47], incidence [48], asthma risk [49, 50], exacerbation rate [51] and severity [52] as well as decreased asthma control [52], response to corticosteroid therapy [53] and quality of life [54].

Recently, Malden et al. (2020) performed a systematic review and meta-analysis of the literature to explore the effects of obesity in relation to medical conditions including asthma. Pooled analysis of 111,784 children up to 19 years from 16 asthma studies (14 cross-sectional, one cohort, one case-control) showed that having childhood obesity significantly increased the odds of asthma diagnosis by 50% in comparison to non-obese children ($p < 0.001$; OR: 1.5, 95% CI: 1.3–1.7). Furthermore, in a subgroup analysis by sex, boys showed higher odds for having asthma and obesity than girls (boys: $p = 0.10$, OR: 2.0; 95% CI: 1.4–2.9; girls: $p = 0.56$, OR: 1.6; 95% CI: 1.2–2.2, respectively), although non-significant.

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The same trend was documented in other two meta-analyses [47, 49]. Sharma et al. estimated the impact of overweight/obesity on asthma morbidity in 4,012 children aged 5-18 years [47]. Meta-analysis of three cross-sectional studies indicated that obese children and adolescents had 1.7 times the prevalence of asthma compared to normo-weight ($p < 0.001$, OR: 1.7, 95% CI: 1.4-2.1); and 1.6 times in the overweight group ($p < 0.001$, OR: 1.6, 95% CI: 1.2-1.9). Although no heterogeneity ($I^2 = 0.0\%$) was observed among studies, population size was small and asthma status was assessed by self-report. Azizpour et al. in a meta-analysis of 16 case-controls including data from 8,397 children and adolescents (2-19 years) revealed that the odds for asthma in overweight children and adolescents was 1.64 times more likely compared to underweight and normo-weight ($p < 0.001$; OR: 1.64; 95% CI: 1.13–2.38); and 1.92 times more likely in obese children and adolescents ($p < 0.001$; 95% CI: 1.39–2.65) [49].

With respect to asthma development, in a retrospective U.S cohort study, Lang et al. (2018) compared asthma incidence among 507, 496 overweight/obese and normo-weight children (2-17 years) [48]. According to the investigators, the risk for incident asthma significantly increased among overweight ($p < 0.001$; RR: 1.17, 95% CI: 1.10–1.25) and obese children ($p < 0.001$, RR: 1.26, 95% CI: 1.18–1.34) after adjusting for age, race, ethnicity, sex, site, insurance status, baseline allergic rhinitis, baseline food allergy, and baseline proton pump inhibitor. However, the adjusted risk for spirometry-diagnosed asthma increased only among obese children ($p < 0.001$, RR: 1.29, 95% CI: 1.16–1.42). Thus, it was concluded that about 23% to 27% of new asthma cases in obese children was attributable to obesity and that 10% of all cases could be avoided in the absence of overweight and obesity.

Additionally, some studies reported sex differences in predicting asthma incidence for overweight and obese subjects [55-58] reporting a dose-response relationship of elevated BMI on asthma incidence [55]. Compared to normo-weight, overweight and obese children were more susceptible to increased incident of asthma risk, (overweight: RR: 1.19, 95% CI: 1.03-1.37, obese: RR: 2.02, 95% CI: 1.16-3.50), predominately in obese boys (boys: RR: 2.47, 95% CI, 1.57-3.87; girls: RR: 1.25, 95% CI: 0.51-3.03) [55]. In

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contrast, the reverse was observed in adolescence [56]. More specifically, in a cohort study, the Tucson Children's Respiratory Study, girls who became overweight or obese between ages 6 and 11 years (OR: 6.8, 95% CI: 2.4, 19.4 and OR: 5.5, 95% CI: 1.3, 23.3) were more likely to develop wheezing compared with normo-weight counterparts [57]. This relationship was not seen in boys. More specifically, Wang et al. (2014), in the Manchester Asthma and Allergy birth cohort study, reported that overweight girls (as measured by percent body fat using Bioelectrical Impedance Analysis (BIA), were three times more likely than boys to have current asthma and 2.5 times more likely to wheeze than normo-weight girls [58]. For every 1% increase in body fat, there was a 10% increase in asthma risk.

Furthermore, overweight/obese girls with early onset of menarche (< 12 years) tend to have 50% increased asthma risk with a more severe asthma phenotype relative to girls with later menarche [59]. A plausible explanation could be the hormonal effect of estrogen and progesterone acting as inflammatory agents triggering airway inflammation and obstruction. Given that adipose tissue is metabolically active and in obesity androgen levels are elevated, it is possible that increased androgenic and estrogenic activity might contribute to increased asthma risk in girls. [60]. During the menstrual cycle, peak estrogen levels have been associated with increased symptoms and decreased pulmonary function in asthmatic women [61]. Contrastingly, low progesterone levels associated with obesity led to reduced β_2-receptors causing decreased bronchial smooth muscle relaxation and activation of symptoms [61]. In another meta-analysis of fourteen studies (8 cross-sectional, 3 respective or cohorts, 3 randomized clinical trials) involving 46,070 asthmatic children and adolescents up to 18 years old, Ahmadizar et al. (2016) reported that severe exacerbation risk (as measured by oral corticosteroid use) increased in obese asthmatics compared to non-obese peers ($p = 0.02$, OR: 1.17, 95% CI: 1.03–1.34) [51]. No association was observed between obesity and asthma control.

With regards to medication, in an earlier study, the Childhood Asthma Management Program (CAMP) trial that included 1,041 pediatric asthma patients aged 5-12 years recruited during 1993-1995, Forno et al. documented decreased corticosteroid response in overweight/obese subjects

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compared to the non-obese [53]. Among non-overweight subjects on corticosteroid therapy there was a 44% significant reduction in emergency visits or hospital admission ($p = 0.001$, 95% CI = 20.7– 60.9%). In contrast, no significant difference was observed among overweight/obese children between treatment arms ($p = 0.97$).

Obesity and Lung Function

Obesity is a complicating factor that causes significant alterations in pulmonary dynamics/mechanics and airway inflammation in asthmatic patients. Disparities exist in pulmonary function tests between obese adult and juvenile patients with asthma. Adults appear to have a restrictive ventilatory deficit exhibiting low FVC and FEV_1 but normal FEV_1/FVC [62]. Excessive accumulation of fat in the thoracic and abdominal cavities leads to limited diaphragm mobility and expansion of the rib cage and consequently reduction in lung volume as evident by reduced total lung capacity (TLC), functional residual capacity (FRC) and expiratory reserve volume (ERV) [63].

Few studies have reported spirometric outcomes in lean and overweight/obese asthmatic children. Extensive research conducted by Forno et al. demonstrated that, unlike in adults, obese asthmatic children tend to have either an obstructive ventilatory deficit or dysanaptic lung growth representing an incongruence between airway growth and that of the lung parenchyma which is reflected by normal or abnormally high FVC and FEV_1 but decreased ratio of FEV_1/FVC [64]. In addition, airway dysanapsis was associated with reduced response to long-term inhaled corticosteroid therapy in obese children [53]. The obstructive ventilatory pattern is most likely due to rapid somatic development as a result of weight gain rather than a marker of poor asthma control [64, 65]. Notably, superior lung development and high spirometric indices FVC, FEV_1 and FEV_1/FVC are observed in swimmers and divers attributed to the effect of regular physical activity rather than respiratory disease [66, 67].

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Consistent with these observations, prior studies have documented the phenomena of dysanapsis and large lungs in both asthmatic and non-asthmatic overweight/obese pediatric patients compared to normo-weight [64, 65, 68-71]. Recently, in our investigation of the effect of weight status on lung function in Greek asthmatic school children, we found that being overweight/obese was correlated to increments in FEV_1, FVC and $FEF_{25-75\%}$ (a measure of small airway caliber) as compared to normo-weight [71]. In addition, lower values of FeNO, marker of eosinophilic airway inflammation [72] were observed in the overweight/obese group. This is in line other cross-sectional studies [69, 70, 72, 73]. Ekstrom et al. (2018) using data from the Swedish BAMSE population-based birth cohort study involving children up to 16 years, demonstrated that overweight/obesity was associated with increased lung volume (FVC, FEV_1) and airway obstruction (reduced FEV_1/FVC), but not for FeNO [69]. Jones et al. (2017) in a case-control study of Brazilian asthmatic and healthy, non-asthmatic children aged 8-16 years old, found an increase in FVC and FEV_1 by 13.6% and 7.6%, respectively, as well as a 3.5% reduction in FEV_1/FVC in overweight asthmatic children [65].

Comparable results were described by Yao et al. (2017) in the PATCH study, a population-based study of Asian children 5-18 years old [70]. BMI disproportionately increased FVC, FEV_1, PEF and $FEF_{25-75\%}$, but decreased FEV_1/FVC and FeNO. In Mediterranean countries, excess bodyweight was positively correlated to FVC and FEV_1, but inversely with FEV_1/FVC and $FEF_{25-75\%}$/ FVC in Italian adolescents [68]. These results indicate pulmonary impairment in overweight/obese children without any history of obstructive respiratory disease. The ratio of $FEF_{25-75\%}$/ FVC has been used as a surrogate measure for dysanapsis. It is conceivable that as BMI increases, there is a reduction in airway size as evident by $FEF_{25-75\%}$/ FVC. The same trend was demonstrated in another study by Schmauck-Gómez et al. (2016) [73]. Normo-weight asthmatic children and adolescents had significantly lower FEV_1/VC and MEF_{50} (Maximal Expiratory Flow at 50% of the forced vital capacity) than overweight/obese children and healthy controls; and FeNO was significantly higher in normo-weight asthmatics than in overweight/obese children and adolescents.

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Han et al. (2014), using data from children 6-17 years participating in the National Health and Nutrition Examination Survey (NHANES) (2007-2010), reported that among non-asthmatic children with low FeNO, BMI, percent body fat and waist circumference were associated with higher FEV_1 and FVC, and lower FEV_1/FVC [72]. Contrastingly, in asthmatic children with high FeNO, all adiposity indicators were associated with decreased FEV_1/FVC only. High BMI or percent body fat was associated with worse asthma severity or control in asthmatic children and increased FeNO, but not in asthmatic children with low FeNO. Thus, obesity may play a role in asthma inception via non-atopic pathways. According to the authors the increments in FEV_1, FVC and decrements in FEV_1/FVC in non-asthmatic children without inflammation could signify dysanapsis. Alternatively, in asthmatic children with high FeNO, no increase was observed in FEV_1 or FVC, but a prominent decrease in FEV_1/FVC was observed as compared to asthmatic children with low FeNO. This is characteristic of true airflow obstruction caused by adiposity in children with asthma and a high FeNO.

However, in contradiction to these findings, other pediatric studies have reported pulmonary dysfunction in children [74, 75]. More specifically, Spathopoulos et al. (2009) documented high BMI reduced spirometry parameters, FVC, FEV_1, $FEF_{25-75\%}$ and FEV_1/FVC in overweight or obese Greek school-children (6-11 years) [74]. Similar outcomes were reported in Turkish children aged 9-15 years old [75]. Ulger et al. noted that values of FVC, FEV_1, PEF and $FEF_{25-75\%}$ were lower in comparison to the control group [75].

Mechanisms of Action

The mechanistic basis for the observed effects of obesity in pediatric pulmonology remains to be established. A number of feasible hypotheses have been postulated to explain the pathological mechanisms that ameliorate lung function in asthmatic children that include mechanical, immunological and hormonal. Abdominal fat tissue accumulation in the thoracic-abdominal cavity increases abdominal pressure that leads to reduced ventilation and

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consequently decreased tidal volume and airway caliber [63]. The mechanical work overload caused by excess adipose tissue deposition in the abdomen decreases expansion of airway smooth muscle cells and eventually increases airway resistance. Over time airway remodeling occurs affecting airway smooth muscle contractibility with increased airway hyperresponsiveness that give rise to reduced lung volumes, expiratory reserve volume (ERV) and functional residual capacity (FRC). These alterations of lung physiology contribute to shallow breathing in obese subjects and higher perception of dyspnea [63].

Secondly, adipose tissue may be related to inflammation and immune responses through production of cytokines, adipokines and macrophage activation that play a crucial role in the inflammatory response milieu. It is well-acknowledged that obesity is associated with a state of chronic low-grade systemic inflammation [76]. Adipose tissue is metabolically active, secreting a variety of hormones that regulate metabolism and inflammation. Leptin is produced and secreted in proportion to the adipocyte mass and functions as a pro-inflammatory hormone inducing neutrophil chemotaxis, production of reactive oxygen species and natural killer cells, activation of macrophages and production of Th1 cytokines, IL-6 and IFN-γ [77]. In addition, leptin and leptin receptors are expressed by human lung cells, epithelial cells, type II pneumocytes and macrophages [77].

Rastogi et al. (2012) documented an inverse correlation between leptin production and the ratio of FEV_1/FVC in obese asthmatic children, probably mediated by Th1 inflammatory cytokines IFN-γ and IFN-γ-inducible protein 10 [78]. According to the authors', excessive production of leptin contributed to the obstructive pattern in subjects driven by Th1 immune response. Airway hyperresponsiveness in obesity, a cardinal feature of asthma, could also be linked to increased production of adipokines (TNF-α, IL-6, IL-8, monocyte chemoattractant protein-1, complement proteins, leptin and adiponectin) secreted from adipose macrophages and adipocytes. Sharma et al. demonstrated that an increment in BMI by 1 kg/m^2 was associated with a 3.1% increase in airway hyperresponsiveness risk [79]. Furthermore, presence of tissue eosinophilia is a hallmark of asthma. Leptin

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directly activated recruitment of eosinophils and delayed apoptosis, thus playing an important role in survival of eosinophilia in airways [77].

In comparison, adiponectin, an anti-inflammatory adipokine, is reduced in obesity. Adiponectin and its receptors are expressed on lung epithelia. This hormone possesses the ability to down-regulate pro-inflammatory cytokines, IL-6 and TNFα and stimulate anti-inflammatory cytokine, IL-10. In the lungs of animal models adiponectin attenuated airway hyperresponsiveness, eosinophils, Th2 cells, whereas leptin increased hyperresponsiveness mediated by Th1-type inflammation rather than a Th2 allergic asthma response [80]. In humans, low serum adiponectin concentrations were found to be associated with greater asthma risk among peri-pubertal girls [81]. In a cross-sectional study, pre-pubertal and peri-pubertal asthmatic boys with higher serum adiponectin concentration showed less severe exercise-induced bronchoconstriction, fewer day symptoms and exacerbations, higher FEV_1/FVC and $FEF_{25–75\%}$ [81].

Hypoxic Inflammation

Adipocyte hypertrophy and hyperplasia lead to local adipose hypoxia that induce circulating monocytes to activate into patrolling monocytes which generate Th1-driven pro-inflammatory cytokines namely monocyte chemoattractant protein 1 (MCP-1), tumor necrosis factor (TNF-α), IL-6 and IFN-γ-inducible protein 10 (IP-10) [82]. The frequency of chemokine receptor type 2 (CCR2+) patrolling monocytes is inversely correlated to FEV_1/FVC [82]. Hence, elevated concentrations of leptin secreted from adipocytes and Th1 polarization of the immune response might be responsible for neutrophilic airway infiltration and low FeNO measurements observed in obese asthmatic children.

Reduced Fractional Exhaled Nitric Oxide Measurements in Obese Asthmatic Children

Collectively, from the data of the aforementioned pediatric studies, it appears that surplus body fat leads to Th1-driven neutrophilic airway inflammation as compared to Th2 eosinophilic inflammation suggesting that obesity-related asthma may be mediated by non-eosinophilic pathways [64].

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In adult patients with asthma, lower cut-offs for FeNO, Ig E levels and blood eosinophil counts were found [83]. A conceivable explanation for lower FeNO levels in obese pediatric asthma patients could be that obesity causes altered trafficking of eosinophils into the airway lumen and as a result sputum eosinophil count and FeNO measurements may underestimate the degree of Th2 inflammation [84]. Increased Th2 sputum cytokine profile in the absence of a sputum eosinophilia suggests that eosinophil function is altered in obesity, such that response to CCR3 chemokines and Th2 cytokines is impaired, or that eosinophils remain in the airway wall and possibly have altered survival [84]. Moreover, the obese state skews CD4 cells towards Th1 polarization rather than Th2 eosinophilic inflammation, thus manifesting as low values of FeNO [85]. Zheng et al. reported increased sputum neutrophil counts in obese-asthmatics of the non-atopic phenotype [86].

From another perspective, reduced FeNO might be the result of limited production of NO in the airways as well as increased NO metabolism [87]. Excessive fat leads to an increase in arginase expression relative to L-arginine concentration and subsequently to lower FeNO levels [87]. Nitric Oxide is produced from L-arginine via the enzymatic action of NO synthase and arginase competes with NO synthase for L-arginine, thus resulting in the limited production of FeNO [87]. In this context, conditions such as obesity and asthma favoring a lower L-arginine/arginase balance could contribute to reducing NO airway bioavailability and increasing oxidative stress [87]. Oxidative stress associated with obesity leads to the formation of reactive nitrogen species (RNS) and an increase in RNS/NO ratio would result in lower measures of exhaled NO levels [87].

Oxidative Stress Related to Obese-Asthma Phenotype

High oxidative stress participates in both obesity and asthma pathologies. Oxidative stress is defined as an imbalance between production of pro-oxidants (Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) and antioxidant defence mechanisms in the body such as glutathione peroxidase, catalase, superoxide dismutase (SOD), vitamins C and E along with glutathione [88]. It has been postulated that high levels of

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pro-oxidant molecules could initiate Th2-dominant immunity and may contribute to the development and progression of allergic airway inflammation. Cross-sectional studies showed that obese subjects had higher levels of oxidative stress biomarkers and lower anti-oxidants than normo-weight counterparts [89-91]. Pediatric subjects with asthma have lower concentrations of vitamins C and E, SOD and catalase along with reduced enzymatic activity in association with decreased lung function [92]. Furthermore, elevated levels of 8-isoprostanes and malondialdehyde (MDA) (markers of oxidative stress and lipid peroxidation in pulmonary disease) in exhaled breath condensate (EBC) were detected in child asthmatic subjects compared to non-asthmatic controls [93-95]. Therefore, increased airway oxidative stress could potentially be the mechanism linking obesity to asthma.

It is well established that the obese state invokes metabolic derangement characterized by insulin resistance and dyslipidemia in humans [82]. Insulin also plays a pivotal role in regulating lung function. Hyperglycemia and hyperinsulinemia can contribute to airway hyperresponsiveness and remodeling through epithelial damage and airway smooth muscle proliferation [96]. Similar to leptin, insulin has pro-inflammatory activity that induces a switch towards a Th1 immune response causing a reduction in FEV_1/FVC by a non-atopic mechanism [82]. Forno et al. using data from the NHANES study showed that overweight/obesity driven insulin resistance and metabolic syndrome in asthmatic and non-asthmatic adolescents were related with worse lung function (decreased FVC, FEV_1 and FEV_1/FVC) [97]. Up to 10% lower FEV_1/FVC was observed in asthmatic adolescents than in non-asthmatics. Obesity was also associated with airway hyperresponsiveness in asthmatic children with insulin resistance, as compared to asthmatics with no insulin resistance [98]. Potential mechanisms for a causal effect of insulin resistance in obesity-driven asthma include Th1 polarization [85], increased allergic sensitization [99], oxidative stress [100], airway smooth muscle dysfunction, and airway epithelial damage [96, 101]. Furthermore, studies have associated dyslipidemia in synergy with obesity [102, 103]. It is common for obese subjects to have high serum concentrations of triglycerides and cholesterol

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but low HDL concentrations. Cottrell et al. (2011), demonstrated that in obese asthmatic children, high serum triglyceride concentrations were correlated with chronic airway hyperresponsiveness, independent of BMI [102].

Strengths/Limitations

Intriguingly, the aforementioned studies reporting on the phenomena of dysanapsis and paradoxically subnormal FeNO values observed in children of the obese-asthmatic phenotype highlight that the effects of overweight is primarily in increasing lung size without any effect on airway resistance. Common to all studies was the application of spirometry to assess asthma status which mainly reflects central airway caliber [104]. Spirometry may have low specificity and sensitivity in these patients. It may not provide an accurate assessment of lung function and the underlying inflammation for this sub-group. Also, the current reference equations used to estimate spirometric indices are generated through population studies of healthy children without respiratory disease [13] and therefore may not be suitable for overweight/obese patients. Notably, the equations proposed by the Global lung Function Initiative (GLI-2012) recommended for 3-95 years, developed by Quanjer et al. take into account age and height but does not adjust for body weight [105]. Hence it is possible that these might lead to inaccurate measurements of lung function in overweight/obese pediatric patients. This brings to our attention that perhaps pediatric pulmonologists should take caution when interpreting spirometry testing in overweight and obese pediatric asthma patients. Impulse oscillometry may be more appropriate to measure lung resistance and reactance (markers of 'true obstruction') in central and peripheral airways, thereby confirming the presence of unequal lung growth in patients of the obese-asthma phenotype [104]. In two studies, impulse oscillometry was applied to examine for true obstructive disorder in children in conjunction to spirometry, and this deserves further investigation [65, 69]. Adding impedance oscillometry to spirometry improves our understanding of the ventilatory abnormalities in overweight children.

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The main weaknesses of these studies are those inherent to all cross-sectional studies in that the causal relationship between obese-asthma in children is not discernable and the direction of causation remains unknown. Furthermore, reverse causation cannot be excluded where obesity related chest wall restriction gives rise to asthma-like symptoms, breathing patterns with higher frequencies at lower tidal volume [106]. Regarding the effect of obesity on increased airway obstruction as reflected by decreased FEV_1/FVC, this could be attributed to the increase in FVC. Alternatively, high BMI may reflect increased lean muscle mass and be correlated with higher lung volumes and therefore influence the observed relationship with FEV_1 and FVC [107].

From another point of view, overweight/obese subjects are less likely to participate in physical activity and sustain less physical exertion than lean counterparts [106]. Overweight and obesity related physical deconditioning can cause dyspnea and exercise limitation which can mimic asthma-like symptoms rather than respiratory disease. Discrepancies among studies regarding observations on the effect of adiposity on pulmonary mechanics can be explained by variability in sample size, number of subjects with obesity, ethnic differences in fat deposition, age of onset, duration and severity of obesity, assessment of asthma outcome (clinical examination vs questionnaire/parent report), measurement of adiposity (BMI, % body fat, waist circumference, BIA, DEXA) as well as adjustment for confounding factors in statistical models. Another drawback, reduced physical activity, has been suggested as one of the mechanisms that connect obesity to asthma. These studies are limited in that physical activity was not measured and therefore a possible effect of regular exercise cannot be excluded. Only a couple of studies accounted for physical activity as a modifying factor in statistical analyses [71, 75]. Even though universally BMI is commonly used as a proxy for surplus body fat in pediatric studies, it is not a direct measure of adiposity since it does not differentiate between lean and fat mass, and consequently could lead to significant misclassification [108]. Waist circumference, waist-to-height ratio, skin-fold thickness or Bio-electrical Impedance Analysis (BIA)-derived % body fat mass rather than BMI might be a better representation of excessive body fat in children that is detrimental

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to health [109]. Then again, Forno et al. in a study exploring the effect of obesity in asthmatic children 6-14 years demonstrated, when using BMI, that percent body fat and waist circumference, as measures of adiposity, were positively associated with asthma risk, severity and FVC [44]. Only waist circumference was associated with decrements in FEV_1/FVC and BMI with FEV_1 [44]. Ulger et al. reported negative correlations between BMI, skin fold thickness, waist to hip circumference and basal FVC, FEV_1 and PEF in healthy children 9-15 years [75].

Regarding airway inflammation as represented by FeNO, few cross-sectional studies have explored the effect of excess body fat in relation to airway inflammation, a characteristic feature of asthma pathology [69-73]. Possible shortcomings in these studies were that blood neutrophils, T-cell composition (ratio of Th1: Th2), cytokines (TNFα, INF-α, IL6, IL13, IL10) and adipokine levels (leptin, adiponectin) were not measured. Given the possible discordance between FeNO and pulmonary inflammation in obese asthmatic patients, the presence of obesity may decrease the ability of this biomarker to predict eosinophilic inflammation in this sub-group. Hence other surrogate markers such as sputum and blood eosinophil counts, as well as Ig E, may be more suitable for identifying the type of inflammation and in predicting response to therapy. This is worth consideration in future studies in order to enrich our understanding of the molecular pathways involved in the inflammatory response of obese-asthma phenotype in children.

Weight Loss, Exercise and Pediatric Asthma

Physical Activity

Physical inactivity and sedentary behavior in children are important asthma risk factors because they are potentially modifiable and therefore an opportunity for prevention. Convincing evidence was published in a Cochrane review performed by Eichenberger et al. (2013) evaluating the effect of exercise training on asthma and exercise endurance in children and adults [110]. Meta-analysis of 17 studies including 599 subjects showed that

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exercise significantly increased days without asthma symptoms, improved FEV_1, exercise capacity, quality of life and decreased airway hyperresponsiveness and exercise-induced bronchoconstriction. Despite the benefits of regular physical activity on cardiovascular health and decreased mortality, both children and adults with asthma are less likely to exercise than non-asthmatic counterparts, for fear of provoking an asthma attack (exercise-induced bronchoconstriction) [111].

However, there is little evidence on the association between physical activity and pediatric asthma risk, with some reporting increased risk with low physical activity [112-115] and increased sedentariness [115, 116] while others, increased physical activity with asthma induction [111, 117, 118]. In ISAAC (Phase 3), Mitchell et al. (2013), using international data from 76, 164 children aged 6-7 found that vigorous exercise ≥ 3 times per week decreased current wheeze by 17% (OR_{adj}: 0.83, 95%CI: 0.76, 0.91) and asthma ever by 18% (OR_{adj}: 0.82, 95%CI: 0.75, 0.90), adjusted for region, language, gross national income, sex, BMI and daily TV viewing [115]. Regarding sedentary behavior as assessed by TV viewing, a dose-effect relationship was found with risk increasing with the number of viewing hours.

Tsai et al. (2007), in the cross-sectional study of 2, 290 school-children aged 11-12 years, demonstrated that being overweight was positively associated with asthma symptoms (OR_{adj}: 1.47, 95% CI: 1.08-2.00 and exercise-induced wheezing (OR_{adj}: 1.80, 95%CI: 1.37–2.38)[114]. However, daily physical activity (≥ 30 minutes) was associated with less frequent occurrence of dyspnea-associated wheezing (OR_{adj}: 0.66, 95%CI: 0.45-0.95) and exercise-induced cough (OR_{adj}: 0.73. 95%CI: 0.55-0.99), adjusting for residential region, gender, and allergy.

In the PANACEA study (Physical Activity, Nutrition and Allergies in children Examined in Athens) which was an epidemiological, cross-sectional survey investigating the prevalence of asthma and allergies in 815 Greek children 10-12 years old, observed that in boys, one hour increase in TV viewing or playing videogames increased asthma odds by 10% [95% CI: 1.01, 1.04] [113]. Boys with asthma symptoms were 2.2-times more likely [95% CI: 1.34, 3.54] to not participate in any physical activity than boys

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without any asthma-related symptoms. Body weight and TV viewing or playing with videogames were not associated with asthma symptoms in girls. Finally, girls that did not participate in any activity during a week were 63% more likely to have asthma symptoms [95% CI 0.86, 3.11] than girls who participated in physical activities.

With respect to sedentary behavior, the Koala birth cohort (Child, Parent and Health: Lifestyle and Genetic Constitution Birth Cohort) that included 1, 838 children with a 10-year follow-up, reported no association between parent reported physical activity and asthma or lung function in children. However, an association was found between sedentary time and lower FEV_1/FVC (increased obstruction) in children at age 6-7 years [116]. In contrast, in an earlier study, Ownby et al. (2007) reported that 38.7% of children with asthma aged 8-10 years, wheezed during physical activity [117]. A similar trend was found by Ostrom et al. (2011) and Lin et al. (2016), documenting that approximately 50% of asthmatic children over 4 years experienced exercised-induced respiratory symptoms of dyspnea, cough and wheeze [111, 118]. The disparities among the aforementioned pediatric studies highlight the need for more well-designed, high powered intervention studies to clarify the duration, intensity, frequency and type of exercise that provides respiratory benefits in asthmatic children.

Weight Loss Interventions

Weight loss in obese asthma patients has overall health benefits including better asthma control, lung function and asthma-related quality of life [119]. Lifestyle changes recommended for addressing overweight/obesity in asthma patients include following a healthy diet, reduced energy intake and increased physical activity [120]. However, there is lack of weight-loss interventions in pediatric asthma. According to a systematic review conducted by Okoniewski et al. (2019) evaluating the effects of weight loss on asthma outcome in obese asthmatics, pooled analysis of data from two pediatric randomized controlled trials (RCTs) [121, 122] showed that weight loss interventions resulted in significant weight loss in the intervention group (-0.14 standard deviation scores to -15.9% BMI), whereas pulmonary function improvements (ERV, TLC, RV

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(Residual Volume)) were observed in both arms [123]. Nonetheless, high heterogeneity existed among studies that included variability in weight-loss interventions regarding calorie restriction and exercise therapy. Thus, it was concluded that additional research is warranted to clarify the clinical impact of weight loss in obese-asthmatic pediatric patients.

More specifically, in a recent RCT, Willeboordse et al. (2016) demonstrated that weight reduction in asthmatic children resulted in significant improvements in pulmonary function indices [122]. The MIKADO clinical trial was designed to evaluate the efficacy of an 18-month diet and exercise intervention program in 87 overweight/obese children aged 6-16 years (intervention group (n = 43) versus control (n = 44) diagnosed with asthma or at high risk of asthma development. The intervention group was given dietary advice and an exercise program, whereas the control received usual asthma care. After 18 months, the investigators noted that a modest decrease in BMI resulted in improvements in $\%FEV_1$, %FVC, %ERV and %TLC as well as in aerobic fitness, asthma control and quality of life for both the intervention and control groups. Although the improvements in % FVC, asthma control and quality of life were more pronounced in the intervention group, there was a 10% improvement in FEV_1 and FVC, which is clinically significant according to ATS/ERS recommendations [124].

Jensen et al. (2013) conducted a 10-week pilot RCT in 28 obese asthmatic children 8-17 years [Intervention group (n = 13) versus control (n = 15)] [121, 122]. The intervention group underwent a dietary plan targeting a 500 kcal energy deficit/day. At follow-up, a 5.7% reduction in body weight was observed in the intervention group along with improvements in asthma control score and quality of life, compared to the control. No significant changes in pulmonary dynamics, eosinophils or neutrophils were observed between the groups. However, in the intervention group, a significant change from baseline was detected for ERV (~0.7 L), RV and % RV/TLC (mean improvement: 0.4L for RV; -6.9% for RV/TLC), suggesting a reduction in obstruction; and a non-significant reduction in eNO, eosinophils, neutrophils following weight loss. Therefore, these studies demonstrate that it is possible

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to improve static lung function and asthma control in obese asthmatic children by a dietary intervention alone.

In another study, Lucas et al. (2017), in the Healthy Hearts Program which was a 12-week dietary and exercise intervention for overweight and obese asthmatic and non-asthmatic children up to 18 years (N = 232), documented that in both groups adherence to the program resulted in a decrease in BMI, marked improvements in cardiorespiratory health and exercise intensity [125].

The clinical implications of these studies are significant because they showed that the adverse effects of overweight/obesity on lung function in pediatric asthma can be reversed to a certain extent via simple lifestyle modifications that are feasible in a real-life setting. Given potential benefits of regular exercise in respiratory health, an intervention study would be required to determine if a physical-activity regimen in children is capable of preventing or modifying the course of asthma.

Obese-Asthma and COVID-19

The pandemic spread of COVID-19, a severe acute respiratory syndrome of coronavirus-2 (SARS-CoV-2), is a major public health crisis of great concern. Epidemiological evidence to date suggests that COVID-19 is less serious in children compared to adults. According to latest data from the UK, including 16,749 patients hospitalized for COVID-19, only 239 (2%) were under 18 years [126]. Very low fatality rates have been documented from Italian and Chinese pediatric casualties [127]. Although COVID-19 is a multi-system disease, it predominately affects the respiratory system [128]. Children and adolescents contract COVID-19 via contamination from family members. A recent systematic review that included international data from 7, 780 pediatric patients, residing in 26 countries, showed that in 50-60% of cases COVID-19 infection was presented by mild symptoms of fever and cough, and approximately 20% were asymptomatic [127]. However, in 50 severe pediatric cases of COVID-19, obesity was a high prevalent comorbidity with greater susceptibility and

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severity of this condition [128, 129]. In the U.S., among 50 pediatric cases of COVID-19, it was reported that obesity was the most significant risk factor for mechanical ventilation, elevated inflammatory markers at admission and during hospitalization including disease severity [129]. A feasible mechanism by which obesity impairs normal pulmonary function could be due to the pressure exerted by abdominal adiposity on the lungs through the diaphragm, thus limiting the movement of respiratory muscles, causing reduced lung volumes, less oxygen saturation which is manifested as worse clinical presentation in these patients [130]. It has been proposed that since obesity is a risk factor for asthma development in children, it could contribute to a higher risk of respiratory infection. Interestingly, the same molecular pathways underlying the obese-asthma link, primarily the state of chronic inflammation [131] is also involved in the severity of COVID-19 [130]. Given that obesity in children may potentiate complications in COVID-19 infection, it is imperative that pediatricians are aware of this issue in order to reduce respiratory burden in asthmatic patients.

Nevertheless, whether obesity in asthmatic children constitutes a risk factor for COVID-19 is unclear. According to a systematic review conducted by Castro-Rodriguez and Forno (2020), examining whether asthma in pediatric patients is related to higher COVID-19 risk and severity, no conclusion was possible due to the lack of data on pediatric asthma [128]. Only one report from the U.S. CDC stated that approximately 11% (40/345) of children had chronic lung diseases including asthma, and one hospital in New York reported that 24% (11/46) hospitalized for COVID-19, had asthma. However, consistent with several studies, there was low asthma prevalence in COVID-19 patients, possibly attributed to a protective effect conferred by the use of inhaled corticosteroids which might suppress replication of COVID-19 and cytokine production [132]. In addition, adult and pediatric patients with asthma and allergies were found to have reduced angiotensin converting enzyme 2 (ACE2) gene expression in airway cells, suggesting another potential mechanism of reduced COVID-19 risk [133]. A possible source of bias worth considering is that asthmatic patients may practice more caution and social distancing as a protective measure, thus leading to under-reporting of asthma prevalence in COVID-19 pediatric

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patients. To summarize, given the limited data on the impact of obese-asthma and COVID-19, future international pediatric studies are needed to provide more concrete evidence as proof of concept, and for more effective targeted therapy and care of these patients.

CONCLUSION

Obesity and asthma are believed to share common etiological determinates and it has been suggested that obesity results in a distinct asthma phenotype characterized by more severe disease, with increased exacerbation, poorer asthma control and steroid responsiveness including pulmonary impairment in children. Effective public health strategies focusing on reducing the increasing obesity trend, have the potential to reduce asthma risk, patient burden and overall cost.

The findings of this review highlight the complexity and heterogeneity of asthma in children and imply that deficits observed in lung function of pediatric asthma patients might be due to obesity-related systemic inflammation, mechanical overload, metabolic dysfunction, oxidative stress and hormonal effects of excess body fat including disproportionate lung growth. Childhood may represent a critical window of opportunity to intervene and prevent long-term consequences. Phenotyping and lifestyle interventions promoting a healthy diet, daily physical activity and outdoor play combined with asthma therapy should be considered key points in the management of obesity in pediatric asthma patients. Future longitudinal studies are recommended to determine the mechanisms underlying the obese-asthma phenotype in children.

REFERENCES

[1] Ferrante G, La Grutta S. The Burden of Pediatric Asthma. *Front Pediatr* 2018;6:186.

Complimentary Contributor Copy

[2] Lehnert T, Sonntag D, Konnopka A, Riedel-Heller S, König HH. Economic costs of overweight and obesity. *Best Pract Res Clin Endocrinol Metab* 2013;27:105-15.

[3] Sahoo K, Sahoo B, Choudhury AK, Sofi NY, Kumar R, Bhadoria AS. Childhood obesity: causes and consequences. *J Family Med Prim Care* 2015;4:187-92.

[4] Melén E, Himes BE, Brehm JM, Boutaoui N, Klanderman BJ, Sylvia JS, et al. Analyses of shared genetic factors between asthma and obesity in children. *J Allergy Clinical Immunol* 2010;126:631-7.e78.

[5] Tantisira KG, Weiss ST. Complex interactions in complex traits: obesity and asthma. *Thorax.* 2001;56:ii64-73.

[6] WHO. *Fact Sheet: Obesity and Overweight.* Geneva, Switzerland: World Health Organization; 2020. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. (Accessed Sep 2020).

[7] Reilly JJ, Kelly J. Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. *Int J Obes* (2005). 2011;35:891-8.

[8] Doeing DC, Solway J. Airway smooth muscle in the pathophysiology and treatment of asthma. *J Appl Physiol* (1985). 2013;114:834-43.

[9] GINA. *Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention* (2020). Fontana, USA: Global Initiative for Asthma (GINA); 2020. https://ginasthma.org. (Accessed July 2020).

[10] Hough KP, Curtiss ML, Blain TJ, Liu R-M, Trevor J, Deshane JS, et al. Airway Remodeling in Asthma. *Front Med* 2020;7.

[11] Lambrecht BN, Hammad H. The immunology of asthma. *Nat Immunol* 2015;16:45-56.

[12] Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? *Lancet* (London). 2015;386:1075-85.

[13] Katsardis CV, Alexandraki S, Paraskakis E. Chapter 2: Spirometry in children 6-16 years old In: Katsardis Ch, Koumbourlis A, Anthracopoulos M & Paraskakis E., editors. *Paediatric Pulmonary*

Complimentary Contributor Copy

Function Testing Indications and Interpretation. New York, USA NOVA Biomedical.; 2015. p. 15–42.

[14] Serebrisky D, Wiznia A. Pediatric Asthma: A Global Epidemic. *Ann Glob Health.* 2019;85:6.

[15] Asher I, Pearce N. Global burden of asthma among children. *Int J Tuberc Lung Dis* 2014;18:1269-78.

[16] Lai C, Beasley R, Crane J, Foliaki S, Shah J, Weiland S. Global variation in the prevalence and severity of asthma symptoms: phase three of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax.* 2009;64:476–83.

[17] Dharmage SC, Perret JL, Custovic A. Epidemiology of Asthma in Children and Adults. *Front Pediatr* 2019;7:246-.

[18] Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995;332:133-8.

[19] van Aalderen WM. Childhood asthma: diagnosis and treatment. *Scientifica (Cairo)* 2012;2012:674204.

[20] Yao TC, Ou LS, Yeh KW, Lee WI, Chen LC, Huang JL. Associations of age, gender, and BMI with prevalence of allergic diseases in children: PATCH study. *J Asthma* 2011;48:503-10.

[21] Fuseini H, Newcomb DC. Mechanisms Driving Gender Differences in Asthma. *Curr Allergy Asthma Rep* 2017;17:19.

[22] Bui DS, Walters HE, Burgess JA, Perret JL, Bui MQ, Bowatte G, et al. Childhood Respiratory Risk Factor Profiles and Middle-Age Lung Function: A Prospective Cohort Study from the First to Sixth Decade. *Ann Am Thorac Soc* 2018;15:1057-66.

[23] Tai A, Tran H, Roberts M, Clarke N, Gibson AM, Vidmar S, et al. Outcomes of childhood asthma to the age of 50 years. *J Allergy Clin Immunol* 2014;133:1572-8.e3.

[24] Iordanidou M, Loukides S, Paraskakis E. Asthma phenotypes in children and stratified pharmacological treatment regimens. *Expert Rev Clin Pharmacol* 2017;10:293-303.

Complimentary Contributor Copy

[25] Hallberg J, Thunqvist P, Schultz ES, Kull I, Bottai M, Merritt A-S, et al. Asthma phenotypes and lung function up to 16 years of age-the BAMSE cohort. *Allergy* 2015;70:667-73.

[26] Garcia-Aymerich J, Benet M, Saeys Y, Pinart M, Basagaña X, Smit HA, et al. Phenotyping asthma, rhinitis and eczema in MeDALL population-based birth cohorts: an allergic comorbidity cluster. *Allergy* 2015;70:973-84.

[27] Howrylak JA, Fuhlbrigge AL, Strunk RC, Zeiger RS, Weiss ST, Raby BA. Classification of childhood asthma phenotypes and long-term clinical responses to inhaled anti-inflammatory medications. *J Allergy Clin Immunol* 2014;133:1289-300, 300.e1-12.

[28] Schatz M, Hsu JW, Zeiger RS, Chen W, Dorenbaum A, Chipps BE, et al. Phenotypes determined by cluster analysis in severe or difficult-to-treat asthma. *J Allergy Clin Immunol* 2014;133:1549-56.

[29] Sánchez-Zauco N, Del Rio-Navarro B, Gallardo-Casas C, Del Rio-Chivardi J, Muriel-Vizcaino R, Rivera-Pazos C, et al. High expression of Toll-like receptors 2 and 9 and Th1/Th2 cytokines profile in obese asthmatic children. *Allergy Asthma Proc* 2014;35:34-41.

[30] de Andrade WC, Lasmar LM, Ricci Cde A, Camargos PA, Cruz Á A. Phenotypes of severe asthma among children and adolescents in Brazil: a prospective study. *BMC Pulm Med.* 2015;15:36.

[31] Reddy MB, Covar RA. Asthma phenotypes in childhood. *Curr Opin Allergy Clin Immunol* 2016;16:127-34.

[32] Mandy M, Nyirenda M. Developmental Origins of Health and Disease: the relevance to developing nations. *Int Health.* 2018;10:66-70.

[33] Forno E, Young OM, Kumar R, Simhan H, Celedón JC. Maternal obesity in pregnancy, gestational weight gain, and risk of childhood asthma. *Pediatrics.* 2014;134:e535-46.

[34] Liu S, Zhou B, Wang Y, Wang K, Zhang Z, Niu W. Pre-pregnancy Maternal Weight and Gestational Weight Gain Increase the Risk for Childhood Asthma and Wheeze: An Updated Meta-Analysis. *Front Pediatr* 2020;8:134.

Complimentary Contributor Copy

[35] Rzehak P, Wijga AH, Keil T, Eller E, Bindslev-Jensen C, Smit HA, et al. Body mass index trajectory classes and incident asthma in childhood: results from 8 European Birth Cohorts--a Global Allergy and Asthma European Network initiative. *J Allergy Clin Immunol* 2013;131:1528-36.

[36] Wilson RM, Marshall NE, Jeske DR, Purnell JQ, Thornburg K, Messaoudi I. Maternal obesity alters immune cell frequencies and responses in umbilical cord blood samples. *Pediatr Allergy Immunol* 2015;26:344-51.

[37] Costa SMR, Isganaitis E, Matthews TJ, Hughes K, Daher G, Dreyfuss JM, et al. Maternal obesity programs mitochondrial and lipid metabolism gene expression in infant umbilical vein endothelial cells. *Int J Obes* (2005). 2016;40:1627-34.

[38] Malti N, Merzouk H, Merzouk SA, Loukidi B, Karaouzene N, Malti A, et al. Oxidative stress and maternal obesity: feto-placental unit interaction. *Placenta* 2014;35:411-6.

[39] Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance in utero. *Diabetes Care* 2009;32:1076-80.

[40] Gross E, Lee DS, Hotz A, Ngo KC, Rastogi D. Impact of Obesity on Asthma Morbidity During a Hospitalization. *Hosp Pediatr* 2018;8:538-46.

[41] Okubo Y, Nochioka K, Hataya H, Sakakibara H, Terakawa T, Testa M. Burden of Obesity on Pediatric Inpatients with Acute Asthma Exacerbation in the United States. *J Allergy Clin Immunol Pract* 2016;4:1227-31.

[42] Stingone JA, Ramirez OF, Svensson K, Claudio L. Prevalence, demographics, and health outcomes of comorbid asthma and overweight in urban children. *J Asthma* 2011;48:876-85.

[43] Manion AB, Velsor-Friedrich B. Quality of Life and Health Outcomes in Overweight and Non-Overweight Children With Asthma. *J Pediatr Health Care* 2017;31:37-45.

[44] Forno E, Acosta-Pérez E, Brehm JM, Han YY, Alvarez M, Colón-Semidey A, et al. Obesity and adiposity indicators, asthma, and atopy

Complimentary Contributor Copy

in Puerto Rican children. *J Allergy Clin Immunol* 2014;133:1308-14, 14.e1-5.

[45] Carroll CL, Stoltz P, Raykov N, Smith SR, Zucker AR. Childhood Overweight Increases Hospital Admission Rates for Asthma. *Pediatrics* 2007;120:734.

[46] Chen YC, Tu YK, Huang KC, Chen PC, Chu DC, Lee YL. Pathway from central obesity to childhood asthma. Physical fitness and sedentary time are leading factors. *Am J Respir Crit Care Med* 2014;189:1194-203.

[47] Sharma V, Coleman S, Nixon J, Sharples L, Hamilton-Shield J, Rutter H, et al. A systematic review and meta-analysis estimating the population prevalence of comorbidities in children and adolescents aged 5 to 18 years. *Obes Rev* 2019;20:1341-9.

[48] Lang JE, Bunnell HT, Hossain MJ, Wysocki T, Lima JJ, Finkel TH, et al. Being Overweight or Obese and the Development of Asthma. *Pediatrics* 2018;142:e20182119.

[49] Azizpour Y, Delpisheh A, Montazeri Z, Sayehmiri K, Darabi B. Effect of childhood BMI on asthma: a systematic review and meta-analysis of case-control studies. *BMC Pediatr* 2018;18:143.

[50] Malden S, Gillespie J, Hughes A, Gibson A-M, Farooq A, Martin A, et al. Obesity in young children and its relationship with diagnosis of asthma, vitamin D deficiency, iron deficiency, specific allergies and flat-footedness: A systematic review and meta-analysis. *Obes Rev* 2020 doi: 10.1111/obr.13129.

[51] Ahmadizar F, Vijverberg SJ, Arets HG, de Boer A, Lang JE, Kattan M, et al. Childhood obesity in relation to poor asthma control and exacerbation: a meta-analysis. *Eur Respir J* 2016;48:1063-73.

[52] Quinto KB, Zuraw BL, Poon KY, Chen W, Schatz M, Christiansen SC. The association of obesity and asthma severity and control in children. *J Allergy Clin Immunol* 2011;128:964-9.

[53] Forno E, Lescher R, Strunk R, Weiss S, Fuhlbrigge A, Celedón JC. Decreased response to inhaled steroids in overweight and obese asthmatic children. *J Allergy Clin Immunol* 2011;127:741-9.

Complimentary Contributor Copy

[54] van Gent R, van der Ent CK, Rovers MM, Kimpen JLL, van Essen-Zandvliet LEM, de Meer G. Excessive body weight is associated with additional loss of quality of life in children with asthma. *J Allergy Clin Immunol* 2007;119:591-6.

[55] Chen YC, Dong GH, Lin KC, Lee YL. Gender difference of childhood overweight and obesity in predicting the risk of incident asthma: a systematic review and meta-analysis. *Obes Rev* 2013;14:222-31.

[56] Lu KD, Billimek J, Bar-Yoseph R, Radom-Aizik S, Cooper DM, Anton-Culver H. Sex Differences in the Relationship between Fitness and Obesity on Risk for Asthma in Adolescents. *J Pediatr* 2016;176:36-42.

[57] Castro-Rodríguez JA, Holberg CJ, Morgan WJ, Wright AL, Martinez FD. Increased incidence of asthmalike symptoms in girls who become overweight or obese during the school years. *Am J Respir Crit Care* 2001;163:1344-9.

[58] Wang R, Custovic A, Simpson A, Belgrave DC, Lowe LA, Murray CS. Differing associations of BMI and body fat with asthma and lung function in children. *Pediatr Pulmonol* 2014;49:1049-57.

[59] Lieberoth S, Gade E, Kyvik KO, Backer V, Thomsen SF. Early menarche is associated with increased risk of asthma: Prospective population-based study of twins. *Respir Med* 2015;109:565-71.

[60] Baer HJ, Colditz GA, Willett WC, Dorgan JF. Adiposity and Sex Hormones in Girls. *Cancer Epidemiol Biomarkers Prev* 2007;16:1880.

[61] Haggerty CL, Ness RB, Kelsey S, Waterer GW. The impact of estrogen and progesterone on asthma. *Ann Allergy Asthma Immunol* 2003;90:284-91.

[62] Melo LC, Silva MAMd, Calles ACdN. Obesity and lung function: a systematic review. *Einstein (São Paulo)* 2014;12:120-5.

[63] Watson RA, Pride NB, Thomas EL, Fitzpatrick J, Durighel G, McCarthy J, et al. Reduction of total lung capacity in obese men: comparison of total intrathoracic and gas volumes. *J Appl Physiol* (1985) 2010;108:1605-12.

Complimentary Contributor Copy

[64] Forno E, Weiner DJ, Mullen J, Sawicki G, Kurland G, Han YY, et al. Obesity and Airway Dysanapsis in Children with and without Asthma. *Am J Respir Crit Care Med* 2017;195:314-23.

[65] Jones MH, Roncada C, Fernandes MTC, Heinzmann-Filho JP, Sarria Icaza EE, Mattiello R, et al. Asthma and Obesity in Children Are Independently Associated with Airway Dysanapsis. *Front Pediatr* 2017;5:270.

[66] Lazovic-Popovic B, Zlatkovic-Svenda M, Durmic T, Djelic M, Djordjevic Saranovic S, Zugic V. Superior lung capacity in swimmers: Some questions, more answers! *Revista Portuguesa de Pneumologia (English Edition)* 2016;22:151-6.

[67] Konarski M, Klos R, Nitsch-Osuch A, Korzeniewski K, Prokop E. Lung function in divers. *Adv Exp Med Biol* 2013;788:221-7.

[68] Cibella F, Bruno A, Cuttitta G, Bucchieri S, Melis MR, De Cantis S, et al. An elevated body mass index increases lung volume but reduces airflow in Italian schoolchildren. *PLoS One* 2015;10:e0127154.

[69] Ekström S, Hallberg J, Kull I, Protudjer JLP, Thunqvist P, Bottai M, et al. Body mass index status and peripheral airway obstruction in school-age children: a population-based cohort study. *Thorax* 2018;73:538-45.

[70] Yao TC, Tsai HJ, Chang SW, Chung RH, Hsu JY, Tsai MH, et al. Obesity disproportionately impacts lung volumes, airflow and exhaled nitric oxide in children. *PLoS One* 2017;12:e0174691.

[71] Papamichael MM, Katsardis C, Tsoukalas D, Erbas B, Itsiopoulos C. Weight Status and Respiratory Health in Asthmatic Children. *Lung* 2019;197:777-82.

[72] Han YY, Forno E, Celedón JC. Adiposity, fractional exhaled nitric oxide, and asthma in U.S. children. *Am J Respir Crit Care Med* 2014;190:32-9.

[73] Schmauck-Gomez JS, Menrath I, Kaiser MM, Herz A, Kopp MV. Children and Adolescents with Asthma Differ in Lung Function Parameters and Exhaled NO from Children and Adolescents with Obesity. *Klin Padiatr* 2016;228:189-94.

Complimentary Contributor Copy

[74] Spathopoulos D, Paraskakis E, Trypsianis G, Tsalkidis A, Arvanitidou V, Emporiadou M, et al. The effect of obesity on pulmonary lung function of school aged children in Greece. *Pediatr Pulmonol.* 2009;44:273-80.

[75] Ulger Z, Demir E, Tanaç R, Gökşen D, Gülen F, Darcan S, et al. The effect of childhood obesity on respiratory function tests and airway hyperresponsiveness. *Turk J Pediatr*. 2006;48:43-50.

[76] Rastogi D, Holguin F. Metabolic Dysregulation, Systemic Inflammation, and Pediatric Obesity-related Asthma. *Ann Am Thorac Soc* 2017;14:S363-s7.

[77] Kim SH, Sutherland ER, Gelfand EW. Is there a link between obesity and asthma? *Allergy Asthma Immunol Res* 2014;6:189-95.

[78] Rastogi D, Canfield SM, Andrade A, Isasi CR, Hall CB, Rubinstein A, et al. Obesity-associated asthma in children: a distinct entity. *Chest* 2012;141:895-905.

[79] Sharma S, Tailor A, Warrington R, Cheang M. Is obesity associated with an increased risk for airway hyperresponsiveness and development of asthma? *Allergy Asthma Clin Immunol* 2008;4:51-8.

[80] Shore SA. Obesity, airway hyperresponsiveness, and inflammation. *J Appl Physiol* 2010;108:735-43.

[81] Sood A, Shore SA. Adiponectin, Leptin, and Resistin in Asthma: Basic Mechanisms through Population Studies. *J Allergy*. 2013;2013:785835.

[82] Sansone F, Attanasi M, Di Pillo S, Chiarelli F. Asthma and Obesity in Children. *Biomedicines* 2020;8:231.

[83] Lugogo N, Green CL, Agada N, Zhang S, Meghdadpour S, Zhou R, et al. Obesity's effect on asthma extends to diagnostic criteria. *J Allergy Clin Immunol* 2018;141:1096-104.

[84] Desai D, Newby C, Symon FA, Haldar P, Shah S, Gupta S, et al. Elevated sputum interleukin-5 and submucosal eosinophilia in obese individuals with severe asthma. *Am J Respir Crit Care Med* 2013;188:657-63.

[85] Rastogi D, Fraser S, Oh J, Huber AM, Schulman Y, Bhagtani RH, et al. Inflammation, metabolic dysregulation, and pulmonary function

Complimentary Contributor Copy

among obese urban adolescents with asthma. *Am J Respir Crit Care Med* 2015;191:149-60.

[86] Zheng J, Zhang X, Zhang L, Zhang HP, Wang L, Wang G. Interactive effects between obesity and atopy on inflammation: A pilot study for asthma phenotypic overlap. *Ann Allergy Asthma Immunol* 2016;117:716-7.

[87] Holguin F. Arginine and nitric oxide pathways in obesity-associated asthma. *J Allergy* 2013;2013:714595.

[88] Holguin F, Fitzpatrick A. Obesity, asthma, and oxidative stress. *J Appl Physiol* (1985). 2010;108:754-9.

[89] Albuali W. Evaluation of oxidant-antioxidant status in overweight and morbidly obese Saudi children. *World J Clin Pediatr* 2014;3:6-13.

[90] Rowicka G, Dyląg H, Ambroszkiewicz J, Riahi A, Weker H, Chełchowska M. Total Oxidant and Antioxidant Status in Prepubertal Children with Obesity. *Oxid Med Cell Longev* 2017;2017:5621989.

[91] Codoner-Franch P, Boix-Garcia L, Simo-Jorda R, Del Castillo-Villaescusa C, Maset-Maldonado J, Valls-Belles V. Is obesity associated with oxidative stress in children? *Int J Pediatr Obes* 2010;5:56-63.

[92] Nadeem A, Siddiqui N, Alharbi NO, Alharbi MM. Airway and systemic oxidant-antioxidant dysregulation in asthma: a possible scenario of oxidants spill over from lung into blood. *Pulm Pharmacol Ther* 2014;29:31-40.

[93] Dut R, Dizdar EA, Birben E, Sackesen C, Soyer OU, Besler T, et al. Oxidative stress and its determinants in the airways of children with asthma. *Allergy* 2008;63:1605-9.

[94] Bodini A, Peroni D, Vicentini L, Loiacono A, Baraldi E, Ghiro L, et al. Exhaled breath condensate eicosanoids and sputum eosinophils in asthmatic children: a pilot study. *Pediatr Allergy Immunol* 2004;15:26-31.

[95] El-Alameey IR, Fathy GA, Shady MMA, Ali A, Fathy HA, Youness ER, et al. Relationship of Oxidant and Antioxidant Markers to

Complimentary Contributor Copy

Asthma Severity in Egyptian Asthmatic Children. *Open Access Maced J Med Sci.* 2017;5:645-50.

[96] Agrawal A, Mabalirajan U, Ahmad T, Ghosh B. Emerging interface between metabolic syndrome and asthma. *Am J Respir Cell Mol Biol* 2011;44:270-5.

[97] Forno E, Han Y-Y, Muzumdar RH, Celedón JC. Insulin resistance, metabolic syndrome, and lung function in US adolescents with and without asthma. *J Allergy Clin Immunol* 2015;136:304-11.e8.

[98] Karampatakis N, Karampatakis T, Galli-Tsinopoulou A, Kotanidou EP, Tsergouli K, Eboriadou-Petikopoulou M, et al. Impaired glucose metabolism and bronchial hyperresponsiveness in obese prepubertal asthmatic children. *Pediatr Pulmonol* 2017;52:160-6.

[99] Sánchez Jiménez J, Herrero Espinet FJ, Mengibar Garrido JM, Roca Antonio J, Peños Mayor S, Peñas Boira Mdel M, et al. Asthma and insulin resistance in obese children and adolescents. *Pediatr Allergy Immunol* 2014;25:699-705.

[100] Singh VP, Aggarwal R, Singh S, Banik A, Ahmad T, Patnaik BR, et al. Metabolic Syndrome Is Associated with Increased Oxo-Nitrative Stress and Asthma-Like Changes in Lungs. *PloS One* 2015;10:e0129850-e.

[101] Dekkers BG, Schaafsma D, Tran T, Zaagsma J, Meurs H. Insulin-induced laminin expression promotes a hypercontractile airway smooth muscle phenotype. *Am J Respir Cell Mol Biol* 2009;41:494-504.

[102] Cottrell L, Neal WA, Ice C, Perez MK, Piedimonte G. Metabolic abnormalities in children with asthma. *Am J Respir Crit Care Med* 2011;183:441-8.

[103] Chen YC, Tung KY, Tsai CH, Su MW, Wang PC, Chen CH, et al. Lipid profiles in children with and without asthma: interaction of asthma and obesity on hyperlipidemia. *Diabetes Metab Syndr* 2013;7:20-5.

[104] McNulty W, Usmani OS. Techniques of assessing small airways dysfunction. *Eur Clin Respir J* 2014;1:10.3402/ecrj.v1.25898.

Complimentary Contributor Copy

[105] Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012;40:1324-43.

[106] Lang JE. Obesity, Nutrition, and Asthma in Children. *Pediatr Allergy Immunol Pulmonol* 2012;25:64-75.

[107] Jensen ME, Gibson PG, Collins CE, Wood LG. Lean mass, not fat mass, is associated with lung function in male and female children with asthma. *Pediatr Res* 2014;75:93-8.

[108] López-Sánchez GF, Sgroi M, D'Ottavio S, Díaz-Suárez A, González-Víllora S, Veronese N, et al. Body Composition in Children and Adolescents Residing in Southern Europe: Prevalence of Overweight and Obesity According to Different International References. *Front Physiol* 2019;10:130.

[109] Guibas GV, Manios Y, Xepapadaki P, Moschonis G, Douladiris N, Mavrogianni C, et al. The obesity-asthma link in different ages and the role of body mass index in its investigation: findings from the Genesis and Healthy Growth Studies. *Allergy* 2013;68:1298-305.

[110] Eichenberger PA, Diener SN, Kofmehl R, Spengler CM. Effects of exercise training on airway hyperreactivity in asthma: a systematic review and meta-analysis. *Sports Med* 2013;43:1157-70.

[111] Lin LL, Huang SJ, Ou LS, Yao TC, Tsao KC, Yeh KW, et al. Exercise-induced bronchoconstriction in children with asthma: An observational cohort study. *J Microbiol Immunol Infect*. 2019;52:471-9.

[112] Firrincieli V, Keller A, Ehrensberger R, Platts-Mills J, Shufflebarger C, Geldmaker B, et al. Decreased physical activity among Head Start children with a history of wheezing: use of an accelerometer to measure activity. *Pediatr Pulmonol.* 2005;40:57-63.

[113] Priftis KN, Panagiotakos DB, Anthracopoulos MB, Papadimitriou A, Nicolaidou P. Aims, methods and preliminary findings of the Physical Activity, Nutrition and Allergies in Children Examined in Athens (PANACEA) epidemiological study. *BMC Public Health* 2007;7:140.

Complimentary Contributor Copy

[114] Tsai HJ, Tsai AC, Nriagu J, Ghosh D, Gong M, Sandretto A. Associations of BMI, TV-watching time, and physical activity on respiratory symptoms and asthma in 5th grade schoolchildren in Taipei, Taiwan. *J Asthma* 2007;44:397-401.

[115] Mitchell EA, Beasley R, Bjorksten B, Crane J, Garcia-Marcos L, Keil U. The association between BMI, vigorous physical activity and television viewing and the risk of symptoms of asthma, rhinoconjunctivitis and eczema in children and adolescents: ISAAC Phase Three. *Clin Exp Allergy* 2013;43:73-84.

[116] Eijkemans M, Mommers M, Remmers T, Draaisma JMT, Prins MH, Thijs C. Physical activity and asthma development in childhood: Prospective birth cohort study. *Pediatr Pulmonol.* 2020;55:76-82.

[117] Ownby DR, Peterson EL, Nelson D, Joseph CCL, Williams LK, Johnson CC. The Relationship of Physical Activity and Percentage of Body Fat to the Risk of Asthma in 8- to 10-year-old Children. *J Asthma* 2007;44:885-9.

[118] Ostrom NK, Eid NS, Craig TJ, Colice GL, Hayden ML, Parsons JP, et al. Exercise-induced bronchospasm in children with asthma in the United States: results from the Exercise-Induced Bronchospasm Landmark Survey. *Allergy Asthma Proc* 2011;32:425-30.

[119] Peters U, Dixon AE, Forno E. Obesity and asthma. *J Allergy Clin Immunol* 2018;141:1169-79.

[120] Styne DM, Arslanian SA, Connor EL, Farooqi IS, Murad MH, Silverstein JH, et al. Pediatric Obesity-Assessment, Treatment, and Prevention: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2017;102:709-57.

[121] Jensen ME, Gibson PG, Collins CE, Hilton JM, Wood LG. Diet-induced weight loss in obese children with asthma: a randomized controlled trial. *Clin Exp Allergy* 2013;43:775-84.

[122] Willeboordse M, van de Kant KDG, Tan FES, Mulkens S, Schellings J, Crijns Y, et al. A Multifactorial Weight Reduction Programme for Children with Overweight and Asthma: A Randomized Controlled Trial. *PloS One.* 2016;11:e0157158-e.

Complimentary Contributor Copy

[123] Okoniewski W, Lu KD, Forno E. Weight Loss for Children and Adults with Obesity and Asthma. A Systematic Review of Randomized Controlled Trials. *Ann Am Thorac Soc* 2019;16:613-25.

[124] Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J* 2005;26:319-38.

[125] Lucas JA, Moonie S, Hogan MB, Evans WN. Efficacy of an exercise intervention among children with comorbid asthma and obesity. *Public Health* 2018;159:123-8.

[126] Docherty AB, Harrison EM, Green CA, Hardwick HE, Pius R, Norman L, et al. Features of 16,749 hospitalised UK patients with COVID-19 using the ISARIC WHO Clinical Characterisation Protocol. medRxiv. 2020:2020.04.23.20076042. doi: https://doi.org/10.1101/2020.04.23.20076042.

[127] Hoang A, Chorath K, Moreira A, Evans M, Burmeister-Morton F, Burmeister F, et al. COVID-19 in 7780 pediatric patients: A systematic review. *EClinicalMedicine* 2020;24.

[128] Castro-Rodriguez JA., Forno E. Asthma and COVID-19 in children – a systematic review and call for data. *Pediatr Pulmonol* 2020. doi: 10.1002/ppul.24909.

[129] Zachariah P, Johnson CL, Halabi KC, Ahn D, Sen AI, Fischer A, et al. Epidemiology, Clinical Features, and Disease Severity in Patients With Coronavirus Disease 2019 (COVID-19) in a Children's Hospital in New York City, New York. *JAMA Pediatr* 2020: e202430.

[130] Rychter AM, Zawada A, Ratajczak AE, Dobrowolska A, Krela-Kaźmierczak I. Should patients with obesity be more afraid of COVID-19? *Obesity Rev* 2020;21:e13083.

[131] De A, Rastogi D. Association of pediatric obesity and asthma, pulmonary physiology, metabolic dysregulation, and atopy; and the role of weight management. *Expert Rev Endocrinol Metab* 2019;14:335-49.

Complimentary Contributor Copy

[132] Yamaya M, Nishimura H, Deng X, Sugawara M, Watanabe O, Nomura K, et al. Inhibitory effects of glycopyrronium, formoterol, and budesonide on coronavirus HCoV-229E replication and cytokine production by primary cultures of human nasal and tracheal epithelial cells. *Respir Investig* 2020;58:155-68.

[133] Jackson DJ, Busse WW, Bacharier LB, Kattan M, O'Connor GT, Wood RA, et al. Association of respiratory allergy, asthma, and expression of the SARS-CoV-2 receptor ACE2. *J Allergy Clin Immunol* 2020;146:203-6.e3.

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Chapter 2

BIOACTIVES OF *LACTUCA SATIVA*: NUTRITIONAL AND CLINICAL IMPORTANCE

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ABSTRACT

Lactuca sativa, commonly called as lettuce, is a well-known leafy vegetable consumed worldwide that possessing high nutritional value. The nutritional and bioactive composition of lettuce differs depending on the origin and the type of lettuce. Lettuce is low in calories and own numerous health benefits mainly due to its high availability of vitamins, phytochemicals (phenolics and carotenoids), macro and micronutrients. Several *in vitro* and *in vivo* studies have demonstrated the antioxidant, cholesterol-lowering, cardioprotective, neuroprotective, anti-diabetic, anti-inflammatory, anti-proliferative, anti-cancer activities of the lettuce bioactives. In addition, clinical studies have shown lettuce consumption markedly increased the bioavailability of phenolics and carotenoids in the plasma, improved glucose metabolism and reduced total lipids in the liver. However, the explication of molecular and metabolic mechanisms of

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lettuce derived bioactives need to be studied further for the health benefits and safety and regular consumption.

Keywords: *Lactuca sativa, bioactives, phenolics, carotenoids, vitamins, nutrition, clinical importance*

1. INTRODUCTION

Lettuce is an herbaceous plant belonging to the Asteraceae family. The lettuce leaves are bright, mostly green and red, with a few variations (Harlan 1986). Lettuce grows in various shapes and textures, such as thick iceberg heads, notched, scalloped, frilly or rough stems. Lettuce usually lives 70-125 days from planting to harvesting before flowering, depending on the variety and time of cultivation (Foteinis and Chatzisymeon 2016). Lettuce is primarily consumed fresh as salads in most parts of the world, however in certain eastern countries such as China and Greece, lettuce stems are eaten as fried, crudely, wicked or as a sauce. In earlier days, Europe and North America have dominated the lettuce market, however presently lettuce consumption and cultivation have expanded worldwide. Studies have demonstrated the effect of different processing environments on phytochemical antioxidants in lettuce. The relationship between light stress resistance, photoacclimatory plasticity and vegetable nutritional quality are considered as convenient model crop for examination (Freeland-Graves and Nitzke 2013).

Lettuce is composed of various phytochemicals such as phenolics, carotenoids, vitamins, minerals and fibre which are all critical for human health and disease prevention (DuPont et al. 2000). In lettuce, chlorogenic acid, caffeic acid and their derivatives are the most common occurring phenolic acids (Zhao et al. 2007). A study by Llorach et al. (2008) documented the occurrence of flavones luteolin, kaempferol derivatives and quercetin in the lettuce varieties. The lettuce carotenoids include β-carotene, lutein, lactucaxanthin, zeaxanthin, neoxanthin and violaxanthin (Saini et al. 2018; Naveen et al. 2020). Among the vitamins, lettuce is rich in vitamin (C,

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E, K, A) and folate (Romani et al. 2002). Raw lettuce is reported to contain minerals such as iron, calcium, magnesium, sodium, potassium, zinc (Santos et al. 2014). Typically the composition of these phytochemicals changes with lettuce varieties.

In recent times, the consumer's choice for fresh foods over preserved foods is mainly to improve the longevity. The study concluded that the consumption of green leafy vegetables was associated with reduced risk of chronic diseases such as cancer, cardiovascular diseases, and aging. A vast number of *in vitro* and *in vivo* studies have shown anti-diabetic, cholesterol-lowering, anti-inflammatory, anti-cancer activities of lettuce's bioactive compounds. Among the less frequent lettuce applications are nicotine-free tobacco derived from lettuce berries and edibles from seeds of primitive lettuce. Also, lactucarium, the dried latex derived from a relative of wild lettuce, *Lactuca virosa* L., is used for the sleep-inducing medication (Trojanowska 2005). This chapter will provide a detailed report on the nutritional and clinical status of bioactives among different lettuce varieties.

2. Lettuce Varieties and Their Nutritional Benefits

Lettuce is reported to protect skin, minimize cold, flues and headaches, improve digestion, decrease arthritis, reduce the risks of osteoporosis and improve one's understanding (Duncan 1821). Lettuce is typically fiber rich, fat free, low calorie food useful for weight reduction. Lettuce has high fiber and cellulose content, which help in eliminating bile salts from the bloodstream eventually contributing to cholesterol breakdown (Hu et al. 2000). There is a higher ratio of omega-3 to omega-6 fatty acids for fat metabolism in Romaine lettuce. This variety has 20% of its calories as protein. (Mou and Ryder 2004). Lettuce consisted of minerals such as phosphorus, calcium, iron, magnesium, sodium, zinc, potassium and vitamins including riboflavin, thiamin, niacin, folate, B6, C, E, A and K (Olszyk et al. 2020). Lettuce is shown to have a glycemic index of 15 and is

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well regarded for its low glycemic index, which helps in maintaining the body weight (Chadchan, Jargar, and Das 2016). Lettuce extracts have shown important regulatory activity against inflammation through lipoxygenase and carrageenan-type biocatalysts. Lettuce's neurological characteristics are another feature of lettuce. Neuronal mortality of physical memory links contributes to memory loss and Alzheimer's disease (Singh et al. 2008). The study has shown that lettuce extracts having influence on neuronal cell death. Researchers demonstrated that lettuce could be used to monitor against neurodegenerative disorders as a neuroprotective agent. Nicolle et al. (2004) showed lettuce intake significantly improved the antioxidant and cholesterol metabolism in experimental rats.

Several studies have shown the antioxidant effect of lettuce in scavenging free radicals and strongly preventing lipid peroxidation. The lettuce latex possessed antimicrobial properties, where *Salmonella typhimurium* and many other yeasts are deformed upon treatment with lettuce latex. Researchers also established the antimicrobial effects of the lettuce phytochemicals, terpenes and carotenoids and perhaps even enzymes like glucanases (S.-Y. Kim et al. 2011). Lettuce has abundant minerals such as copper, calcium, magnesium, zinc and potassium that are important to the body's metabolism. Lettuce minerals chiefly helped in reducing contaminants and maintaining the equilibrium of acid and alkaline. Manganese is a co-factor of the superoxide dismutase, an antioxidant enzyme (Altunkaya et al. 2009). For the development of red blood cells, copper is essential and iron is required to shape the red blood cells. The lettuce salads shown to combat illnesses such as osteoarthritis, anaemia, cardiovascular diseases, Parkinson disease and various cancers (Slavin and Lloyd 2012). Lettuce water extract regulated certain cancer forms. Treatment of lettuce extracts showed marked influence on leukaemia and breast cancer (Sakthivel and Ganesan 2017).

Lettuce salad is a very healthy source of vitamins such as vitamin A and β-carotene. It is estimated that 100g of fresh lettuce provided 247% of daily vitamin A and 4.443kg of carotene, which is essential to preserving a healthy respiratory tract, scalp, and sight.

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Zeaxanthin was confirmed to be a significant dietary carotenoid in the lettuce, specifically ingested into retinal macula lutea, where it is believed to have an antioxidant defense against macular diseases correlated with age in the elders (Simko 2019). Lettuce's high iron content aids in increasing blood production and the carriage of oxygen and nutrients around the body. Lettuce salad has nutrients such as potassium, which avoid a rise in blood pressure. The vitamins A, E and C in lettuce protects skin from sun damage and aging and stimulate collagen development to maintain the skin. Lettuce serves as a natural treatment for body detoxification because of its high nutritional content, which tends to purify the body by washing the toxic components. It tends to make the bones safe as a rich calcium supply (Lazic, Lazic, and Sekulic 2002). Romaine lettuce has strong anti-allergy properties because of its folate content that helps avoid and relieve allergy symptoms, so it is essential for asthma patients. It includes fiber that enhances blood supply to the heart. The 8-carotene component of lettuce inhibits plaque accumulation in the heart (San Miguel-Moncin et al. 2003).

2.1. Crisphead Lettuce

Crisphead lettuce is also known as Iceberg lettuce. Unlike Romaine lettuce about the nutritional benefit, it offers an attired supply of certain vital nutrients and minimal calorie content. The calories of iceberg lettuce are due to the number of carbohydrates. Carbohydrate maintains the nervous system and facilitates muscle contraction, while dietary fibers aid prevents constipation. Like other lettuce types, iceberg lettuce is a vital source of vitamin K, which helps protect skeletal integrity, bone health and osteoporosis (Gurdon et al. 2019). A diced cup of iceberg lettuce reportedly contained 0.8 kcal, 17mg of starch, 50mg of protein, 70mg of fiber and almost negligible fat. Iceberg lettuce is low among other leafy vegetables. 95% of iceberg lettuce is water with various levels of phosphorous, iron, sodium and potassium.

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The lowest nutrient profile of all lettuce type is the iceberg lettuce, with very few calories and little vitamins A, C and K and folate (Akbar and Akbar 2020).

2.2. Butterhead Lettuce

Butterhead lettuce is rich in different nutrients and is frequently added to burgers, rolls and sandwiches. Butterhead salad is considered to be smooth, leafy and delicate with dark green leaves. Butterhead type appear as bright yellow and crunchy and is highly appealing in lettuce. The fragile leaves can also be used as a low-calorie nutrient-rich wrapper to pack other foods. Butterhead lettuce is typically low in calorie, fat and sodium; however, it is rich in other nutrients. Each serving cup contained less than a half gram of fat, 363µg of carbohydrate, 74µg of protein, 1.53µg of sugar and only 0.8g of sodium (El-Nakhel et al. 2019). A cup of butterhead lettuce contained 0.91 µg of vital nutrients in the manner of leafy green butterhead lettuce high in vitamin K and one cup of chopped butterhead lettuce with a vitamin K content of more than 56pg. Butterhead lettuce is indeed a vital source of phosphorus that helps the human body's essential nerve functioning. A cup of shredded butter salad produces 33mg phosphorus per serve (Viacava, Roura, and Agüero 2015).

2.3. Romaine or Cos lettuce

Romaine is a common type of lettuce well-known as a great complement to a diet for weight loss. Romaine salad contains 1.7 kilocalories per cup; however, romaine salad is high in moisture. The fiber content of 2g per serving is a good complement for eliminating the digestive tract (Kim et al. 2007). Romaine lettuce is a rich source of Vitamin C and is a cardioprotective vegetable; a serving of Romaine lettuce can give up to 40% of the per day needs (Mou 2012).

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In particular, vitamin C prevented cholesterol from binding and sticking to the wall of blood vessels. One or two portions of romaine salad in the regular diet is suggested for a healthy heart. Romaine lettuce is a rich source of vitamin K, which has a beneficial effect on bone growth, muscle strength and blood clotting factors. One cup consumption of romaine salad contains 0.247g or 1/10 portion of daily needs. Fiber present in this leafy vegetable binds with bile acids so that the bowels absorb them. This role stimulates the liver to generate more bile acids, reducing the body's total amount of cholesterol. Research has shown that romaine lettuce has brain-protective effects, mainly caffeic acids, due to phenolic substances. The isochlorogenic add was precisely the most critical effect in shielding the brain from oxidative stress. Studies have shown that Romaine lettuce has an anxiolytic impact on animals when exposed to a series of conduct tests. They recommended romaine salad as an alternative way to relieve anxiety and panic disorders (López et al. 2014).

2.4. Leaf Lettuce

Leaf salad is fragile and not approved for regular usage because it is pricey and bitter in high temperatures. Low-calorie leaf lettuce is nevertheless abundant in numerous other micronutrients. A cup of sliced leaf lettuce has almost 7 calories and it has even less dietary fat. Each cup of blended leaf salad has 0.1g of fat or residues of saturated fat. Leaf lettuce is low in carbohydrates (1.2g) in each cup of chopped leaves. "Archives of Internal Medicine" literature has suggested that low carbon diets can achieve a higher losing weight rate than low-fat meals (Patrícia and Oliveira 2015). A cup of chopped leaf lettuce produced 0.6g dietary fiber and is the primary source of loose-leaf lettuce carbohydrate. Lettuce from the leaf has a relatively large vitamin A and K quality. Various tests have shown that leaf lettuce is a good source of antioxidants and folic acid (Riga et al. 2019).

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Table 1. Nutritional profile of lettuce varieties

Nutritive Value / Lettuce types		Crisphead	Butterhead	Romaine	Leaf Lettuce
Vitamins	Vitamin A	94.8 mcg	546.6 mcg	156.9 mcg	105.6 mcg
	Alpha Carotene	0.0029 mg	0.0 mg	0.0 mg	0.0 mg
	Retinol	0.0 mg	0.0 mg	0.0 mg	0.0 mg
	β-Carotene	0.215 mg	1.093 mg	0.314 mg	0.211 mg
	β-Cryptoxanthin	0.0 mg	0.0 mg	0.0 mg	0.0 mg
	Lycopene	0.0 mg	0.0 mg	0.0 mg	0.0 mg
	Vitamin C	2.0 mg	2.0 mg	1.4 mg	0.9 mg
	Vitamin D	-	-	-	-
	Lutein+ Zeaxanthin	0.199 g	0.0 g	0.139 g	0.0822 g
	Vitamin K	0.0174 mg	0.0563 mg	0.0062 mg	0.0082 mg
	Thiamine	0.0 mg	0.0 mg	0.0 mg	0.0 mg
	Riboflavin	0.0 mg	0.0 mcg	0.0 mg	0.0 mg
	Niacin	0.1 mg	0.2 mg	0.0 mg	0.0 mg
	Vitamin B6	0.0 mg	0.0 mg	0.0 mg	0.0 mcg
	Folate	0.0209 mg	0.0401 mg	0.0082 mg	0.0018 mg
	Vitamin B12	0.0 mg	0.0 mg	0.0 mg	0.0 mg
	Pantothenic Acid	0.1mg	0.1mg	0.0 mg	0.0 mg
	Choline	4.8 mg	4.6 mg	0.6 mg	0.06 mg
	Betaine	0.1 mg	0.1 mg	0.0 mg	0.0 mg
Minerals	Iron	300 mcg	700 mcg	100 mcg	0.0 mcg
	Calcium	13000 mcg	19300 mcg	2000 mcg	1700 mcg
	Phosphorus	14400 mcg	18200 mcg	1800 mcg	1400 mcg
	Magnesium	5000 mcg	7200 mcg	800 mcg	600 mcg
	Sodium	7200 mcg	2800 mcg	500 mcg	1300 mcg
	Potassium	0.102 g	0.131 g	0.0148 g	0.0092 mg
	Copper	0.0 g	0.0 g	0.0 g	0.0 g
	Zinc	100 mcg	100 mcg	100 mcg	100 mcg
	Manganese	100 mcg	100 mcg	0.0 mcg	0.0 mcg
	Fluoride	-	-	-	-
	Selenium	100 mcg	0.3 mcg	0.0 mcg	0.0 mcg
Carbohydrate	Total carbohydrate	2300 mg	1200 mg	200 mg	100 mg
	Dietary fiber	900 mg	600 mg	100 mg	100 mg
	Starch	0.0 mg	-	0.0 mg	0.0 mg
	Sugars	1400 mg	500 mg	100 mg	0.0 mg
	Sucrose	36.0 mg	0.0mg	0.0 mg	0.0 mg
	Glucose	655 mg	236mg	24.4 mg	17.1 mg
	Fructose	720 mg	281mg	48.0 mg	20.4 mg

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Nutritive Value \ Lettuce types		Crisphead	Butterhead	Romaine	Leaf Lettuce
Fatty Acids	Total Fat	100 mg	100 mg	-	-
	Total Omega-3 fatty acid	37.4 mg	45.7 mg	-	-
	Total Omega-6 fatty acid	15.1 mg	18.7 mg	-	-
	Polyunsaturated Fat	100 mg	100 mg	-	-
Proteins & Amino acids	Protein	600 mg	700 mg	100 mg	100 mg
	Tryptophan	6.5 mg	7.2mg	0.6 mg	0.4 mg
	Phenylalanine	16.6 mg	29.1mg	3.9 mg	2.6 mg
Calorie Information	Calories	10.1 (42.3 kJ)	7.2 (30.1 kJ)	1.0 (4.2 kJ)	0.7 (2.9 kJ)
	Carbohydrate	7.7 (32.2 kJ)	4.3 (18.0 kJ)	0.7 (2.9 kJ)	0.5 (2.1 kJ)
	Fat	0.8 (3.3 kJ)	1.0 (4.2 kJ)	0.2 (0.8 kJ)	0.1 (0.4 kJ)
	Protein	1.6 (6.7 kJ)	1.8 (7.5 kJ)	0.2 (0.8 kJ)	0.2 (0.8 kJ)
	Alcohol	0.0 (0.0 kJ)	0.0 (0.0 kJ)	0.0 (0.0 kJ)	0.0 (0.0 kJ)
References		("Lettuce, Iceberg (Includes Crisphead Types), Raw Nutrition Facts & Calories" 2020) (M. J. Kim et al. 2016)	("Lettuce, Butterhead (Includes Boston and Bibb Types), Raw Nutrition Facts & Calories" 2020) (Mou 2012)	Konstantopoulou et al. 2010; (Nutrition Data 2020)	("Lettuce, Green Leaf, Raw Nutrition Facts & Calories" 2020); (Koudela and Petříková 2008)

3. BIOACTIVE COMPOUNDS OF *LACTUCA SATIVA*

Chronic disorders, such as coronary disease, asthma and obesity are increasing worldwide. Consumption of some foods may minimize chronic disease risk. In particular, the richness of fruits, legumes, green leafy vegetables are considered health benefits because they are high in vitamins and bioactive compounds. Carotenoids and phenolic substances are the major phytochemicals found in lettuce. *In vitro*, clinical trials, therapeutic and pharmacological trials demonstrated to know the bioactive substances researched for their chemical properties, application in food and health consequences (NEGRÃO et al. 2020).

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To minimize the incidence of oxidative stress-related disorders, higher doses of these nutrients are advised because of the antioxidant effects of ascorbic acid, carotenoids, and α-tocopherol. Baby butterhead lettuce recorded 0.1035–0.121g of ascorbic acid content. Baby romaine lettuce was much more robust having 0.21–0.40g of ascorbic acid (Aćamović-Djoković et al. 2011). The ascorbic acid level in baby green Romaine lettuce was higher than in mature Romaine lettuce and mature spinach indicating that baby green Romaine lettuce is a rich source of ascorbic acid. The content of baby butterhead 'Red Cross' for carotenoids was far higher, β-carotene 0.015–0.14g, than the total phenolic content of baby romaine lettuce. The phenolic content was 650–670 µg in the baby green leaf lettuce (Llorach, Tomás-Barberán, and Ferreres 2004).

Total phenolic content of lettuce differs among lettuce varieties, where crisphead iceberg was lower than other standard US-based lettuce. Moreover, the total phenolic content of lettuce is greatly influenced by the leaf coloration. In red leaf and red romaine lettuce, the total phenolic content was higher than in the green lettuce. This may be attributed to the red color, mainly due to anthocyanins, a subset of phenolic compounds. In particular, experiments have shown that lettuce contained several bioactive compounds. The collection of red-pigmented leaves or manipulating cultural conditions could lead to higher concentrations of these bioactives (Mulabagal et al. 2010).

Five lettuce varieties were tested for their flavonoid content and flavonols, which are three continental green icebergs, Romaine and green leaf subtypes, and two red leaf subtypes are the oak leaf and lollo rosso (Becker et al. 2015). Quercetin, Kaempferol, Anthocyanins and Luteolin are the major phenolics reported in lettuce. However, in all varieties, these substances were not reliably identified, with varying content of different forms of lettuce. The average flavonoid content of red leaf was higher in red Rosso 280µg and red oak leaf 1.1µg, accompanied by green continental 200µg and 170µg, green Romaine iceberg 10µg. The higher phenolic content in red variety compared with green lettuce is mainly attributed to the prevalence of phenolic acids and flavonols (Damerum et al. 2015).

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Baby lettuce also supplies many nutritional minerals, comparable to mature lettuce. Studies found that 500µg of sodium and 600µg of Baby green romaine, and 100 µg of lollo rosso leaf lettuce are the sodium's sums. For baby leaf lettuces, the sodium levels reported by the USDA is 500µg, which was usually higher than that reported usual sodium content. The potassium content of baby green leaf lettuce was reported as 2.2mg. The potassium content of Romaine lettuce ranged between 1.1 to 1.6mg, which was closer to rose baby lettuce. Mature green leaf lettuce has a more excellent potassium range from 1.9 to 4.1mg was recorded in a study (Sublett, Barickman, and Sams 2018).

4. Bioavailability and Clinical Importance of Lettuce Bioactives

A significant number of phenolic compounds in lettuce have been recorded. The aqueous lettuce extracts exhibited inhibition of oxygen radical-scavenging ability in low-density lipoproteins, oxyradicals-induced inhibition of ethylene formation, hydroxyl radical-scavenging potential and regulating activity against copper-induced protein oxidation (Liu et al. 2007). There are claims that the flavonoids and polyphenols in lettuce are particularly susceptible to environmental circumstances. Studies have shown that post-harvest storage and processing in many lettuce cultivars caused significant loss of flavonoids and phenolic acids. In changing the antioxidant properties and quality of lettuce varieties, differences in the genotype and breeding conditions have enormous consequences (DuPont et al. 2000).

It's indeed vitamin A present in two forms: retinoids and provitamin A. Retinols are usually present in animal sources, while carotenoids are found in foods obtained from plants. Pro-vitamin A function is found in carotenoids a and b-carotene, and b-cryptoxanthin. The concentrations of retinol in b-carotene are more significant than in a-carotene and b-cryptoxanthine (Irwig et al. 2002). For vision, fertility, embryogenic growth, and

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immune function, Vitamin A is essential. Carotenoid use was proposed due to its antioxidant properties correlated with a lower incidence of chronic diseases. Carotenoids are various lipid-soluble pigments commonly found in yellow-orange fruits and vegetables and gloomy leafy green vegetables (Cruz et al. 2012). The main carotenoids in lettuce are β-carotene and lutein. Carotenoid content differs though with the varieties of lettuce. The Romaine variety had the maximum β-carotene content of 0.0523g, while the crisphead was the lowest (Mou 2005). The amount of β-carotene and lutein was carefully examined in 22 cultivars of crisphead, five of the butterhead, the romaine and green leaf varieties, and four red leaf lettuces recorded 0.11–0.569 to 0.13–0.543g. β-carotene and lutein were the highest combined amount in green leaves and the romaine types accompanied by red leaf type followed by the smallest in crisphead (Llorach et al. 2008).

5. Factors Influencing Bioactive Content of Lettuce

Genotype variation, environmental variation and agronomic conditions are the major factors that affect the bioactive content of lettuce. Phytonutrient rich fruits and vegetables offer protection against various diseases, but the range of phytonutrients in fruits and vegetables is shallow. Allelic variations in the genes responsible for the phytonutrient quantity are accountable for fruits and vegetables (Hounsome et al. 2008). Lettuce is a rich source of phenolic compounds such as vitamins C and E, carotenoids, etc. Studies in rats showed consuming lettuce improved cholesterol metabolism and antioxidant blood plasma capacity in humans and rats. Beneficial phenolic compounds present in lettuce include chicoric acid, chlorogenic acid and derivatives of flavonoids - quercetin and kaempferol (Mulabagal et al. 2010). Homocysteine, a toxic by-product of long-term high blood pressure, is known to inhibit lipid peroxidation, which can cause the damage DNA under the influence of heat and other chemicals. Also, Cyclooxygenase is known to reduce hypermethylation of DNA, which

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prevents tumor formation. It was advised to consume whole leafy vegetables rather than depending on supplements due to difficulties in isolating the phytonutrients and poor stability of the extracted phytonutrients. Hence the growth of horticulture to produce natural fruits has become an efficient strategy to raise human nutrition richly in phytonutrients. This led to the development of several novel methods to enhance the nutritional content of fruits and vegetables. The genetic basis of lettuce's antioxidant potential was studied by Damerum et al. in a recombinant inbred line obtained from a cross between cultivated lettuce and wild-type lettuce. He observed a quantitative difference in the levels of CTA derivatives, caffeoylquinic acid (CQA), Quercetin-3-glucuronide and Quercetin-3-malonylglucoside compared to the parent crop (Mulabagal et al. 2010).

The antioxidant content is a significant parameter that determines fruit and vegetable quality. In lettuce, phytochemicals produced in various environmental conditions impart antioxidant potential. Light is an important abiotic factor that determines phytochemical synthesis. Studies by Merzlyak and Chivkunova (2000) reported that the intensity of light determines antioxidant anthocyanin synthesis. Light leads to changes in plants, like changes to metabolism and pigment composition to cope with changing environmental conditions (Li and Kubota 2009). Studies in baby lettuce by Li and Kubota (2009) showed that exposure to auxiliary red light increased the phenolic content by 6% compared to white light exposure. At the same time, supplementation of far-red light has been reported to have harmful effects due to decreased anthocyanin, carotenoid, and chlorophyll content, leading to decreased antioxidant activity. Zhou et al. showed that supplementation of artificial light and normal light could increase the phytochemical content in green lettuce (Zhou, Liu, and Yang 2012). The nitrogen content of the soil is an essential factor that determines the health benefits of leafy vegetables. High nitrogen content has harmful health effects on humans. Supplementation of nitrogen externally is reported to reduce essential nutrients (Barker, Peck, and MacDonald 1971). Therefore, low nitrogen content is known to increase the nutritional quality of lettuce. Low nitrate content increases Mn, Zn, Mg, Ca, etc. (Mareczek, daržininkystė, and 2005 2020). The effect of temperature and growth conditions are other

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environmental factors that determine the antioxidant potential of lettuce. Low temperature leads to the accumulation of anthocyanins in fruits and lettuce (Boo et al. 2011).

Light intensity, temperature, CO2 level, water supply, and pathogens are environmental factors that influence stress response in plants. Studies by Koyama et al. showed that drought stress induces lettuce roots to produce more ascorbic acid, polyphenols, and sugars (Uno et al. 2016). Air temperature also affects metabolic pathways in lettuce leaves. They accelerate anthocyanin and chlorophyll synthesis in red lettuce leaves reported by Gazula et al. (Gazula et al. 2005). Weather and surrounding growth conditions also affect the enzymatic and antioxidant activity of lettuce. It was reported that the lettuce roots and fruits accumulated more anthocyanin content (Romani et al. 2002). Abscisic acid is a hormone that lets plants deal with tension, such as under drought conditions. A study was done on the role of Abscisic acid on the antioxidant enzyme potential in lettuce leaves. (Al Muhairi et al. 2015).

CONCLUSION

Lettuce absorbs low Na and offers reasonably good Fe, depending on the varieties and cultivars. Crisphead lettuce, classified as an iceberg, was the lowest source of dietary minerals among lettuce forms popularly eaten in the U.S. In contrast, leaf-type lettuces were usually higher in these minerals. Lettuce is healthy in moisture content and low in calories, so the lettuce's nutrient benefit was not acknowledged. Lettuce has health advantages because of its addition to dietary fiber, the availability of many essential dietary minerals, antioxidants, and secondary metabolites such as carotenoids and polyphenols. Among the lettuce varieties used in the U.S., butterhead, iceberg lettuce, and red leaf types proved strong in folate. For butterhead, Romaine and leaf lettuces, higher b-carotene and lutein content were recorded than crisphead. Phenolic content was usually higher in red lettuce than in green lettuce, although vitamin C was higher in green leaf lettuce. Baby leaf lettuce can also have a rich vitamin C supply with a higher

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baby green iceberg lettuce than adult iceberg lettuce. Despite the general opinion that lettuce has low nutritional value, nutritional evidence from numerous studies supports that lettuce may provide considerable quantities of vital nutrients, especially folate, anthocyanins, and carotenoids. Baby green leaf lettuce had similar Fe but lowered K and Mg than the mature green leaf. Proportionally, baby lettuces got more or fewer phytonutrients than mature lettuces. Lettuce is often high in folate (vitamin B9), vitamin C, b-carotene, and phenolics. Besides, lettuce's nutritional benefit may be increased by exploiting cultural activities such as a light-emitting diode, strengthened hydroponic method, and sufficient fertilization. Therefore, lettuce merits more studies to analyse nutrient composition in a standardized growing setting, implement techniques to improve lettuce nutritional value, and elucidate mechanisms liable for bioactive substances beneficial effects.

REFERENCES

Aćamović-Djoković, Gordana, Radoš Pavlović, Jelena Mladenović, and Milena Djurić. 2011. "Vitamin C Content of Different Types of Lettuce Varieties 1." *Acta Agriculturae Serbica*. Vol. XVI.

Akbar, Shahid, and Shahid Akbar. 2020. "Lactuca Sativa L. (Asteraceae/Compositae)." In *Handbook of 200 Medicinal Plants*, 1067–75. Springer International Publishing. doi:10.1007/978-3-030-16807-0_115.

Altunkaya, Arzu, Eleonora Miquel Becker, Vural Gökmen, and Leif H. Skibsted. 2009. "Antioxidant Activity of Lettuce Extract (Lactuca Sativa) and Synergism with Added Phenolic Antioxidants." *Food Chemistry* 115 (1). Elsevier: 163–68. doi:10.1016/j.foodchem.2008.11.082.

Barker, A. V., N. H. Peck, and G. E. MacDonald. 1971. "Nitrate Accumulation in Vegetables. I. Spinach Grown in Upland Soils [1]." *Agronomy Journal* 63 (1). Wiley: 126–29. doi:10.2134/agronj1971.00021962006300010039x.

Complimentary Contributor Copy

Becker, Christine, Branimir Urlić, Maja Jukić Špika, Hans-Peter Kläring, Angelika Krumbein, Susanne Baldermann, Smiljana Goreta Ban, Slavko Perica, and Dietmar Schwarz. 2015. "Nitrogen Limited Red and Green Leaf Lettuce Accumulate Flavonoid Glycosides, Caffeic Acid Derivatives, and Sucrose While Losing Chlorophylls, B-Carotene and Xanthophylls." Edited by Wagner L. Araujo. *PloS One* 10 (11). Public Library of Science: e0142867. doi:10.1371/journal.pone.0142867.

Boo, Hee Ock, Buk Gu Heo, Shela Gorinstein, and Sang Uk Chon. 2011. "Positive Effects of Temperature and Growth Conditions on Enzymatic and Antioxidant Status in Lettuce Plants." *Plant Science* 181 (4). Elsevier: 479–84. doi:10.1016/j.plantsci.2011.07.013.

Chadchan, Kailash S., Jameel G. Jargar, and Swastika N. Das. 2016. "Anti-Diabetic Effects of Aqueous Prickly Lettuce (Lactuca Scariola Linn.) Leaves Extract in Alloxan-Induced Male Diabetic Rats Treated with Nickel (II)." *Journal of Basic and Clinical Physiology and Pharmacology* 27 (1). Walter de Gruyter GmbH: 49–56. doi:10.1515/jbcpp-2015-0038.

Cruz, Rebeca, Paula Baptista, Sara Cunha, José Alberto Pereira, and Susana Casal. 2012. "Carotenoids of Lettuce (Lactuca Sativa L.) Grown on Soil Enriched with Spent Coffee Grounds." *Molecules* 17 (2): 1535–47. doi:10.3390/molecules17021535.

Damerum, Annabelle, Stacey L. Selmes, Gaia F. Biggi, Graham J. J. Clarkson, Steve D. Rothwell, Maria José Truco, Richard W. Michelmore, et al. 2015. "Elucidating the Genetic Basis of Antioxidant Status in Lettuce (Lactuca Sativa)." *Horticulture Research* 2 (1). Nature Publishing Group: 1–13. doi:10.1038/hortres.2015.55.

Duncan, Andrew. 1821. "XIII. Additional Observations on the Use of Lactucarium, or Lettuce Opium; Particularly in a Case of Cynanche Laryngea, or Croup." *The Philosophical Magazine* 57 (274). Informa UK Limited: 84–87. doi:10.1080/14786442108652464.

DuPont, M. Susan, Zofia Mondin, Gary Williamson, and Keith R. Price. 2000. "Effect of Variety, Processing, and Storage on the Flavonoid Glycoside Content and Composition of Lettuce Endive." *Journal of*

Complimentary Contributor Copy

Agricultural and Food Chemistry 48 (9). American Chemical Society: 3957–64. doi:10.1021/jf0002387.

El-Nakhel, Christophe, Antonio Pannico, Marios C Kyriacou, Maria Giordano, Stefania De Pascale, and Youssef Rouphael. 2019. "Macronutrient Deprivation Eustress Elicits Differential Secondary Metabolites in Red and Green-pigmented Butterhead Lettuce Grown in a Closed Soilless System." *Journal of the Science of Food and Agriculture* 99 (15). John Wiley and Sons Ltd: 6962–72. doi:10.1002/jsfa.9985.

Foteinis, Spyros, and Efthalia Chatzisymeon. 2016. "Life Cycle Assessment of Organic versus Conventional Agriculture. A Case Study of Lettuce Cultivation in Greece." *Journal of Cleaner Production* 112 (January). Elsevier Ltd: 2462–71. doi:10.1016/j.jclepro.2015.09.075.

Freeland-Graves, Jeanne H., and Susan Nitzke. 2013. "Position of the Academy of Nutrition and Dietetics: Total Diet Approach to Healthy Eating." *Journal of the Academy of Nutrition and Dietetics* 113 (2). Elsevier: 307–17. doi:10.1016/j.jand.2012.12.013.

Gazula, Aparna, Matthew D Kleinhenz, John G Streeter, and A Raymond Miller. 2005. "Temperature and Cultivar Effects on Anthocyanin and Chlorophyll b Concentrations in Three Related Lollo Rosso Lettuce Cultivars." *Hortscience*. Vol. 40.

Gurdon, Csanad, Alexander Poulev, Isabel Armas, Shukhratdzhon Satorov, Meg Tsai, and Ilya Raskin. 2019. "Genetic and Phytochemical Characterization of Lettuce Flavonoid Biosynthesis Mutants." *Scientific Reports* 9 (1). Nature Publishing Group: 3305. doi:10.1038/s41598-019-39287-y.

Harlan, Jack R. 1986. "Lettuce and the Sycomore: Sex and Romance in Ancient Egypt." *Economic Botany* 40 (1). Springer-Verlag: 4–15. doi:10.1007/BF02858936.

Hounsome, N., B. Hounsome, D. Tomos, and G. Edwards-Jones. 2008. "Plant Metabolites and Nutritional Quality of Vegetables." *Journal of Food Science*. *J Food Sci.* doi:10.1111/j.1750-3841.2008.00716.x.

Hu, F. B., E. B. Rimm, M. J. Stampfer, A. Ascherio, D. Spiegelman, and C. W. Willett. 2000. "Prospective Study of Major Dietary Patterns and Risk

Complimentary Contributor Copy

of Coronary Heart Disease in Men." *American Journal of Clinical Nutrition* 72 (4). American Society for Nutrition: 912–21. doi:10.1093/ajcn/72.4.912.

Irwig, Michael S., Ahmed El-Sohemy, Ana Baylin, Nader Rifai, and Hannia Campos. 2002. "Frequent Intake of Tropical Fruits That Are Rich in β-Cryptoxanthin Is Associated with Higher Plasma β-Cryptoxanthin Concentrations in Costa Rican Adolescents." *Journal of Nutrition* 132 (10). American Institute of Nutrition: 3161–67. doi:10.1093/jn/131.10.3161.

Kim, Hyun Jin, Jorge M. Fonseca, Ju Hee Choi, and Chieri Kubota. 2007. "Effect of Methyl Jasmonate on Phenolic Compounds and Carotenoids of Romaine Lettuce (Lactuca Sativa L.)." *Journal of Agricultural and Food Chemistry* 55 (25). American Chemical Society: 10366–72. doi:10.1021/jf071927m.

Kim, Moo Jung, Youyoun Moon, Janet C. Tou, Beiquan Mou, and Nicole L. Waterland. 2016. "Nutritional Value, Bioactive Compounds and Health Benefits of Lettuce (Lactuca Sativa L.)." *Journal of Food Composition and Analysis*. Academic Press Inc. doi:10.1016/j.jfca.2016.03.004.

Kim, Sung-Youn, Dong-Hyun Kang, Jin-Ki Kim, Yong-Geun Ha, Ju Young Hwang, Taewan Kim, and Seon-Ho Lee. 2011. "Antimicrobial Activity of Plant Extracts Against Salmonella Typhimurium, Escherichia Coli O157:H7, and Listeria Monocytogenes on Fresh Lettuce." *Journal of Food Science* 76 (1). John Wiley & Sons, Ltd: M41–46. doi:10.1111/j.1750-3841.2010.01926.x.

Koudela, Martin, and K. Petříková. 2008. "Nutrients Content and Yield in Selected Cultivars of Leaf Lettuce (*Lactuca Sauva* L. Var Crispa)." *Horticultural Science* 35 (3). Czech Academy of Agricultural Sciences: 99–106. doi:10.17221/3/2008-hortsci.

Lazic, B., S. Lazic, and P. Sekulic. 2002. "Effect of Species and Variety on the Content of Macroelements and Micronutrients in Lettuce." In *Acta Horticulturae*, 579:609–12. International Society for Horticultural Science. doi:10.17660/ActaHortic.2002.579.107.

Complimentary Contributor Copy

"Lettuce, Butterhead (Includes Boston and Bibb Types), Raw Nutrition Facts & Calories." 2020. Accessed December 10. https://nutritiondata.self.com/facts/vegetables-and-vegetable-products/2474/2.

"Lettuce, Green Leaf, Raw Nutrition Facts & Calories." 2020. Accessed December 10. https://nutritiondata.self.com/facts/vegetables-and-vegetable-products/2477/2.

"Lettuce, Iceberg (Includes Crisphead Types), Raw Nutrition Facts & Calories." 2020. Accessed December 10. https://nutritiondata.self.com/facts/vegetables-and-vegetable-products/2476/2.

Li, Qian, and Chieri Kubota. 2009. "Effects of Supplemental Light Quality on Growth and Phytochemicals of Baby Leaf Lettuce." *Environmental and Experimental Botany* 67 (1): 59–64. doi:10.1016/j.envexpbot.2009.06.011.

Liu, Xiangfei, Shane Ardo, Marisa Bunning, John Parry, Kequan Zhou, Cecil Stushnoff, Frank Stoniker, Liangli Yu, and Patricia Kendall. 2007. "Total Phenolic Content and DPPH {radical Dot} Radical Scavenging Activity of Lettuce (Lactuca Sativa L.) Grown in Colorado." *LWT - Food Science and Technology* 40 (3). Academic Press: 552–57. doi:10.1016/j.lwt.2005.09.007.

Llorach, Rafael, Ascensión Martínez-Sánchez, Francisco A. Tomás-Barberán, María I. Gil, and Federico Ferreres. 2008. "Characterisation of Polyphenols and Antioxidant Properties of Five Lettuce Varieties and Escarole." *Food Chemistry* 108 (3). Elsevier: 1028–38. doi:10.1016/j.foodchem.2007.11.032.

Llorach, Rafael, Francisco A. Tomás-Barberán, and Federico Ferreres. 2004. "Lettuce and Chicory Byproducts as a Source of Antioxidant Phenolic Extracts." *Journal of Agricultural and Food Chemistry* 52 (16). American Chemical Society: 5109–16. doi:10.1021/jf040055a.

López, Alicia, García Alonso Javier, Jose Fenoll, Pilar Hellín, and Pilar Flores. 2014. "Chemical Composition and Antioxidant Capacity of Lettuce: Comparative Study of Regular-Sized (Romaine) and Baby-Sized (Little Gem and Mini Romaine) Types." *Journal of Food Composition and Analysis* 33 (1). Academic Press: 39–48. doi:10.1016/j.jfca.2013.10.001.

Complimentary Contributor Copy

Mareczek, A, M Leja - Sodininkystė ir daržininkystė, and undefined 2005. 2020. “Effect of Urea Foliar Application on Antioxidative Properties of Lettuce and Broccoli.” *Cabdirect.Org*. Accessed December 9. https://www.cabdirect.org/cabdirect/abstract/20063014201.

Mou, Beiquan. 2005. “Genetic Variation of Beta-Carotene and Lutein Contents in Lettuce.” *Journal of the American Society for Horticultural Science* 130 (6). American Society for Horticultural Science: 870–76. doi:10.21273/jashs.130.6.870. 2012. “Nutritional Quality of Lettuce.” *Current Nutrition & Food Science* 8 (3). Bentham Science Publishers Ltd.: 177–87. doi:10.2174/157340112802651121.

Mou, Beiquan, and Edward J. Ryder. 2004. “Relationship between the Nutritional Value and the Head Structure of Lettuce.” In *Acta Horticulturae*, 637:361–67. International Society for Horticultural Science. doi:10.17660/ActaHortic.2004.637.45.

Muhairi, Mohamed A. Al, Abdul J. Cheruth, Shyam S. Kurup, Gabriel A. Rabert, and Mohammad S. Al-Yafei. 2015. “Effect of Abscisic Acid on Biochemical Constituents, Enzymatic and Non Enzymatic Antioxidant Status of Lettuce (Lactuca Sativa L.) under Varied Irrigation Regimes.” *Cogent Food & Agriculture* 1 (1). Informa UK Limited: 1080888. doi:10.1080/23311932.2015.1080888.

Mulabagal, Vanisree, Mathieu Ngouajio, Ajay Nair, Yanjun Zhang, Aditya L. Gottumukkala, and Muraleedharan G. Nair. 2010. “In Vitro Evaluation of Red and Green Lettuce (Lactuca Sativa) for Functional Food Properties.” *Food Chemistry* 118 (2). Elsevier: 300–306. doi:10.1016/j.foodchem.2009.04.119.

NEGRÃO, Leonardo Dias, Paulo Víctor de Lima SOUSA, Ana Maria BARRADAS, Amanda de Castro Amorim Serpa BRANDÃO, Marcos Antônio da Mota ARAÚJO, and Regilda Saraiva dos Reis MOREIRA-ARAÚJO. 2020. “Bioactive Compounds and Antioxidant Activity of Crisphead Lettuce (Lactuca Sativa L.) of Three Different Cultivation Systems.” *Food Science and Technology*, no. AHEAD (August). FapUNIFESP (SciELO). doi:10.1590/fst.04120.

Complimentary Contributor Copy

Nutrition Data. 2020. *Lettuce, Cos or Romaine, Raw Nutrition Facts & Calories.* Accessed December 10. https://nutritiondata.self.com/facts/vegetables-and-vegetable-products/2475/2.

Olszyk, David M., Tamotsu Shiroyama, Jeffrey M. Novak, Keri B. Cantrell, Gilbert Sigua, Donald W. Watts, and Mark G. Johnson. 2020. "Biochar Affects Essential Nutrients of Carrot Taproots and Lettuce Leaves." *Hort Science* 55 (2). American Society for Horticultural Science: 261–71. doi:10.21273/HORTSCI14421-19.

Patrícia, Márcia, and Sousa Oliveira. 2015. *Microbial Safety of Lettuce: Foodborne Pathogens Incidence, Their Pathogenic Potential and Biopreservative Strategies.* http://hdl.handle.net/10803/307379.

Riga, Patrick, Leyre Benedicto, Ángel Gil-Izquierdo, Jacinta Collado-González, Federico Ferreres, and Sonia Medina. 2019. "Diffuse Light Affects the Contents of Vitamin C, Phenolic Compounds and Free Amino Acids in Lettuce Plants." *Food Chemistry* 272 (January). Elsevier Ltd: 227–34. doi:10.1016/j.foodchem.2018.08.051.

Romani, Annalisa, Patrizia Pinelli, Carlotta Galardi, Graziano Sani, Antonio Cimato, and Daniela Heimler. 2002. "Polyphenols in Greenhouse and Open-Air-Grown Lettuce." *Food Chemistry* 79 (3): 337–42. doi:10.1016/S0308-8146(02)00170-X.

Sakthivel, Kunnathur Murugesan, and Padmavathi Ganesan. 2017. "Cancer Preventive and Therapeutic Properties of Fruits and Vegetables: An Overview." doi:10.1142/9789814508896_0001.

San Miguel-Moncin, M., M. Krail, S. Scheurer, E. Enrique, R. Alonso, A. Conti, A. Cistero-Bahima, and S. Vieths. 2003. "Lettuce Anaphylaxis: Identification of a Lipid Transfer Protein as the Major Allergen." *Allergy* 58 (6). John Wiley & Sons, Ltd: 511–17. doi:10.1034/j.1398-9995.2003.00097.x.

Simko, Ivan. 2019. "Genetic Variation and Relationship among Content of Vitamins, Pigments, and Sugars in Baby Leaf Lettuce." *Food Science & Nutrition* 7 (10). Wiley-Blackwell: 3317–26. doi:10.1002/fsn3.1196.

Singh, Manjeet, Madeleine Arseneault, Thomas Sanderson, Ven Murthy, and Charles Ramassamy. 2008. "Challenges for Research on Polyphenols from Foods in Alzheimer's Disease: Bioavailability,

Complimentary Contributor Copy

Metabolism, and Cellular and Molecular Mechanisms." *Journal of Agricultural and Food Chemistry*. American Chemical Society. doi:10.1021/jf0735073.

Slavin, Joanne L., and Beate Lloyd. 2012. "Health Benefits of Fruits and Vegetables." *Advances in Nutrition* 3 (4). Oxford Academic: 506–16. doi:10.3945/an.112.002154.

Sublett, William, T. Barickman, and Carl Sams. 2018. "Effects of Elevated Temperature and Potassium on Biomass and Quality of Dark Red 'Lollo Rosso' Lettuce." *Horticulturae* 4 (2). MDPI Multidisciplinary Digital Publishing Institute: 11. doi:10.3390/horticulturae4020011.

Trojanowska, Anna. 2005. "Lettuce, Lactuca Sp., as a Medicinal Plant in Polish Publications of the 19th Century." *Kwartalnik Historii Nauki i Techniki : Kwartal'nyĭ Zhurnal Istorii Nauki i Tekhniki* - 50 (3–4): 123–34. https://europepmc.org/article/med/17153150.

Uno, YuichiReduction of leaf lettuce tipburn using an indicator cultivar, Hiroshi Okubo, Hiromichi Itoh, and Ryohei Koyama. 2016. "Reduction of Leaf Lettuce Tipburn Using an Indicator Cultivar." *Scientia Horticulturae* 210 (October). Elsevier B.V.: 14–18. doi:10. 1016/ j.scienta.2016.07.001.

Viacava, Gabriela Elena, Sara Inés Roura, and María Victoria Agüero. 2015. "Optimization of Critical Parameters during Antioxidants Extraction from Butterhead Lettuce to Simultaneously Enhance Polyphenols and Antioxidant Activity." *Chemometrics and Intelligent Laboratory Systems* 146 (August). Elsevier: 47–54. doi:10.1016/j.chemolab. 2015.05.002.

Zhou, W. L., W. K. Liu, and Q. C. Yang. 2012. "Quality Changes in Hydroponic Lettuce Grown under Pre-Harvest Short-Duration Continuous Light of Different Intensities." *Journal of Horticultural Science and Biotechnology* 87 (5). Headley Brothers Ltd: 429–34. doi:10.1080/14620316.2012.11512890.

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Chapter 3

THE ROLE OF METABOLIC SYNDROME IN THE CHANGING EVOLUTION OF HEPATOCELLULAR CARCINOMA: A RAPIDLY EVOLVING LANDSCAPE

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ABSTRACT

During the past decades, the globally increasing incidence of metabolic syndrome and obesity has attained the character of an epidemic. Additionally, Non Alcoholic Fatty Liver Disease (NAFLD), strongly associated with type 2 diabetes mellitus and metabolic syndrome, has emerged as a major contributor to chronic liver disease and cirrhosis, with a global prevalence of about 25,2%. In the meantime, despite the development of anti-viral therapies and organized vaccination programs, emerging data demonstrate that the incidence of hepatocellular carcinoma

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(HCC) is continuously rising. However, the current epidemiological landscape of HCC is believed to be quite different than that of the past century, with obesity, metabolic syndrome, type 2 diabetes mellitus and NAFLD acting as the leading modifiers in the development of HCC. This review attempts to shed light into the pathogenetic mechanisms connecting metabolic syndrome and NAFLD to the emergence and progression of hepatocellular carcinoma, emphasizing on the evolving epidemiological profile of this type of cancer.

INTRODUCTION

Despite current advances in surgery and chemotherapy strategies, hepatocellular carcinoma (HCC) remains one of the most lethal forms of cancer worldwide, and the most common type of primary liver cancer, representing about 85% of all primary liver tumors [1]. During the past decades, there has been a significant increase in the incidence of hepatocellular carcinoma [2], notably during the 2000-2010 decade, which seems to be followed by a plateau [2, 3] of the rhythm of increase after 2010. According to US databases, both incidence and mortality rates were higher in the age group of 50-64 years old, while the disease was three times more common between men than women [3]. The reported incidence of HCC in the US in 2012 was 6,7/ 100,000, compared to 4,4/100,000 in 2000 [2]. Even though several emerging risk factors for HCC have been identified throughout years of scientific and clinical research [5], numerous recent studies attempt to associate the current increase in the incidence of HCC to the epidemic proportions of obesity and metabolic syndrome worldwide [9]. Other important modifiers of HCC risk involve Hepatitis B and C infection and related cirrhosis, Alcoholic Liver Disease (ALD) and Nonalcoholic Fatty Liver Disease (NAFLD) and Steatohepatitis (NASH). In terms of mortality, even though overall liver cirrhosis mortality seems to be decreasing in Europe [4], the total number of deaths related to HCC remains high. Recent data analyses suggest that in Europe, Italy presents the highest HCC death rate, followed by France and Greece [4]. The global prognosis of hepatocellular carcinoma remains poor, with average five-year survival

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rate of 10% [1, 6] for all stages of HCC, which can reach up to 25% in case of locally advanced tumors. In addition to its important fatality, HCC presents a recurrence rate of about 70% in 5 years [7, 8], either by metastases or de novo tumors, furthermore complicating the potential therapeutic strategies.

Consequently, HCC remains a significantly fatal and multifactorial disease, of important social and economic cost, that needs to be better understood. Although numerous risk factors have been identified and studied in the past decades, there is evidence that the epidemiologic characteristics of HCC are evolving. More precisely, several studies demonstrate a decrease [12, 13, 14] in the incidence of HCC in areas with extremely high HBV infection rates, such as East Asia and sub- Saharan Africa [10], a fact that could be attributed to the development of vaccination programs since childbirth and the establishment of more efficient antiviral therapy. In the meantime, obesity and metabolic syndrome [15] are emerging as major factors in the development of HCC, an element that could significantly change the landscape in the future.

EPIDEMIOLOGY OF HEPATOCELLULAR CARCINOMA; RISK FACTORS

Hepatocellular carcinoma is the sixth most commonly diagnosed cancer and fourth in terms of mortality- it is responsible for 8,2% of cancer related mortalities according to data published in 2020 [29]. In addition, other epidemiologic data suggest that the incidence of HCC is more than three times higher in men than women [30] as well as among Asians and Pacific Islanders in comparison to whites [13]. Since its emergence, various risk factors have been linked to the appearance and progression of HCC, the most important of which are mentioned below.

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Hepatitis B Virus (HBV)

HBV infection is one of the most thoroughly identified risk factors for liver cirrhosis and HCC. The first worldwide assessment of chronic HBV infection suggested that in 2010, about 248 million people were tested positive for Hepatitis B superficial Antigen (HBsAg), with higher prevalence in sub- Saharan Africa and Eastern Asia [31]. It is estimated that about 15-25% of individuals with chronic HBV infection die from cirrhosis or hepatocellular carcinoma [32]. As a result of the high HBV infection incidence in Eastern Asia (about 8% of the general population), these areas present the highest HCC incidence worldwide (about 35 in 100.000 in China and 395.000 cases per year [33]). The mechanisms by which HBV infection results in the appearance of HCC not only involve development of cirrhosis, but also direct carcinogenic impact of HBV DNA on liver cell proliferation and genetic mutations, mechanisms that can lead to HCC development on a non-cirrhotic liver [33, 34].

Hepatitis C Virus (HCV)

Along with HBV, HCV infection has been studied as a major risk factor for HCC, especially in the population of "baby-boomers," individuals who were born between 1945-1965. With more than 170 million people being chronically infected with HCV [35] and an annual incidence of about 6.19 per 100,000 habitants in Europe [36], HCV infection accounts for about 20% of viral etiology HCC [37], primarily in countries with intermediate HCC incidence, such as Western Europe and Japan [38], where HBV prevalence is lower. Most studies conclude that the major mechanism by which HCV, an RNA virus, leads to HCC is by causing liver cirrhosis, with an annual risk of 2-7% [38].

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Alcoholic Liver Disease

Alcoholic liver disease (ALD) is a major contributor to the development of HCC, especially in western countries where the prevalence of viral hepatitis is lower; specifically, it is estimated that about 60-80% of liver related mortality in Europe is due to chronic alcohol consumption [39]. ALD involves various pathological situations, such as liver steatosis, fibrosis, alcoholic hepatitis and cirrhosis [9]. Additionally, recent studies have demonstrated that alcohol consumption acts in a synergistic manner with other important risk factors in the development and progression of liver damage and HCC, such as viral hepatitis B and C, obesity and Non Alcoholic Fatty Liver Disease (NAFLD) [4, 39]. In this context, emerging data indicates that the amount of daily alcohol consumption may have a dose-related effect on the development of HCC and the stage of the diagnosed disease [40], although more studies are requested in order to shed light into the pathogenetic mechanisms of this multifactorial disease. A recent meta-analysis of 19 cohorts [41] has demonstrated that there is a 16% increased risk for the development of HCC for heavy-drinkers, compared to non-drinkers.

Non Alcoholic Fatty Liver Disease (NAFLD) and Non Alcoholic Steatohepatitis (NASH)

Etiologically connected to obesity, excess lipid accumulation, sedentary lifestyle and metabolic syndrome [42], NAFLD is being recently recognized as the second leading etiology of HCC resulting in liver transplant in the US [43], behind HCV- related HCC. Moreover, with the prevalence of obesity and metabolic syndrome increasing rapidly during the past decades- even reaching the proportions of an epidemic-, studies have raised the hypothesis that NAFLD is the most rapidly growing etiology of HCC and is expected to become the dominating cause of liver transplantation in the close future [44]. As the main topic of this article, the complex association between

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NAFLD, metabolic syndrome and HCC is discussed more thoroughly below.

METABOLIC SYNDROME AND HEPATOCELLULAR CARCINOMA; POSSIBLE CONNECTIONS

Metabolic Syndrome Definition and Epidemiology

Metabolic syndrome is defined as an assemblance of clinical and biological abnormalities that can lead to significantly increased risk for cardiovascular disease. According to the ATP III [16] (National Cholesterol Education Program's Adult Treatment Panel III report), the diagnosis of Metabolic Syndrome can be established when at least three of the five following criteria are present:

During the last few decades, metabolic syndrome is evolving into a major cause of morbidity as well as mortality, with social and financial costs that are impossible to calculate. Data from a European study [17] estimate the prevalence of metabolic syndrome at about 24,3% (23,9% in men and 24,6% in women), while the Portuguese PORMETS [18] study suggests that between 36,5% and 49,6% of the participating population satisfy the criteria of the metabolic syndrome, depending on different definitions used. About 64,3% of participants also presented with high blood pressure, pursuing the hypothesis that hypertension and metabolic syndrome (MS) are strongly connected. In China, a meta-analysis [19] raises the hypothesis that urban lifestyle increases the risk of MS compared to rural, estimating the overall prevalence in the study group at about 24,5%. Therefore, it becomes obvious that metabolic syndrome and its components (central obesity, insulin resistance, hyperglycemia, hypertension and dyslipidemia) represent a significant threat to health systems worldwide, whose link to cancer development is yet to be established.

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Table 1. Components of metabolic syndrome according to ATP III

Risk factor	Defining level
Abdominal obesity, given as waist circumference	Men: >102 cm (>40 in) Women: >88 cm (>35 in)
Triglycerides	≥150 mg/dL
HDL cholesterol	Men: <40 mg/dL Women: <50 mg/dL
Blood pressure	≥130/≥85 mm Hg
Fasting glucose	≥110 mg/dL

Metabolic Syndrome and Cancer; Illuminating Possible Pathways

The excess of adipose tissue and free fatty acids (FFAs), basic characteristics of metabolic syndrome, can possibly lead to a chronic inflammatory state, by activating pathways that involve pro-inflammatory cytokines, insulin resistance, activation of macrophages and dysregulation of apoptotic mechanisms. Supporting the hypothesis of chronic inflammatory reaction, emerging evidence from several studies [21] suggest that these pathways may be held accountable for the connection between metabolic syndrome and several common forms of cancer, such as endometrial [22] and post- menopausal breast cancer [23], colorectal [24], thyroid [25], prostate [26], pancreatic [27] and liver cancer [28]. In the meta-analysis of Esposito et al. [21], which included 38,940 cancer patients diagnosed with different types of tumors, liver cancer emerged as having the strongest association with metabolic syndrome in men's cohorts (Relative Risk 1.43, $P < 0{,}0001$), while in women's cohorts the highest relative risk was attributed to endometrial carcinoma (Relative Risk: 1.61, $P = 0{,}01$), firmly supporting the hypothesis that birth control pills and their side- effects act as major risk factors for this type of cancer.

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Table 2. Data from recent studies assessing the risk for hepatocellular carcinoma (HCC) and other common types of cancer in patients with metabolic syndrome (MS). MS: Metabolic Syndrome; RR: Risk Ratio; CI: Confidence Interval; OR: Odds Ratio

References	Type of study	Type of cancer	Results
Esposito K. et al. [22]	Meta-analysis	Endometrial cancer	MS was associated with an increased cancer risk (RR:1,89, 95% CI 1.34-2.67, P < 0,001)
Agnoli C. et al. [23]	Prospective nested case-control	Breast cancer	MS was significantly associated with development of breast cancer (RR:1,58, 95% CI 1.07-2.33, P = 0,004)
Esposito K. et al. [24]	Meta-analysis	Colorectal cancer (CRC)	MS was associated with an increased risk of CRC (Men: RR: 1.33, 95% CI 1.18-1.50 Women: RR:1.41, 95% CI 1.18-1.70)
Yin D. et al. [25]	Meta-analysis	Thyroid cancer	Increased risk for thyroid cancer for patients with insulin resistance (RR:1.59, 95% CI 1.12-2.27, P = 0.01), elevated blood glucose (RR: 1.40, 95%CI 1.15-1.70, P < 0.001), high BMI (RR: 1.35,95% CI 1.23-1.48, P < 0.001) and hypertension (RR: 1.34, 95%CI 1.22-1.47, P < 0.001)
Esposito K. et al. [26]	Meta-analysis	Prostate cancer	Increased risk for prostate cancer for patients with waist circumference >102 cm (by 56%, P = 0,007) and hypertension (by 15%, P = 0.035)
Rosato V. et al. [27]	Case-control study and meta-analysis of 3 cohort studies	Pancreatic cancer	Case-control: OR of 2.36 for pancreatic cancer for diabetic patients (95% CI, 1.43-3.90) Meta-analysis: Sumary RR of 1.55 (95% CI, 1.19-2.01) for patients with MS
Jinjuvadia R. et al. [28]	Meta-analysis of three cohorts and one case-control study	Hepatocellular carcinoma (HCC)	Overall increased risk for HCC of 81% (RR: 1,81 95% CI, 1.37-2.41) in subjects with MS

Although the exact mechanisms responsible for the correlation between metabolic syndrome and carcinogenesis remain to be further illuminated, it

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becomes more and more obvious that this syndrome and its components may act as important modifiable risk factors for several types of cancer. As a result, they could be potentially targeted by lifestyle and metabolic health changes in order to primarily prevent the development of numerous malignancies, for some of which- such as pancreatic cancer- the available treatment options in late stages remain scarce.

Metabolic Syndrome and Hepatocellular Carcinoma

In this context, and with more and more studies demonstrating the implication of metabolic syndrome (MS) and Non-alcoholic Fatty Liver Disease (NAFLD) in the pathogenesis of hepatocellular carcinoma, it is important to highlight some responsible mechanisms for this association.

Defining NAFLD

NAFLD is a complex hepatic disorder that involves isolated fatty infiltration (steatosis) of >5% of liver cells [44, 45] and, in more advanced stages, fatty infiltration plus inflammation (Non-Alcoholic Steatohepatitis, NASH), fibrosis and cirrhosis, in the absence of important alcohol consumption (<20g per day for women and <30g per day for men). Concerning the diagnosis, NAFLD is often discovered as an incidental increase in liver enzymes –notably in alanine aminotransferase (ALT) compared to asparate aminotransferase (AST)- or when hepatic fat is incidentally detected in ultrasound, CT or MRI abdominal imaging [46]. However, in cases of progressing NAFLD, including NASH, liver biopsy is needed, which will reveal steatosis, hepatocellular ballooning, lobular inflammation and varying degrees of fibrosis [46]. Interestingly, the majority of patients with NAFLD or compensated cirrhosis remain asymptomatic, thus undiagnosed until the advanced stages of the disease [47] and, as result, NAFLD may be held responsible for about 30-75% of "cryptogenic cirrhosis" cases. Nevertheless, it is essential to highlight the fact that the diagnosis of NAFLD/ NASH should be considered in case of an incidental rise in aminotransferases that co-exists with metabolic syndrome

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or its components, thus an organized screening strategy for HCC should be established.

NAFLD and Metabolic Syndrome; A Bidirectional Association

As mentioned above, metabolic syndrome and obesity affect a rapidly growing part of the general population; following these epidemic proportions, a global prevalence of 25,24% is attributed to NAFLD according to a recent meta-analysis [48]. According to some authors, NAFLD represents the hepatic manifestation of metabolic syndrome [49], having as common pathogenetic mechanisms insulin resistance [44] and the subsequent increased delivery of Free Fatty Acids (FFAs) to the liver, leading to steatosis and NASH progression [50]. The results of the increased lipotoxicity in the liver involve β-oxidation of FFAs in mitochondria and ROS (Reactive Oxygen Species) production, leading to oxidative and endoplasmic reticulum (ER) stress [51, 52]. These events are believed to be playing a significant role in the activation of apoptotic pathways that result in liver injury, inflammation and fibrosis in NASH patients [53].

However, rather than identifying NAFLD and NASH as the mere results of metabolic syndrome, it is essential to highlight the bidirectional character of this association, especially between NAFLD and type 2 Diabetes Mellitus. Specifically, apart from being a result of chronic lipotoxicity, inflammation and insulin resistance, NAFLD could also act as the onset for the development of type 2 diabetes mellitus [44, 54]. This hypothesis is supported by the higher prevalence of NAFLD among diabetic patients, in comparison to the general population; in a study involving 2,839 diabetic individuals, 69% of them had NAFLD, diagnosed by abdominal imaging [55]. Additionally, accumulation of excess triglycerides in the liver is much more common in diabetic patients, and sometimes irrelevant of their blood aminotransferases levels [56], and is also likely to make their glycemic control much more difficult [57]. Consequently, it is essential to fathom that Type 2 Diabetes Mellitus as well as NAFLD patients are in increased risk for liver injury, steatosis, cirrhosis and even HCC, facts that make a close gastroenterological surveillance of these patients crucial.

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NAFLD and HCC

As mentioned above, NAFLD related cirrhosis is increasingly linked to the development of hepatocellular carcinoma, as the missing puzzle piece in the association between metabolic syndrome and liver cancer. In a recent cohort study of 510 patients with HCV or NASH-cirrhosis, a cumulative annual risk of HCC that was comparable to HCV cirrhosis was attributed to NASH- 2,6% per year vs 4% per year, respectively [58]. The real percentage of NAFLD related HCC is possibly underestimated, not only due to the fact that these cases are often attributed to cryptogenic cirrhosis, but also because they remain asymptomatic for a long time and they do not undergo organized screening. However, in parallel with obesity and metabolic syndrome epidemic, a significant increase in NAFLD-related HCC has been documented, with NAFLD cirrhosis being the underlying etiology of 34,8% of HCC cases in a 2010 analysis [66].

Interestingly, emerging data indicates that NAFLD is more likely to result in HCC on a non-cirrhotic liver, in comparison to other HCC etiologies, such as viral hepatitis [59], thus suggesting that the progression of NAFLD to NASH and HCC may be more rapid. Supporting the same hypothesis, a national cohort study from the US indicates that in 35% of NAFLD- related HCC and 33,8% of idiopathic HCC cases there was no evidence of cirrhosis, while the non- cirrhotic cases were significantly fewer if HCV (8.9%, $P < 0,01$) or alcohol abuse (10,9%, $P < 0,01$) were the major risk factors for HCC [60]. Possible mechanisms for the development of HCC in the absence of cirrhosis involve malignant transformation of hepatic adenoma [61], which is more likely to occur in male patients, as well as those with metabolic syndrome [62]. Additionally, a recent study indicates that patients with NAFLD and metabolic syndrome-related HCC tend to have a shorter survival time and are also often diagnosed with larger/ advanced stage tumors, less likely to receive liver transplant in comparison to other HCC etiologies [63]. Consistent with these data are the findings of an epidemiological analysis of 11,187 HCC cases, suggesting that patients with NAFLD (1.11 times) and ALD (1.19 times) were more likely to die within 2 years of diagnosis than HBV patients (0,94 times) [64].

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Consequently, and bearing in mind that NAFLD patients are usually not included in HCC screening programs, thus presenting with poor prognosis and in advanced stages of cancer, an adjustment of surveillance programs may be necessary [65].

The Role of Genetic Predisposition

Regarding the molecular and pathophysiological mechanisms connecting NAFLD and HCC, a number of genetic predispositions has been recently identified. Specifically, using Genome-Wide Association Studies (GWAS), a single- nucleotide variant in *PNPLA3* (rs738409) was identified [68], as the most important genetic variant associated with significantly higher hepatic fat levels in its carriers ($P = 5.9 * 10^{-10}$), as well as with important susceptibility to hepatic inflammation ($P = 3.7 * 10^{-10}$). *PNPLA3* encodes a 481 amino acid protein of the patatin-like phospholipase family, whose activity is associated with lipid acyl hydrolysis; specifically, the rs738409 variant is associated with increased circulating levels of ICAM-1 and decreased levels of adiponectin. Interestingly, the same studies concluded that this variant was most commonly found in Hispanics, a group that is most susceptible to NAFLD and HCC, according to recent epidemiological data [2, 5]. Firmly supportive of these findings, a meta-analysis of 16 studies [69] indicates that homozygotes for this allele tend to have a 28% increase in serum ALT levels, 3.24-fold greater risk of higher necro inflammatory scores and 3.2-fold greater risk of developing fibrosis when compared with non-carriers ($P < 10*10^{-9}$). Consequently, the emergence of this evidence leads to the conclusion that several genetic modifiers- most of which remain unidentified- may account for certain families' and populations' predisposition to liver injury and carcinogenesis.

The Role of Obesity

As highlighted above, a plethora of studies have indicated that HCC has one of the strongest associations to obesity and metabolic syndrome when compared to other types of cancer. In a prospective study by the American Cancer Society [71], overweight individuals had significantly higher mortality rates from several types of cancer in comparison to normal weight

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participants (52% higher for men and 62% for women), while a linear increase in death rates was associated with increasing BMI (Body Mass Index). Regarding liver cancer, a strong association was discovered, with a Relative Risk for HCC-related death of 1.90 (95% CI, 1.46–2.47) for male participants with BMI between 30-34,9. For BMI between 35-39,9, the relative risk was significantly higher (RR: 4.52, 95% CI, 2.94–6.94) in men's cohorts, while for women RR for HCC related mortality was 1.68 (95% CI, 0.93–3.05).

This data, in accordance with emerging data from multiple studies connecting HCC to metabolic syndrome [72, 73], highlight the importance of better comprehension of the underlying mechanisms. The excess of adipose tissue that characterizes obesity contributes to the establishment of a low-grade chronic inflammatory state [74]. Indeed, lipid peroxidation and accumulation of ROS, along with the production of pro-inflammatory cytokines (adipocytokines) create fertile soil for cell injury and carcinogenesis (Figure 1). More precisely, TNF-α (Tumor Necrosis Factor α), a pro-inflammatory cytokine, is believed to be playing a critical role in the development of several types of cancer, such as multiple myeloma and other hematological malignancies, HCC, gastric and breast cancer [75]. The molecular mechanisms of this association involve NO (Nitric Oxide) and ROS mediated DNA damage [76], as well as increased angiogenesis and tumor cell survival, via genetic inductions, that lead to increased expression of antiapoptotic molecules [77].

Acting additively to TNF-α, IL-6 is considered as a multifunctional pro-inflammatory cytokine, inducing excessive cell proliferation and preventing tumor cell apoptosis via multiple pathways [78]. Indeed, IL-6 secretion by adipose tissue and its binding to the IL-6 receptor induce tyrosine phosphorylation of STAT1 and STAT3 proteins, via JAK1 activation. This pathway is suggested to maintain a pluripotent role in tumor cell proliferation and survival [79]. As a result, IL-6 secretion and JAK-STAT activation are strongly connected to the appearance and progression of multiple malignant diseases, such as multiple myeloma, Kaposi sarcoma and breast cancer. Studies performed on mice confirmed the hypothesis that IL-6 and TNF-α increase the risk of obesity-induced HCC, via inflammatory

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and carcinogenic pathways [80]. Consequently, it becomes obvious that IL-6 and STAT3, an oncogenic transcription factor, potentially play a significant role in the association of chronic inflammation and cancer in the case of HCC [81].

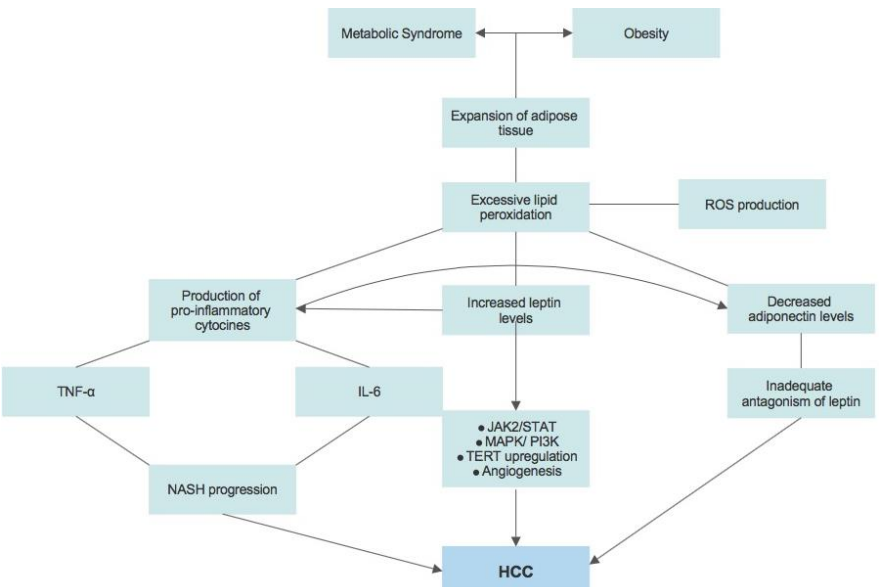


Figure 1. The role of obesity and excess fat tissue in the development of HCC. TNF-a: Tumor Necrosis Factor; IL-6: Interleukin 6; ROS: Reactive Oxygen Species; NASH: Non-Alcoholic Steatohepatitis.

Additionally, besides the secretion of pro-inflammatory cytokines that predispose to a carcinogenic microenvironment, oxidative stress and DNA damage also contribute to carcinogenesis in the context of obesity. B-oxidation of fatty acids, as well as decreased adiponectin levels result in excessive production of ROS (Reactive Oxygen Species) and, thus, oxidative stress and potential DNA damage. According to experimental studies performed on mouse NAFLD models, ROS generated DNA damage increases the risk of HCC and other types of cancer, such as cholangiocarcinoma and gastric cancer [82, 83].

Moreover, adiponectin, an anti-inflammatory cytokine that targets HCC development by inducing cell apoptosis, is expressed inadequately in

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patients with NAFLD and metabolic syndrome [9, 84]. On the other hand, a pro-inflammatory and pro-fibrogenic cytokine called leptin is expressed at higher levels in obese patients, potentially promoting the development of HCC [85]. Emerging data indicates that leptin is implicated in the development of steatosis, fibrosis, angiogenesis and even oncogenesis by targeting molecular pathways such as JAK2/STAT3 and PI-3K/Akt [65, 86]. Although adiponectin and leptin interact in an antagonistic manner, the inadequate levels of adiponectin are not sufficient in order to suppress TNF-α, IL-6 and leptin mediated inflammation and prevent its progression to NASH and HCC.

The Key Role of Diabetes and Insulin Resistance

Insulin resistance (IR), a basic component of type 2 diabetes mellitus (T2DM) as well as metabolic syndrome, is believed to be playing a significant role in the appearance and progression of NAFLD; nevertheless, fatty infiltration of the liver is not the only mechanism by which IR can lead to HCC [70] (Figure 2).

Numerous studies have established the hypothesis that T2DM acts as an independent risk factor for the development of HCC. A large prospective study from Greece demonstrated a strong association between history of diabetes and HCC, with an Odds Ratio of 1.83 (95% CI 1.18–2.84) for the development of HCC in diabetic individuals. This risk was not altered when adjusted for other common etiological factors of HCC, such as alcohol and viral hepatitis [87]. Additionally, a large cohort study from the US Department of Veteran Affairs [88] indicated that diabetes as a variable in the development of HCC had an adjusted hazard ratio of 2.16 (95% CI, 1.86-2.52, P < 0,0001).

The mechanisms that connect insulin resistance and diabetes mellitus to the appearance and progression of hepatocellular carcinoma are complex and interrelated. Specifically, the first and most obvious pathway is that of NAFLD (Figure 2). Indeed, the increased lipolysis induced by hyperinsulinemia and insulin resistance leads to high circulating levels of Free Fatty Acids (FFAs), and, consequently, to accumulation of triglycerides in the liver, thus favoring liver steatosis and NAFLD [89].

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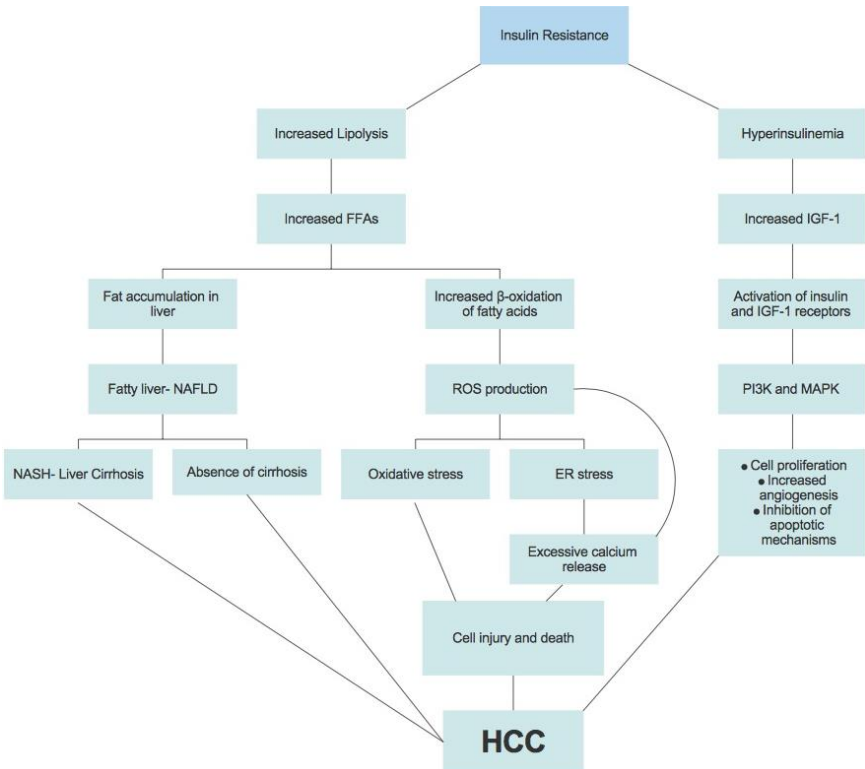


Figure 1. The role of insulin resistance in the development of HCC. HCC: Hepatocellular Carcinoma; NAFLD: Non-Alcoholic Fatty Liver Disease; NASH: Non-Alcoholic Steatohepatitis; FFAs: Free Fatty Acids; ROS: Reactive Oxygen Species; ER: Endoplasmic Reticulum; IFG-1: Insulin Growth Factor 1.

Simultaneously, hyperinsulinemia leads to increased β-oxidation of fatty acids, thus favoring the development of oxidative and endoplasmic reticulum stress; this process contributes to the establishment of a chronic inflammatory state and the production of C-Reactive Protein, fibrinogen and other coagulative factors that is observed in patients with liver damage [90]. As a result, this chronic impaired metabolic and inflammatory state make liver cells more susceptible to injury and oncogenesis. Finally, another important pathway in the association between hyperinsulinemia and HCC is the upregulation of IGF-1 (Insulin Growth Factor 1) and activation of IRS-1 (Insulin Receptor Substrate 1). Indeed, the increased expression of insulin

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also provokes the expression of IGF-1, a peptide hormone that stimulates cell proliferation [91]. When insulin and IGF-1 bind to their receptors, the MAPK and PI3K pathways are activated. Interestingly, these pathways are believed to play a significant role in liver carcinogenesis, by stimulating cell proliferation and inhibiting apoptosis [92, 93]. More precisely, it is suggested that the MAPK pathway leads to carcinogenesis by inducting proto-oncogenes and the Wnt/ β-catenin cascade [94], while PI3K interferes in the cyclin D1-dependent cell cycle [52, 89].

Consequently, it is important to note that insulin resistance and diabetes mellitus contribute to HCC development, not only by affecting lipid and glycose metabolism, but also via complex genetic and molecular pathways, that request further studies in order to be better understood.

CONCLUSION

In conclusion, metabolic syndrome and NAFLD are evolving into leading etiologies of chronic liver disease and hepatocellular carcinoma, either on cirrhotic or non- cirrhotic background, and are expected to overcome viral hepatitis. The pathogenetic mechanisms implicated in this complex association involve DNA mutations and damage, metabolic and hormonal disorders, as well as intricate inflammatory pathways. Furthermore, due to the current epidemic proportions of metabolic syndrome and obesity, more prospective and epidemiological studies are needed in order to illuminate the global burden of NAFLD and metabolic syndrome-related HCC and its consequences. Consequently, better understanding the rapidly evolving landscape of HCC and its etiologies is crucial in the development of novel screening and prevention programs, as well as of therapeutic strategies better adjusted to the growing threat that metabolic syndrome is presenting to global health.

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REFERENCES

[1] Haute Autorité de Santé. *ALD n° 30 - Cancer primitif du foie*. Saint-Denis La Plaine: HAS; 2012. [*ALD n ° 30 - Primitive cancer of the foil*]

[2] White DL, Thrift AP, Kanwal F, Davila J, El-Serag HB. Incidence of Hepatocellular Carcinoma in All 50 United States, From 2000 Through 2012. *Gastroenterology*. 2017;152(4):812-820.e5. doi:10.1053/j.gastro.2016.11.020.

[3] Altekruse SF, Henley SJ, Cucinelli JE, McGlynn KA. Changing hepatocellular carcinoma incidence and liver cancer mortality rates in the United States. *Am J Gastroenterol*. 2014;109(4):542-553. doi:10.1038/ajg.2014.11.

[4] Ascione A, Fontanella L, Imparato M, Rinaldi L, De Luca M. Mortality from cirrhosis and hepatocellular carcinoma in Western Europe over the last 40 years. *Liver Int*. 2017 Aug;37(8):1193-1201. doi: 10.1111/liv.13371. Epub 2017 Mar 5. PMID: 28111883.

[5] Kim HS, El-Serag HB. The Epidemiology of Hepatocellular Carcinoma in the USA. *Curr Gastroenterol Rep*. 2019;21(4):17. Published 2019 Apr 11. doi:10.1007/s11894-019-0681-x.

[6] Alqahtani A, Khan Z, Alloghbi A, Said Ahmed TS, Ashraf M, Hammouda DM. Hepatocellular Carcinoma: Molecular Mechanisms and Targeted Therapies. *Medicina (Kaunas)*. 2019;55(9):526. Published 2019 Aug 23. doi:10.3390/medicina55090526.

[7] Liu CY, Chen KF, Chen PJ. Treatment of Liver Cancer. *Cold Spring Harb Perspect Med.* 2015 Jul 17;5(9):a021535. doi: 10.1101/cshperspect.a021535. PMID: 26187874; PMCID: PMC4561392.

[8] Llovet JM, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. Semin Liver Dis. 2005;25(2):181-200. doi: 10.1055/s-2005-871198. PMID: 15918147.

[9] Rahman R, Hammoud GM, Almashhrawi AA, Ahmed KT, Ibdah JA. Primary hepatocellular carcinoma and metabolic syndrome: An

Complimentary Contributor Copy

update. *World J Gastrointest Oncol*. 2013;5(9):186-194. doi:10.4251/wjgo.v5.i9.186.

[10] Wallace MC, Preen D, Jeffrey GP, Adams LA. The evolving epidemiology of hepatocellular carcinoma: a global perspective. Expert Rev GastroenterolHepatol. 2015 Jun;9(6):765-79. doi: 10.1586/17474124.2015.1028363. Epub 2015 Apr 1. PMID: 25827821.

[11] Kumar, Vinay, Abbas, Abul K., Aster, Jon C. Robbins, Stanley L., eds. *Robbins Basic Pathology*. Philadelphia, PA: Elsevier/Saunders, 2013. Print.

[12] Alexander J, Torbenson M, Wu TT, Yeh MM. Non-alcoholic fatty liver disease contributes to hepatocarcinogenesis in non-cirrhotic liver: a clinical and pathological study. *J Gastroenterol Hepatol*. 2013 May;28(5):848-54. doi: 10.1111/jgh.12116. PMID: 23302015.

[13] Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol*. 2009 Mar 20;27(9):1485-91. doi: 10.1200/JCO.2008.20.7753. Epub 2009 Feb 17. PMID: 19224838; PMCID: PMC2668555.

[14] Gao S, Yang WS, Bray F, et al. Declining rates of hepatocellular carcinoma in urban Shanghai: incidence trends in 1976-2005. *Eur J Epidemiol* 2012;27:39-46.

[15] Tsoulfas G. Hepatocellular carcinoma and metabolic syndrome: The times are changing and so should we. *World J Gastroenterol*. 2019;25(29):3842-3848. doi:10.3748/wjg.v25.i29.3842.

[16] Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Final report. *Circulation*. 2002; 106: 3143–3421.

[17] Scuteri A, Laurent S, Cucca F, Cockcroft J, Cunha PG, Mañas LR, MattaceRaso FU, Muiesan ML, Ryliškytė L, Rietzschel E, Strait J, Vlachopoulos C, Völzke H, Lakatta EG, Nilsson PM; Metabolic Syndrome and Arteries Research (MARE) Consortium. Metabolic syndrome across Europe: different clusters of risk factors. *Eur J Prev*

Complimentary Contributor Copy

Cardiol. 2015 Apr;22(4):486-91. doi: 10.1177/2047487314525529. Epub 2014 Mar 19. PMID: 24647805; PMCID: PMC4544872.

[18] Raposo L, Severo M, Barros H, Santos AC. The prevalence of the metabolic syndrome in Portugal: the PORMETS study. *BMC Public Health*. 2017 Jun 8;17(1):555. doi: 10.1186/s12889-017-4471-9. PMID: 28595618; PMCID: PMC5465455.

[19] Li R, Li W, Lun Z, Zhang H, Sun Z, Kanu JS, Qiu S, Cheng Y, Liu Y. Prevalence of metabolic syndrome in Mainland China: a meta-analysis of published studies. *BMC Public Health*. 2016 Apr 1;16:296. doi: 10.1186/s12889-016-2870-y. PMID: 27039079; PMCID: PMC4818385.

[20] Braun S, Bitton-Worms K, LeRoith D. The link between the metabolic syndrome and cancer. *Int J Biol Sci* 2011;7:1003–15.

[21] Esposito K, Chiodini P, Colao A, Lenzi A, Giugliano D. Metabolic syndrome and risk of cancer: a systematic review and meta-analysis. *Diabetes Care* 2012;35:2402–11.

[22] Esposito K, Chiodini P, Capuano A, Bellastella G, Maiorino MI, Giugliano D. Metabolic syndrome and endometrial cancer: a meta-analysis. *Endocrine*. 2014 Feb;45(1):28-36. doi: 10.1007/s12020-013-9973-3. Epub 2013 May 3. PMID: 23640372.

[23] Agnoli C, Berrino F, Abagnato CA, Muti P, Panico S, Crosignani P, Krogh V. Metabolic syndrome and postmenopausal breast cancer in the ORDET cohort: a nested case-control study. *Nutr Metab Cardiovasc Dis.* 2010 Jan;20(1):41-8. doi: 10.1016/j.numecd.2009.02.006. Epub 2009 Apr 10. PMID: 19361966; PMCID: PMC2819536.

[24] Esposito K, Chiodini P, Capuano A, Bellastella G, Maiorino MI, Rafaniello C, Panagiotakos DB, Giugliano D. Colorectal cancer association with metabolic syndrome and its components: a systematic review with meta-analysis. *Endocrine*. 2013 Dec;44(3):634-47. doi: 10.1007/s12020-013-9939-5. Epub 2013 Apr 2. PMID: 23546613.

[25] Yin DT, He H, Yu K, Xie J, Lei M, Ma R, Li H, Wang Y, Liu Z. The association between thyroid cancer and insulin resistance, metabolic syndrome and its components: A systematic review and meta-analysis.

Complimentary Contributor Copy

Int J Surg. 2018 Sep;57:66-75. doi: 10.1016/j.ijsu.2018.07. 013. Epub 2018 Aug 3. PMID: 30081182.

[26] Esposito K, Chiodini P, Capuano A, Bellastella G, Maiorino MI, Parretta E, Lenzi A, Giugliano D. Effect of metabolic syndrome and its components on prostate cancer risk: meta-analysis. *J Endocrinol Invest*. 2013 Feb;36(2):132-9. doi: 10.1007/BF03346748. PMID: 23481613.

[27] Rosato V, Tavani A, Bosetti C, Pelucchi C, Talamini R, Polesel J, Serraino D, Negri E, La Vecchia C. Metabolic syndrome and pancreatic cancer risk: a case-control study in Italy and meta-analysis. *Metabolism*. 2011 Oct;60(10):1372-8. doi: 10.1016/j. metabol.2011.03.005. Epub 2011 May 6. PMID: 21550085.

[28] Jinjuvadia R, Patel S, Liangpunsakul S. The association between metabolic syndrome and hepatocellular carcinoma: systemic review and meta-analysis. *J ClinGastroenterol*. 2014;48(2):172-177. doi:10.1097/MCG.0b013e3182a030c4.

[29] Erratum: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2020 Jul;70(4):313. doi: 10.3322/caac.21609. Epub 2020 Apr 6. *Erratum for: CA Cancer J Clin*. 2018 Nov;68(6): 394-424. PMID: 32767693.

[30] McGlynn KA, London WT. The global epidemiology of hepatocellular carcinoma: present and future. *Clin Liver Dis*. 2011 May;15(2):223-43, vii-x. doi: 10.1016/j.cld.2011.03.006. PMID: 21689610; PMCID: PMC4141529.

[31] Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet*. 2015 Oct 17;386(10003):1546-55. doi: 10.1016/S0140-6736(15)61412-X. Epub 2015 Jul 28. PMID: 26231459.

[32] Nelson NP, Easterbrook PJ, McMahon BJ. Epidemiology of Hepatitis B Virus Infection and Impact of Vaccination on Disease. *Clin Liver Dis*. 2016 Nov;20(4):607-628. doi: 10.1016/j.cld.2016. 06.006.

Complimentary Contributor Copy

Erratum in: Clin Liver Dis. 2017 May;21(2):xiii. PMID: 27742003; PMCID: PMC5582972.

[33] Yang JD, Kim WR, Coelho R, et al. Cirrhosis is present in most patients with hepatitis B and hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2011; 9(1): 64-70.

[34] Bonilla Guerrero R, Roberts LR. The role of hepatitis B virus integrations in the pathogenesis of human hepatocellular carcinoma. *J Hepatol* 2005;42:760–777.

[35] Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. *World J Gastroenterol* 2016; 22(34): 7824-40.

[36] Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. *J Hepatol*. 2013 Mar;58(3):593-608. doi: 10.1016/j.jhep.2012.12.005. PMID: 23419824.

[37] Petruzziello, A. (2018). Epidemiology of Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) Related Hepatocellular Carcinoma. *The Open Virology Journal, 12(Suppl-1, M3), 26–32.* doi:10.2174/1874357901812010026.

[38] Di Bisceglie AM. Hepatitis C and hepatocellular carcinoma. *Hepatology*. 1997 Sep;26(3 Suppl 1):34S-38S. doi: 10.1002/hep.510260706. PMID: 9305661.

[39] Ramadori P, Cubero FJ, Liedtke C, Trautwein C, Nevzorova YA. Alcohol and Hepatocellular Carcinoma: Adding Fuel to the Flame. *Cancers*. 2017; 9(10):130.

[40] Corrao G, Bagnardi V, Zambon A, Arico S. Exploring the dose-response relationship between alcohol consumption and the risk of several alcohol-related conditions: a meta-analysis. *Addiction*. 1999 Oct;94(10):1551-73. doi: 10.1046/j.1360-0443.1999.9410155111.x. PMID: 10790907.

[41] Turati F, Galeone C, Rota M, Pelucchi C, Negri E, Bagnardi V, Corrao G, Boffetta P, La Vecchia C. Alcohol and liver cancer: a systematic review and meta-analysis of prospective studies. *Ann Oncol*. 2014

Complimentary Contributor Copy

Aug;25(8):1526-35. doi: 10.1093/annonc/mdu020. Epub 2014 Mar 14. PMID: 24631946.

[42] Ogunwobi OO, Harricharran T, Huaman J, et al. Mechanisms of hepatocellular carcinoma progression. *World J Gastroenterol*. 2019;25(19):2279-2293. doi:10.3748/wjg.v25.i19.2279.

[43] Wong RJ, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology*. 2014 Jun;59(6):2188-95. doi: 10.1002/hep.26986. Epub 2014 Apr 25. PMID: 24375711.

[44] Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol*. 2013 Jun;10(6):330-44. doi: 10.1038/nrgastro.2013.41. Epub 2013 Mar 19. PMID: 23507799.

[45] Anstee QM, McPherson S, Day CP. How big a problem is non-alcoholic fatty liver disease? *BMJ*. 2011 Jul 18;343:d3897. doi: 10.1136/bmj.d3897. PMID: 21768191.

[46] Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med*. 2018 Jul;24(7):908-922. doi: 10.1038/s41591-018-0104-9. Epub 2018 Jul 2. PMID: 29967350; PMCID: PMC6553468.

[47] Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol*. 2010 Aug;53(2):372-84. doi: 10.1016/j.jhep. 2010.04.008. Epub 2010 May 7. PMID: 20494470.

[48] Younossi, ZM et al. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 64, 73–84 (2016).

[49] Tang R, Liu H, Yuan Y, et al. Genetic factors associated with risk of metabolic syndrome and hepatocellular carcinoma. *Oncotarget*. 2017;8(21):35403-35411. doi:10.18632/oncotarget.15893.

[50] Utzschneider KM, Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab*. 2006

Complimentary Contributor Copy

Dec;91(12):4753-61. doi: 10.1210/jc.2006-0587. Epub 2006 Sep 12. PMID: 16968800.

[51] Hirsova, P, SH Ibrabim, GJ Gores, and H Malhi, "Thematic review series: Lipotoxicity: Many roads to cell dysfunction and cell death lipotoxic lethal and sublethal stress signaling in hepatocytes: Relevance to NASH pathogenesis," *Journal of Lipid Research*, vol. 57, no. 10, pp. 1758–1770, 2016.

[52] Kutlu O, Kaleli HN, Ozer E. Molecular Pathogenesis of Nonalcoholic Steatohepatitis- (NASH-) Related Hepatocellular Carcinoma. *Can J Gastroenterol Hepatol*. 2018;2018:8543763. Published 2018 Aug 29. doi:10.1155/2018/8543763.

[53] Afonso MB, PM Rodrigues, T Carvalho et al. "Necroptosis is a key pathogenic event in human and experimental murine models of non-alcoholic steatohepatitis," *Clinical Science*, vol. 129, no. 8, pp. 721–739, 2015.

[54] Vanni, E. et al. From the metabolic syndrome to NAFLD or vice versa? *Dig. Liver Dis.* 42, 320–330 (2010).

[55] Targher, G. et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 30, 1212–1218 (2007).

[56] Portillo-Sanchez, P. et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *J. Clin. Endocrinol. Metab.* 100, 2231–2238 (2015).

[57] Jimba, S. et al. Prevalence of non-alcoholic fatty liver disease and its association with impaired glucose metabolism in Japanese adults. *Diabet. Med.* 22, 1141–1145 (2005).

[58] Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology*. 2010;51(6): 1972-1978. doi:10.1002/hep.23527.

[59] Kawada N, Imanaka K, Kawaguchi T, Tamai C, Ishihara R, Matsunaga T, Gotoh K, Yamada T, Tomita Y. Hepatocellular carcinoma arising from non-cirrhotic nonalcoholic steatohepatitis. *J*

Complimentary Contributor Copy

Gastroenterol 2009; 44: 1190-1194 [PMID: 19672551 doi: 10.1007/s00535-009-0112-0].

[60] Mittal S, El-Serag HB, Sada YH, et al. Hepatocellular Carcinoma in the Absence of Cirrhosis in United States Veterans is Associated With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol*. 2016;14(1):124-31.e1. doi:10.1016/j.cgh.2015.07.019.

[61] Cholankeril G, Patel R, Khurana S, Satapathy SK. Hepatocellular carcinoma in non-alcoholic steatohepatitis: Current knowledge and implications for management. *World J Hepatol*. 2017;9(11):533-543. doi:10.4254/wjh.v9.i11.533.

[62] Farges O, Ferreira N, Dokmak S, Belghiti J, Bedossa P, Paradis V. Changing trends in malignant transformation of hepatocellular adenoma. *Gut*. 2011 Jan;60(1):85-9. doi: 10.1136/gut.2010.222109. PMID: 21148580.

[63] Younossi ZM, Otgonsuren M, Henry L, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology* 2015;62: 1723–30.

[64] Golabi P, Fazel S, Otgonsuren M, Sayiner M, Locklear CT, Younossi ZM. Mortality assessment of patients with hepatocellular carcinoma according to underlying disease and treatment modalities. *Medicine* (Baltimore). 2017 Mar;96(9):e5904. doi: 10.1097/MD.0000000000005904. PMID: 28248853; PMCID: PMC5340426.

[65] Marengo A, Rosso C, Bugianesi E. Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis. *Annu Rev Med*. 2016;67:103-117. doi:10.1146/annurev-med-090514-013832.

[66] Dyson J, Jaques B, Chattopadyhay D, et al. 2014. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J. Hepatol.* 60:110–17.

[67] Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, Gudnason V, Eiriksdottir G, Garcia ME, Launer LJ, Nalls MA, Clark JM, Mitchell BD, Shuldiner AR, Butler JL, Tomas M, Hoffmann U, Hwang SJ, Massaro JM, O'Donnell CJ, Sahani DV, Salomaa V, Schadt EE, Schwartz SM, Siscovick DS; NASH CRN;

Complimentary Contributor Copy

GIANT Consortium; MAGIC Investigators, Voight BF, Carr JJ, Feitosa MF, Harris TB, Fox CS, Smith AV, Kao WH, Hirschhorn JN, Borecki IB; GOLD Consortium. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet*. 2011 Mar;7(3):e1001324. doi: 10.1371/journal.pgen.1001324. Epub 2011 Mar 10. PMID: 21423719; PMCID: PMC3053321.

[68] Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet*. 2008;40:1461- 1465.

[69] Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology*. 2011 Jun;53(6):1883-94. doi: 10.1002/ hep.24283. Epub 2011 May 14. PMID: 21381068.

[70] Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit patho- genesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 2016;65:1038–1048. https://doi.org/10.1016/j.metab ol.2015.12.012.

[71] Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med*. 2003 Apr 24;348(17):1625-38. doi: 10.1056/NEJMoa021423. PMID: 12711737.

[72] Wolk A, Gridley G, Svensson M, et al. A prospective study of obesity and cancer risk (Sweden). *Cancer Causes Control* 2001; 12:13-21.

[73] Moller H, Mellemgaard A, Lindvig K, Olsen J. Obesity and cancer risk: a Danish record-linkage study. Eur J Cancer 1994; 30A:344-50.

[74] Mendonça FM, de Sousa FR, Barbosa AL, et al. Metabolic syndrome and risk of cancer: which link? *Metabolism*. 2015;64(2):182-189. doi:10.1016/j.metabol.2014.10.008.

[75] Mocellin S, Rossi CR, Pilati P, Nitti D. Tumor necrosis factor, cancer and anticancer therapy. *Cytokine Growth Factor Rev*. 2005 Feb;16(1): 35-53. doi: 10.1016/j.cytogfr.2004.11.001. Epub 2004 Dec 19. PMID: 15733831.

Complimentary Contributor Copy

[76] Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. *J Clin Invest*. 2007 May;117(5): 1175-83. doi: 10.1172/JCI31537. PMID: 17476347; PMCID: PMC 1857251.

[77] Luo, JL, Maeda, S, Hsu, LC, Yagita, H, and Karin, M. 2004. Inhibition of NF-kB in cancer cells con- verts inflammation-induced tumor growth mediat- ed by TNF-α to TRAIL-mediated tumor regression. *Cancer Cell*. 6:297–305.

[78] Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. *Cytokine Growth Factor Rev*. 2002 Aug-Oct;13(4-5):357-68. doi: 10.1016/s1359-6101(02)00027-8. PMID: 12220549.

[79] Hodge DR, Hurt EM, Farrar WL. The role of IL-6 and STAT3 in inflammation and cancer. *Eur J Cancer*. 2005 Nov;41(16):2502-12. doi: 10.1016/j.ejca.2005.08.016. Epub 2005 Sep 30. PMID: 16199153.

[80] Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer RG, Osterreicher CH, Takahashi H, Karin M. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell*. 2010 Jan 22;140(2):197-208. doi: 10.1016/j.cell. 2009.12.052. PMID: 20141834; PMCID: PMC2836922.

[81] H. Min, F. Mirshahi, A. Verdianelli et al. "Activation of the GP130-STAT3 axis and its potential implications in nonalcoholic fatty liver disease," *American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 308, no. 9, pp. G794–G803, 2015.

[82] Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol*. 2019;16(7):411-428. doi:10.1038/s41575-019-0145-7.

[83] Teoh NC, Dan YY, Swisshelm K, Lehman S, Wright JH, Haque J, Gu Y, Fausto N. Defective DNA strand break repair causes chromosomal instability and accelerates liver carcinogenesis in mice. *Hepatology*. 2008 Jun;47(6):2078-88. doi: 10.1002/hep.22194. PMID: 18506893.

[84] Xing SQ, Zhang CG, Yuan JF, Yang HM, Zhao SD, Zhang H. Adiponectin induces apoptosis in hepatocellular carcinoma through

Complimentary Contributor Copy

differential modulation of thioredoxin proteins. *Biochem Pharmacol*. 2015 Jan 15;93(2):221-31. doi: 10.1016/j.bcp.2014.12.001. Epub 2014 Dec 13. PMID: 25514170.

[85] Ikejima K, Okumura K, Kon K, Takei Y, Sato N. Role of adipocytokines in hepatic fibrogenesis. *J Gastroenterol Hepatol*. 2007 Jun;22Suppl 1:S87-92. doi: 10.1111/j.1440-1746.2007.04961.x. Erratum in: *J Gastroenterol Hepatol*. 2008 Mar;23(3):501-2. PMID: 17567476.

[86] Jiang N, Sun R, Sun Q. Leptin signaling molecular actions and drug target in hepatocellular carcinoma. *Drug Des Devel Ther*. 2014 Nov 14;8:2295-302. doi: 10.2147/DDDT.S69004. PMID: 25484575; PMCID: PMC4238752.

[87] Lagiou P, Kuper H, Stuver SO, Tzonou A, Trichopoulos D, Adami HO. Role of diabetes mellitus in the etiology of hepatocellular carcinoma. *J Natl Cancer Inst*. 2000 Jul 5;92(13):1096-9. doi: 10.1093/jnci/92.13.1096. PMID: 10880555.

[88] El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology*. 2004 Feb;126(2):460-8. doi: 10.1053/j.gastro. 2003.10.065. PMID: 14762783.

[89] Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology*. 2010;51(5):1820-1832. doi:10.1002/hep.23594.

[90] Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. *Nutrients*. 2013 May 10;5(5):1544-60. doi: 10.3390/nu5051544. PMID: 23666091; PMCID: PMC3708335.

[91] Tanaka S, Mohr L, Schmidt EV, Sugimachi K, Wands JR. Biological effects of human insulin receptor substrate-1 overexpression in hepatocytes. *Hepatology*. 1997 Sep;26(3):598-604. doi: 10.1002/hep. 510260310. PMID: 9303488.

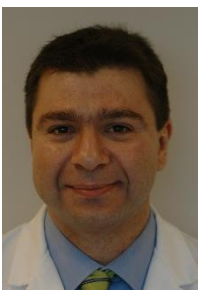
Complimentary Contributor Copy

[92] Mansour SJ, Matten WT, Hermann AS, Candia JM, Rong S, Fukusawa K, Van de Woude GF, et al. Transformation of mammalian cells by constitutively active MAP kinase kinase. *Science* 1994;265:966-970.

[93] Janku F, AO Kaseb, AM Tsimberidou, RA Wolff, and R Kurzrock, "Identification of novel therapeutic targets in the PI3K/AKT/mTOR pathway in hepatocellular carcinoma using targeted next generation sequencing," *Oncotarget*, vol. 5, no. 10, pp. 3012–3022, 2014.

[94] Chettouh H, M Lequoy, L Fartoux, C Vigouroux, and C Desbois-Mouthon, "Hyperinsulinaemia and insulin signalling in the pathogenesis and the clinical course of hepatocellular carcinoma," *Liver International*, vol. 35, no. 10, pp. 2203–2217, 2015.

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Chapter 4

NUTRITIONAL FACTS OF LEAFY VEGETABLE *LACTUCA SATIVA*

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ABSTRACT

Lactuca sativa (lettuce) is one of the major leafy vegetable widely grown across the world. There are numerous varieties of lettuce and it remains to be the most widely consumed vegetable, mainly in the form of salads, as it is rich in nutrition. Lettuce is commonly eaten in raw form without cooking or processing; hence the quality and quantity of the bioactives in lettuce are not compromised. Bioactives of lettuce includes

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carotenoids, phenolics, fibers, vitamins and minerals that generally differ among the lettuce varieties. Green lettuce is rich in Vitamin C, whereas red lettuce is rich in phenolic content. Lactucaxanthin, a xanthophyll carotenoid, also the isomer of lutein, is a novel bioactive carotenoid abundantly present in lettuce. Studies have shown that the lettuce bioactives exhibited antioxidant, anti-diabetic, cholesterol lowering, anti-inflammatory and anticancer activities. In this book chapter, lettuce's nutritional composition and health benefits among the different varieties of lettuce are summarized.

Keywords: lettuce, nutrition, carotenoids, phenolics, vitamins, minerals, health benefits

1. INTRODUCTION

Lactuca sativa (Lettuce) is an important green leafy vegetable originated from Egypt and is now cultivated worldwide. Lettuce crop consists of various cultivars, differentiated on its morphology, phenotypical and genetic variation. Lettuce is an annual plant often grown as a leafy vegetable with a suitable temperature of below 25°C and with the pH of 6.0 to 6.8. The hydroponic system's practice of growing lettuce is preferable because the yield is greater than the soil-grown lettuce (Cruz et al., 2012). Another advantage of the hydroponic practice is water usage, reuse of nutrient in the water and no risk of soil-borne diseases. A leaf is a central part used from the lettuce crop; hence, it is commonly called as leafy vegetable. Lettuce is an economically crucial green vegetable regularly used next to tomato (FAOSTAT, 2012). Lettuce leaves are used as raw and processed dishes like salads, soups, wraps and sandwiches. Lettuce leaves are also used to prepare cooked dishes in different nations of the world. The general practice of eating lettuce in its raw form helps the ingredients/bioactives to remain intact. However, processing like cooking may degrade the temperature-sensitive bioactives. Lettuce is progressively used worldwide because of its rich nutrient and nutraceuticals (Gopalan et al., 2017; Naveen et al., 2020).

The health-promoting compounds of lettuce include phenolic compounds (Materska et al., 2019), flavonoids (Zivcak et al., 2017),

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carotenoids (Kimura et al., 2002), lipids (Kaur et al., 2014), dietary fibers (Xueping et al., 2015), vitamins (A & K), iron (Fe) and folate. Different coloured lettuce have been increased in recent past, where darker lettuce (romaine, red leaf and green leaf) are rich sources of riboflavin, vitamin A, thiamine, niacin, Fe, manganese (Mn), potassium (K), calcium (Ca), selenium (Se) and carotenoids (β-carotene, lutein and lactucaxanthin) (Bunning et al., 2010; Naveen et al., 2020). Taste, texture, color and size are the critical parameters for the successful marketing of lettuce. Lettuce is packaged and marketed as a whole or as cut pieces for salad (Martinez-Sanchez et al., 2012). The global production of lettuce in 2017 was 27 million tonnes, and China stood as the largest producer of lettuce, contributing 56% followed by United States, India and Spain (FAOSTAT, 2018).

The commonly grown varieties of lettuce are romanie, Iceberg (Crisp head), Green, Stem and Butterhead. The composition of bioactives differ based on combinations, and it is necessary to select a type of lettuce variety rich in bioactives. The screening of bioactives of different lettuce varieties helps to expand its usage by mankind as a diet. Even though there are no detailed guidelines concerning bioactive composition in many nations, nutrition and nutraceuticals' rights on the tags must contain the information (Mampholo et al., 2013).

Lettuce has several health beneficial effects and regular intake proved to improve the health by reducing the risk of cataracts, heart disease, cancer, and stroke (USDA, 2004). The dietary fibers present in the lettuce help in improving digestion. Carotenoids, phenolics, and vitamin C of lettuce are reported for their radical scavenging activities (Lopez et al., 2014). The chapter's objective was to list out the bioactive compounds of the lettuce, practice of lettuce usage as food, and the health benefits of the lettuce bioactives.

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2. NUTRITIONAL AND NUTRACEUTICAL FACTORS OF LETTUCE

2.1. Phenolic Content

Phenolic compounds are plant secondary metabolites of the phenylpropanoid biosynthetic pathway. Phenolic compounds play crucial physiological roles throughout the plant life cycle. The accumulation of polyphenols is the plant's defense response against the pathogen and pests (Naveen et al., 2013a; 2013b; 2019; 2020). However, for human application, these phenolic compounds are used as antioxidants. Free radicals cause oxidative stress leading to various human complications, and the intake of phenolic compounds reduce the stress created by the radicals. Phenolic compounds have been reported to show beneficial effect against diabetes, inflammation, cancer, neurodegeneration, and cardiovascular diseases (Seeram et al., 2006; Shukitt-Hale et al., 2009; Cuevas-Rodríguez et al., 2010; Slatnar et al., 2012; You et al., 2012; Naveen et al., 2018).

There are several reports on the total phenolic content of various fruits and vegetables and their individual profile. The phenolic content differs between lettuce varieties (Kim et al., 2016). Studies revealed that red romaine lettuce's phenolic content is higher than the green lettuce because of anthocyanins (Zhao et al., 2007; Llorach et al., 2008; Luna et al., 2013). Green romaine and crisphead lettuce varieties contained 70 to 94% phenolic acids, whereas red leaf lettuce contained only 35 to 45% (Llorach et al., 2008). Chlorogenic acid, caffeic acid, and their derivatives are the common, occurring phenolic acids in lettuce (Zhao et al., 2007; Llorach et al., 2008). Ferreres et al. (1997) showed higher caffeic acid derivatives (1.8 mg/g fresh weight (FW)) in the red tissue when compared with the green (0.6 mg/g FW) and white (0.2 mg/g FW) tissues of lettuce.

The total phenolics in iceberg variety was lower (0.05 mg/g FW) than red variety (0.3 to 0.4 mg/g FW), frozen spinach (0.1 mg/g FW) and broccoli (0.2 mg/g FW) (Mattila and Hellstrom, 2007). However, alteration in culture conditions was reported to enhance phenolic acid content. The open-field

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production system of lettuce showed higher chlorogenic acid content (3.5 to 7.9 mg/g DW) in 'Red Sails' (cultivar of red loose leaf lettuce) and in 'Kalura' (cultivar of romaine lettuce) (0.6 to 2.0 mg/g DW) when compared to high tunnel production system (Zhao et al., 2007; Tsormpatsidis et al., 2008).

Zhou et al., (2019) examined the influence of low nitrogen (LN) source on the phenolic accumulation in lettuce and the phenolic extract's antioxidant potential. An increase in 100.5% of phenolics and 153.06% of flavonoid content were evident after 3 days of LN treatment. Increased phenolic compound accumulation was also analysed using HPLC and found chicoric acid (156.7%) as abundant polyphenol followed by chlorogenic acid (309.0%), quercetin (406.7%) and luteolin (166.3%) after 5 days of LN treatment. The content of quercetin and chlorogenic acid increased to 19.2% and 22.7% in LN compared to control (10.3% and 15.1% respectively). But, no significant effect in caftaric acid content and decrease in the percentage of coumaroylquinic acid were observed upon LN treatment.

Mampholo et al. (2016) screened 16 different lettuce varieties for their total phenolic content grown in a closed hydroponic system. It was shown that the multi-red 4 variety possessed higher phenolic acids (2.81 mg/100 gm FW). Recently, Materska et al. (2019) studied the quantitative and qualitative analysis of individual phenolic compound in lettuce treated with $CaCl_2$ before harvest and cold storage using HPLC, LC-MS, and NMR. The relation between the individual compound, total phenolics and antioxidant activities were analysed and determined the correlation between lettuce storage time and compound concentration. The increase in the levels of caffeoyltartaric (CTA; 120%), 2,3-dicaffeoyltartaric (2.3-diCTA; 65%) and 5-O-caffeoylquinic acid (5-OCQA; 57%) was noticed after application of $CaCl_2$ and stored for 7 days.

2.1.1. Flavonoid

The flavonoid content changes among lettuce varieties and pigmentation pattern on lettuce leaves (Kim et al., 2016). The red lettuce (lollo rosso) contains higher total phenolic and is due to the presence of the phenolic acid, anthocyanins and flavonols. In case of green lettuce, only phenolic acids

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were the major group of phenolic component. Zhao et al. (2007) screened the lettuce's flavonol content grown in open field system and high tunnel system. They found higher flavonol content in the lettuce grown in open field system due to the higher solar radiation level. In case of lollo rosso variety, the higher flavonoid was observed in red (1.38 mg/g FW), followed by green (0.24 mg/g FW) and later white (0.04 mg/g FW) leaves (Ferreres et al., 1997).

Five different lettuce cultivars, of which three were green leaf lettuce (romaine, iceberg and green lettuce) and two red lettuce, were analyzed for total flavonoid content (Llorach et al., 2008). This study identified flavones luteolin, kaempferol derivatives and quercetin in the lettuce varieties. Changes in the total flavonoid content among the cultivars were observed and were found higher in red leaf lettuce subtype lollo rosso (2.8 mg/g FW), followed by red oak lettuce (1.1 mg/g FW), green lettuce (0.2 mg/g) and romaine lettuce (0.17 mg/g FW). The iceberg variety contained lower total flavonoid (0.01 mg/g FW).

2.1.2. Anthocyanins

Anthocyanins are red pigment components and come under the subgroup of flavonoids. Anthocyanin such as cyanidin 3-(6-malonylglucoside) is liable for the red colour of in the red oak leaf and lollo rosso leaf lettuce (Ferreres et al., 1997; Llorach et al., 2008). Cultivation conditions play a central role on determining the lettuce anthocyanin content. By varying the UV exposure (high UV transparency film and low UV transparency film), an increase in total phenolics, quercetin, and anthocyanin levels were observed in red leaf subtype lollo rosso lettuce (Zhao et al., 2007). In contrast, no effect on the concentration of the phenolic of green leaf subtype lollo biondo lettuce was observed (Ordidge et al., 2010). Overall results of the study revealed that phenolic concentration was more in red lettuces when compared to green lettuces (Zhao et al., 2007; Llorach et al., 2008) and UV enhanced the concentration of phenolics by stimulating the synthesis of phenolics (Zhao et al., 2007; Ordidge et al., 2010). Llorach et al. (2008) screened the total anthocyanin content and found

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a higher level (0.5 mg/g FW) in lollo rosso lettuce than (0.3 mg/g FW) in red oak leaf lettuce.

2.2. Carotenoid Content

Carotenoids are lipid-soluble yellow (xanthophyll), orange (carotene) pigments and are a diverse group of molecules present widely in vegetables, fruits, and leafy vegetables (Maiani et al., 2009). β-carotene is the major carotenoid present in lettuce, followed by lutein and lactucaxanthin. Lactucaxanthin is an isomer of lutein present mainly in lettuce, hence the name lactucaxanthin. The concentration of lactucaxanthin and lutein percentage was 46 and 54% respectively and the higher concentration was observed in green lettuce compared to romaine and iceberg lettuce (Naveen et al., 2020). The chemical and spectroscopic evidence confirmed the structure of lactucaxanthin validating the (3R,6R,3'R,6'R)-E,E-carotene-3,3'-diol as a novel carotenoid (Siefermann-Harm et al., 1981). In addition to these, the presence of other carotenoids like neoxanthin and violaxanthin were also reported (Saini et al., 2018). The carotenoid content differs among the lettuce cultivar type. The maximum content of the β-carotene (52.3 mg/g FW) was observed in romaine lettuce, and the lowest was observed in iceberg lettuce (3.0 mg/g FW) (USDA, 2015).

Mou (2005) screened the β-carotene and lutein content in 22 crisphead lettuce varieties (5 butterhead varieties, 5 romaine varieties, 5 green leaf varieties, and 4 red leaf varieties). The study revealed lutein and β-carotene levels were more in green lettuce followed by romaine and red leaf lettuce, and the lower content was observed in crisphead variety. The concentration of lutein and β-carotene ranged from 1.3-54.3 and 1.1-56.9 mg/g FW, respectively, in romaine lettuce. β-carotene and lutein concentration in romaine lettuce was 20.1 to 41.8 mg/g FW and 7.7 to 14.9 mg/g FW (López et al., 2014). Baslam et al. (2013) reported the 9.0 to 24.0 mg/g in FW of β-carotene and 12.5 to 31.0 mg/g FW of lutein.

Marineau et al. (2012) reported 44 to 69 mg/g FW of lutein and 33.3 to 55.3 mg/g FW of β-carotene in butter head lettuce. Another research group

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said 22.7 to 68.3 mg/g FW of β-carotene and lutein content ranged 30.6 to 85.8 mg/g FW in butterhead lettuce. Lutein concentration was usually more in red oak leaf lettuce (16.9 mg/g FW) comparison to butterhead lettuce (9.4 to 14.3 mg/g FW), green oak lettuce (8.5 to 15.2 mg/g FW), and Batavia (7.5 to 12.0 mg/g FW) (Nicolle et al., 2004b). But, the concentration of β-carotene was reported low in green lettuce (6.0 to 9.1 mg/g FW) in comparison to the red oak lettuce (10.1 mg/g FW), the result of the study was in contrast to the finding of Mou (2005). But according to USDA (2015), the quantity of β-carotene in both red lettuce (45 mg/g FW) and green lettuce (44.4 mg/g FW) was almost the same.

Naveen et al., (2020) screened lutein and lactucaxanthin contents in three different lettuce varieties *i.e*., green, romania and iceberg. The study revealed that the lactucaxanthin and lutein percentage was 46% and 54%, respectively and more in green lettuce than romania and iceburg lettuce. The quantity of lactucaxanthin was 0.36 ± 0.06, 0.42 ± 0.05 and 3.05 ± 0.12 mg/100 g of dry weight (DW) and lutein was 0.43 ± 0.08, 0.50 ± 0.03 and 3.63 ± 0.15 mg/100 g DW in iceburg, romania and green lettuce respectively.

Zeaxanthin, neoxanthin, and violaxanthin are other carotenoids present in lettuce. In red oak leaf lettuce, the zeaxanthin concentration was 2 mg/g FW, while in green lettuce it ranged from 0.7 to 2.7 mg/g FW (Nicolle et al., 2004b). Martineau et al., (2012) reported neoxanthin (1.3 to 2.1 mg/g FW) and violaxanthin (2.2 to 4.4 mg/g FW) contents in butterhead lettuce. The range of neoxanthin and violaxanthin was 20 to 39.5 and 22.5 to 50.5 mg/g FW, respectively (Baslam et al. 2013). The concentration of carotenoids in external leaves was more than the internal leaves, due to the greater sunlight intensity, that increases the biosynthesis of carotenoid in external leaves (Mou, 2005; Baslam et al., 2013).

Martineau et al. (2012) reported antheraxanthin (32.7 mg/g FW) as a rich carotenoid in butterhead lettuce. López et al. (2014) found lactucaxanthin in romaine lettuce (4.0 to 11.1 mg/g FW) of light-harvesting complexes of Photosystem II. Lettuce is rich in xanthophyll and carotenoids, in specific β-carotene, lutein and lactucaxanthin, and is normally maximum in green, romaine and butterhead varieties. The β-carotene concentration in green leaf, romaine, and butterhead lettuce was equivalent to that in spinach

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(56.3 mg/g FW) (USDA, 2015). Mampholo et al. (2016) analyzed the β-carotene and ascorbate content by screening 16 lettuce varieties grown in closed hydroponic condition, out of which red oak lettuce (multired 4) showed higher content of 16.45 and 30.61 mg/100 gm FW, respectively.

Improvement of lettuce genetically may enrich the carotenoid levels. The genetic variation in lettuce type and the difference in carotenoid content were determined. The lutein content was more than the β-carotene content in crisphead type, while in other lettuce the β-carotene was higher. During summer, carotenoids' concentration was more in lettuce comparably than the less sunny season or the plant grown on raised bed (Mou, 2005).

Cruz et al. (2012) compared the content of carotenoid and chlorophyll in lettuce grown in spent coffee grounds and on soil under greenhouse conditions. The study showed marked increase in levels of lutein (90%), β-carotene (72%), and chlorophyll (61%). Also, increase in biomass of 2.5 to 10% and bioactive compounds was noticed in lettuce variety grown on coffee spent.

2.3. Vitamin Contents

Lettuce contained vitamins such as vitamin (C, E, K, A) and folate. Lettuce consumption in raw form provides significant source of vitamins such as A, E, K, C and folate (Romani et al., 2002). Lettuce contained α- and γ-tocopherols as major forms of Vitamin E. Vitamin E's content ranged between 2.1 to 7.6 mg/g FW in butterhead, Batavia, green and oak leaf lettuce (Nicolle et al., 2004b). The range of α-tocopherol is higher in green lettuce and lower in romaine lettuce, and the content varies from 3 to 2.2 mg/g FW (USDA, 2015). Chun et al. (2006) reported that the content of α-tocopherol was higher in romaine lettuce compared to the crisphead variety.

Vitamin C is an essential organic substance that helps to induce immune system and has antioxidant property (Carr and Frei, 1999; Carr and Vissers, 2013). In Batavia, butterhead and oak lettuce Vitamin C's content ranged from 61 to 99 mg/g FW (Nicolle et al., 2004b). Iceberg, green leaf and romaine lettuce contained 42, 195, and 28 mg/g FW of vitamin C

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respectively (Lolrach et al., 2008). Lopez et al. (2014) reported that vitamin C content ranged from 61 to 109 mg/g FW in seven different lettuce cultivars of romaine and two cultivars of mini romaine lettuce. Among seven romaine lettuce, 'AR-29213' showed higher vitamin C concentration (109 mg/g FW), and Isasa revealed lesser vitamin C concentration (70 mg/g FW). As per their previous reports, lettuce subtypes like lollo rosso, red oak, and continental lettuce have also shown high vitamin C levels (Llorach et al., 2008). According to USDA (2015), green lettuce is rich in vitamin C that fulfils 22% of the required content recommended by the World Health Organization (WHO).

Folate (Vitamin B9) is rich in leafy vegetables and functions as cofactors in single-carbon transfer reactions (Scott et al., 2000). The deficiency of folate lead to decrease in DNA synthesis, decreasing cellular growth, development, and other functions (Taruscio et al., 2011; Shohag et al., 2012). Moreover, insufficient folate consumption may increase certain cancers risk (Bailey and Gregory, 1999). The recommended dietary allowance of folate for pregnant women and adults is 600 and 400 mg/day, respectively (Institute of Medicine, 1998). The intake of lettuce (100 g) makes up to 18% of folate. Romaine lettuce contained a higher quantity of folate (1.4 mg/g FW) compared to crisphead lettuce (0.3 mg/g FW) as per USDA (2015) (Table 1). The report also documented 1.9 mg/g FW folate content in spinach, which is lesser than the romaine lettuce (2.2 mg/g FW) (Wang et al., 2013). Lopez et al. (2014) showed folate content in romaine (subtype mini romaine) to be 0.3 to 0.6 mg/g FW.

Table 1. Recommended lettuce types for each nutrient/phytochemical

Lettuce type/cultivar	**Rich nutrient source**
Romaine	Dietary fiber
Butterhead, baby green leaf and red leaf lettuce	Iron
Romaine, butterhead and red leaf lettuce	Folate
Green leaf and baby green romaine lettuce	Vitamin C
Green leaf, romaine and butter head lettuce	β-carotene, lutein and lactucaxanthin
Red romaine and red leaf lettuce	Phenolic compounds

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2.4. Mineral Content

Consumption of lettuce in raw form is known to provide minerals such as iron (Fe), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), zinc (Zn) (Tsormpatsidis et al., 2008; Santos et al., 2014). The mineral composition varied among the lettuce cultivar types and based on leaf maturity, cultivation and culture conditions. Santos et al. (2014) showed that the baby leaf lettuce contained lower K, Mg and Fe than mature lettuce leaves. Na and K concentration in green lettuce were 0.5 mg/g FW and 2.2 mg/g FW, respectively. Lollo rosso baby leaf and green romaine lettuce contained the same amount of Na (0.1 mg/g FW) (Neocleous et al., 2014). The concentration of K in romaine lettuce (1.1 to 1.6 mg/g FW) and red leaf lollo rosso baby leaf lettuce (1.1 to 1.2 mg/g FW) were found similar. However, Fallovo et al. (2009) reported higher K content (1.9 to 4.1 mg/g FW) in matured green lettuce.

According to USDA (2015) report, the Na content was higher (0.05 to 0.3 mg/g FW) in baby leaf lettuce than the matured leaf lettuce. Several research groups have screened Na levels in various lettuce types, Kawashima and Soares (2003) reported 0.05 mg/g FW in butterhead lettuce. While, Baslam et al. (2013) reported 0.04 to 0.21 mg/g FW Na content in crisphead and romaine lettuce. Green leaf lettuce contained lesser Na levels (0.04 to 1.4 mg/g FW) compared to red lettuce (0.05 to 2.2 mg/g FW) (Koudela and Petríková, 2008). Green and red lettuce are generally considered as rich sources of K. Reports suggest that 100 g of fresh lettuce provide 4 to 8% of K, and the recommended intake of K is 4.7 g/day/adults (Institute of Medicine, 2005). K content ranged between 2.7 to 4.4 mg/g FW in different types of lettuce like romaine and crisphead (Baslam et al., 2013). USDA (2015) report demonstrated romaine type as the richest K source than the crisphead lettuce. A study by Kawashima and Soares (2003) have shown the K levels in butterhead lettuce to be 2.4 to 3.2 mg/g FW. Likewise, Koudela and Petríková (2008) estimated the K levels in green lettuce (2.4 to 6.0 mg/g FW) and red lettuce (2.5 to 6.5 mg/g FW). The Ca levels in Batavia Rubia Munguía, Cogollos de Tudela and Maravilla de Verano lettuces ranged from 0.2 to 1.0 mg/g FW (Baslam et al., (2013). Koudela and

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Petrikova (2008) did not found any major differences in the Ca levels between the green leaf (0.2 to 0.8 mg/g FW) and red leaf (0.2 to 0.5 mg/g FW) varieties. Altogether, the leaf type/age variety and environmental condition of cultivation influenced Ca's content in lettuce.

P and Mg are the other important minerals present in the lettuces. The recommended intake value of Mg and P are 310 to 420 mg/day and 700 mg/day, respectively and in fresh lettuce (100 g) provided 3 to 9% and 0.3 to 3% of RDA of Mg and P, respectively (Institute of Medicine, 1997). The level of P in Batavia Rubia Munguía, Cogollos de Tudela and Maravilla de Verano lettuces was found to be 0.05 to 0.13 mg/g FW and that of Mg was 0.11 to 0.48 mg/g FW (Baslam et al., 2013). P and Mg levels in butterhead, iceberg, romaine, green and red leaf lettuces ranged from 0.2 to 0.3 mg/g FW and 0.07 to 0.14 mg/g FW, respectively (USDA, 2015). According to the results of Kawashima and Soares (2003), high levels of Mg (0.2 mg/g FW) was noticed in butterhead lettuce. Koudela and Petríková (2008) measured the Mg levels in mature green lettuce to be 1.1 to 4.1 mg/g FW. In another report from Santos et al. (2014), the baby green lettuce contained 0.3 mg/g FW of Mg, which was relatively higher than that of red leaf lettuce (0.12 to 0.14 mg/g FW). Neocleous et al. (2014) also determined the green romaine lettuce's Mg concentration to be 0.12 to 0.17 mg/g FW.

Fe and Zn are the other two important minerals required for healthy wellbeing as per WHO recommendation. The recommended intake of Fe is 8-18 mg/day/adult. However, the fresh lettuce (100 g) provides 15% of the recommended content (Institute of Medicine, 2001). The amount of Fe ranged from 3.0 to 5.6 mg/g FW, as reported by Baslam et al. (2013). USDA (2015) report presented the Fe content to be 9.7, 8.6 and 12 mg/g FW in the mature romaine, green leaf, and red leaf lettuces, respectively. Fe concentration of 8.6 to 12.4 mg/g FW was higher in the US lettuce varieties, whereas in crisphead type, the content was less (4.1 mg/g FW). Higher Fe content was observed in romaine lettuce than in crisphead lettuce (Mou and Ryder, 2004), but in the butterhead case, the Fe content was 5 mg/g FW (Kawashima and Soares, 2003). Zn is also an essential mineral for cellular activity and is also considered an immune booster (Klouberta and Rink, 2015). In green to red lettuce type, Zn's content was 1.5 to 2.3 mg/g FW

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(USDA, 2015). Baslam et al. (2013) reported 2.5 to 8 mg/g FW of Zn in crisphead type compared to butterhead, green, romaine, and red lettuce. According to the literature, the maximum Fe concentration was observed in the baby green lettuce (14.6 mg/g FW) (Santos et al., 2014), which was more than baby red lettuce (lollo rosso) (8.1 to 13.4 mg/g FW) and green romaine (8.9 to 12.0 mg/g FW) (Neocleous et al., 2014).

Busari et al. (2016) screened the mineral content between the freshly harvested wild lettuce leaves, sundried and blanched-sundried wild lettuce leaves and found that the stored dried and fresh lettuce are quite good sources of Na, Mg, Ca, Fe and K. 100g fresh wild lettuce contained Na (488.33 mg), Ca (430.00 mg), Mg (100 mg), K (51.67 mg) and Fe (13.30 mg). Sublett et al. (2018) studied the effect of red lollo variety to growing temperature and determined the mineral and nutrient content. The study revealed that 25°C is more suitable for higher mineral accumulation in lettuce than 33°C.

Besides nutrient availability and nutraceuticals, lettuce also contained few non-caloric nutrients, dietary fibers and polyunsaturated fatty acids (PUFA). These nutrients play a very important role in improving the health by reducing body weight. Although lettuce contained less fat and less calorie, it is a poor source of protein. Romaine lettuce possessed higher PUFA content the lettuce types (butterhead, iceberg, green and red leaf lettuce) (USDA, 2015). α-linolenic acid (60%) and linolenic acid (20%) are the two major fatty acids reported in lettuce (Le Guedard et al., 2008). Lettuce also served as a good source of dietary fibre *i.e*., carbohydrates with less digestibility, providing less calories. Among the US population, dietary fiber is quite significant because of the lettuce consumption in raw from (The United States Census Bureau, 2012; USDA, 2012). Lettuce (100 g) provides (21 to 38 g/day) of fiber for adults, 10% of the recommended intake (Institute of Medicine, 2002).

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3. HEALTH BENEFITS OF LETTUCE BIOACTIVES

Studies have shown that the lettuce bioactives exhibited antioxidant, anti-diabetic, cholesterol lowering, anti-inflammatory, anti-cancer activities. Lutein and zeaxanthin are the major xanthophyll carotenoids in green leafy vegetables such as lettuce, kale, spinach, yellow-colored carrots, etc. Studies showed lutein and zeaxanthin protected the retina from UV radiation, and a lutein-rich diet prevented age-related macular degeneration and cataract (Sommerburg et al., 1998; Feskanich et al., 1999). β-carotene, another important carotenoid of lettuce, reported to preventing plaque deposition in the heart, thereby reducing cardiological disorders (Caballero et al., 2015). Violaxanthin, a epoxyxanthophyll pigment with orange colour found in lettuce, is reported for free radical scavenging activity and shown to inhibit lipid peroxidation. A study conducted in our laboratory showed lactucaxanthin from green lettuce exhibiting α-amylase and α-glucosidase activities in diabetic rats (Gopal et al., 2017). Recently, we demonstrated the antioxidant effect of lactucaxanthin extracted from green lettuce (Naveen et al., 2020). Neoxanthin, an epoxy xanthophyll carotenoid of lettuce, exhibits a pivotal role in photooxidative stress (Giossi et al., 2020). The anticancer effect of xanthophyll carotenoids from lettuce was studied in the HeLa and A549 cells. Among the different carotenoids, 9-z-neoxanthin showed potent cytotoxicity against the cancer cells (Saini et al., 2018). A human trail conducted by Fernandez et al. (1997) with 1584 colorectal cancer and 2879 control subjects demonstrated the beneficial effect of lettuce rich diet on the risk of colorectal cancer. In another clinical trial conducted on non-smokers, the increased consumption of fresh vegetables such as lettuce, carrots, and tomatoes protected from lung cancer among nonsmokers by 25% (Brennan et al., 2000).

CONCLUSION

Lettuce is an important green leafy vegetable cultivated across different parts of the world. Several lettuce varieties are grown: romaine, iceberg

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(crisphead), green, stem and butterhead. Lettuce is typically composed of phenolic compounds, flavonoids, carotenoids, lipids, dietary fibres, vitamins (A & K), iron (Fe), and folate. Some of the main pharmacological activities of lettuce bioactives include antioxidant, cardioprotective, neuroprotective, anti-inflammatory, anti-cancer. Lettuce is being used traditionally to cure ailments such as anemia, cough, infectious diseases, sleep disorders, muscle pains, etc. Recent clinical studies confirm the beneficial effects of lettuce consumption for several chronic diseases, including cancer. Altogether lettuce derived phytochemicals could be employed as an attractive option to synthetic drugs. Still, additional investigations are essential to promote lettuce phytochemicals as dietary supplements to other clinical diseases.

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REFERENCES

Bailey, L.B., and Gregory, J.F., 1999. Folate metabolism and requirements. *J. Nutr*. 129(4):779-782.

Baslam, M., Morales, F., Garmendia, I., Goicoechea, N. 2013. Nutritional quality of outer and inner leaves of green and red pigmented lettuces (*Lactuca sativa* L.) consumed as salads. *Sci. Hort.* 151:103-111.

Benjamin, C. Finglas, P. and Toldra. F. 2015. Encyclopedia of Food and Health - 1st Edition. In *Encyclopedia of Food and Health*, 1st Edition. Academic Press. https://www.elsevier.com/books/encyclopedia-of-food-and-health/caballero/978-0-12-84947-2.

Complimentary Contributor Copy

Bunning, M.L., Kendall, P.A., Stone, M.B., Stonaker, F.H. and Stushnoff, C. 2010. Effects of seasonal variation on sensory properties and total phenolic content of 5 lettuce cultivars. *J. Food Sci.*75:156-161.

Busari, K.R. Oyeyinka, S.A. Akinoso, R. Aworh O.C. 2016. Nutritional and sensory properties of wild lettuce (*Lactuca taraxacifolia*) leaves as affected by sun drying alone or in combination with blanching Croatian Journal of Food Technology, *Biotechnology and Nutrition* 11 (1-2):28-35.

Carr, A.C., and Frei, B. 1999. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am. J. Clin. Nutr.* 69 (6):1086-1107.

Carr, A.C., and Vissers, M.C.M. 2013. Synthetic or food-derived vitamin C-are they equally bioavailable? *Nutrients* 5(11):4284-4304.

Chiara, G, Cartaxana, P. and Cruz. S. 2020. "Photoprotective Role of Neoxanthin in Plants and Algae. *Molecules.* MDPI AG. https://doi.org/10.3390/molecules25204617.

Chun, J., Lee, J., Ye, L., Exler, J., and Eitenmiller, R.R. 2006. Tocopherol and tocotrienol contents of raw and processed fruits and vegetables in the United States diet. *J. Food Compos. Anal.* 19(2–3):196-204.

Cruz, R. Baptista, P. Cunha, S. Pereira, J.A. and Casal, S. 2012. Carotenoids of Lettuce (*Lactuca sativa* L.) Grown on Soil Enriched with Spent Coffee Grounds. *Molecules*, 17:1535-1547.

Cuevas-Rodríguez, E.O., Dia, V.P., Yousef, G.G., García-Saucedo, P.A., López-Medina, J., Paredes-López, O., De Mejia, E.G., and Lila, M.A., 2010. Inhibition of pro-inflammatory responses and antioxidant capacity of Mexican blackberry (*Rubus* spp.) extracts. *J. Agric. Food Chem.* 58(17):9542-9548.

Fallovo, C., Rouphael, Y., Rea, E., Battistelli, A., and Colla, G., 2009. Nutrient solution concentration and growing season affect yield and quality of *Lactuca sativa* L. var: *acephalain* floating raft culture. *J. Sci. Food Agric.* 89:1682-1689.

FAOSTAT. 2012. *FAOSTAT Database for Crops* [Online], Food and Agricultural Organization of the United Nations (FAO), Rome, Italy.

Complimentary Contributor Copy

http://faostat.fao.org/site/567/DesktopDefault (accessed August 30, 2012).

FAOSTAT. 2018. FAOSTAT Database for Crops [Online], *"Lettuce (with chicory) production in 2017; Countries/Regions/Production Quantity from pick lists"*. UN Food & Agriculture Organization, Statistics Division (FAOSTAT). 2018

Fernandez, E., La Vecchia, C. D'Avanzo, B. Negri, E. and Franceschi. S. 1997. "Risk Factors for Colorectal Cancer in Subjects with Family History of the Disease." *British Journal of Cancer* 75(9):1381-84. https://doi.org/10.1038/bjc.1997.234.

Ferreres, F., Gil, M.I., Castañer, M., and Tomás-Barberán, F.A., 1997. Phenolic metabolites in red pigmented lettuce (*Lactuca sativa*). Changes with minimal processing and cold storage. *J. Agric. Food Chem.* 45(11):4249-4254.

Feskanich, Diane, Peter Weber, Walter C. Willett, Helaine Rockett, Sarah L. Booth, and Graham A. Colditz. 1999. Vitamin K Intake and Hip Fractures in Women: A Prospective Study. *American Journal of Clinical Nutrition* 69(1):74-79. https://doi.org/10.1093/ajcn/69.1.74.

Gopal, S.S. Lakshmi, M.J. Sharavana, G. Gunaseelan S. Sreerama Y.N. and Baskaran V. 2017. Lactucaxanthin – a potential anti-diabetic carotenoid from lettuce (*Lactuca sativa*) inhibits α-amylase and α-glucosidase activity *in vitro* and in diabetic rats. *Food Funct.,* 8:1124-1131.

Institute of Medicine, 1997. *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. Institute of Medicine, Washington, D.C.

Institute of Medicine, 1998. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline.* Institute of Medicine, Washington, D.C.

Institute of Medicine, 2000. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids.* Institute of Medicine, Washington, D.C.

Institute of Medicine, 2001. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron,*

Complimentary Contributor Copy

Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Institute of Medicine, Washington, D.C.

Institute of Medicine, 2002. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids.* Institute of Medicine, Washington D.C.

Institute of Medicine, 2005. *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate.* Institute of Medicine, Washington D.C.

Kaur, N., Chugh, V., and Gupta, A.K., 2014. Essential fatty acids as functional components of foods-a review. *J. Food Sci. Technol.* 51(10):2289-2303.

Kawashima, L.M., and Soares, L.M.V., 2003. Mineral profile of raw and cooked leafy vegetables consumed in Southern Brazil. *J. Food Compos. Anal.* 16 (5):605-611.

Kim, M. Moon, J.Y. Tou, J.C. Mou, B. and Waterland N.L. 2016. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). *Journal of Food Composition and Analysis.* 49:19–34.

Kimura, M. and Rodriguez-Amaya D.B. 2002. A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoids. *Food Chemistry.* 78:389-398.

Klouberta, V., Rink, L., 2015. Zinc as a micronutrient and its preventive role of oxidative damage in cells. *Food Funct.* 6(10):3195-3204.

Koudela, M., and Petríková, K., 2008. Nutrients content and yield in selected cultivars of leaf lettuce (*Lactuca sativa* L. var. *crispa*). *Hortic. Sci.* 35(3):99-106.

Le Guedard, M., Schraauwers, B., Larrieu, I., and Bessoule, J.-J., 2008. Development of a biomarker for metal bioavailability: the lettuce fatty acid composition. *Environ. Toxicol. Chem.* 27(5):1147-1151.

Llorach, R., Martínez-Sánchez, A., Tomás-Barberán, F.A., Gil, M.I., and Ferreres, F., 2008. Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. *Food Chem.* 108(3):1028-1038.

López, A., Javier, G.A., Fenoll, J., Hellin, P., and Flores, P., 2014. Chemical composition and antioxidant capacity of lettuce: Comparative study of

Complimentary Contributor Copy

regular-sized (Romaine) and baby-sized (Little Gem and Mini Romaine) types. *J. Compos. Anal.* 33(1):39-48.

Luna, M.C., Martínez-Sánchez, A., Selma, M.V., Tudela, J.A., Baixauli, C., and Gil, M.I., 2013. Influence of nutrient solutions in an open-field soilless system on the quality characteristics and shelf life of fresh-cut red and green lettuces (*Lactuca sativa* L.) in different seasons. *J. Sci. Food Agric.* 93(2):415-421.

Maiani, G., Periago Castón, M.J., Catasta, G., Toti, E., Goñi Cambrodon, I., Bysted, A., Granado-Lorencio, F., Olmedilla-Alonso, B., Knuthsen, P., Valoti, M., Böhm, V., Mayer-Miebach, E., Behsnilian, D., and Schlemmer, U., 2009. Carotenoids: actual knowledge on food sources, intakes: stability and bioavailability and their protective role in humans. *Mol. Nutr. Food Res.* 53:S194-S218.

Mampholo, B.M., Sivakumar, D., Beukes, M. and Jansen Van Rensburg, W.S. 2013. Effect of modified atmosphere packaging on the quality and bioactive compounds of Chinese cabbage (*Brassica rapa* Lssp. *chinensis*). *J. Sci. Food Agric.* 93:2008-2015

Mampholo, B.M. Maboko, M.M. Soundy P. and Sivakumar D. 2016. Phytochemicals and overall quality of leafy lettuce (lactuca sativa l.) Varieties grown in closed hydroponic system. *Journal of Food Quality*. 39:805-815.

Martineau, V., Lefsrud, M., Naznin, M.T., and Kopsell, D.A., 2012. Comparison of light-emitting diode and high-pressure sodium light treatments for hydroponics growth of Boston lettuce. *Hort Science*. 47(4):477-482.

Martinez-Sanchez, A., Luna, M.C., Selma, M.V., Tudela, J.A., Abad, J. and Gil, M.I. 2012. Baby-leaf and multi-leaf of green and red lettuces are suitable raw materials for the fresh-cut industry. *Postharvest Biol.* Technol. 63:1-10.

Materska, M. Olszówka, K. Chilczuk, B. Stochmal, A. Pecio, L. Pacholczyk-Sienicka, B. Piacente, S. Pizza, C. and Masullo. M. 2019. Polyphenolic profiles in lettuce (*Lactuca sativa* L.) after CaCl2 treatment and cold storage. *European Food Research and Technology*. 245:733-744.

Complimentary Contributor Copy

Mattila, P., and Hellstrom, J., 2007. Phenolic acids in potatoes, vegetables, and some of their products. *J. Food Compos. Anal.* 20(3–4): 152-160.

Mou, B., and Ryder, E.J., 2004. Relationship between the nutritional value and the head structure of lettuce. *Acta Hortic.* 637:361-367.

Naveen, J., Hariprasad, P., Chandra Nayaka, S. and Niranjana S.R. (2013a) Cerebroside mediated elicitation of defense response in chilli (*Capsicum annuum* L.) against *Colletotrichum capsici* infection, *Journal of Plant Interactions,* 8(1):65-73.

Naveen, J., Hariprasad, P., Navya, H.M. and Niranjana, S.R. 2013b. Induction of resistance in chilli seedlings against anthracnose disease by crude cell wall elicitors from *Colletotrichum capsici* isolate uom-18. *Int J Pharm Bio Sci* 4(4):304-314.

Naveen, J., and Baskaran, V. 2018. Antidiabetic plant-derived nutraceuticals: a critical review. *Eur J Nutr* 57:1275-1299.

Naveen, J., Anitha, R.E., Sachin, R.C., and Baskaran, V. 2020. Cytotoxicity and 3T3-L1 cell uptake of lactucaxanthin purified and characterized by LC-MS and NMR from lettuce (*Lactuca sativa*), *Journal of Liquid Chromatography & Related Technologies.* 43(7-8): 233-246.

Naveen, J., Navya, H.M., Hariprasad, P., Hithamani, G. and Niranjana, S.R. 2019. Rhizobacteria *Bacillus* spp. Induce resistance against anthracnose disease in chili (*Capsicum annuum* L.) through activating host defense response. *Egyptian Journal of Biological Pest Control.* 29:45 1-9.

Naveen, J., Navya, H.M., Hariprasad, P., Hithamani, G. and Niranjana, S.R. 2020. Acibenzolar-S-methyl and β-amino butyric acid-induced upregulation of biochemical defense against *Colletotrichum capsici* infection in chilli (*Capsicum annuum*), *Archives of Phytopathology and Plant Protection.* 53(3-4):141-161.

Neocleous, D., Koukounaras, A., Siomos, A.S. and Vasilakakis, M., 2014. Assessing the salinity effects on mineral composition and nutritional quality of green and red baby lettuce. *J. Food Qual.* 37(1):1-8.

Nicolle, C., Carnat, A., Fraisse, D., Lamaison, J.L., Rock, E., Michel, H., Amouroux, P., and Remesy, C., 2004b. Characterization and variation of antioxidant micronutrients in lettuce (*Lactuca sativa* folium). *J. Sci. Food Agric.* 84(15):2061-2069.

Complimentary Contributor Copy

Ordidge, M., García-Macías, P., Battey, N.H., Gordon l.H. Hadley, P., John, P., Lovegrove, J.A., Vysini, E., and Wagstaffe, A., 2010. Phenolic contents of lettuce, strawberry, raspberry, and blueberry crops cultivated under plastic films varying in ultraviolet transparency. *Food Chem.* 119(3):1224-1227.

Paul, B. Fortes, C. Butler, J. Agudo, A. Benhamou, S. Darby, S. Gerken, M. et al. 2000. A Multicenter Case-Control Study of Diet and Lung Cancer among Non-Smokers. *Cancer Causes and Control* 11 (1):49-58. https://doi.org/10.1023/A:1008909519435.

Romani, A., Pinelli, P., Galardi, C., Sani, G., Cimato, A., and Heimler, D. 2002. Polyphenols in Greenhouse and Open-Air-Grown Lettuce. *Food Chem.,* 79:337-342.

Saini, R.K. Moon, S.H. Gansukh, E. and Keum. Y.S. 2018. An efficient one-step scheme for the purification of major xanthophyll carotenoids from lettuce, and assessment of their comparative anticancer potential. *Food Chemistry.* 266:56-65.

Santos, J., Oliva-Teles, M.T., Delerue-Matos, C. and Oliveira, M.B.P.P., 2014. Multi-elemental analysis of ready-to-eat baby leaf vegetables using microwave digestion and high-resolution continuum source atomic absorption spectrometry. *Food Chem.* 151:311-316.

Scott, J., Rebeille, F. and Fletcher, J., 2000. Folic acid and folates: the feasibility for nutritional enhancement in plant foods. *J. Sci. Food Agric.* 80 (7):795-824.

Seeram, N.P., Adams, L.S., Zhang, Y.J., Lee, R., S. and, D., Scheuller, H.S., Heber, D., 2006. Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells *in vitro*. *J. Agric. Food Chem.* 54 (25):9329-9339.

Shohag, M.J.I., Wei, Y., and Yang, X. 2012. Changes of folate and other potential health-promoting phytochemicals in legume seeds as affected by germination. *J. Agric. Food Chem.* 60(36):9137-9143.

Shukitt-Hale, B., Cheng, V., and Joseph, J.A. 2009. Effects of blackberries on motor and cognitive function in aged rats. *Nutr. Neurosci.* 12(3):135-140.

Complimentary Contributor Copy

Siefermann-Harm, D. Hertzberg, S. Borch. G. and Veliaaen-Jensen. S. 1981. Lactucaxanthin, an Ɛ,Ɛ-Carotene-3,3'-Diol from *Lactuca sativa*. *Phytochemisrty.* 20:85-88.

Slatnar, A., Jakopic, J., Stampar, F., Veberic, R. and Jamnik, P. 2012. The effect of bioactive compounds on in vitro and in vivo antioxidant activity of different berry juices. *Plos One* 7 (10):e47880.

Sublett, W.L. Barickman T.C. and Sams C.E. 2018. Effects of Elevated Temperature and Potassium on Biomass and Quality of Dark Red 'Lollo Rosso' Lettuce. *Horticulturae,* 4(11):1-11.

Taruscio, D., Carbone, P., Granata, O., Baldi, F., Mantovani, A. 2011. Folic acid and primary prevention of birth defects. *Biofactors.* 37(4):280-284.

The United States Census Bureau, 2012. *Health and Nutrition, Statistical Abstract of the United States: 2012.* The United States Census Bureau, Suitland, MD.

Tsormpatsidis, E., Henbest, R.G.C., Davis, F.J., Battey, N.H., Hadley, P., Wagstaffe, A., 2008. UV irradiance as a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce 'Revolution' grown under polyethylene films. *Environ. Exp. Bot.* 63(1-3):232-239.

USDA, 2012. *Commodity Consumption by Population Characteristics.* USDA, Washington, D.C.

USDA, 2015. *National Nutrient Database for Standard Reference Release* 28. USDA, Washington, D.C.

USDA. 2004. U.S. Department of Agriculture, Agricultural Research Service, National nutrient data base for standard Reference, *Release* 17. http://www.nal.usda.gov/fnic/foodcomp/Data/SR17/sr17.

Wang, C., Riedl, K.M., and Schwartz, S.J. 2013. Fate of folates during vegetable juice processing-deglutamylation and interconversion. *Food Res. Int.* 53(1):440-448.

Xueping, S. Yu, F. Jiang, C. Wang, H. and Yukui. R. Comparison of nutrient components of Chinese lettuce *Lactuca sativa* L.) and American lettuce. *Emirates Journal of Food and Agriculture.* 2015. 27(10):790-792.

You, Q., Chen, F., Wang, X., Jiang, Y.M., and Lin, S.Y. 2012. Anti-diabetic activities of phenolic compounds in muscadine against alpha-

Complimentary Contributor Copy

glucosidase and pancreatic lipase. *LWT-Food Sci. Technol.* 46(1):164-168.

Zhao, X., Carey, E.E., Young, J.E., Wang, W.Q., and Iwamoto, T. 2007. Influences of organic fertilization, high tunnel environment, and postharvest storage on phenolic compounds in lettuce. *Hort. Science* 42(1):71-76.

Zhou, W. Liang, X. Dai, P. Yao C., Zhang, Y. Zhang, M. Lu, L. Jin C. and Lin X. 2019. Alteration of phenolic composition in lettuce (*Lactuca sativa* L.) by reducing nitrogen supply enhances its anti-proliferative effects on colorectal cancer cells. *Int. J. Mol. Sci.*, 20, 4205.

Zivcak, M., Brückova, K., and Sytar, O. et al. 2017. Lettuce flavonoids screening and phenotyping by chlorophyll fluorescence excitation ratio. *Planta* 245:1215-1229.

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Chapter 5

HEALTH PROMOTING EFFECTS OF ONION (*ALLIUM CEPA L.*) – THE SPICY VEGETABLE

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ABSTRACT

Several medicinal properties of onion have been recognized since ancient times and used in traditional systems of medicine for treating diseases. Bioactive phytochemicals present in this spicy vegetable have now been understood to promote human health in many ways. The mechanism of such an action has also been elucidated by researchers in clinical studies and experimental models. Insulin secretagogue effect, hypoglycemic influence, hypolipidemic action, antioxidant effect, cardioprotective effect, gallstone prevention, cancer preventive potential, and digestive stimulant action are among the scientifically validated health effects of onion consumption. Its ability to suppress oxidative stress and the hypolipidemic potential have far-reaching implications in the prevention of several degenerative diseases. Generally, the antidiabetic

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influence of dietary onion was higher when consumed along with dietary fiber-rich fenugreek seeds, which also exerts an antidiabetic effect by an independent mechanism. Onion also enhances the bioavailability of micronutrients from plant foods.

Keywords: antioxidant influence, hypocholesterolemic effect, antidiabetic effect, cardio-protection, gallstone prevention, cancer prevention, digestive stimulant

INTRODUCTION

Onion (*Allium cepa*) bulbs belong to the *Allium* genus of flowering plants among others including garlic (*Allium sativum*), leeks (*Allium ampeloprasum*), and chives (*Allium schoenoprasum*). While onions are believed to have originated from Western and Eastern parts of Asia, they are cultivated and consumed across the world today. Onions have a strong taste and a sharp-pungent flavor. Onion is very widely used in any culinary dish, often served cooked, but can also be eaten raw and used in pickles and chutneys. There are numerous species of onions found around the world, viz., *Yellow onions – Sweet onions – White onions – Red onions – Green onions – Shallots – Leeks.* The historical records suggest the use of onions by the Chinese as back as 5000 years. The diverse medicinal properties of onion have been recognized since prehistoric times when they were used to treat ailments such as headaches, common cold, fever, heart disease, and mouth sores. This spicy vegetable contains various minerals (particularly potassium), vitamins (particularly Vitamin C and B vitamins), and potent phytochemicals (such as quercetin) that have been understood to promote human health in many ways. Besides its use as a culinary vegetable, the essential oil of onion has antiseptic and antibacterial properties. Diverse health beneficial attributes of onion have now been scientifically validated and the mechanisms of such an action are more or less understood.

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Table 1. Nutritional composition of fresh onion bulb (expressed as amount/ 100 g)

Major nutrient		**Micronutrient**	
Energy, kcal	40	Calcium, mg	23
Protein, g	1.1	Iron, mg	0.21
Total lipid, g	0.1	Magnesium, mg	10
Carbohydrates, g	9.3	Phosphorus, mg	29
Total dietary fiber, g	1.7	Potassium, mg	146
Sugars, total, g	4.2	Selenium, µg	0.5
		Vitamin C, mg	7.4
		Folate, µg	19



Figure 1. Onion bulb.

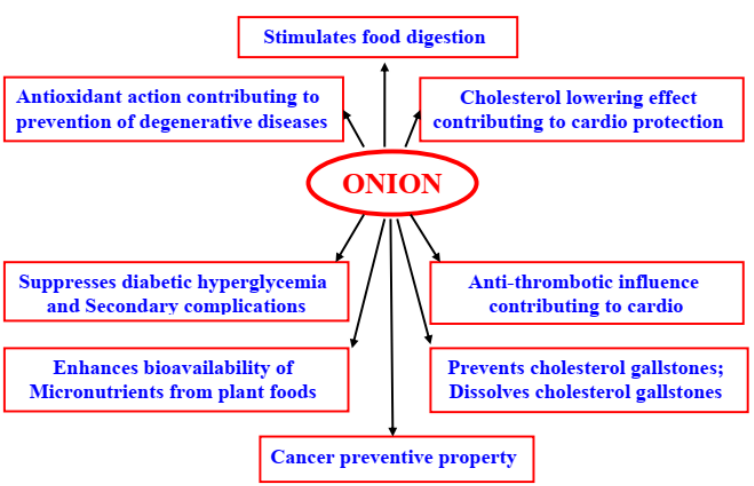


Figure 2. Diverse health benefits of onion consumption.

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BENEFICIAL EFFECTS ON HUMAN HEALTH

Onion Exerts Digestive Stimulant Action

The influence of spices including onion on the digestive secretions, and the digestive enzymes of gastric, pancreatic, and intestinal juices has been reviewed (Platel & Srinivasan, 2004). An animal study has evidenced that consumption of onion similar to several other spices induces higher secretion of bile acids in the bile (as much as 50% increase when fed at a 3% level for 8 weeks), (Sambaiah & Srinivasan, 1991; Platel & Srinivasan, 2000a). Even a single oral administration of onion also produced a significantly increased bile acid output. Bile acids play a vital role in fat digestion and absorption. Onion consumption is also understood to produce a significant stimulation of the activities of digestive enzymes originating from the pancreas, *viz.*, lipase, amylase, and proteases (Platel & Srinivasan, 2000b). Pancreatic amylase activity is observed to be stimulated by dietary onion (by about 30%). Dietary intake of onion also significantly enhanced trypsin and chymotrypsin activity. Dietary onion is also shown to have a beneficial effect on the terminal digestive enzymes of small intestinal mucosa (Platel & Srinivasan, 1996). An appreciable increase in intestinal lipase activity was observed in animals given a single oral dose of onion. The activity of intestinal amylase was also significantly enhanced by single-dose administration of onion (by 124%). Among the terminal digestive enzymes, disaccharidases of the small intestinal mucosa complete the digestion of carbohydrates. The intake of onion either dietary or as a single oral dose is shown to stimulate all the disaccharidases (maltase, sucrose, and lactase), the effect being more pronounced with continued intake of this spice. Dietary onion also enhanced the activity of intestinal alkaline phosphatase. Onion exerted a stimulatory effect on the activities of all the small intestinal terminal digestive enzymes when given as a single oral dose. The influence of this spice on lipase and amylase being over 100% stimulation, the beneficial effect on the other enzymes was in the range of 20-35%.

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Concomitant with such stimulation of bile secretion and the activity of digestive enzymes by onion, leading to accelerated digestion, a reduction (of about 15%) in the food transit time in the gastrointestinal tract has also been evidenced (Platel & Srinivasan, 2001). Thus, the digestive stimulant action of onion seems to be mediated through 1) by stimulating the liver to secrete bile rich in bile acids, components that are vital for fat digestion and absorption, and 2) by stimulation of activities of enzymes that are responsible for digestion. Onion has shown a favorable influence on most of the digestive enzymes of both the pancreas and small intestine. This beneficial influence of onion on pancreatic digestive enzymes was more evident by a continued intake of this spice, while intestinal enzymes were stimulated even with one-time exposure. This observation suggests that onion has great potential as a digestive stimulant by its action on digestive enzymes.

Onion Has Potential Antidiabetic Influences

Onion bulbs are used in traditional medicine and folk remedies for treating diabetes. Onion is documented to be hypoglycemic in experimental diabetic animal models and diabetic patients. Petroleum ether and diethyl ether extracts of onion have produced hypoglycemic activities (comparable to 70% efficiency of a standard dose of tolbutamide) in alloxan-induced diabetic rabbits[21]. Feeding 3% freeze-dried onion powder to streptozotocin-induced diabetic rats significantly improved the metabolic status of these animals and exerted its antihyperglycemic effects (Babu & Srinivasan, 1997). Administration of essential oil of onion to streptozotocin–induced diabetic rats (100 mg.kg body weight^{-1} for 3 weeks) significantly countered the compromised insulin level as well as lowered blood glucose (El-Soud & Khalil, 2010). Feeding 1% onion peel extract or oral administration of onion juice to experimental diabetic rats for 4 – 6 weeks showed remarkable hypoglycemic and antioxidant influences (Campos et al., 2003; Pal et al., 2013). Aqueous extract of onion (200, 250, and 300 mg.kg^{-1}) exerted dose-

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dependent antihyperglycemic effects in alloxan-induced diabetic rats (Echi et al., 2011).

Oral administration of aqueous onion extract for 4 weeks resulted in significant antihyperglycemic effects as well as antioxidant effects in alloxan-induced diabetic rats and rabbits besides alleviation of the liver and renal damage (El-Demerdash et al., 2005; Ogunmodede et al., 2012). SMCS isolated from onion is reported to have shown hypoglycemic and antioxidant effect similar to the standard drugs (glibenclamide and insulin) in alloxan-induced diabetic rats (Sheela et al., 1995; Kumari & Augusti, 2002). The antidiabetic potential of onion has also been demonstrated in clinical trials. The effect of orally administered (25 - 200 g) aqueous onion extract in healthy volunteers (Sharma et al., 1977), a dose-dependent effect on fasting blood glucose levels in both Type 1 and Type 2 diabetic patients (Eldin & Ahmed, 2010), increased serum insulin levels and hypoglycemic effect of 2-propenyl propyl disulfide isolated from onion (2.5 mg/kg) in healthy subjects (Augusti & Benaim, 1975) have been documented.

The blood glucose-lowering effect has been attributed to the presence of sulfur compounds in onion [di-(2-propenyl) disulfide and 2-propenyl propyl disulfide]. The mechanism of hypoglycemic action of onion involves direct stimulation of the secretion of insulin by the pancreas (secretagogue activity) and also, stimulation of glucose uptake in peripheral tissues by improving insulin sensitivity or stimulating GLUT4 expression. It is also suggested that the disulfide compounds of onion exert an insulin-sparing action by preventing insulin from -SH inactivation in the body, thus increasing the life span of the hormone. The beneficial antidiabetic action of onion could also be a result of scavenging of the free radicals generated during diabetes-induced oxidative stress, being a rich source of the flavonoids particularly (quercetin and kaempferol) and anthocyanins, thus promoting a countering of oxidative stress.

Dietary intervention with onion (3%) for 6 weeks countered hyperglycemia and associated metabolic abnormalities (in blood and urine) in streptozotocin-induced diabetic rats mediated through a significant insulinotropic action, countering of pancreatic pathology, antioxidant potential, and hypolipidemic influence (Pradeep & Srinivasan, 2017a,

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2017b). Oxidative stress plays a crucial role in the progression of diabetic secondary complications and pathogenesis of diabetic nephropathy. Dietary intervention with onion is reported to effectively counter the diabetes-induced nephromegaly, increase in glomerular filtration rate, and functional abnormalities of renal tissue in streptozotocin-induced diabetic rats (Pradeep & Srinivasan, 2018a; Babu & Srinivasan, 1997). This beneficial influence was also indicated by the extent and quality of proteinuria, by the extent of leaching of renal tubular enzymes into the urine, and by the modulation of the activities of marker enzymes in the renal tissue (Babu & Srinivasasn, 1999). Further studies on the mechanistic aspects of the nephroprotective influence of dietary onion on renal lesions associated with diabetes (Pradeep et al., 2019) has revealed countering of the up-regulation of renal glucose transporters, down-regulation of the polyol pathway, nitric oxide, inducible nitric oxide synthase, and N-acetyl-β-D-glucosaminidase activity, and normalization of podocyte damage.

Hyperglycemia in the diabetic conditions is associated with increased oxidative stress, cholesterol, and triglyceride concentrations in the cardiac tissue which precipitate cardiovascular complications. Dietary intervention with onion is reported to significantly counter hypercholesterolemia, elevated heart cholesterol, and triglycerides under diabetic conditions (Pradeep & Srinivasan, 2018c). Additionally, the cardioprotective influence of onion was also mediated through a blocking of the renin-angiotensin system, specifically a consequence of reduced activation of Angiotensin-converting enzyme (ACE) and Angiotensin Type 1 receptor (AT_1) in the heart tissue. This was also corroborated by a restoration of histopathological abnormalities of heart tissue.

Hyperglycemia-induced osmotic and oxidative stress in the eye lens is responsible for the pathogenesis of diabetic cataracts. Animals maintained on onion-supplemented diet significantly countered oxidative stress markers, AGEs, and expression of their receptor (RAGE) in the eye lens (Pradeep & Srinivasan, 2018d). Increased activity of polyol pathway enzymes, altered crystallin protein distributional profile, their expression, the activity of carbohydrate metabolizing enzymes, and antioxidant status was significantly annulled by dietary intervention with onion. Dietary

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intervention with onion significantly delayed cataractogenesis in diabetic rats.

Dietary onion was also observed to partially reverse the deformity, fragility, and reduced fluidity of erythrocytes accompanying diabetes in rats (Pradeep & Srinivasan, 2018b). This was achieved through countering the altered cholesterol: phospholipid ratio in the erythrocyte membrane and echinocyte formation resulting from diabetic hyperlipidemia and increased lipid peroxidation. The antisickling potency of onion was accomplished by a substantial decrease in the echinocyte population, nitric oxide metabolites, and AGEs in erythrocytes of diabetic animals.

Higher Antidiabetic Influence of Onion in Combination with Fenugreek Seeds

Dietary onion and the fiber-rich fenugreek (*Trigonella foenum-graecum*) seeds and are independently known to be effective in the management of diabetes and its complications. These two possess significant hypoglycemic, hypolipidemic, and antioxidant potential in diabetic situation. Since the mechanism of action of these two spices is dissimilar concerning the modulation of glucose homeostasis, the combined use of these two ingredients possibly has an additive antidiabetic influence; this has been verified in the streptozotocin-induced diabetic rat model.

A higher beneficial influence of dietary intervention for 6 weeks with onion (3%) and fenugreek seeds (10%) on hyperglycemia and its associated metabolic abnormalities has been observed in streptozotocin-induced diabetic rats, which was characterized by an improvement in peripheral insulin resistance and insulin secretion, and ameliorative effects on pancreatic pathology (Pradeep & Srinivasan, 2017a). The restoration of antioxidant status and countering of the lipid abnormalities in blood and liver of diabetic rats were higher in the case of dietary intervention with the combination onion and fenugreek amounting to an additive effect (Pradeep & Srinivasan, 2017b). While oxidative stress has a crucial role in the progression of diabetic complications, the combination of onion and fenugreek showed an additive effect concerning restoring the circulating

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antioxidants, along with lowering of the circulating oxidative stress markers (Pradeep & Srinivasan, 2017b).

While dietary intervention with either onion or fenugreek seeds effectively countered the diabetes-induced structural and functional abnormalities of renal tissue in streptozotocin-induced diabetic rats, the beneficial influence of dietary onion was higher when the intervention was combined with fenugreek seeds on oxidative stress-mediated renal injury (Pradeep & Srinivasan, 2018a, Pradeep et al., 2019). While dietary intervention with onion or fenugreek seeds were independently found to ameliorate the pathological changes in the cardiac tissue in diabetic animals, the beneficial effect on CVD was observed to be higher with the combined intervention with onion and fenugreek seeds, amounting to an additive or sometimes even a synergistic beneficial effect (Pradeep & Srinivasan, 2018c). The beneficial anti-cataractogenic influence of supplemental onion in streptozotocin-induced diabetic rats was higher when combined with dietary intervention with fenugreek seeds (Pradeep & Srinivasan, 2018d). Similarly, the beneficial hematoprotective effect of dietary onion on the deformation of red blood cells was higher when this intervention was made in combination with fenugreek seeds (Pradeep & Srinivasan, 2018b).

Hypolipidemic Effects of Onion in Hyperlipidemic and Diabetic Situations Contributing to Cardioprotection

Onion has been extensively studied for hypocholesterolemic potential in various experimental animal models as well as in clinical trials (Srinivasan, 2013). Cholesterol-lowering in blood and liver has been evidenced following the intake of onion or its essential oil in several studies involving high fat- / high sucrose- fed rats. Similarly, a reduction in circulatory cholesterol has been observed in rabbits maintained on a cholesterol-enriched diet and simultaneously feeding onion or its essential oil. Reduction in blood cholesterol following onion consumption in several human trials involving normal subjects/ hyperlipidemic patients has also been documented. Onion juice (equivalent of 25 g of onion.kg^{-1}.day^{-1}) is reported

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to lower the elevated blood cholesterol in rabbits maintained on a high-cholesterol diet (Lin et al., 2002; Kleijnen et al., 1989).

The lipid-lowering influence of S-methyl cysteine sulfoxide (SMCS) isolated from onion (200 $mg.kg^{-1}$ for 45 days) has been observed in experimental rats maintained on a cholesterol-enriched diet (Kumari & Augusti, 2002). The authors further provide the mechanism of hypolipidemic action of SMCS to be through a reduction of endogenous lipogenesis and increased catabolism of lipids. The deleterious effect of a high-cholesterol diet on rabbit erythrocytes and the beneficial effect of onions in reversing this has been studied in rabbits and rats (Vatsala & Singh, 1981; Ahluwalia & Mohindroo, 1989). Onion extract administration is shown to significantly reduce triglycerides in the aorta besides in circulation and liver. This beneficial effect of onion has been ascribed to its sulfur-containing compounds, which oxidize thiol compounds that are either free or associated with proteins (Sebastian et al., 1979). The hypolipidemic influence of dietary onion has also been demonstrated in streptozotocin-induced diabetic rats (Babu & Srinivasan, 1997; Pradeep & Srinivasan, 2017a).

Cardioprotective Effect

Onion is cardioprotective chiefly by its hypocholesterolemic influence and its ability to reduce oxidative stress as seen in hyperlipidemic/ hypercholesterolemic and diabetic situations as discussed in previous sections (Srinivasan, 2019). Besides, onion is also reported to exert a significant antithrombotic effect in experimental rats (Bordia et al., 1996; Yamada et al., 2004). Prevention of arterial thrombotic diseases assumes importance in developed countries through adopting an appropriate dietary strategy to minimize the risk factor for thrombotic events. Administration of rats with aqueous extract of onion (500 mg/kg), orally or intraperitoneally, daily for 4 weeks significantly decreased the level of thromboxane B2 (TXB2) in the serum amounting to an antithrombotic effect ((Bordia et al., 1996). Onion has also been shown to exert a significant antithrombotic

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activity both *in vitro* and *in vivo* by platelet aggregation test using hemostometry (Yamada et al., 2004). Onion showed thrombolytic activity in addition to the antiplatelet effect.

Onion Prevents Cholesterol Gallstone Disease Primarily by Modulating Cholesterol Homeostasis

Dietary Onion Reduces the Incidence of Lithogenic Diet-Induced Cholesterol Gallstones in Experimental Studies

Experimental mice fed with a diet containing high (0.5%) cholesterol for ten weeks resulted in cholesterol supersaturation in the gallbladder bile which promoted the formation of cholesterol gallstones (CGS) (Vidyashankar et al., 2009). In the same study, when onion (both raw or heat-processed) was included in the diet at 2.0% level along with the lithogenic diet for ten weeks, there was a significant reduction in the CGS incidence (15-39% decrease), the effect being higher in the heat-processed onion group. Dietary onion markedly reduced cholesterol concentration in the bile, cholesterol: phospholipid ratio, and hence, the biliary cholesterol saturation index (around 0.90 from 1.90 in the onion-free lithogenic diet group). Dietary onion increased the activities of hepatic cholesterol-7α-hydroxylase and sterol-27-hydroxylase that participate in cholesterol catabolism. Serum and liver cholesterol were decreased by feeding onion in this situation. Thus, dietary onion (either raw or heat-processed) exerts a preventive influence on cholesterol gallstone formation by decreasing the cholesterol hypersecretion into bile and also increasing the bile acid secretion thus decreasing the risk of formation of lithogenic bile.

Dietary Onion Accelerates the Regression of the Pre-Established Cholesterol Gallstones in Experimental Studies

In a separate study, the potential of dietary onion in regressing the pre-established cholesterol gallstones was also verified in experimental mice (Vidyashankar et al., 2010a). When the animals were maintained on onion containing (2% dehydrated onion) diet for 10 weeks after inducing CGS in

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mice with a cholesterol-enriched diet, cholesterol gallstones were dissolved by more than 50% by both raw or heat-processed, as compared to only 10% regression in the onion-free control group. Biliary cholesterol and cholesterol saturation index were significantly lowered by dietary onion, concomitant with a reduction in serum and liver cholesterol levels in the post-CGS induction period. Activities of cholesterol degrading enzymes–cholesterol-7α-hydroxylase and sterol 27-hydroxylase were higher in onion fed animals. This suggests that onion consumption effectively accelerates the regression of pre-established cholesterol gallstones by promoting cholesterol desaturation in bile. This nutraceutical potential of dietary onion could find an application in the dietary intervention strategy to address the regression of cholesterol gallstones and stopping its possible recurrence.

Beneficial Effect of Dietary Onion on Biliary Proteins and Lipid Peroxidation Which Influence Cholesterol Nucleation in Bile

Precipitation of cholesterol to form gallstones in the gallbladder is controlled by procrystallizing and anticrystallizing factors present in the bile, besides its supersaturation. While dietary onion has been observed to possess antilithogenic potential in experimental mice, the role of biliary proteins from rats fed lithogenic diet or garlic/ onion containing diet in the formation of cholesterol gallstones has also been studied (Vidyashankar et al., 2010b). The observations of this study suggest that apart from beneficially modulating the biliary cholesterol saturation index, onion consumption also influences the titres of cholesterol nucleating and antinucleating protein factors that contribute to onion's antilithogenic potential.

Antioxidant Effect of Onion Contributing to the Prevention of Degenerative Diseases

Cumulative oxidative stress produced in body tissues has a crucial role in degenerative changes ultimately leading to diseases such as diabetes, CVD, inflammatory diseases, and cancer. Dietary antioxidants are a big help

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in countering the progressive oxidative stress, which supplements the innate antioxidant system. Onion is a major source of flavonoids, especially the two well recognized bioactive compounds, quercetin 4'-*o*-β-glucoside and quercetin 3,4'-*o*-β-diglucoside. The onion extract containing various flavonoids or onion oil are understood to stimulate the cellular antioxidant system by significantly increasing the intracellular concentration of glutathione, activities of glutathione-dependent antioxidant enzymes such as glutathione peroxidase under induced stress conditions. The antioxidant effect of onion oil (100 $mg.kg^{-1}$ for 3 weeks) on nicotine-induced lipid peroxidation studied in rat tissues has indicated that onion oil is an effective antioxidant against the oxidative damage caused by nicotine, its efficacy being higher than vitamin E (Helen et al., 1999). The aqueous extract of onion is shown to inhibit 5-lipoxygenase of human PMNL cells, the key enzyme involved in the biosynthesis of leukotrienes in a concentration-dependent manner (Prasad et al., 2004).

Increased oxidative stress accompanying diabetes mellitus has a precipitative role in the progression of all secondary complications of this condition. Treatment of experimentally induced diabetic rats (for 2 months) with S-methyl cysteine sulfoxide (SMCS) isolated from onion produced a significant antioxidant effect (Kumudkumari & Augusti, 2002). SMCS lowered the levels of lipid peroxidation products similar to insulin in experimental diabetes through stimulation of the inherent antioxidant enzymes, viz., superoxide dismutase and catalase. It has been observed that dietary onion (included at 5% level) reduces the virulent superoxide generation by suppressing the angiotensin-II production, increasing the NO production in the aorta, and lowering the blood pressure in high-fat fed rats (Yamamoto et al., 2005).

Anticancer Properties of Onion

The anticancer property is the latest among the health benefits linked to the consumption of onions. Onions contain flavonols and organosulfur compounds, which are understood to possess anticarcinogenic properties.

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onions may have some protective effect on certain cancers, particularly those of the gastrointestinal tract. Consumption of onion is associated with a lower risk of breast cancer. Researchers have recently identified a sulfur-containing bioactive compound called onionin A (ONA), and it is believed it can suppress a tumor microenvironment. Treatment of mouse epithelial ovarian cancer (EOC) with ONA resulted in a suppression of cancer cell proliferation, which also revealed the abolishment of the signals for chemo-resistance (Tsuboki et al., 2016).

Studies show an inverse association between onion intake and risk of cancers of the lung, prostate, and stomach. There is also recent evidence on the association between onion intake and breast cancer (Desai et al., 2020). In a population-based, case-control study in Puerto Rico, involving 314 primary breast cancer cases, an association of high onion consumption with protection against breast cancer was evidenced in this population.

Onion as Bioavailability Enhancer of Micronutrients from Food Grains

Deficiencies of iron and zinc among the population dependent on plant foods are most common because the bioavailability of these micronutrients is particularly low from plant foods. This necessitates an adoption of viable food-based strategies to maximize their bioavailability to prevent wide-spread deficiencies of these minerals in plant foods dependent population. In the backdrop of dietary sulfur-containing amino acids having been reported to improve the mineral status of experimental animals, sulfur compounds-rich *Allium* spice onion has been examined for a similar beneficial modulation of the mineral bioavailability (Gautam et al., 2010). Exogenously added onion (1.5 and 3 g/10 g grain) generally significantly increased the bioaccessibility of iron from the tested cereal grains (Rice and Sorghum) and pulses (Green gram and Chickpea) in both raw and cooked conditions. Exogenously added onion also similarly enhanced the bioaccessibility of zinc from these cereal and pulse food grains. Thus, onion by its promoting influence on the bioaccessibility of iron and zinc from food

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grains finds potential application in evolving food-based strategy to improve the bioavailability of trace minerals and hence contributes to the human health benefit.

The promoting influence of specific combinations of a food acidulant, β-carotene-rich vegetables, and onion, all of which are known promoters of iron and zinc bioaccessibility has also been evaluated for a possible additive/ synergistic effect on the bioaccessibility of zinc and iron from food grains (Gautam et al., 2011). In this context, each of the individual combinations of the food acidulant *amchur* (0.75 g.10 g^{-1}), β-carotene-rich vegetable (2.5 g.10 g^{-1}), and onion (1.5 g.10 g^{-1}) were evaluated. The positive influence on the bioaccessibility of iron was additive in the case of onion-carrot and onion-amaranth in a few food grains. The positive influence on the bioaccessibility of iron was even synergistic in the case of a*mchur*-onion in a few grains. The combination of *amchur*-onion produced an additive promoting influence on zinc bioaccessibility in a few instances.

While, the sulfur compounds-rich onion is understood to improve the bioaccessibility of iron and zinc from food grains in *in vitro* situation, the beneficial effect *in vivo* of dietary onion along with β-carotene-rich carrot, and the food acidulant ─ *amchur* on zinc status has been assessed during repletion in zinc-deficient rats (Gautam et al., 2012). In this animal study on rats rendered zinc deficient for four weeks, feeding of onion or onion + *amchur* for subsequent two weeks for zinc replenishment, showed a better zinc status in body tissues and bones compared to those repleted with control stock diet.

BIOACCESSIBILITY OF BIOACTIVE POLYPHENOLS FROM ONION

Polyphenols from plant foods are now increasingly understood to be important because of their diverse health beneficial influences. Total polyphenol and flavonoid contents of onion and their bioaccessibility as influenced by heat processing and the presence of food acidulants have been

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evaluated (Hithamani & Srinivasan, 2016). Roasting of onion was found to result in a significant (≈50%) increase in total polyphenols (2.17 $mg.g^{-1}$ fresh onion), while total flavonoids in onion (0.27 $mg.g^{-1}$) remains unchanged as a result of domestic heat processing. Bioaccessible polyphenols and flavonoids from onion are reported to be 0.96 and 0.02 $mg.g^{-1}$, respectively, and open-pan boiling of onion increased the bioaccessible portion of polyphenols from the same. There was a qualitative as well as a quantitative change in the phenolic composition in onion upon addition of the food acidulants particularly, lime and *amchur*. Total bioaccessible polyphenols of onion decreased significantly in presence of lime juice, while the same was generally increased in presence of *amchur*. Bioaccessibility of quercetin from onion increased 6-fold in presence of *amchur*, while a few polyphenols viz., protocatechuic acid, syringic acid, rutin, and myricetin became bioaccessible in presence of this food acidulant. *Amchur* enhanced the bioaccessible polyphenols from onion more than lime juice. Since *amchur* enhances the concentration of bioaccessible polyphenols, its use in food preparations could be a strategy to maximize the bioavailability of polyphenols, especially flavonoids from onion.

CONCLUSION

Onion exerts digestive stimulant action by promoting bile acids-rich bile juice secretion, and by stimulating the digestive enzymes contributed by the pancreatic juice. Onion has potential antidiabetic influences, exerted through reversing the pancreatic pathology and compromised insulin secretion and sensitivity. The antioxidant and hypolipidemic effects of onion in hyperlipidemic and diabetic situations are well documented. Onion is cardioprotective by hypocholesterolemic, antioxidant potential, and anti-thrombotic influence. Onion prevents Cholesterol gallstone disease primarily by modulating cholesterol homeostasis. Generally, the antidiabetic influence of dietary onion was higher when consumed along with dietary fiber-rich fenugreek seeds, which also exerts an antidiabetic effect by an

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independent mechanism. Thus, diabetes and its associated secondary complications can be beneficially ameliorated by consuming these two functional food constituents in right combination to derive an additive/ higher benefit. Onion also enhances the bioavailability of micronutrients from food grains.

REFERENCES

Ahluwalia, P. & Mohindroo, A. 1989. Effect of oral ingestion of different fractions of *Allium cepa* on the blood and erythrocyte membrane lipids and certain membrane-bound enzymes in rats. *J. Nutr. Sci. Vitaminol*. (Tokyo) 35: 155–61.

Augusti, K. T. & Benaim, M. E. 1975. Effect of essential oil of onion on blood glucose, free fatty acid and insulin levels of normal subjects. *Clin. Chim. Acta* 60: 121–23.

Babu, P. S. & Srinivasan, K. 1999. Renal lesions in streptozotocin induced diabetic rats maintained on onion and capsaicin containing diets. *J. Nutr. Biochem*. 10: 477–83.

Babu, P. S. & Srinivasan, K. 1997. Influence of dietary capsaicin and onion on the metabolic abnormalities associated with streptozotocin induced diabetes mellitus. *Mol. Cell. Biochem*. 175: 49–57.

Bordia, T., Mohammed, N., Thomson, M. & Ali, M. 1996. An evaluation of garlic and onion as antithrombotic agents. *Prostaglandins Leukot. Essent. Fatty Acids*, 54: 183–86.

Campos, K. E., Diniz, Y. S., Cataneo, A. C., Faine, L. A., Alves, M. J. & Novelli, E. B. 2003. Hypoglycaemic and antioxidant effects of onion, *Allium cepa*: dietary onion addition, antioxidant activity and hypoglycaemic effects on diabetic rats. *Int. J. Food Sci. Nutr*. 54: 241–46.

Desai, G., Schelske-Santos, M., Nazario, C. M., Rosario-Rosado, R. V., Mansilla-Rivera, I., Ramarez-Marrero, F., Nie, J., Myneni, A. A., Zhang, Z-F., Freudenheim, J. L. & Mu, L. 2020. Onion and garlic intake

Complimentary Contributor Copy

and breast cancer, a case-control study in Puerto Rico. *Nutr. Cancer*, 72: 791-800.

Echi, P. C., Ozougwu, J. C. & Eyo, J. E. 2011. Hypoglycaemic effects of *Allium Cepa, Allium Sativum* and *Zingiber Officinale* aqueous extracts on alloxan induced diabetic *Rattus novergicus*. *Med. J. Islamic World Acad. Sci.* 19: 121–26.

El-Demerdash, F. M., Yousef, M. I. & El-Naga, N. A. 2005. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. *Food Chem. Toxicol*. 43: 57–63.

Eldin, I. M. T. & Ahmed, E. M. 2010. Preliminary study of the clinical hypoglycemic effects of *Allium cepa* (red onion) in type 1 and type 2 diabetic patients. *Environ. Health Insights* 4: 71–77.

El-Soud, N. & Khalil, M. 2010. Antioxidative effects of *Allium cepa* essential oil in streptozotocin induced diabetic rats. *Macedonian J. Med. Sci*. 3: 344–51.

Gautam, S., Platel, K. & Srinivasan, K. 2010. Higher bioaccessibility of iron and zinc from food grains in presence of garlic and onion. *J. Agric. Food Chem.* 58: 8426–29.

Gautam, S., Platel, K. & Srinivasan, K. 2011. Promoting influence of combinations of *amchur*, β-carotene-rich vegetables and *Allium* spices on the bioaccessibility of zinc and iron from food grains. *Int. J. Food Sci. Nutr*. 62: 518–24.

Gautam, S., Platel, K. & Srinivasan, K. 2012. Assessment of zinc deficiency and effect of dietary carrot, amchur and onion on zinc status during repletion in zinc deficient rats. *J. Sci. Food Agri*. 92: 165–70.

Helen, A., Krishnakumar, K., Vijayammal, P. L. & Augusti, K. T. 2000. Antioxidant effect of onion oil (*Allium cepa*. Linn) on the damages induced by nicotine in rats as compared to α-tocopherol. *Toxicol. Lett.* 116: 61–68.

Hithamani, G. & Srinivasan, K. 2016. Bioaccessibility of polyphenols from onion (*Allium cepa*) as influenced by domestic heat processing and food acidulants. *Indian J. Nutr. Dietet*. 53: 391–404.

Kleijnen J, Knipschild P, Terriet G. (1989) Garlic, onions and cardiovascular risk factors. *Br. J. Clin. Pharmacol*. 28: 535–44.

Complimentary Contributor Copy

Kumari, K. & Augusti, K. T. 2002. Antidiabetic and antioxidant effects of S-methyl cysteine sulfoxide isolated from onions (*Allium cepa* Linn) as compared to standard drugs in alloxan diabetic rats. *Indian J. Exp. Biol.* 40: 1005–09.

Lin, M. C., Wang, E. J., Lee, C., Chin, K. T., Liu, D., Chiu, J. F. & Kung, H. F. 2002. Garlic inhibits microsomal triglyceride transfer protein gene expression in human liver and intestinal cell lines and in rat intestine. *J. Nutr*. 132: 1165–68.

Ogunmodede, O. S., Saalu, L. C., Ogunlade, B., Akunna, G. G. & Oyewopo, A. O. 2012. An evaluation of the hypoglycemic, antioxidant and hepatoprotective potentials of onion (*Allium cepa* L.) on alloxan-induced diabetic rabbits. *Int. J. Pharmacol*. 8: 21–29.

Pal, M., Roy, U. K., Datta, S., Ghosh, T., Harlalka, S. & Biswas, L. 2013. Onion peel extracts ameliorate oxidative stress in streptozotocin-induced diabetic rats. *Serbian J. Exp. Clin. Res*. 14: 101–08.

Platel, K. & Srinivasan, K. 1996. Influence of dietary spices or their active principles on digestive enzymes of small intestinal mucosa in rats. *Int. J. Food Sci. Nutr*. 47: 55–59.

Platel, K. & Srinivasan, K. 2000a. Stimulatory influence of select spices on bile secretion in rats. *Nutr. Res.* 20: 1493–1503.

Platel, K. & Srinivasan, K. 2000b. Influence of dietary spices or their active principles on pancreatic digestive enzymes in albino rats. *Nahrung* 44: 42–46.

Platel, K. & Srinivasan, K. 2001. Studies on the influence of dietary spices on food transit time in experimental rats. *Nutr. Res*. 21: 1309–14.

Platel, K. & Srinivasan, K. 2004. Digestive stimulant action of spices: A myth or reality? *Indian J. Med. Res*. 119: 167–79.

Pradeep, S. R. & Srinivasan, K. 2017a. Amelioration of hyperglycemia and associated metabolic abnormalities by a combination of fenugreek (*Trigonella foenum-graecum*) seeds and onion (*Allium cepa*) in experimental diabetes. *J. Basic Clin. Physiol. Pharmacol*. 28: 493–505.

Pradeep, S. R. & Srinivasan, K. 2017b. Amelioration of oxidative stress by dietary fenugreek (*Trigonella foenum-graecum* L.) seeds is potentiated

Complimentary Contributor Copy

by onion (*Allium cepa* L.) in streptozotocin-induced diabetic rats. *Appl. Physiol. Nutr. Metab*. 42: 816–28.

Pradeep, S. R. & Srinivasan, K. 2018a. Alleviation of oxidative stress-mediated renal injury by dietary fenugreek (*Trigonella foenum-graecum*) seeds and onion (*Allium cepa*) in streptozotocin-induced diabetic rats. *Food Funct*. 9: 134–48.

Pradeep, S. R. & Srinivasan, K. 2018b. Haemato-protective influence of dietary fenugreek (*Trigonella foenum-graecum* L.) seeds and onion (*Allium cepa* L.) in streptozotocin-induced diabetic rats. *Biomed. Pharmacother*. 98: 372─81.

Pradeep, S. R. & Srinivasan, K. 2018c. Alleviation of cardiac damage by dietary fenugreek (*Trigonella foenum-graecum*) seeds is potentiated by onion (*Allium cepa*) in experimental diabetic rats *via* blocking renin-angiotensin system. *Cardiovasc. Toxicol*. 18: 221–31.

Pradeep, S. R. & Srinivasan, K. 2018d. Ameliorative influence of dietary fenugreek (*Trigonella foenum-graecum*) seeds and onion (*Allium cepa*) on eye lens abnormalities *via* modulation of crystallin proteins and polyol pathway in experimental diabetes. *Curr. Eye Res*. 43: 1108─18.

Pradeep, S. R. & Srinivasan, K. 2019. Synergy among dietary spices in exerting antidiabetic influences. In: Second edition of "*Bioactive Foods as Dietary Interventions for Diabetes",* Victor R. Preedy & Ronald R. Watson (Eds), Academic Press, London, UK; Chapter-28; pp. 407–24.

Pradeep, S. R., Barman, S. & Srinivasan, K. 2019. Attenuation of diabetic nephropathy by dietary fenugreek seeds (*Trigonella foenum-graecum*) and onion (*Allium cepa*) *via* suppression of glucose transporters and rennin-angiotensin system. *Nutrition* 67–68: 110543.

Prasad, N. S., Raghavendra, R., Lokesh, B. R., & Naidu, K. A. 2004. Spice phenolics inhibit human PMNL 5-lipoxygenase. *Prostaglandins Leukot. Essent. Fatty Acids*, 70: 521–28.

Sambaiah, K. & Srinivasan, K. 1991. Secretion and composition of bile in rats fed diets containing spices. *J. Food Sci. Technol*. 28: 35–38.

Sebastian, K. L., Zacharias, N. T., Philip, B. & Augusti, K. T. 1979. The hypolipidemic effect of onion (*Allium cepa*) in sucrose fed rabbits. *Indian J. Physiol. Pharmacol*. 23: 27–30.

Complimentary Contributor Copy

Sharma, K. K., Gupta, R. K., Gupta, S. & Samuel, K. C. 1977. Antihyperglycemic effect of onion: effect on fasting blood sugar and induced hyperglycemia in man. *Indian J. Med. Res*. 65: 422–29.

Sheela, C. G., Kumari, K. & Augusti, K. T. 1995. Anti-diabetic effects of onion and garlic sulfoxide amino acids in rats. *Planta Med*. 61: 356–57.

Srinivasan, K. 2013. Dietary spices as beneficial modulators of lipid profile in conditions of metabolic disorders and diseases. *Food Funct.* 4: 503–21.

Srinivasan, K. 2014. Antioxidant potential of spices and their active constituents. *Crit. Rev. Food Sci. Nutr*. 54(3): 352–72.

Srinivasan, K. 2019. Cardio protective influence of dietary spices mediated through their hypolipidemic and antioxidant potential. In: "*Oxidative Stress in Heart Diseases*," Chakraborti, S., Dhalla, N. S., Ganguly, N. K. & Dikshit, M. (Eds), Springer, Germany; Chapter-8; pp. 173–89.

Tsuboki, J., Fujiwara, Y., Horlad, H., Shiraishi, D., Nohara, T., Tayama, S., Motohara, T., Saito, Y., Ikeda, T., Takaishi, K., Tashiro, H., Yonemoto, Y., Katabuchi, H., Takeya, M. & Komohara, Y. 2016, Onionin A inhibits ovarian cancer progression by suppressing cancer cell proliferation and the protumour function of macrophages. *Scientific Reports*, 6: 29588

Vatsala, T. M. & Singh, M. 1981. Relationship between plasma cholesterol level and erythrocytes shape in rabbits on atherogenic diet and onion extracts. *Curr. Sci.* 50: 211–13.

Vidyashankar, S., Sambaiah, K. & Srinivasan, K. 2009. Dietary garlic and onion reduce the incidence of atherogenic diet induced cholesterol gallstone in experimental mice. *Br. J. Nutr*. 101: 1621–29.

Vidyashankar, S., Sambaiah, K. & Srinivasan, K. 2010a. Regression of preestablished cholesterol gallstones by dietary garlic and onion in experimental mice. *Metabolism* 59: 1402–12.

Vidyashankar, S., Sambaiah, K. & Srinivasan, K. 2010b. Effect of dietary garlic and onion on biliary proteins and lipid peroxidation which influence cholesterol nucleation in bile. *Steroids* 75: 272–81.

Complimentary Contributor Copy

Yamada, K., Naemura, A., Sawashita, N., Noguchi, Y. & Yamamoto, J. 2004. An onion variety has natural antithrombotic effect as assessed by thrombosis/thrombolysis models in rodents. *Thromb. Res*. 114: 213–20.

Yamamoto, Y., Aoyama, S., Hamaguchi, N. & Rhi, G. S. 2005. Antioxidative and antihypertensive effects of Welsh onion on rats fed with a high-fat high-sucrose diet. *Biosci. Biotechnol. Biochem*. 69: 1311–17.

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Chapter 6

OCCAM'S RAZOR, HICKAM'S DICTUM, AND THE ODYSSEY OF RARE DISEASE DIAGNOSIS

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ABSTRACT

Occam's razor proffers guidance in the diagnosis of rare diseases, suggesting that the most likely diagnosis is that which can explain every symptom. This logic should lead a clinician who is attuned to their patient to arrive at a short list of the compatible causes which can explain all of—even the slightest—findings of their patient's physical exam. In conflict with this enduring philosophical principle, however, Hickam's dictum observes that it is not always so straightforward: one patient can experience multiple conditions simultaneously. With respect to the very rarest of diseases, statistics appear to support Hickam's dictum in that two relatively common ailments may in fact be a more likely explanation for a patient's constellation of symptoms than a singular and remarkably atypical disease.

Consequently, the reality of rare disease diagnosis is defined by a process commonly referred to as a "diagnostic odyssey": a journey fraught

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with diagnostic delays and misdiagnoses, which a patient is forced to negotiate even while experiencing the symptoms of their illness. Intuitively, medical training and clinical practice afford greater exposure to more common diseases, necessarily resulting in many uncommon and unusually maladies—simply by virtue of their rarity—resisting easy diagnosis.

Despite the obstacles therefore inherent in rare disease diagnosis, several systems-level interventions can be expected to limit the diagnostic delays experienced by patients with rare diseases, and the frustration associated with years or decades of untreated symptoms. These interventions include increased integration at the levels of medical training and practice, the utilization of emerging digital resources designed to assist with rare disease diagnosis, and the adoption of patient navigators to assist and advocate for patients during their diagnostic odyssey. While many of the challenges intrinsic to diagnosing rare diseases are therefore ineradicable, interventions designed to ameliorate the diagnostic odyssey can nonetheless be meaningful for patients.

Keywords: Occam's razor, Hickam's dictum, diagnostic odyssey, diagnostic challenges, rare diseases

OCCAM'S RAZOR

The law of parsimony, or Occam's razor, is a logical principle attributed to the 14th century English theologian and philosopher William of Occam (Re and Bellini 2002). It states: "do not multiply entities without necessity" (Schaffer 2015), representing the attitude that the simpler of two explanations (or that with the fewest assumptions) is most likely to be the right one. In other words, the most probably explanation for any event is the one which most closely aligns with the available information, contradicting the least of what is already known and requiring the least of what is not. This maxim has found many applications in modern medicine, beginning with the adaptation dubbed "Osler's rule" which holds that a single unifying diagnosis that can explain each of a patient's symptoms is the most likely to be correct (Michelson 2004).

Occam's razor and its derivatives are irreplaceable for lending economy to the process of developing theories and explanations, securing their place

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in the toolbox of a diagnostician: numerous reports exist of the principle's successful application to identify a variety of ailments without necessitating an exhaustive work-up to rule out every other possible cause (Foussias and Remington 2010; Gomes et al. 2003; Lui et al. 2015). Indeed, the razor has a high predictive value in many circumstances: a patient who presents to the clinic with exertional shortness of breath, lightheadedness, and chest pain *could* be suffering concurrently from a pneumonia, dehydration, and a peptic ulcer, but it is much more likely that they have aortic stenosis (a single, common heart valve disease which classically causes all three symptoms).

Occam's razor is therefore also a mainstay of rare disease diagnosis, and clinicians can apply it fruitfully in the assessment of patients experiencing unique constellations of symptoms with no know cause. For instance, although the diagnosis of a pheochromocytoma is rather rare, it may be the best explanation for a patient every one of the dozens of symptoms associated with this tumor. Each of these symptoms is shared between pheochromocytoma and a multitude of other conditions, but when all of these symptoms are experienced in concert the rare diagnosis paradoxically becomes a common explanation. Even in circumstances where no single disease is implicated by the full list of signs and symptoms present (and not present), Occam's razor can offer a limited pool of possible diagnoses which is able to guide further testing to acquire information which can distinguish them.

Despite the demonstrable utility of Occam's razor, it is not a perfect diagnostic tool. Occam's logic is elegant and easy to apply in practice, but it has one critical weakness intrinsic to its reliance on probabilities: even when a single explanation is the most likely, the reality of multiple explanations is possible.

HICKAM'S DICTUM

This criticism levied against Occam's razor was first attributed to the 20th century American physician John Hickam in what has become infamously known as Hickam's dictum: "a patient can have as many

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diseases as they damn well please" (Mani et al. 2011). Hickam's dictum identifies that at least some patients are bound to experience multiple diseases simultaneously, therefore finding itself in direct opposition with Occam's razor (Gahl et al. 2016; Gupta et al. 2012). This, too, is reflected in countless reports of patients whose diagnosis (of two or more conditions) was wrongly oversimplified by Occam's razor (Balci et al. 2017; Drachman 2000; Wendy et al. 2019).

With respect to the rarest of diseases, probability actually favours Hickam. Even if a patient presents with fatigue, frequent nosebleeds, peripheral neuropathy, and blurred vision—essentially the classic symptoms of Waldenström's macroglobulinemia, a rare disease with an estimated incidence of approximately three people per million per year (Kyle et al. 2018)—a combined diagnosis of diabetes (which has a worldwide prevalence of almost 10%; (Forouhi and Wareham 2014) and anemia secondary to a nosebleed (which has an estimated lifetime incidence of 60%; (Gifford and Orlandi 2008) is more likely.

Unfortunately, Hickam's dictum is unable to provide guidance for rare disease diagnosis beyond the portent that limiting possible explanations to a single diagnosis risks premature diagnostic closure and misdiagnosis. Taken together, however, Hickam's dictum and Occam's razor remain useful in illustrating the natural history of rare disease diagnosis: there are no shortcuts. In the absence of a simple principle to guide the delivery of an uncommon diagnosis, clinicians are left to rely on gathering as much information as possible by way of obtaining a patient history, performing physical exams, and conducting tests. The patient, for their part, often has no choice but to tolerate their undiagnosed illness for the duration of this long and taxing process.

THE DIAGNOSTIC ODYSSEY

In the context of rare diseases, the journey to receiving a correct diagnosis is drawn-out and weathered by ailing patients who sometimes

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spend years or decades seeking validation in the form of an explanation for their symptoms (Austin et al. 2018). The average delay from symptom onset to diagnosis of cblC cobalamin metabolism defects, to provide one example, is reported to range from 3 months to 20 or more years (Huemer et al. 2014). This same diagnostic delay for early-onset dementia averages 4.4 years (Bakker et al. 2013). In these cases, reconciling Occam's razor and Hickam's dictum to arrive at an accurate diagnosis takes considerable time and patience.

Rare diseases are generally defined as those conditions with a population frequency of 1 in 2000 or less (Richter et al. 2015), but as a collective this group of diseases is estimated to effect almost half a million people worldwide (Wakap et al. 2020). The diagnostic odyssey, a common pathway overrun with misdiagnoses and diagnostic delays which many patients with rare diseases find themselves forced to negotiate, is a widespread, frustrating, and often intrinsically harmful misadventure (Brenna 2020; Esquivel-Sada and Nguyen 2018).

The etiology of the diagnostic odyssey is multifactorial and, regrettably, many of its causes resist elimination. Intuitively, physicians will be better prepared to recognize common diseases: this is because, by virtue of Occam's razor, medical training can be expected to focus on those afflictions which physicians can expect to see with the greatest frequency, and also because physicians who have already entered into practice will statistically be afforded less clinical exposure to uncommon diseases (Purohit 2019). As a consequence, one cause of the diagnostic odyssey is that physicians are inherently not as well-prepared to diagnose and treat rare diseases.

NAVIGATING THE ODYSSEY

Despite these innate barriers to diagnosis, the diagnostic odyssey for patients with rare diseases is nevertheless actionable from a systems perspective. Even if some delay in the diagnosis of these conditions cannot be altogether extinguished, there are several levels at which assiduously

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crafted interventions can contribute to arriving at an end point for this diagnostic odyssey.

There is already evidenced effectiveness for several interventions, including: the incorporation of probabilistic diagnostic decision support systems—such as IBM's artificially-intelligent supercomputer, Watson (Brenna 2019)—in the clinical setting, which effectively combine Occam's razor, Hickam's dictum, and supercomputing to suggest the possibility of rare diseases based on the consistency of diagnostic criteria and case presentations (Ronicke et al. 2019; Svenstrup et al. 2015); the use of devoted rare disease search engines such as FindZebra, which are demonstrated effectively assist with the diagnosis of rare diseases more readily than non-specific search engines such as Google or PubMed (Svenstrup et al. 2015); the creation of medical centers specifically focused on the diagnosis and treatment of rare diseases, which have been trialed in China and are credited with accelerating the diagnostic odyssey (Kang et al. 2018); and new and emerging technologies such as DNA sequencing which have demonstrated efficacy for diagnosing rare diseases with high sensitivity and specificity, presenting—especially as costs decrease—a possible future for panel testing in cases of ambivalent diagnosis (Warman Chardon et al. 2015). In parallel with these advances, there is a growing body of evidence to suggest a role for education in alleviating the diagnostic odyssey: an international survey performed in 2013 indicated that a majority of physicians, intuitively, agreed that additional training around rare diseases would support quicker diagnosis (Engel et al. 2013).

The enduring diagnostic odyssey warrants further considerations of new means for reducing the time-to-diagnosis for patients with rare diseases. However, the demonstrated utility of these interventions which have already been adopted does illuminate several of the core causes of diagnostic delay in these circumstances: a lack of integrated clinician education, limited resources which can provide easy access to information relating to rare diseases, and a healthcare system structure in which patients can "fall through the cracks" and suffer longer odysseys due to a lack of follow-up. The remainder of this chapter provides perspective on the future of this task by way of suggesting several novel and promising interventions which have

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not been evidenced in the context of rare disease diagnosis. For clinicians, these interventions include updates in medical education with a greater focus on integration between subjects, as well as the utilization of new in-practice diagnostic tools designed to help address rare diseases (similar to those described above, which have previously been successful). Beyond clinicians, there is also an emerging and unique role in rare disease medicine for patient navigators who are able to aid patients on their diagnostic odyssey.

Medical Education

Medical education bears risks of perpetuating the diagnostic odyssey by presenting medicine in silos. In traditional block-based medical curricula, such as that described by Li and colleagues with reference to Chinese undergraduate medical education (representative of many other countries' training programs), students learn topics related to one body system or one specialty before moving on to the next (Li et al. 2018). In the US, this same format has been demonstrated to effectively teach the information within each block, but it discourages integration between these blocks (Muller et al. 2008). The training of clinicians therefore appears to divide them into experts of disparate fields in a landscape where few have full understanding of the interfaces between them (Muller et al. 2008; Vergel et al. 2017). As a consequence, students graduate into a practice in which they may struggle to form cohesive teams with specialists of other areas. This is compounded structures within the healthcare system which preserve the division between these silos clinicians practice in, where there may not be consensus on who is the most responsible provider in charge of a patients' care. Physicians fax referrals and seldom follow up to make sure they were received—a large proportion of referred patients do not even appear to ultimately see the provider they were referred to (Chameides and Yamamoto 1973; Garneau et al. 2018). At times, it is likely that the tension between resource stewardship (which includes limiting referrals which are not obviously necessary) and

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quality patient care is also contributing to diagnostic delays (Ndeikoundam Ngangro et al. 2012).

It is inevitable that no one provider can have expertise about everything within medicine, and therefore be well-situated to easily diagnose rare diseases with multi-system presentations. However, it can be expected that through improved connectedness, we will be able to limit those spaces between specialists where patients with undiagnosed rare diseases and on their diagnostic odyssey are temporarily or permanently lost to follow-up. At the level of medical education, this warrants a greater focus on healthcare systems integration. It is possible that this would require a departure from block-based curricula toward an alternative such as the "spiral curriculum" (a method characterized by repetitive exposure to topics on a longitudinal basis, which has been evidenced in some settings but not yet studied in the context of rare disease education; (Fraser et al. 2019; Masters and Gibbs 2007). Additionally, clinicans have indicated that rare disease diagnosis would be augmented by increased training around what circumstances would warrant casting a wide diagnostic net to appropriately bring in specialists from many fields (Engel et al. 2013).

Novel Technologies

For clinicians already in practice, there are several extant tools with the novel ability to aid physicians in recognizing and diagnosing rare diseases. Orphanet, one example of these aids, is a digital resource dedicated to mapping out rare diseases to make their identification and management accessible to health providers, patients and families, and researchers (Rath et al. 2012; Weinreich et al. 2008). By leveraging technological innovations like mass information storage and intelligent search algorithms, these types of databases are able to put a wealth of information about rare disease at the fingertips of every participant in the diagnostic odyssey (Rath et al. 2012). Orphanet and its comrades vet experts to contribute information on rare diseases, and make this information freely available to everyone through search engines specific to uncommon diseases (Weinreich et al. 2008). For

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physicians in practice, utilizing these resources when the possibility of a rare disease arises can shorten the time from symptom onset to diagnosis for patients with unexplained presentations (and who have already, unfruitfully, seen several specialists).

More actively, novel genome sequencing technologies are finding application as auxiliary services in the diagnostic odyssey. Next-generation sequencing is an increasingly affordable and rapid means of identifying rare diseases with genetic causes or associations (Fernandez-Marmiesse et al. 2017). To do so, a patient's DNA (in part or in entirety) is rapidly sequenced to identify any of thousands of genes known to cause rare diseases, allowing for a molecular diagnosis to be made without delay (Boycott et al. 2013). Whole-exome and whole-genome sequencing offer an even more thorough search for diagnosis, and the resource-intensive nature of these tests is offset by their unprecedented ability to 'screen' for an enormous number of diseases with genetic associations, securing the position of these tests as emerging flagship technologies in the diagnostic odyssey of paediatric patients (Wright et al. 2018). In 2019, the molecular diagnostic rate of whole-exome sequencing was estimated at 50% (Frésard et al. 2019). DNA sequencing technology is therefore emerging as a frontrunner among technologies which can enable the diagnosis of rare diseases. As these technologies become more accessible, it can be anticipated that there may be a role for next-generation sequencing in newborn screenin, in addition to investigation prompted by symptomology (Botkin and Rothwell 2016).

Looking even further ahead, machine learning technology is beginning to contribute to the diagnosis of rare diseases. Artificially-intelligent systems offer diagnostic assistance by "training" on large datasets and then using encoded patterns to make predictions from new data (Schaefer et al. 2020). In one study by Garg and colleagues, machine learning was applied to retrospectively review electronic medical records and attempt to identify patients with cardiac amyloidosis on the basis of features extracted from the records of patients known to have this rare condition (Garg et al. 2016). Machine learning has been applied experimentally in the context of nearly 100 unique rare diseases and the role it will play in the future of rare disease diagnosis continues to take shape (Schaefer et al. 2020).

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Patient Navigators

A third intervention which shows promise in alleviating the diagnostic odyssey is the implementation of patient navigators—particularly those with specialized training in the context of rare diseases—in the healthcare system. These professionals are trained to provide individualized assistance to patients and their families, helping them to negotiate the labyrinthian medical system in which it is easy to get lost between providers (Ranaghan et al. 2016)Patient navigators comprise a relatively new profession within the medical system, but one already evidenced to bolster the continuity of patient care and help to connect patients with the right resources—whether that is the appropriate doctors or reliable sources of information (Walkinshaw 2011). Patient navigators are able to contribute to greater integration of care between disciplines and settings (Doucet et al. 2019), reflected in significant increases in patient satisfaction (Tho and Ang 2016).

In the context of rare diseases, patient navigators are well-situated to accompany patients on their diagnostic journey, advocating for those patients who are temporarily forgotten within the system (Freeman 2006). It is known that patients with undiagnosed rare diseases tend to have many physicians involved in their care, which can make for a particularly confusing medical record and increasing diagnostic uncertainty (Kliegman et al. 2017). In these circumstances, there is a unique role for patient navigators to forge therapeutic relationships with patients so that they do not have to navigate the diagnostic odyssey alone.

CONCLUSION

The diagnostic odyssey associated with rare disease is in some ways inevitable, as identified in tensions between Occam's razor and Hickam's dictum: a single, unifying diagnosis may be less probably than the coexistence of several common diseases, relegating the correct explanation to the category of diagnoses by exclusion. As a result, many patients with

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rare diseases engage on a consuming journey, the diagnostic odyssey, which begins surreptitiously with the onset of a first and often non-specific symptom. This odyssey is a major contributor to dissatisfaction with the healthcare system, as well as to increased morbidity and mortality in the context of diseases for which treatment delays can be of great consequence (Budych et al. 2012; Zurynski et al. 2017).

Despite the intuitive nature of delayed diagnoses for diseases which are unfamiliar to physicians, the diagnostic odyssey remains an actionable target. Through broad health systems reform clinicans can develop their environment into one which more effectively serves the patients negotiating the corkscrewing path of an elusive diagnosis. These proposed interventions include: embracing integration in medical education; encouraging the further creation and utilization of high-quality resources geared towards rare disease diagnosis;continuing to develop DNA sequencing, machine learning, and other novel diagnostic technologies; and accepting help from other professionals such as patient navigators.

Responding to Occam's razor and Hickam's dictum, we can infer a more tempered logical principle that the most likely explanation for a patient's presenting symptoms is that—whether it is one or several diagnoses, on balance of cumulative probabilities—which is most consistent with the available information. In many cases, this approach will not provide a definitive diagnosis without first pursuing additional information, which is a fundamental part of medical diagnosis in any context. With respect to rare diseases, however, the investigations required (and the length of time which can elapse between them) can be components of the diagnostic odyssey, representing points at which providers can intervene to make a meaningful difference for their patients.

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REFERENCES

Austin, C. P., Cutillo, C. M., Lau, L. P. L., Jonker, A. H., Rath, A., Julkowska, D., et al. (2018). Future of Rare Diseases Research 2017–2027: An IRDiRC Perspective. *Clinical and Translational Science.* Blackwell Publishing Ltd.

Bakker, C., de Vugt, M. E., van Vliet, D., Verhey, F. R. J., Pijnenburg, Y. A., Vernooij-Dassen, M. J. F. J., & Koopmans, R. T. C. M. (2013). Predictors of the Time to Institutionalization in Young- Versus Late-Onset Dementia: Results from the Needs in Young Onset Dementia (NeedYD) Study. *Journal of the American Medical Directors Association*, 14(4), 248–253.

Balci, T. B., Hartley, T., Xi, Y., Dyment, D. A., Beaulieu, C. L., Bernier, F. P., et al. (2017). Debunking Occam's razor: Diagnosing multiple genetic diseases in families by whole-exome sequencing. *Clinical Genetics*, 92(3), 281–289.

Botkin, J. R., & Rothwell, E. (2016). Whole Genome Sequencing and Newborn Screening. *Current Genetic Medicine Reports*, 4(1), 1–6.

Boycott, K. M., Vanstone, M. R., Bulman, D. E., & MacKenzie, A. E. (2013). Rare-disease genetics in the era of next-generation sequencing: Discovery to translation. *Nature Reviews Genetics*. Nature Publishing Group.

Brenna, C. (2019). Watson. *University of Toronto Medical Journal*, 96(1), 25–26.

Brenna, C. (2020). The Journey to Ancient Ithaca: Addressing the diagnostic odyssey of rare disease. *University of Toronto Medical Journal*, 97(3), 37–39.

Budych, K., Helms, T. M., & Schultz, C. (2012). How do patients with rare diseases experience the medical encounter? Exploring role behavior and

Complimentary Contributor Copy

its impact on patient-physician interaction. *Health Policy*, 105(2–3), 154–164.

Chameides, W. A., & Yamamoto, J. (1973). Referral failures: a one year follow up. *American Journal of Psychiatry*, 130(10), 1157–1158.

Doucet, S., Luke, A., Splane, J., & Azar, R. (2019). Patient navigation as an approach to improve the integration of care: The case of navicare/soinsnavi. *International Journal of Integrated Care*, 19(4).

Drachman, D. A. (2000). *Occam's razor, geriatric syndromes, and the dizzy patient. Annals of Internal Medicine*. American College of Physicians.

Engel, P. A., Bagal, S., Broback, M., & Boice, N. (2013). Physician and patient perceptions regarding physician training in rare diseases: The need for stronger educational initiatives for physicians. *The Journal of Rare Disorders*, 1(2), 1–15.

Esquivel-Sada, D., & Nguyen, M. T. (2018). Diagnosis of rare diseases under focus: impacts for Canadian patients. *Journal of Community Genetics*, 9(1), 37–50.

Fernandez-Marmiesse, A., Gouveia, S., & Couce, M. L. (2017). NGS Technologies as a Turning Point in Rare Disease Research, Diagnosis and Treatment. *Current Medicinal Chemistry*, 25(3).

Forouhi, N. G., & Wareham, N. J. (2014). *Epidemiology of diabetes*. *Medicine* (United Kingdom). Elsevier Ltd.

Foussias, G., & Remington, G. (2010). Negative symptoms in schizophrenia: Avolition and Occam's razor. *Schizophrenia Bulletin*.

Fraser, S., Wright, A. D., van Donkelaar, P., & Smirl, J. D. (2019). Cross-sectional comparison of spiral versus block integrated curriculums in preparing medical students to diagnose and manage concussions. *BMC Medical Education*, 19(1), 1–8.

Freeman, H. P. (2006). Patient navigation: A community based strategy to reduce cancer disparities. *Journal of Urban Health*. Springer Science and Business Media Deutschland GmbH.

Frésard, L., Smail, C., Ferraro, N. M., Teran, N. A., Li, X., Smith, K. S., et al. (2019). Identification of rare-disease genes using blood transcriptome sequencing and large control cohorts. *Nature Medicine*. Nature Publishing Group.

Complimentary Contributor Copy

Gahl, W. A., Mulvihill, J. J., Toro, C., Markello, T. C., Wise, A. L., Ramoni, R. B., et al. (2016). The NIH Undiagnosed Diseases Program and Network: Applications to modern medicine. *Molecular Genetics and Metabolism*. Academic Press Inc.

Garg, R., Dong, S., Shah, S., & Jonnalagadda, S. R. (2016). *A Bootstrap Machine Learning Approach to Identify Rare Disease Patients from Electronic Health Records*. http://arxiv.org/abs/1609.01586.

Garneau, J. C., Wasserman, I., Konuthula, N., & Malkin, B. D. (2018). Referral patterns from emergency department to otolaryngology clinic. *Laryngoscope*, 128(5), 1062–1067.

Gifford, T. O., & Orlandi, R. R. (2008). *Epistaxis. Otolaryngologic Clinics of North America*. Elsevier. https://doi.org/10.1016/j.otc.2008.01.003.

Gomes, M. E. R., Deinum, J., Timmers, H. J. L. M., & Lenders, J. W. M. (2003). Occam's razor; anaemia and orthostatic hypotension. *Lancet*, 362(9392), 1282.

Gupta, N., Aragaki, A., Wikenheiser-Brokamp, K. A., Benzaquen, S., & Panos, R. J. (2012). Occam's razor or hickam's dictum? *Journal of Bronchology and Interventional Pulmonology*, 19(3), 216–219.

Huemer, M., Scholl-Bürgi, S., Hadaya, K., Kern, I., Beer, R., Seppi, K., et al. (2014). Three new cases of late-onset cblC defect and review of the literature illustrating when to consider inborn errors of metabolism beyond infancy. *Orphanet journal of rare diseases*. BioMed Central.

Kang, Q., Hu, J., Song, P., & He, J. (2018). System building and improvement for the diagnosis and treatment of rare diseases in Shanghai, China. *Intractable and Rare Diseases Research*, 7(4), 291–294.

Kliegman, R. M., Ruggeri, B. E., & Smith, M. M. (2017). The Team-Based Approach to Undiagnosed and Rare Diseases. *Pediatric Clinics of North America*. W. B. Saunders.

Kyle, R. A., Larson, D. R., McPhail, E. D., Therneau, T. M., Dispenzieri, A., Kumar, S., et al. (2018). Fifty-Year Incidence of Waldenström Macroglobulinemia in Olmsted County, Minnesota, From 1961 through 2010: A Population-Based Study with Complete Case Capture and Hematopathologic Review. *Mayo Clinic Proceedings*, 93(6), 739–746.

Complimentary Contributor Copy

Li, C., Wang, L., Zhang, Y., Li, C., Xu, Y., Shang, L., & Xia, J. (2018). Assessment of a block curriculum design on medical postgraduates' perception towards biostatistics: A cohort study. *BMC Medical Education*, 18(1), 1–10.

Lui, J. K., Gillespie, W., Gaudet, R., & Brettler, D. B. (2015). Occam's razor and the diagnosis of mantle cell lymphoma. *American Journal of Medicine*, 128(12), e25–e26.

Mani, N., Slevin, N., & Hudson, A. (2011). The Three Wise Men on diagnosis. *BMJ*, 343(7837), 1301–1304.

Masters, K., & Gibbs, T. (2007). The Spiral Curriculum: Implications for online learning. *BMC Medical Education*, 7(1), 52.

Michelson, J. (2004). Critique of (im)pure reason: Evidence-based medicine and common sense. *Journal of Evaluation in Clinical Practice.*

Muller, J. H., Jain, S., Loeser, H., & Irby, D. M. (2008). Lessons learned about integrating a medical school curriculum: Perceptions of students, faculty and curriculum leaders. *Medical Education*, 42(8), 778–785.

Ndeikoundam Ngangro, N., Chauvin, P., & Halley des Fontaines, V. (2012). Les déterminants du délai de diagnostic de la tuberculose dans les pays aux ressources limitées. Revue d'Epidemiologie et de Sante Publique. *Rev Epidemiol Sante Publique*.

Purohit, K. (2019). *The Baader-Meinhof Phenomenon in Radiology. Academic Radiology*. Elsevier USA. https://doi.org/10.1016/j.acra.2019.01.025.

Ranaghan, C., Boyle, K., Meehan, M., Moustapha, S., Fraser, P., & Concert, C. (2016). Effectiveness of a patient navigator on patient satisfaction in adult patients in an ambulatory care setting: a systematic review. *JBI database of systematic reviews and implementation reports.*

Rath, A., Olry, A., Dhombres, F., Brandt, M. M., Urbero, B., & Ayme, S. (2012). Representation of rare diseases in health information systems: The orphanet approach to serve a wide range of end users. *Human Mutation*, 33(5), 803–808.

Re, V. lo, & Bellini, L. M. (2002). *William of Occam and Occam's razor. Annals of Internal Medicine.* American College of Physicians.

Complimentary Contributor Copy

Richter, T., Nestler-Parr, S., Babela, R., Khan, Z. M., Tesoro, T., Molsen, E., & Hughes, D. A. (2015). Rare Disease Terminology and Definitions-A Systematic Global Review: Report of the ISPOR Rare Disease Special Interest Group. *Value in Health*, 18(6), 906–914.

Ronicke, S., Hirsch, M. C., Türk, E., Larionov, K., Tientcheu, D., & Wagner, A. D. (2019). Can a decision support system accelerate rare disease diagnosis? Evaluating the potential impact of Ada DX in a retrospective study. *Orphanet Journal of Rare Diseases*, 14(1), 1–12.

Schaefer, J., Lehne, M., Schepers, J., Prasser, F., & Thun, S. (2020). The use of machine learning in rare diseases: a scoping review. *Orphanet journal of rare diseases. NLM* (Medline). https://doi.org/10.1186/ s13023-020-01424-6.

Schaffer, J. (2015). What Not to Multiply Without Necessity. *Australasian Journal of Philosophy*, 93(4), 644–664.

Svenstrup, D., Jørgensen, H. L., & Winther, O. (2015). Rare disease diagnosis: A review of web search, social media and large-scale data-mining approaches. *Rare Diseases*, 3(1), e1083145.

Tho, P. C., & Ang, E. (2016). The effectiveness of patient navigation programs for adult cancer patients undergoing treatment: a systematic review. *JBI database of systematic reviews and implementation reports*, 14(2), 295–321.

Vergel, J., Stentoft, D., & Montoya, J. (2017). Extending the theoretical framework for curriculum integration in pre-clinical medical education. *Perspectives on Medical Education*, 6(4), 246–255.

Wakap, S. N., Lambert, D. M., Olry, A., Rodwell, C., Gueydan, C., Lanneau, V., et al. (2020). Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database. *European Journal of Human Genetics*, 28(2), 165–173.

Walkinshaw, E. (2011). Patient navigators becoming the norm in Canada. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne*.

Warman Chardon, J., Beaulieu, C., Hartley, T., Boycott, K. M., & Dyment, D. A. (2015). Axons to Exons: the Molecular Diagnosis of Rare

Complimentary Contributor Copy

Neurological Diseases by Next-Generation Sequencing. Current Neurology and Neuroscience Reports. *Current Medicine Group LLC 1*.

Weinreich, S. S., Mangon, R., Sikkens, J. J., Teeuw, M. E. en, & Cornel, M. C. (2008). [Orphanet: a European database for rare diseases]. *Nederlands tijdschrift voor geneeskunde*, 152(9), 518–9.

Wendy, C., Steven, D., & Sharp, L. (2019). When Occam's razor fails: A McArdle disease diagnosis following idiopathic transverse myelitis. *Neurology*, 92(15 Supplement 1).

Wright, C. F., FitzPatrick, D. R., & Firth, H. v. (2018). Paediatric genomics: Diagnosing rare disease in children. *Nature Reviews Genetics*. Nature Publishing Group.

Zurynski, Y., Deverell, M., Dalkeith, T., Johnson, S., Christodoulou, J., Leonard, H., & Elliott, E. J. (2017). Australian children living with rare diseases: experiences of diagnosis and perceived consequences of diagnostic delays. *Orphanet Journal of Rare Diseases*, 12(1), 1–9.

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Chapter 7

IRON DEFICIENCY ANEMIA

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ABSTRACT

Recently, iron deficiency and anemia due to iron deficiency is one of the most common medical conditions in clinical practice worldwide. Since in emerging countries mostly malnutrition, insufficient uptake of iron, and parasitic infestations are main cause, in developed countries iron deficiency is still not rare due to vegaterian diet and malignancies. Iron deficiency is usually asymptomatic but also can be seen with widespread clinical features, such as; weakness, fatigue, exortional dyspnea, restless leg syndrome. To diagnose iron deficiency, traditional laboratory tests are complete blood count and peripheral blood smear tests. Transferrin

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saturation and ferritin tests are the most commonly used in diagnosis. Rarely, bone marrow biopsy is needed. Oral iron administration is first line treatment for iron deficiency but intravenous administration of iron can be preferred in conditions such as: drug intolerance, deep anemia, chronic renal failure. Reticulocyte count and complete blood count can be used for evaluating therapy response. Iron deficiency anemia is one of the most common medical condition and important cause of mortality and morbidity so that diagnosis, treatment and follow up is important.

Keywords: iron deficiency, anemia

DEFINITION AND GENERAL INFORMATION

Iron deficiency anemia is defined as hemoglobin <13 mg/dL in men and < 12 mg /dL in women with a decrease in total body iron.

Decrease in total body iron is added to iron deficiency, hemoglobin in men <13 in women and hemoglobin <12 in women, it is defined as iron deficiency anemia [1].

Iron is a mineral that plays an active role in many metabolic processes in human and acts as a cofactor of vital enzymes. Human have 4-5 grams of iron, 70% of which is in the structure of hemoglobin. Human loses 1 gram of iron each day, and in some cases such as menorrhagia iron loss is increased to 20-90 mg. The body's need for iron increases during adolescence, pregnancy and infection status [2].

Iron is mainly absorbed in jejunum, acid substances increase absorption. Finnate, phosphate and antacids reduces the absorption.

Iron is in the structure of ferritin as a storage form and transferrin plays a role in its transport from intestinal epithelial cells to the bone marrow.

Iron is found in the intestinal mucosa with +3 valence and is converted to the +2 valance form by ferric reductase. It is absorbed with DMT1 (divalent metal transporter 1) and converted from +2 valance to +3 form with Hefastin on the basolateral surface and transported with transferrin.

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Increase in Hepcidin levels in such cases inflammation or chronic ilnesses decreases the absorption of iron [3].

Total iron binding capacity, transferrin saturation and ferritin levels are taken into account in calculating iron deficiency. Total iron binding capacity varies between 240-435 ug/dl. Transferrin saturation is calculated as serum iron x100 /Total Iron Binding Capacity. Transferrin Saturation <12% strongly suggests iron deficiency anemia. Ferritin level <12 ng/ml is in favor of iron deficiency anemia, but a low ferritin levels also expected in hypothyroidism and vitamin C deficiency [4].

ETIOLOGY

People with high iron needs; infants, pre-school children, young and productive women, women in the second or third trimester of pregnancy are the groups most affected by iron deficiency. Due to rapid growth and development, adolescents are also among the risky groups for iron deficiency [5].

Other healthy groups may also be at risk in Western countries. People who are vegetarian (especially Vegans) are at risk due to dietary restrictions and blood donors. In the RISE study, in a study conducted with more than 2000 blood donors in the United States, iron deficiency was found in 2/3 of the women and of the men. Athletes who play endurance sports are at risk from inflammation-related increase in hepcidin and blood loss. In all the groups listed, women are more affected than men [5].

Iron deficiency can occur alone or may be due to another underlying disease. As in the elderly population, it can also be seen with many pathologies. Iron deficiency is usually acquired, rarely hereditary [5].

The most common causes of iron deficiency anemia are shown in the Table 1 using reference number 6.

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Table 1. Causes of iron deficiency

Poor intake (malabsorbtion, poverty or vegans)	Increased menstrual bleeding	Benign lesions or malign tumors of gastrointestinal system
Infections (h. pylori, ascaris etc)	Genitourinary or gastrointestinal system bleeding	Hemorrhagic telangiectasis
Genetic	Drugs (proton pomp inhibitors, corticosteroids etc)	Surgery

ACQUIRED IRON DEFICIENCY

Poverty, malnutrition and drought are the obvious causes of anemia and iron deficiency in developing countries [6]. Infective pathologies that cause bleeding, such as schistosomiasis and hookworm infections, often lead to iron deficiency [6].

Leaving aside people at risk in Western societies, chronic bleeding or a decrease in iron intake and absorption may be more serious than anemia itself. For this reason, one of the most important parts in the patient follow up; more investigation is required to find the underlying causes for differential diagnosis considering with each patient's gender, age, history, and clinical findings [5].

Iron deficiency can not be recognized due to other diseases in patients with chronic kidney failure or with multiple comorbidities. There are multiple causes of anemia in the elderly. Low oral intake, decreased absorption (atrophic gastritis, proton pump inhibitor use) or gastrointestinal blood loss (antiagregant-anticoagulant use, angiodysplasias, peptic ulcer, hemorrhoids, colorectal cancers) responsible for 30% of cases. However, it remains undiagnosed due to comorbidities [7].

Patients with chronic renal failure are more vulnerable to iron deficiency due to decreased absorption, blood loss (2-3 grams / dl per year) during

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hemodialysis, high hepcidin levels and inflammation. High hepcidin levels and inflammation can mobilize iron from the stores [8].

Another well-known reason that impairs the regulation of iron metabolism is obesity by subclinical inflammation, increased hepcidin level, and decreased absorption of iron, especially after bariatric surgery [9].

Considering the necessity of balance between iron need and intake, some special clinical situations arise. (For example: acute restriction of iron for erythropoiesis) The best known conditions are treatment with erythropoiesis stimulating agents, postoperative anemia after major surgery. Recovery from anemia may be delayed or an undiagnosed iron deficiency may have occurred after surgery. In addition, cytokine-related defective iron mobilization may play a role in this situation.

Some studies have shown a high prevalence of iron deficiency in patients with congestive heart failure. (approximately 30-50%) Impaired iron absorption, inflammation, increased hepcidin levels have been shown in the early stages of the disease [6].

GENETIC IRON DEFICIENCY

IRIDA, a rare autosomal recessive disease with impairment of systemic iron homeostasis, has been described. (Iron refractory iron deficiency anemia).

IRIDA is caused by the TMPRSS6 mutation encoding the transmembrane protease that inhibits the signaling pathway that activates hepcidin. Function-losing mutation in TMPRSS6 progresses with increased hepcidin production and impaired intestinal absorption of iron [6].

The high hepcidin levels in IRIDA prevents iron absorption and creates a situation opposite to the compensatory mechanism necessary to maintain erythropoiesis. It is unresponsive to oral iron therapy. In cases where anemia is severe, intravenous iron treatments can be given, but this is also partially effective.

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In recent studies, it has been shown that genetic iron deficiency anemia in mouse models can also be caused by the loss of bisphosphate-3 nucleotidase (BPNT-1) enzyme in sulfur metabolism. It has been demonstrated that iron deficiency anemia may also be due to the relationship between intestinal BPNT-1 loss and sulfur and iron balance [10].

SYMPTOMS

Iron deficiency may cause symptoms such as weakness, fatigue, muscle weakness, tachycardia, effort intolerance due to anemia or lack of iron itself. Pica (desire to eat soil or ice) may occur.

Even patients with iron deficiency without anemia is also symptomatic. Because of the key effect of iron on cellular proteins, enzymes (cytochrome and myoglobin), it is not surprising that patients are symptomatic even before they become anemic [11].

Fatigue, the most common symptom, can occur due to even the slightest drop in iron stores [14]. Two studies have shown that women with iron deficiency but non-anemic ferritin levels below 50 ng / ml improve fatigue with iron replacement [12-13]. In another study, improvement in fatigue with parenteral iron treatment was shown in women with ferritin level below 15 and transferrin saturation below 20% [10]. In another recent study, adolescents who were not anemic but who were deficient in iron reported fatigue improvement with iron therapy [15].

Another symptom of iron deficiency is cold intolerance. Although it is secondary to anemia in some patients, it resolves when anemia is resolved. Cold intolerance may also occur in non-anemic iron deficiency. Cold intolerance may occur in hypothyroidism because thyroid hormones also effect iron stores.

An increased incidence of iron deficiency has been found in patients with heart failure. An improvement in cardiac function and quality of life and a decrease in hospitalization rates were found with iron replacement. Iron deficiency also plays a critical role in pulmonary hypertension. Cellular

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response to hypoxia is based on iron and iron plays a critical role in regulating pulmonary vascular resistance, and a decrease in pulmonary artery pressure has been achieved with iron replacement [11].

Restless leg syndrome; leg cramps are a pathology with an uneasy feeling in the leg and the desire to move the leg at night, and in most patients with low iron stores, symptoms are controlled with iron replacement [11].

A common complaint in women with iron deficiency is hair loss. Diffuse non-scarring alopecia can be seen in patients with ferritin <50 ng / dl.

Table 2. The symptoms of iron deficiency anemia

Weakness	Hair loss
Fatigue	Restless leg
Headache	Chest pain
Shortness of breath	Palpitation
Koilonychia	Dry skin

Severe iron deficiency anemia during pregnancy was found to be associated with an increased risk of preterm labor, low neonatal weight and delivery, and an increase in neonatal and maternal mortality.

The symptoms of iron deficiency anemia are shown in the Table 2 using reference number 19.

DIAGNOSIS

According to the World Health Organization, if hemoglobin levels <13 mg/dL in men over the age of 15, hemoglobin levels <12 mg/dL in non-pregnant women and <11 mg/dL in pregnant women is called anemia [16].

Detailed history, physical examination and laboratory tests are essential in patients with iron deficiency anemia.

While evaluating the history of the patients, their diet (how much meat they take, daily tea or coffee consumption and supplementary food intake) should be thoroughly examined. Pica and ice eating history should be

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questioned, surgery history is also important (iron deficiency anemia is frequently seen in patients who have undergone bariatric surgery) and prescribed drugs should be listed. For example, in patients using aspirine or corticosteroids, the frequency of gastric bleeding has increased and iron deficiency anemia may develop due to chronic blood loss. Difficulty in swallowing should be questioned, and if accompanying, endoscopy evaluation should be planned in terms of Plummer-Vinson Syndrome.

Trauma history, haemoptysis, hematemesis, hematuria, melena, menstrual bleeding severity, pregnancy or miscarriage history should be questioned. Further investigation should be done in terms of intestinal parasites in patients with diarrhea. Family history has importance in terms of Celiac disease, Osler- Weber- Rendu syndrome and hereditary iron malabsorption disorders. The presence of a recent blood donation history is also important. The restless leg should also be questioned.

Hemorrhagic or ecchymotic area should be evaluated during physical examination. Face pallor, dry skin, atrophic glossitis, cheliosis and alopecia etc. (Table 2) Telangiectasias could be a warning sign for the Osler Weber Rendu syndrome. On palpation, attention should be paid in terms of the palpable mass in the abdomen and should be confirmed with imaging tests when necessary.

In laboratory tests, hemogram, peripheral smear and ferritin are among the tests that will be requested at the first stage. Low hemoglobin levels and MCV in the complete blood count (<80) suggests iron deficiency anemia, while microcytosis and hypochromia in peripheral smear also suggest iron deficiency anemia. Although ferritin below 15 ng/mL (sensitivity 59%, specificity 99%) is in favor of iron deficiency anemia, there are also guidelines that show cut points of ferritin for iron deficiency anemia is below 30 ng/mL [17].

If complete blood counts and peripheral blood smears suggest iron deficiency, but ferritin levels normal or high; chronic infection should be suspected. If patient have malignancy or kidney disease, serum iron, serum total iron binding capacity and transferrin saturation should be tested. Low serum iron level, high iron binding capacity (>13.1 μmol/l) and low

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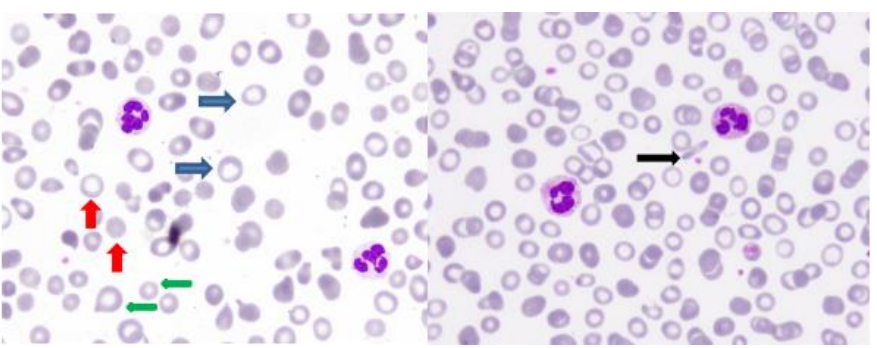
transferrin saturation below 15% are significant findings for iron deficiency anemia [18].

Soluable transferrin receptor and ferritin index, erythrocyte protoporphyrin level and reticulocyte hemoglobin capacity have not been mentioned in this article, since many laboratories have not been studied.

If an increase of 1-2 g/dL in hemoglobin levels occurs within 2-4 weeks after iron treatment given to patients who are considered to have no bleeding and malabsorption, iron deficiency anemia can be diagnosed.

Drugs that can change laboratory results; such as; iron supplements or multivitamin-mineral preparations containing iron. It should be discontinued at least 10 days before testing.

Colonoscopy and endoscopy recommended for menstruating or menopausal women with normal gynecological examination, determining the site of bleeding and early diagnosis of cancer. Occult blood in feces and fecal parasite must be determined. Bone marrow tests are rarely used.



Peripheral smear findings in iron deficiency anemia:

1) Hypochromia in erythrocytes (blue arrow), anisocytosis (green arrow), microcytosis (upper green arrow) and poikilocytosis (red arrow) may seen.
2) Pen cells can also be seen in severe anemias. (black arrow).
3) Reactive thrombocytosis can be seen.

Figure 1 and 2. From the archive of Prof. Dr. İsmet AYDOĞDU.

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DIFFERENTIAL DIAGNOSIS

Since some symptoms in iron deficiency can be seen in other diseases, so that differential diagnosis is important. It should be kept in mind that sometimes patients may have iron deficiency before anemia develops or patients with anemia may have more than one etiology at the same time.

For example, although the patient with fatigue may have iron deficiency, there may be any other system problems such as endocrinology, cardiac, pulmonary or psychiatric. Pica can also be seen in Zinc deficiency and lead poisoning. Restless leg syndrome can also be seen in neurological diseases, sleep disorders, pregnancy, electrolyte disorder and psychiatric diseases.

While mean corpuscular volüme (MCV) may be normal in the early stage in iron deficiency anemia, however the MCV in advanced iron deficiency anemia is generally below 80. Since chronic disease anemia, thalassemia and sideroblastic anemia can be included in the differential diagnosis in hypochromic microcytic anemias, the differences are mentioned in the Table 3 [20].

Peripheral blood smear usually shows hypochromia and microcytosis, but may be normal. Reticulocyte levels are low. In anemia due to chronic hemolysis; for G6PD level should be checked. It can not be seen in acute hemolysis. In thalassemia; MCV below 80, high levels of HbA2 or HbF in hemoglobin electrophoresis, family history and high erythrocyte count is expected. The Mentzer index can be helpful in differential diagnosis. Microcytosis with ovalocytosis and unresponsive to iron therapy suggests thalassemia. IgA anti-endomisium antibodies (IgA anti-EmA) and/or IgA anti tissue transglutaminase antibodies should be checked for Celiac disease. TSH levels should be checked to avoid hypothyroidism. Untreated hypothyroidism causes unsuccessful treatment of iron deficiency anemia.

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Table 3. Differential diagnosis

	Iron Deficiency Anemia	**Chronic Disease Anemia**	**Sideroblastic Anemia**	**Thalassemia**
Serum Iron	Decreased	Decreased	Normal or ıncreased	Normal
Saturation Of Transferrin	Decreased	Normal or decreased	Normal or ıncreased	Normal
Ferritin	Decreased	Normal or ıncreased	Normal or ıncreased	Normal or ıncreased
Rdw	Increased	Normal	Normal	Normal
Bone Marrow Iron Reserve	None or decreased.	Normal or ıncreased	Increased	Normal
Mcv/Rbc	<13	-	-	>13
Number Of Eritroside	Normal or decreased	Normal or decreased	Normal or decreased	Normal or ıncreased
Eritroside Morphology	Hypochromia in erythrocytes, anisocytosis, microcytosis and poikilocytosis	Sımılar to ıron deficıency anemıa	Sımılar to ıron deficıency anemıa	Target cells and basophılıc poınts.

Acute or chronic liver disease, Hodgkin's disease, acute leukemia, solid tumors, fever, acute inflammation and renal replacement therapies can hide iron deficiency anemia by making false ferritin elevation.

Smoking, chronic hypoxia, and high altitude cause high hemoglobin levels, making it difficult to diagnose iron deficiency anemia.

TREATMENT

Treatment in individuals with iron deficiency anemia generally consists of 4 stages.

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Diet Therapy

Although oral intake is poor in the vast majority of patients, additional treatment support is required even if diet is generally followed. Patients should increase their consumption of red meat, fish, poultry, iron-added bread / cereals, beans, dark green vegetables, lentils, raisins and avoid the consumption of substances with high caffeine content. Foods containing vitamin C increase the absorption of iron. There should be 2 hours between milk and dairy products and iron intake. A restriction of <500 ml milk is recommended for adults. Foods with high levels of chemicals should be limited (phytates and polyphenols) [21].

Oral Therapy

Oral therapy in patients is generally 100-200 mg of elemental iron per day. Ferrous sulphate is prescribed as iron furamate and ferrous gluconate. Oral iron therapy causes side effects in patients such as gastric discomfort, bloating, constipation, diarrhea and staining the stool color to black. To prevent or reduce these side effects, treatment should be started with a low dose and the dose should be increased gradually. Since its absorption with other foods and drugs may decrease, it should be given at least 2 hours apart. Although studies have been conducted that vitamin C increases absorption when given 250-500 mg per day, no definitive evidence has been shown (20). Since the hemoglobin value is expected to increase by 1-2 g / dl 2-4 weeks after starting oral iron therapy, the control complete blood count should be tested 2-4 weeks later. If the treatment progresses smoothly in terms of patient compliance and other factors, it can be completed in 2-4 months. After the hemoglobin level is normal, the treatment is continued for 3-6 months to saturate the stores. Ferrous sulphate 325 mg Tablet 3 tımes a day, ferrous gluconate 300 mg Tablet 2 or 3 times a day and ferrous fumarat 324 mg is given as 1 tablet altough given 1 hour before a meal. It can also happen in 25% of patients with constipation. The anti-constipation medication can be given as long as the iron is taken. H2 receptor antagonists-

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proton pump inhibitors reduce iron absorption. Enteric coated drugs are expensive and reduce symptoms but may cause iron deficiency [22].

3 + valent ferric polymaltose preparations have few side effects but it is not recommended to be preferred in the treatment due to poor oral absorption [23].

IV Therapy

Intravenous (IV) iron therapy provides faster absorption of iron and faster recovery of anemia than oral iron therapy, but is more expensive than oral iron. Although less common than before, clinicians are reluctant to administer IV iron because of the higher risk of allergies than oral iron [24-25].

IV iron treatment ındications shown in the Table 4 using reference number 4.

The IV iron requirement of the patients should be calculated as total iron dose (mg) = weight (kg) x normal-patient hemoglobin difference x 2.4 + 500mg. The total dose can be given at once or in divisions. The IV iron preparations used in the world are shown in Table 5 using reference number 26.

Table 4. IV iron treatment indications

Patient who did not respond or tolerate oral therapy
Exacerbation of the underlying disease with oral iron intake, such as inflammatory bowel disease or infections
In patients with chronic kidney failure using EPO or hemodialysis
Severe anemia
Malabsorbsion syndromes (celiac etc)

In cases who's hemoglobin level needs to be increased rapidly, or if oral iron therapy is unsuccessful due to chronic blood loss, IV iron therapy should be given. It has also been shown to exacerbate local inflammation as well as failure of oral iron therapy in inflammatory bowel disease [27].

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In chronic renal failure patients using EPO, iv iron should be given instead of oral iron to fill iron stores beforehand [27].

Table 5. IV iron preparations

DRUG
Iron dextran
Ferric carboxymaltose
Ferric gluconate
Iron sucrose
Iron isomaltoside
Ferumoxytol

In pregnant women, if hemoglobin is below 10.5 g / dL in the 2'nd trimester or at any time in the 3rd trimester, IV iron treatment should be given instead of oral iron. It has been shown that iv iron treatment is superior to oral iron in terms of hematological, maternal and fetal outcomes [28-29-30].

During intravenous iron therapy, temporary side effects such as nausea, vomiting, headache, chest pain, flushing, back pain may occur up to 48 hours after the end of the infusion [31].

It includes giving the infusion at a slow rate to minimize the risk, careful patient observation and administration by trained health personel in an environment with access to resuscitation facilities [32].

RESPONSE CRITERIA

Reticulocytosis should be observed within 72 hours of starting iron supplement therapy (according to some authors this is evident at 7 days). Reticulocytes rise within 7 to 10 days and hemoglobin levels increase 1-2 g / dL every 2 weeks or 2 g / dL every 3 weeks. After normalization of hemoglobin levels, treatment can be continued for 3-6 months to refill the iron reserves.

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If there is no adequate response to iron therapy, the diagnosis of the patient may be wrong, the presence of subclinical hypothyroidism or its treatment may be incomplete. Therefore, it should be evaluated for the second time.

If there is no response to 4-6 weeks of treatment, the patient should be referred to the hematology department.

RESULT

Iron deficiency anemia is widely observed in daily life practice. It appears in a wide spectrum of dietary habits, depending on the underlying chronic conditions. Postmenapozal spelling is very important in the case of iron deficiency anemia in women. Diagnosis and treatment of iron deficiency and iron deficiency anemia significantly improves the quality of life.

REFERENCES

[1] Cook JD. Diagnosis and management of iron-deficiency anaemia. *Best Pract Res Clin Haematol* 2005;18:319e32.

[2] Rebecca F, et al: ABC of Clinical haematology: Iron deficiency anaemia. Clinical review. *BMJ* 1997; 314:360.

[3] Andrews NC. Disorders of iron metabolism. N Engl J Med. 1999 Dec 23;341(26):1986-95. doi: 10.1056/NEJM199912233412607. Erratum in: *N Engl J Med* 2000 Feb 3;342(5):364. PMID: 10607817.

[4] Ülkü B. İ. Ü. Cerrahpaşa Tıp Fakültesi Sürekli Tıp E¤itimi Etkinlikleri Anemiler Sempozyumu 19-20 Nisan 2001 (*Cerrahpaşa Faculty of Medicine Continuous Medical Education Activities Anemias Symposium 19-20 April 2001*), İstanbul, s. 23-32.

[5] Camaschella C, Iron deficiency. *Blood* 2019; 133 (1): 30–39.

Complimentary Contributor Copy

[6] Camaschella C, Iron-Deficiency Anemia *N Engl J Med* 2015; 372:1832-1843.

[7] Girelli D, Marchi G, Camaschella C. Anemia in the elderly. *HemaSphere.* 2018;2(3):e40.

[8] Macdougall IC, Bircher AJ, Eckardt KU, et al; Conference Participants. Iron management in chronic kidney disease: conclusions from a "Kidney Disease: Improving Global Outcomes" *(KDIGO) Controversies Conference. Kidney Int*. 2016;89(1):28-39.

[9] Steenackers N, Van der Schueren B, Mertens A, et al. Iron deficiency after bariatric surgery: what is the real problem? *Proc Nutr Soc.* 2018;77(4):445-455.

[10] Hudson BH, Hale AT, Irving RP, Li S, York JD. Modulation of intestinal sulfur assimilation metabolism regulates iron homeostasis. *Proc Natl Acad Sci USA*. 2018; 115(12):3000-3005.

[11] Thomas G. DeLoughery, Iron Deficiency Anemia, *Medical Clinics of North America*, Volume 101, Issue 2, 2017, Pages 319-332, ISSN 0025-7125, ISBN 9780323509800, https://doi.org/10.1016/j.mcna. 2016.09.004.

[12] Vaucher P, Druais PL, Waldvogel S, Favrat B. Effect of iron supplementation on fatigue in nonanemic menstruating women with low ferritin: a randomized controlled trial. *CMAJ*. 2012 Aug 7;184(11):1247-54. doi: 10.1503/cmaj.110950. Epub 2012 Jul 9. PMID: 22777991; PMCID: PMC3414597.

[13] Verdon F, Burnand B, Stubi CL, et al. Iron supplementation for unexplained fatigue in non-anaemic women: double blind randomised placebo controlled trial. *BMJ* 2003;326(7399):1124.

[14] Krayenbuehl PA, Battegay E, Breymann C, et al. Intravenous iron for the treatment of fatigue in nonanemic, premenopausal women with low serum ferritin concentration. *Blood* 2011;118(12):3222–7.

[15] Sharma R, Stanek JR, Koch TL, et al. Intravenous iron therapy in non-anemic irondeficient menstruating adolescent females with fatigue. *Am J Hematol* 2016; 91(10):973–7.

[16] D. Wayne Thomas et al. Guideline for the laboratory diagnosis of functional iron deficiency *Br J Heamotol*. 2013; 161:639-48.

Complimentary Contributor Copy

[17] Van den Broek NR, Letsky EA, White SA, Shenkin A, Iron status in pregnant women: which measurements are valid? Br J Haematol. 1998;103(3):817. / Pavord S et al. UK guidelines on the management of iron deficiency in pregnancy. *Br J Haematol*. 2020;188(6):819. Epub 2019 Oct 2.

[18] Susan F Clark, Iron deficiency anemia: diagnosis and management *Curr Opin Gastroenterol*. 2009 Mar;25(2):122-8. doi: 10.1097/MOG. 0b013e32831ef1cd.

[19] Salma AlDallal Iron Deficiency Anaemia: A Short Review *J Cancer Res Immunooncol* 2016, 2:1.

[20] Massey, A. C. (1992). Microcytic anemia: Differential Diagnosis and Management of Iron Deficiency Anemia. *Medical Clinics of North America*, 76(3), 549–566. doi:10.1016/s0025-7125(16)30339-x.

[21] M. Gillooly et al. The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. *Br. J. Nutr*. (1983), 49, 331.

[22] Berber I, Diri H, Erkurt MA, Aydogdu I, Kaya E, Kuku I Evaluation of ferric and ferrous iron therapies in women with iron deficiency anaemia *Adv Hematol*. 2014;2014:297057. doi: 10.1155/ 2014/297057. Epub 2014 Jun 11.

[23] Dinlen N et al. Efficiency of Ferrous Sulphate and Ferric Polymaltose Treatment in Iron Deficiency Anemia and The Effects of Multivitamin Support on *Relevant Therapies pediatric journal* 12(2):72-77, 2012 doi:10.5222/j.child.2012.072.

[24] Onken JE, Bregman DB, Harrington RA, et al. A multicenter, randomized, active-controlled study to investigate the efficacy and safety of intravenous ferric carboxymaltose in patients with iron deficiency anemia. *Transfusion* 2014; 54: 306-15.

[25] Vadhan-Raj S, Strauss W, Ford D, et al. Efficacy and safety of IV ferumoxytol for adults with iron deficiency anemia previously unresponsive to or unable to tolerate oral iron. *Am J Hematol* 2014; 89: 7-12.

Complimentary Contributor Copy

[26] Auerbach M, Ballard H. Clinical use of intravenous iron: administration, efficacy, and safety. *Hematol Am Soc Hematol EducProgram.* 2010;2010:338---47.38.

[27] Reinisch W, Chowers Y, Danese S, et al. The management of iron deficiency in inflammatory bowel disease — an online tool developed by the RAND/UCLA appropriateness method. *Aliment Pharmacol Ther* 2013; 38: 1109-18.

[28] Auerbach M, Macdougall I. The available intravenous iron formulations: History, efficacy, and toxicology. *Hemodial Int* 2017; 21(Suppl 1): S83–S92.

[29] Bencaiova G, von Mandach U, Zimmermann R. Iron prophylaxis in pregnancy: intravenous route versus oral route. *Eur J Obstet Gynecol Reprod Biol* 2009; 144: 135–9.

[30] Breymann C, Milman N, Mezzacasa A, Bernard R, Dudenhausen J. investigators F-A. Ferric carboxymaltose vs. oral iron in the treatment of pregnant women with iron deficiency anemia: an international, open-label, randomized controlled trial (FER-ASAP). *J Perinat Med* 2017; 45: 443–53.

[31] Auerbach M, Strauss W, Auerbach S, Rineer S, Bahrain H. Safety and efficacy of total dose infusion of 1,020 mg of ferumoxytol administered over 15 min. *Am J Hematol* 2013; 88: 944-7.

[32] *New recommendations to manage risk of allergic reactions with intravenous iron-containing medicines*. London: European Medicines Agency, 2013 (http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2013/06/WC500144874.pdf).

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Chapter 8

NUTRITIONAL AND ANTI-CANCER EFFECTS OF CAROTENOIDS FROM *LACTUCA SATIVA*

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ABSTRACT

Lactuca sativa (Lettuce) is one of the most common green leafy vegetable cultivated across the world. Lettuce is mostly consumed in raw form and used to prepare curries, salads and soups. Lettuce is less in calories and is a good source of iron, folate, vitamin C and fiber. Wide varieties of lettuce include romaine, loose-leaf, iceberg, butterhead-leaf (green/red) and stem lettuce. Depending on the variety, the composition of lettuce also changes. Among all the varieties, romaine lettuce is rich in dietary fiber while the butterhead is rich in iron and folate content. Green leaf and baby romaine are rich in Vitamin C levels while β-carotene and lutein are rich in butterhead and romaine lettuce. Phenolic compounds are typically high in red romaine and red leaf varieties. Lutein, β-carotene,

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violaxanthin, neoxanthin, zeaxanthin and lactucaxanthin are the major carotenoids of lettuce. Lettuce is well documented for its numerous biological activities such as antioxidant, anti-diabetic, cholesterol-lowering, anti-inflammatory, anti-cancer, etc.

Keywords: *Lactuca sativa,* carotenoids, lutein, lactucaxanthin, nutritional status, anti-cancer

1. INTRODUCTION

Lactuca sativa (*L. sativa*) commonly known as lettuce is an annual plant belonging to the Asteraceae family. Lettuce originated in Europe and Southwest Asia, however presently widely cultivated globally for its nutrient-rich fleshy leaves used for culinary preparations like salads, soups and curries (Alconero, 1988, Hart, 2005). The demand for lettuce has increased worldwide due to its high nutritional value and therapeutic importance. In 2014, China became the first global cultivator of lettuce, producing 13,500,000 tons four times higher produced by the USA followed by India, Spain, Italy, Iran, Japan, Turkey, Mexico and Germany (Figure 1). Lettuce consisted of seven morphotypes that include butterhead, crisphead, cos, cutting, stalk and the Latin lettuce. Lettuce is low in calories and rich in carotenoids, Vitamins (A, C, K, niacin and folate) and minerals like calcium, phosphorous, iodine, iron, copper and arsenic (Noumedem et al., 2017). Depending on the variety, the nutritional profile of lettuce also changes. Among the varieties, romaine lettuce is rich in dietary fiber while the butterhead is rich in iron and folate content. Green leaf and baby romaine are rich in Vitamin C whereas β-carotene and lutein are rich in butterhead, romaine and leafy lettuce. The phenolic compounds are high in red romaine and red leaf varieties (Lebeda, 2006). Lettuce intake helped in reducing the infections and improving anemia. Lettuce's medicinal properties are secondary metabolites like terpenoids, flavonoids and phenolics synthesized as a natural defense mechanism.

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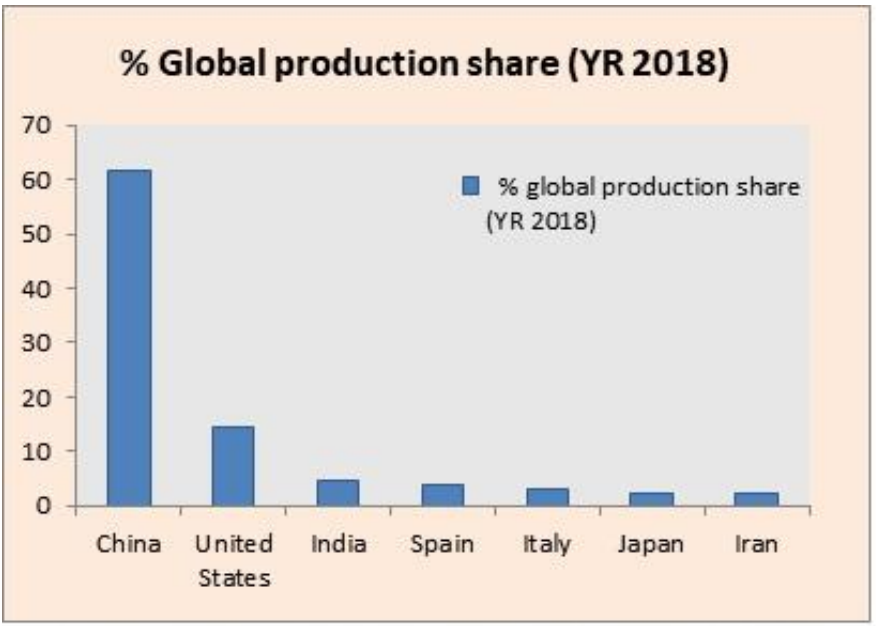


Figure 1. Percentage of global production share of lettuce.

They have been used conventionally for diseases like bronchitis, urinary tract infection, loss of appetite, inflammation, indigestion, etc (Ismail and Mirza, 2015). Several studies on domesticated lettuce varieties were reported for antioxidant, antimicrobial and neuroprotective effects (Moharib et al., 2014). In this chapter, the nutritional and anti-cancer effects of carotenoids from lettuce will be discussed.

2. Carotenoid Profile of Lettuce

Carotenoids are yellow-orange pigments containing eight isoprenoid units linked to form a conjugated double bond system. Carotenoids having only isoprene units are termed as carotenes, examples are lycopene and β-carotene. Carotenoids containing oxygen in addition to hydrocarbon chain are called xanthophylls or oxygenated carotenoids; examples include lutein and zeaxanthin (Abdel-Aal and Young, 2009). Various lettuce varieties are

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grown in different corners of the world, mainly butterhead lettuce, crisphead or iceberg lettuce, romaine lettuce, cutting lettuce, stalk lettuce and Latin lettuce are some of the major types grown widely (Smith, 2011). Of these different types, the three common varieties are head lettuce, loose-leaf lettuce and romaine lettuce (Bennett and Hollister, 2006). Typically all lettuce types are good source of vitamins, phenolics and carotenoids (Damerum et al., 2015). Li and Kubota (2009) showed supplement of far red light to baby lettuce affected carotenoid concentration by 11%.

Lettuce is a rich source of β-carotene and it is reported that 100g of fresh lettuce contained 247% of vitamin A and 4443µg of β-carotene. Recent study by Serafini et al., (2020) showed consumption of 250g of fresh lettuce resulted in a significant increase in plasma β-carotene levels. Zeaxanthin, another major carotenoid of lettuce, constituted around 15% of green lettuce. In 100g fresh green lettuce, zeaxanthin content is estimated as 1730µg (Das and Bhattacharjee, 2020). We also showed the occurrence of major carotenoids such as lutein, lactucaxanthin and β-carotene in lettuce (Gopal et al., 2017). A study by Dorothea siefermann first reported the occurrence of lactucaxanthin in lettuce along with major carotenoids such as β, β-carotene, β, ε-carotene, lutein, violaxanthin and neoxanthin. The levels of lactucaxanthin varied among different lettuce strains such as curly, french, boston and freelice and were found to be 8.2, 11.9, 11.8 and 6.9µg/g of lettuce (Siefermann-Harms et al., 1981; Kimura and Rodriguez-Amaya, 2002). Green lettuce has higher lactucaxanthin content than romania and iceberg varieties (Jayapala et al., 2020). Lettuce also contained neoxanthin, a potential anti-cancer carotenoid constituting 0.5 -2.2mg/100 g of lettuce (Table 1). Kim et al., (2018) identified a total of six carotenoids namely all-E- violaxanthin, 9'-z-neoxanthin, all-E-luteoxanthin, all-E-lactucaxanthin, all-E-lutein and all-E-β-carotene in lettuce (Figure 2).

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Table 1. Carotenoid content in lettuce varieties

Name of the variety	β-carotene (mg/100g fresh weight)	Lutein/ Zeaxanthin(mg/100 g fresh weight)	Lactucaxan-thin (mg/100g dry weight)	Neoxanthin (µg/g fresh weight)	Violaxanthin (µg/g fresh weight)	Zeaxanthin (µg/g fresh weight)	References
Butterhead	1987	1223	-	1.3-2.1	2.2-4.4	1.0-2.4	(Martineau et al., 2012)
Iceberg/ Crisphead	229	277	0.36 ± 0.06	14.0-29.0	22.5-45.5	0.8-2.7	(Baslam et al., 2013), (Jayapala et al., 2020)
Red leaf	4495	1724	-	-	-	2.0	(Das and Bhattacharjee 2020), (Nicolle et al., 2004)
Green leaf	4443	1730	3.05 ± 0.12	-	-	0.7-1.9	(Jayapala et al., 2020), (Nicolle et al., 2004)
Romaine	5226	2312	0.42 ± 0.05	2.3-4.6	5.0-7.5	-	(López et al., 2014), (Jayapala et al., 2020)

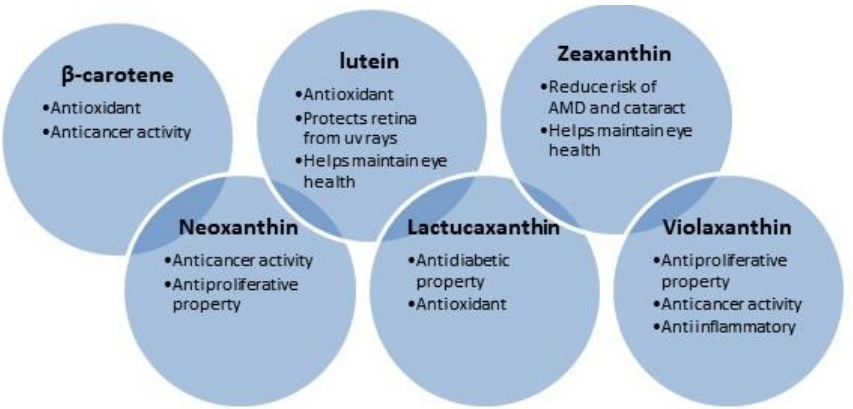


Figure 2. Carotenoids of lettuce and their nutritional importance

3. NUTRITIVE VALUE OF CAROTENOIDS FROM LETTUCE

It is mainly due to the high nutritional and medicinal value that the lettuce's consumption increased across the world in recent years. Reports on the lettuce's chemical composition revealed that 100g of fresh lettuce consisted of 96% water, 1.4g protein, 2.2g carbohydrate, 1.1g dietary fiber, 0.2g fat and 1.2g ash. In addition to carotenoids, lettuce also served as a concentrated source of several minerals such as calcium, iodine, phosphorus, copper, arsenic and iron. Because of the high iron content, lettuce is good for anemic patients (Noumedem et al., 2017). Lettuce comprises vitamins, especially vitamins A, vitamin C, vitamin K, folate and niacin. Major sesquiterpene lactones present in lettuce varieties are germacranolide lactuside A, guaianolides lactucin, lactucopicrin, etc., (Michalska et al., 2009). Ethanol extracts (95% and 50%) of *L. sativa* seeds consisted of four flavonol glycosides namely Japonicin A, Isoquercitrin, caffeic acid and lactucasativoside A (Xu et al., 2012) (Figure 3).

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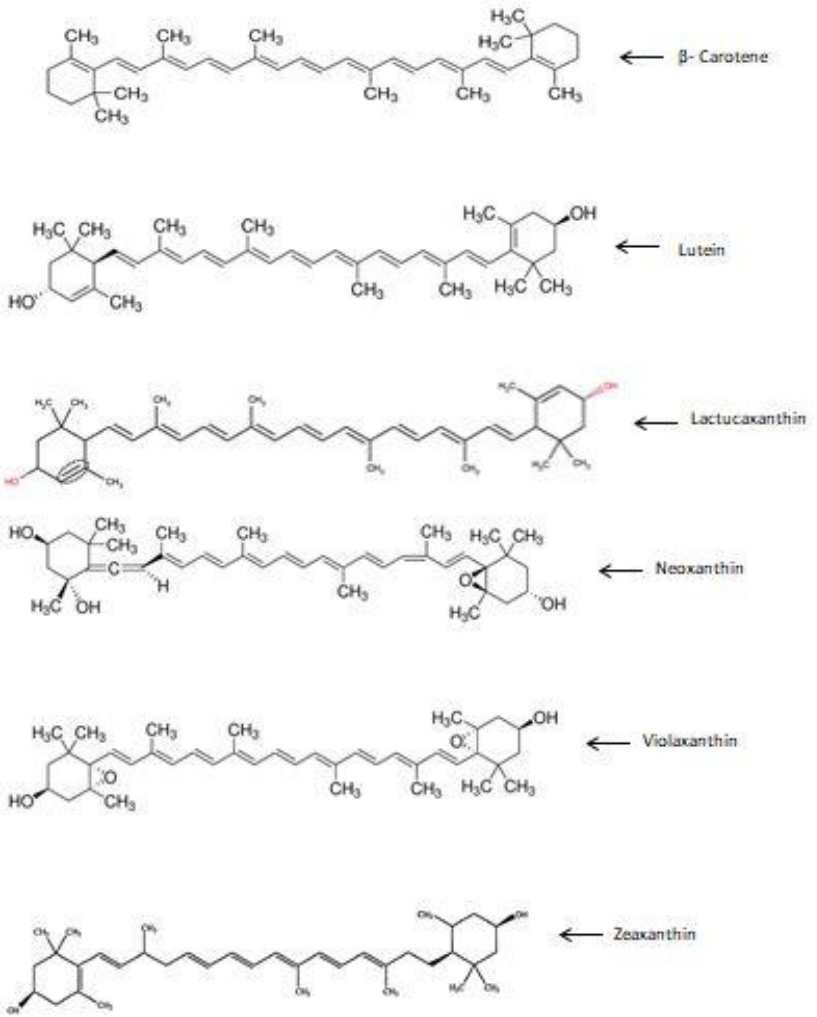


Figure 3. Structure of major carotenoids present in lettuce

3.1. β-Carotene

β-carotene act along with vitamin C and help in the prevention of oxidation of cholesterol. β-carotene prevented plaque deposition in the heart and reduced cardiological disorders (Caballero et al., 2015). It also helped

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in the wellbeing of mucous membranes, skin and vision. Carotenes are the source of vitamin A, like 2µg of carotene can convert to 1 IU of vitamin A. Carotenes enhanced collagen production, ameliorated skin tanning age-related skin problems.

3.2. Lactcucaxanthin

Lactucaxanthin extracted from lettuce exhibited an anti-diabetic effect by inhibiting α-amylase and α-glucosidase activities in diabetic rats. The study clearly highlighted lactucaxanthin as a natural hypoglycemic inhibitor where it slowed down the metabolism of dietary carbohydrates and reduced blood glucose levels (Gopal et al., 2017). In another recent study, we showed lactucaxanthin extracted from green lettuce exhibited antioxidant and free radical scavenging activities (Jayapala et al., 2020)

3.3. Lutein and Zeaxanthin

Lutein and zeaxanthin are dietary xanthophyll carotenoids that are rich in green leafy vegetables such as kale, lettuce, spinach, yellow-colored carrots, corn saffron, etc. Lutein and zeaxanthin can be interconverted in the body by the intermediate mesozeaxanthin form. Lutein and zeaxanthin are used in the food industry as a food colorant (Lutein E number - E161b, Zeaxanthin E number - E161h). Studies revealed that lutein and zeaxanthin selectively absorb into retinal macula lutea from blood and protected the retina from UV radiation. Hence having a lutein-rich diet shown to prevent age-related macular degeneration and cataract (Sommerburg et al., 1998; Feskanich et al., 1999).

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3.4. Violaxanthin

Violaxanthin is an epoxyxanthophyll pigment found in lettuce. Violaxanthin and zeaxanthin are interconvertible pigments, violaxanthin converts to zeaxanthin by depoxidation under light stress. This xanthophyll cycle works based on the photoprotective role of zeaxanthin (Eskling et al., 2001). Violaxanthin is reported to exhibit high ABTS free radical scavenging activity and also inhibited lipid peroxidation. Violaxanthin isolated from microgreen algae showed anti-inflammatory, anti-proliferative and pro-apoptotic activities *in vitro* (Wang et al., 2018).

3.5. Neoxanthin

Neoxanthin is an epoxy xanthophyll carotenoid that plays vital role during photooxidative stress. In nature, neoxanthin is available in two forms; all-trans neoxanthin and 9'-cis-neoxanthin (Giossi et al., 2020). Along with other pharmacological activities, neoxanthin is shown to exhibit antioxidant, anti-proliferative, pro-apoptotic, etc., (Kotake-Nara et al., 2005).

4. Anti-cancer Effects of Carotenoids from Lettuce

A vast number of studies conducted with carotenoids from natural sources shown to modulate various types of cancers such as stomach, cervical, colon, bladder, etc. Carotenoids at lower concentrations exhibited anti-proliferative activity which further prevented the spreading of cancer cells. Anticancer effect of carotenoids was mainly studied in cervical (HeLa), prostate (LNCaP), stomach (HGC27) cancer cells. The anti-cancer activity of carotenoids is contributed mainly due to its higher antioxidant potential followed by immunomodulatory effects, modulation of key

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transcription factors and reversing multidrug resistance of anti-cancer drugs (Chew and Park, 2004).

Saini et al., 2018 conducted a study in which, anti-cancer activities of xanthophyll carotenoids such as lactucaxanthin, violaxanthin, lutein and neoxanthin from lettuce were studied in cervical (HeLa) and lung (A549) cancer cells. The study showed that among the carotenoids tested neoxanthin exhibited potent cytotoxicity (Saini et al., 2018) (Table 2). Among the major carotenoids of lettuce, neoxanthin exhibited potent anti-proliferative and anti-cancer activities (Kotake-Nara et al., 2001). Similar result was reported with neoxanthin from spinach, where neoxanthin with fucoxanthin induced apoptosis of human prostate cancer cells. The pro-apoptotic effect of neoxanthin was mainly due to caspase-3 activation and cleavage of PARP (Kotake-Nara et al., 2005).

A case-control study conducted by Fernandez et al., (1997) in 1584 colorectal cancer and 2879 control subjects highlighted that lettuce rich diet reduced familial colorectal risk cancer. Further research showed that increased consumption of β-carotene and ascorbic acid lowered the risk significantly. In a multicenter case-control study performed with non-smokers, the association between dietary nutrients and lung cancer was elucidated. It was evident that consumption of lettuce, carrots and tomatoes protected from lung cancer. Consumption of carotenoids such as β-carotene and retinol also showed a protective effect at higher concentrations. Diet rich in vegetables and fruits reduced lung cancer incidence among nonsmokers by 25% (Brennan et al., 2000). Mignone et al., (2009) conducted a case control study in US population to determine the association between carotenoid intake and invasive breast cancer risk. The study performed with 5700 women with breast cancer and 6400 control subjects showed that consumption of vitamin A and certain carotenoids reduced breast cancer risk. In another study by Slattery et al., (2000) carotenoid intake and colon cancer risk relation were analyzed. This study was conducted with 1993 colon cancer and 2410 control subjects and showed that lutein from lettuce, spinach, tomatoes reduced the risk of colon cancer development.

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Table 2. Anti-cancer potential of lettuce carotenoids

Name of the carotenoid	Source	System studied	Mode of action	References
β-carotene	Synthetic	Human breast cancer cells (MCF-7)	Induced apoptosis, cell cycle arrest, Inhibited cell proliferation	(Gloria et al., 2014)
Lutein	*Chlorella vulgaris*	Human colon cancer cells (HCT-116)	Induced apoptosis, enhanced fluorescence intensity of the early apoptotic populations, reduced cell viability with IC_{50} (40.31µg/ml)	(Kwang, Song, and Lee 2008)
	Green lettuce (*L. sativa*)	Cervical cancer cells (HeLa) Lung cancer cells (A549)	Reduced cell viability, IC_{50} 9.1µM (HeLa), 17.4µM (A549)	(Saini et al., 2018)
Zeaxanthin	Plant source	Human gastric cancer cells	Induced apoptosis by reducing mitochondrial membrane potential, increased cytochrome C expression, upregulated Bax, caspase -3 and cleaved PARP, downregulated expression of Bcl-2 and induced cell cycle arrest at G2/M phase	(Sheng et al., 2020)

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Table 2. (Continued)

Name of the carotenoid	Source	System studied	Mode of action	References
Violaxanthin	*Chlorella ellipsoidea*	Human colon cancer cells (HCT-116)	Enhanced fluorescence intensity of the early apoptotic populations, reduced cell viability with IC_{50} (40.73µg/ml)	(Kwang, Song, and Lee 2008)
	Green lettuce (*L. sativa*)	Cervical cancer cells (HeLa) Lung cancer cells (A549)	Reduced cell viability, IC_{50} 23.1µM (HeLa), 22.3µM (A549)	(Saini et al., 2018)
Neoxanthin	Green lettuce (*L. sativa*)	Cervical cancer cells (HeLa) Lung cancer cells (A549)	Reduced cell viability, IC_{50} 3.8 µM (HeLa), 7.5 µM (A549)	(Saini et al., 2018)
	Spinach	Human prostate cancer cells (PC-3, DU 145 and LNCaP)	Induced apoptosis, downregulated expression of Bax and Bcl-2, upregulated caspase-3 expression	(E. Kotake-Nara et al., 2001), (Eiichi Kotake-Nara, Asai, and Nagao 2005)
Lactucaxanthin	Green lettuce (*L. sativa*)	Cervical cancer cells (HeLa) Lung cancer cells (A549)	Reduced cell viability, IC_{50} 12.6 µM (HeLa), 15.2 µM (A549)	(Saini et al., 2018)

Even though there was no report about the anti-cancer activity of violaxanthin's from lettuce, anti-cancer effects of violaxanthin from other sources such as marine green algae were well studied. Violaxanthin rich extracts of *Chlorella ellipsoidea* showed inhibition of human colon cancer (HCT-116) cell growth in a dose-dependent manner. Lutein rich extract of freshwater *Chlorella vulgaris* extract also showed anti-proliferative effect towards HCT-116 cells (Kwang et al., 2008).

CONCLUSION

Lettuce is well-known for its nutrients and antioxidant which together contributed to its excellent medicinal value. Lettuce production and consumption increasing at a fast rate across almost all the countries. As lettuce is mostly consumed as fresh, the storage and preservation of bioactives are also necessary. So, there is also a need for the development of more innovative techniques for the retention and preservation of lettuce bioactives. Major pharmacological properties of lettuce carotenoids include antioxidant, antimicrobial, neuroprotective, anti-inflammatory, anti-cancer, etc. The higher antioxidant capacity of lettuce is mainly contributed by phenolic compounds and carotenoids such as lutein, lactucaxanthin, β-carotene, zeaxanthin, neoxanthin, violaxanthin. Lettuce is employed in traditional medicine for many years to cure ailments such as anemia, cough, infectious diseases, sleep disorders, muscle pains, etc. Henceforth, lettuce derived phytochemicals could be used as an alternative for synthetic drugs and more research is required to develop drugs and dietary supplements from lettuce.

REFERENCES

Abdel-Aal, El-sayed M, and Christopher J Young. 2010. "Chapter -8 Carotenoids." In *Healthgrain Methods*, edited by Peter Shrewry, 1st ed.,

Complimentary Contributor Copy

69–94. Elsevier. https://www.elsevier.com/books/healthgrain-methods/shrewry/978-1-891127-70-0.

Alconero, R. 1988. ("Lactuca Sativa" 6 Whitaker 1969).

Bennett, J M, and C W Hollister. 2006. *Medieval Europe: A Short History*. http://www.academia.edu/download/42693482/medieval-europe-samenvatting.pdf.

Brennan, Paul, Cristina Fortes, Joel Butler, Antonio Agudo, Simone Benhamou, Sarah Darby, Michael Gerken, et al., 2000. *"A Multicenter Case-Control Study of Diet and Lung Cancer among Non-Smokers." Cancer Causes and Control* 11 (1): 49–58. https://doi.org/10.1023/A:1008909519435.

Caballero, Benjamin, Paul finglas, and Fidel Toldra. 2015. "Encyclopedia of Food and Health - 1st Edition." In *Encyclopedia of Food and Health*, 1st Edition. Academic Press. https://www.elsevier.com/books/encyclopedia-of-food-and-health/caballero/978-0-12-384947-2.

Chew, Boon P., and Jean Soon Park. 2004. "Carotenoid Action on the Immune Response." In *Journal of Nutrition*, 134:257S-261S. American Institute of Nutrition. https://doi.org/10.1093/jn/134.1.257s.

Damerum, Annabelle, Stacey L. Selmes, Gaia F. Biggi, Graham J. J. Clarkson, Steve D. Rothwell, Maria José Truco, Richard W. Michelmore, et al., 2015. "Elucidating the Genetic Basis of Antioxidant Status in Lettuce (*Lactuca Sativa*)." *Horticulture Research* 2 (1): 1–13. https://doi.org/10.1038/hortres.2015.55.

Das, Ranjana, and Chiranjib Bhattacharjee. 2020. "Chapter 9. Lettuce." In *Nutritional Composition and Antioxidant Properties of Fruits and Vegetables*, edited by Amit K Jaiswal, 143–55. Elsevier science. https://www.google.co.in/books/edition/Nutritional_Composition_and_Antioxidant/j4TzDwAAQBAJ?hl=en&gbpv=0.

Eskling, Marie, Anna Emanuelsson, and Hans-Erik Åkerlund. 2001. *Enzymes and Mechanisms for Violaxanthin-Zeaxanthin Conversion*. In, 433–52. https://doi.org/10.1007/0-306-48148-0_25.

Fernandez, E., C. La Vecchia, B. D'Avanzo, E. Negri, and S. Franceschi. 1997. "Risk Factors for Colorectal Cancer in Subjects with Family

Complimentary Contributor Copy

History of the Disease." *British Journal of Cancer* 75 (9): 1381–84. https://doi.org/10.1038/bjc.1997.234.

Feskanich, Diane, Peter Weber, Walter C. Willett, Helaine Rockett, Sarah L. Booth, and Graham A. Colditz. 1999. "Vitamin K Intake and Hip Fractures in Women: A Prospective Study." *American Journal of Clinical Nutrition* 69 (1): 74–79. https://doi.org/10.1093/ajcn/69.1.74.

Giossi, Chiara, Paulo Cartaxana, and Sónia Cruz. 2020. "Photoprotective Role of Neoxanthin in Plants and Algae." *Molecules*. MDPI AG. https://doi.org/10.3390/molecules25204617.

Gopal, Sowmya Shree, Magisetty Jhansi Lakshmi, Gurunathan Sharavana, Gunaseelan Sathaiah, Yadahally N. Sreerama, and Vallikannan Baskaran. 2017. "Lactucaxanthin-a Potential Anti-Diabetic Carotenoid from Lettuce (*Lactuca Sativa*) Inhibits α-Amylase and α-Glucosidase Activity in Vitro and in Diabetic Rats." *Food and Function* 8 (3): 1124–31. https://doi.org/10.1039/c6fo01655c.

Hart, George. 2005. The Routledge Dictionary of Egyptian Gods and Goddesses. *The Routledge Dictionary of Egyptian Gods and Goddesses*. Routledge. https://doi.org/10.4324/9780203023624.

Ismail, Hammad, and Bushra Mirza. 2015. "Evaluation of Analgesic, Anti-Inflammatory, Anti-Depressant and Anti-Coagulant Properties of Lactuca Sativa (CV. Grand Rapids) Plant Tissues and Cell Suspension in Rats." *BMC Complementary and Alternative Medicine* 15 (1). https://doi.org/10.1186/s12906-015-0742-0.

Jayapala, Naveen, Anitha Rani Elavarasan, Sachin R. Chaudhari, and Baskaran Vallikannan. 2020. "Cytotoxicity and 3T3-L1 Cell Uptake of Lactucaxanthin Purified and Characterized by LC-MS and NMR from Lettuce (*Lactuca Sativa)*." *Journal of Liquid Chromatography & Related Technologies* 43 (7–8): 233–46. https://doi.org/10.1080/10826076.2020.1719414.

Kim, Dae Eun, Xiaomin Shang, Awraris Derbie Assefa, Young Soo Keum, and Ramesh Kumar Saini. 2018. "Metabolite Profiling of Green, Green/Red, and Red Lettuce Cultivars: Variation in Health Beneficial Compounds and Antioxidant Potential." *Food Research International* 105 (March): 361–70. https://doi.org/10.1016/j.foodres.2017.11.028.

Complimentary Contributor Copy

Kimura, Mieko, and Delia B. Rodriguez-Amaya. 2002. "A Scheme for Obtaining Standards and HPLC Quantification of Leafy Vegetable Carotenoids." *Food Chemistry* 78 (3): 389–98. https://doi.org/10.1016/S0308-8146(02)00203-0.

Kotake-Nara, E., M. Kushiro, H. Zhang, T. Sugawara, K. Miyashita, and A. Nagao. 2001. "Carotenoids Affect Proliferation of Human Prostate Cancer Cells." *Journal of Nutrition* 131 (12): 3303–6. https://doi.org/10.1093/jn/131.12.3303.

Kotake-Nara, Eiichi, Akira Asai, and Akihiko Nagao. 2005. "Neoxanthin and Fucoxanthin Induce Apoptosis in PC-3 Human Prostate Cancer Cells." *Cancer Letters* 220 (1): 75–84. https://doi.org/10.1016/j.canlet.2004.07.048.

Kwang, Hyun Cha, Y. I. Koo Song, and Dong Un Lee. 2008. "Antiproliferative Effects of Carotenoids Extracted from Chlorella Ellipsoidea and Chlorella Vulgaris on Human Colon Cancer Cells." *Journal of Agricultural and Food Chemistry* 56 (22): 10521–26. https://doi.org/10.1021/jf802111x.

Lebeda, A. 2006. "Lettuce (Asteraceae; Lactuca Spp.)." In *Genetic Resources, Chromosome Engineering, and Crop Improvement*, edited by Ram J. Singh, 3:393–488. CRC Press. https://doi.org/10.1201/9781420009569-14.

Li, Qian, and Chieri Kubota. 2009. "Effects of Supplemental Light Quality on Growth and Phytochemicals of Baby Leaf Lettuce." *Environmental and Experimental Botany* 67 (1): 59–64. https://doi.org/10.1016/j.envexpbot.2009.06.011.

Michalska, Klaudia, Anna Stojakowska, Janusz Malarz, Ivana Doležalová, Aleš Lebeda, and Wanda Kisiel. 2009. "Systematic Implications of Sesquiterpene Lactones in Lactuca Species." *Biochemical Systematics and Ecology* 37 (3): 174–79. https://doi.org/10.1016/j.bse.2009.02.001.

Mignone, L. I., Giovannucci, E., Newcomb, P. A., Titus-Ernstoff, L., Trentham-Dietz, A., Hampton, J. M., Willett, W. C., & Egan, K. M. (2009). Dietary carotenoids and the risk of invasive breast cancer. *International journal of cancer*, 124(12), 2929–2937. https://doi.org/10.1002/ijc.24334

Complimentary Contributor Copy

Moharib, Sorial A., Nabila Abd El Maksoud, Halla M. Ragab, and Mahmoud M. Shehata. 2014. "Anticancer Activities of Mushroom Polysaccharides on Chemicallyinduced Colorectal Cancer in Rats." *Journal of Applied Pharmaceutical Science* 4 (7): 54–63. https://doi.org/10.7324/JAPS.2014.40710.

Noumedem, Jaures A. K., Doriane E. Djeussi, Lucian Hritcu, Marius Mihasan, and Victor Kuete. 2017. "Lactuca Sativa." In *Medicinal Spices and Vegetables from Africa: Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases*, 437–49. Elsevier Inc. https://doi.org/10.1016/B978-0-12-809286-6.00020-0.

Saini, Ramesh Kumar, So Hyun Moon, Enkhtaivan Gansukh, and Young Soo Keum. 2018. "An Efficient One-Step Scheme for the Purification of Major Xanthophyll Carotenoids from Lettuce, and Assessment of Their Comparative Anticancer Potential." *Food Chemistry* 266 (November): 56–65. https://doi.org/10.1016/j.foodchem.2018.05.104.

Serafini, Mauro, Rossana Bugianesi, Monica Salucci, Elena Azzini, Anna Raguzzini, and Giuseppe Maiani. 2020. *"Effect of Acute Ingestion of Fresh and Stored Lettuce (Lactuca Sativa) on Plasma Total Antioxidant Capacity and Antioxidant Levels in Human Subjects."* https://doi.org/10.1079/BJN2002722.

Siefermann-Harms, Dorothea, Sissel Hertzberg, Gunner Borch, and Synnøve Liaaen-Jensen. 1981. "Lactucaxanthin, an ε,ε-Carotene-3,3′-Diol from Lactuca Sativa." *Phytochemistry* 20 (1): 85–88. https://doi.org/10.1016/0031-9422(81)85222-3.

Slattery, M.L, Benson, J., Curtin, K., Ma. K., Schaeffer. D., Potte, R.J.D. 2000. Carotenoids and colon cancer. *Am. J. Clin. Nutr.* 71:575–582.https://doi.org/10.1093/ajcn/71.2.575.

Smith, Steven. 2011. "Genetic Resources, Chromosome Engineering, and Crop Improvement. Volume 5: Forage Crops." *African Journal of Range & Forage Science* 28 (1): 43–43. https://doi.org/10.2989/10220119.2011.571407.

Sommerburg, Olaf, Jan E. E. Keunen, Alan C. Bird, and Frederik J. G. M. Van Kuijk. 1998. "Fruits and Vegetables That Are Sources for Lutein and Zeaxanthin: The Macular Pigment in Human Eyes." *British Journal*

Complimentary Contributor Copy

of Ophthalmology 82 (8): 907–10. https://doi.org/ 10.1136/ bjo.82.8.907.

Wang, Feifei, Luodong Huang, Baoyan Gao, and Chengwu Zhang. 2018. "Optimum Production Conditions, Purification, Identification, and Antioxidant Activity of Violaxanthin from *Microalga Eustigmatos* Cf. Polyphem (Eustigmatophyceae)." *Marine Drugs* 16 (6). https://doi.org/ 10.3390/md16060190.

Xu, F., G. A. Zou, Y. Q. Liu, and H. A. Aisa. 2012. "Chemical Constituents from Seeds of *Lactuca Sativa*." *Chemistry of Natural Compounds* 48 (4): 574–76. https://doi.org/10.1007/s10600-012-0314-1.

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Chapter 9

DIAGNOSTIC APPLICATION OF CO-OXIMETER FOR HYPOTHERMIA IN FORENSIC PRACTICE

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ABSTRACT

Hypothermia is one of the external causes of death due to a cold environment. There are few or no specific autopsy findings in cases of fatal hypothermia. However, it has been reported that, in cases of hypothermic death, blood in the left cardiac chamber is bright red compared to that in the right cardiac chamber, which might be a characteristic autopsy sign of hypothermic death. This color difference is due to the difference in oxygen content in blood in the two chambers. Examination of this difference using a CO-oximeter would provide valuable information in cases of hypothermia.

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In the present paper, we discuss application of the CO-oximeter for the forensic diagnosis of hypothermia.

Keywords: hypothermia, CO-oximeter, forensic diagnosis

1. INTRODUCTION

Hypothermia is defined as a decrease in core body temperature below 35°C [1-3]. Death by hypothermia is primarily reported in winter season [3], but is sometimes observed even in summer in mountainous areas [2]. It occurs not only outdoors, but even indoors, at an ambient temperature of approximately 15-20°C [3].

Hypothermia is caused by a negative balance between heat production and heat transfer. In case of decrease in heat production, such as due to starvation, malnutrition, metabolic disorders and aging, or increase in heat transfer due to a lower ambient temperature, wetting of the body and cloths, emaciation and alcohol consumption, death by hypothermia can occur.

There are few or no specific morphological findings in cases of fatal hypothermia [2, 4-11], and the diagnosis would be made by exclusion [9, 11]. It has been reported that, in cases of hypothermic death, blood in the left cardiac chamber is bright red compared to that in the right cardiac chamber (Figure 1). This finding is commonly observed at autopsy in cases of hypothermic death [5-10, 12, 13]. This color difference is due to the difference in oxygen content in blood in the two chambers, and is easily measurable by the CO-oximeter [5-8, 10, 12, 13].

CO-oximeter is widely used for evaluation of oxygenation not only in clinical laboratories, emergency departments and intensive care units [14-16], but also in forensic practice [6-8, 10, 12, 13, 17-37]. In the present chapter, we discuss about the forensic diagnostic applications of the CO-oximeter in cases of death by hypothermia.

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Figure 1. Color difference between blood in the left and right cardiac chambers in cases of death by hypothermia.

2. Pathophysiology and Risk Factors of Hypothermia

Body temperature of humans is regulated and remains relatively unchanged under various environmental conditions. If the body is exposed to cold temperatures, heat transfer is lowered by vasoconstriction and piloerection to prevent heat loss, and heat production is increased by shivering and increase in metabolic activity [1, 2]. However, the thermoregulatory mechanism is disrupted by various conditions, such as severe cold environmental conditions, malnutrition, fatigue, trauma and the influence of alcohol or drugs, which can lead to hypothermic death on exposure to cold ambient temperatures.

Low environmental temperature, damp conditions and wind are risk factors for hypothermia [1-4]. Heat transfer is potentiated by strong winds. Further, since water conducts heat 20-25 times faster than air, wet conditions of the body or clothes enhance heat loss as compared to dry conditions [1, 2]. Poor nutritional status is another risk factor of hypothermia, since the low muscle mass results in decreased heat production [2].

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Alcohol is a risk factor for hypothermia through a variety of mechanisms. It causes peripheral vasodilation and increases sweating, both of which increase heat loss from the body. Alcohol also causes muscle relaxation and inhibition of shivering. Further, it has a direct effect on themoregulation in the brain [2]. These mechanisms increase the risk of hypothermia. Various drugs such as central nervous system depressants, are risk factors for hypothermia, as they can cause unconsciousness or immobility. Alcohol and drug intoxication cause cognitive and motor function impairment, decreasing the ability to move away from cold exposure [2]. This decrease in the ability to move away from cold is also observed in cases of trauma, some kinds of natural disease, such as cerebral dysfunction, dementia, hypothyroidism, hypoglycemia (due to food depletion or diabetes) and psychiatric diseases. Elderly people have a higher risk of both immobility and natural disease. Children have an increased risk of hypothermia due to a high body surface area-body mass ratio [2].

The morphological findings in cases of death due to hypothermia depend on the duration of exposure to cold before death [1]. If the duration of the cooling process is short, such as hypothermia due to immersion in cold water, autopsy features might be completely lacking [1].

The final cause of death in hypothermia is either ventricular fibrillation or asystole, that may be due to various factors, such as hypoxia, decrease of enzyme activity and electrolyte dysregulation [1].

3. Forensic Diagnosis of Hypothermia

Diagnosis of fatal hypothermia is based not only on autopsy findings, but also on the circumstances of the victim's surroundings [4]. In the case of death by hypothermia, the following findings are observed.

As external findings [1-4, 8, 9], frost erythema might be observed as a red to brownish discoloration of the skin that is distributed over the extensor surfaces of the large joints such as the knees and elbows, and sometimes the shoulder. Postmortem lividity is often bright red rather than purple.

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Additionally, Wischnewsky's spots might be observed as autopsy findings [1-4, 8, 9, 38, 39] in 40-90% of fatal hypothermia cases [2, 39, 40]. These spots occur due to hemorrhagic erosion of the gastric mucosa. Pancreatic hemorrhage and hemorrhage in the iliopsoas muscle are also sometimes observed. Fatty changes in hepatocytes, cardiac myocytes and renal tubular cells are observed as histological findings [1-4, 8, 9]. Differences in blood color between the left and right cardiac chambers are also observed [1, 5-10, 12, 13].

Scene investigation should also be performed. Ambient air and water temperatures and environmental conditions, such as wind speed and humidity, must also be investigated. The clothing of the victim and its condition (wet or dry), evidence of intoxication, such as empty bottles and packets of drugs, should also be collected and documented [1-4].

4. Application of the CO-Oximeter for Forensic Diagnosis of Hypothermia

We have investigated the oxyhemoglobin saturation ratio (%O_2Hb) in hypothermic death case using a portable CO-oximeter AVOX4000 (AVOX; International Technidyne Corporation, NJ, USA) (Figure 2) [12]. Left and right heart blood were examined routinely at medicolegal autopsy of 13 cases (26 samples) diagnosed as death by hypothermia, based on the autopsy findings and circumstances of the victims' surroundings (hypothermia group). Seventeen cases (five natural deaths, two asphyxias, seven drownings and three trauma deaths) were evaluated as a control group. The subjects included 18 males and 12 females, 23-96 years of age [12]. Since the value of %O_2Hb could have increased as a result of cardiopulmonary resuscitation or administration of oxygen, we excluded cases who received cardiopulmonary resuscitation [10].

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Figure 2. Portable oximeter (AVOX 4000).

Figure 3 shows $\%O_2Hb$ values in blood from the left and right cardiac chambers in each group. In the hypothermia group, the $\%O_2Hb$ value in left heart blood was significantly higher than that in right heart blood. Additionally, $\%O_2Hb$ values of right heart blood in the hypothermia group were significantly higher than those in the control group.

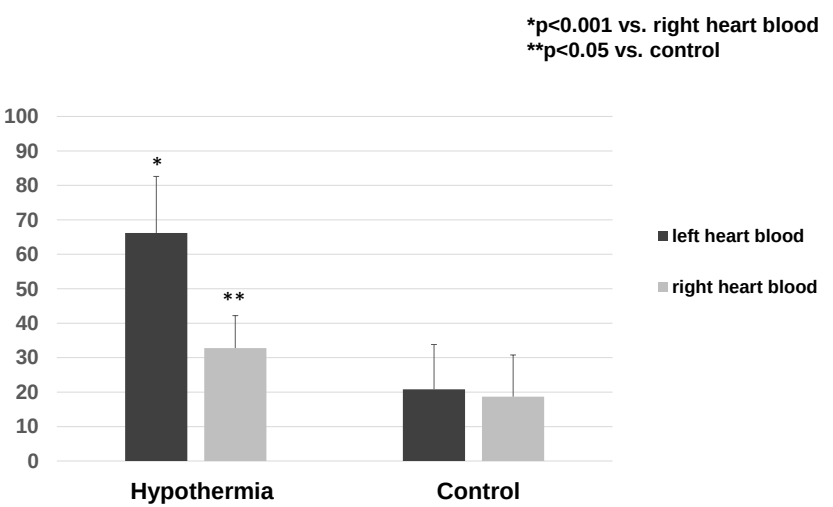


Figure 3. Mean values of oxyhemoglobin saturation ratio ($\%O_2Hb$) of left and right heart blood in cases of death due to hypothermia and the control group.

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Blood in the left cardiac chamber is bright red in color compared to that in the right cardiac chamber in hypothermic death cases [6-8, 10, 13]. As this finding was observed in over 90% of hypothermic death cases [6, 8, 10], this might be one of the characteristic signs of hypothermic death. Our results also support these previous reports.

The higher level of $\%O_2Hb$ in left heart blood might be due to many factors, such as decrease in body temperature, increased binding of oxygen to hemoglobin (Hb) at lower temperatures, and inhalation of cold air before death [6, 8, 10]. Blood in the left heart usually has a higher oxygen content than that in the right heart. In cases of hypothermic death, the lower body temperature maintains the antemortem composition of Hb following death, while also enhancing binding of oxygen to Hb [6, 8, 10].

In our study, $\%O_2Hb$ values in right heart blood in the hypothermia group were significantly higher than those in the control group. Oxygen consumption in peripheral tissues, except skeletal muscle, decreases along with the decrease in body temperature. Reportedly, total oxygen consumption decreases by 6-9% with a decrease of 1℃ in body temperature [41]. This might be due to vasoconstriction in peripheral tissues. Further, the affinity between oxygen and Hb increases approximately 1.7 times following a 10℃ decrease in temperature [42]. The higher $\%O_2Hb$ values in right heart blood in the hypothermia versus the control group might thus reflect a decrease in total oxygen consumption and inhibition of oxygen dissociation from Hb in the hypothermia group.

Yajima et al. proposed designating an oxyhemoglobin (O_2Hb) value in left cardiac blood of ≧ 36% as a basic condition in the diagnostic criteria of death by hypothermia, with a difference in the O_2Hb saturation level between left and right heart blood of ≧ 13% or O_2Hb saturation ratio between left and right heart blood of ≧1.8, as a complementary condition [10]. This finding reflects the final balance of oxygen uptake and consumption in the dying process, and can be estimated using the CO-oximeter, for forensic diagnosis of the pathophysiological status of the victim at the time of death.

As O_2Hb saturation in postmortem heart blood is usually very low (approximately 10-20%) due to diffusion of oxygen out of the blood in the

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postmortem interval between death and postmortem examination, postmortem blood gas analysis is less valuable for interpretation of cause of death in most cases except hypothermia [23]. However, the composition of Hb in hypothermia cases provides valuable information for forensic diagnosis.

The portable CO-oximeter AVOX4000 uses seven wavelengths of light (488.4, 520.1, 562.4, 585.2, 597.5, 621.7, 671.7 nm) for determination of Hb derivatives (O_2Hb, reduced hemoglobin, carboxyhemoglobin and methemoglobin) in blood [32]. Since its use has many advantages, such as lack of the need for sample preparation, easy handling and the fact that its results are obtainable within one minute, it is widely used as routine examination in daily forensic practice.

CONCLUSION

We discussed here about the application of CO-oximeters in the forensic diagnosis of death due to hypothermia. The oximetric profiles of such cases are considered to reflect the final balance of oxygen uptake and consumption [23]. Besides their use in forensic medicine, CO-oximeters might be applicable as a monitoring tool in therapeutic hypothermia [43]. Further applications of the CO-oximeter in various fields of medicine can be expected.

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REFERENCES

[1] Madea, B. (2014). Injuries due to cold. In: Madea B. editor. *Handbook of Forensic Medicine.*, pp. 468-476. West Sussex, Oxford, John Wiley & Sons, Ltd.

[2] Turk, E. E. (2010). Hypothermia. *Forensic Sci Med Pathol*, *6*, 106-115.

[3] Dettmeyer, R. B., Verhoff, M. A. & Schütz, H. F. (2014). Hypothermia, cold and frost. In: *Forensic Medicine*., pp. 209-212. Berlin, Heidelberg, Springer.

[4] Saukko, P. & Knight, B. (2016). *Knight's forensic pathology* (4th Ed). Boca Raton, FL, CRC Press.

[5] Shimizu, K., Shiono, H., Fukushima, T. & Sasaki, M. (1996). Diagnosis of fatal hypothermia -Differences in color between blood from the right and left ventricles and prevalence of Wischnewski's Spot-. *Acta Crim Japon*, *62*, 157-160.

[6] Shimizu, K., Mizukami, H., Fukushima, T., Sasaki, M. & Shiono, H. (1998). Use of a CO-oximeter for forensic diagnosis of hypothermia. *Nihon Houigaku Zasshi*, *52*, 196–201.

[7] Mizukami, H., Shimizu, K., Shiono, H., Uezono, T. & Sasaki, M. (1999). Forensic diagnosis of death from cold. *Leg Med* (Tokyo), *1*, 204-209.

[8] Shiono, H. & Shimizu, K. (2007). Necropsy and autopsy diagnosis of fatal hypothermia. *Res Pract Forens Med*, *50*, 1-7.

[9] Funayama, M. (2008). Diagnosis of hypothermia. *Nihon Hoigaku Zasshi*, *62*, 145-154.

[10] Yajima, D., Asari, M., Okuda, K., Maseda, C., Yamada, H., Ichimaru, C., Matsubara, K., Shiono, H., Iwase, H., Makino, Y. & Shimizu, K. (2015). An objective approach using three indexes for determining fatal hypothermia due to cold exposure; statistical analysis of oxyhemoglobin saturation data. *Leg Med* (Tokyo), *17*, 451–458.

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[11] Türk, E. E., Sperhake, J. P., Pueschel, K. & Tsokos, M. (2005). An approach to the evaluation of fatal hypothermia. *Forensic Sci Med Pathol*, *1*, 31-35.

[12] Tanaka, N., Ameno, K., Jamal, M., Kumihashi, M. & Kinoshita, H. (2011). Application of oximeter AVOX 4000 in the forensic practice (report 2) –Diagnosis for hypothermia-. *Res Pract Forens*, *54*, 205-209.

[13] Kanoto-Nishimaki, Y., Saito, H., Watanabe-Aoyagi, M., Toda, R. & Iwadate, K. (2014). Investigation of oxyhemoglobin and carboxyhemoglobin ratios in right and left cardiac blood for diagnosis of fatal hypothermia and death by fire. *Leg Med* (Tokyo)., *16*, 321–325.

[14] Zijlstra, W. G., Buursma, A. & Zwart, A. (1988). Performance of an automated six-wavelength photometer (Radiometer OSM3) for routine measurement of hemoglobin derivatives. *Clin Chem*, *34*, 149-152.

[15] Mahoney, J. J., Vreman, H. J., Stevenson, D. K. & Van Kessel, A. L. (1993). Measurement of carboxyhemoglobin and total hemoglobin by five specialized spectrophotometers (CO-oximeters) in comparison with reference methods. *Clin Chem*, *39*, 1693-1700.

[16] Bailey, S. R., Russell, R. N. & Martinez, A. (1997). Evaluation of the avoximeter: precision, long-term stability, and use without heparin. *J Clin Monit*, *13*, 191-198.

[17] Okada, M., Okada, T. & Ide, K. (1985). Utilization of a CO-oximeter in the medico-legal field. *Nihon Houigaku Zasshi*, *39*, 318-325.

[18] Okada, M., Okada, T., Kawaguchi, N. & Ide, K. (1986). Studies of methemoglobin production in the blood of dead burn victims. *Nihon Houigaku Zasshi*, *40*, 124-128.

[19] Ohshima, T., Takayasu, T., Nishigami, J., Lin, Z., Kondo, T. & Nagano T. (1992). Application of hemoglobin analysis by CO-oximeter to medico-legal practice with special reference to diagnosis of asphyxia. *Nihon Houigaku Zasshi*, *46*, 382–388.

Complimentary Contributor Copy

[20] Higuchi, T., Noguchi, K. & Maeda, H. (1992). An evaluation of analyzed data of hemoglobin derivatives by CO-oximeter in medico-legal autopsy. *Nihon Houigaku Zasshi*, *46*, 416–418.

[21] Maeda, H., Fukita, K., Oritani, S., Nagai, K. & Zhu, B. L. (1996). An evaluation of post-mortem oxymetry in fire victims. *Forensic Sci Int*, *81*, 201–209.

[22] Oritani, S., Nagai, K., Zhu, B. L. & Maeda, H. (1996). Estimation of carboxyhemoglobin concentrations in thermo-coagulated blood on a CO-oximeter system: an experimental study. *Forensic Sci Int*, *83*, 211–218.

[23] Maeda, H., Fukita, K., Oritani, S., Ishida, K. & Zhu, B. L. (1997). Evaluation of post-mortem oxymetry with reference to the causes of death. *Forensic Sci Int*, *87*, 201–210.

[24] Lee, C. W., Tam, J. C. N., Kung, L. K. & Yim, L. K. (2003). Validity of CO-oximetric determination of carboxyhaemoglobin in putrefying blood and body cavity fluid. *Forensic Sci Int*, *132*, 153–156.

[25] Brehmer, C. & Iten, P. X. (2003). Rapid determination of carboxyhemoglobin in blood by Oximeter. *Forensic Sci Int*, *133*, 179–181.

[26] Watanabe, N., Terazawa, K. & Sakaihara, M. (2003). Blood storage for forensic hemoglobin analysis using CO-oximeter. *Hokkaido Igaku Zasshi*, *78*, 289-295.

[27] Watanabe, N. (2003). Medico-legal application of hemoglobin analysis using CO-oximeter. *Hokkaido Igaku Zasshi*, *78*, 557-566.

[28] Olson, K. N., Hillyer, M. A., Kloss, J. S., Geiselhart, R. J. & Apple, F. S. (2010). Accident or arson: Is Co-oximetry reliable for carboxyhemoglobin measurement postmortem? *Clin Chem*, *56*, 515-520.

[29] Tanaka, N., Ameno, K., Jamal, M., Ohkubo, E., Kumihashi, M. & Kinoshita, H. (2010). Application of oximeter AVOX 4000 for the determination of CO-Hb in the forensic practice. *Res Pract Forens Med*, *53*, 39-43.

[30] Tanaka, N., Ameno, K., Jamal, M., Kumihashi, M. & Kinoshita, H. (2011). Application of oximeter AVOX 4000 in the forensic practice

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(report 2) –Diagnosis for hypothermia-. *Res Pract Forens Med*, *54*, 205-209.

[31] Tanaka, N., Ameno, K., Jamal, M., Kumihashi, M., Miyatake, N. & Kinoshita, H. (2012). Effects of sampling methods and storage on the value of oxyhemoglobin ratio and carboxyhemoglobin ratio. *Res Pract Forens Med, 55,* 51-55.

[32] Fujihara, J., Kinoshita, H., Tanaka, N., Yasuda, T. & Takeshita, H. (2013). Accuracy and usefulness of the AVOXimeter 4000 as routine analysis of carboxyhemoglobin. *J Forensic Sci*, *58*, 1047-1049.

[33] Fujihara, J., Hasegawa, M., Kato, T., Miura, M., Iida, K., Kinoshita, H., Tanaka, N. & Takeshita, H. (2013). A case of drowning lacking typical autopsy findings of carbon monoxide poisoning despite the high CO concentration. *Forensic Toxicol*, *31*, 180-182.

[34] Nakagawa, H., Maebashi, K., Fukui, K., Ochiai, E. & Iwadate, K. (2013). Applicability of a new CO oximeter to forensic autopsy cases. *Acta Crim Japon*, *79*, 16-21.

[35] Tanaka, N., Ameno, K., Jamal, M., Kumihashi, M., Miyatake, N. & kinoshita, H. (2014). Effects of sample dilution on the value of carboxyhemoglobin ratio. *Japanese Journal of Forensic Pathology*, *20*, 39-40.

[36] Tanaka, N., Kinoshita, H., Jamal, M., Takakura, A., Kumihashi, M., Miyatake, N. & Ameno, K. (2015). Squeezed splenic blood sampling as an alternative method for carboxyhemoglobin measurement. *Rom J Leg Med*, *23*, 106-108.

[37] Tanaka, N., Takakura, A., Kumihashi, M., Jamal, M., Ito, A., Kimura, S., Tsutsui, K., Ameno, K. & Kinoshita, H. (2018). Application of portable oximeter for the determination of methemoglobin in forensic practice. *Rom J Leg Med*, *26*, 295-297.

[38] Mant, A. K. (1969). Autopsy diagnosis of accidental hypothermia. *J Forens Med, 16*, 126-129.

[39] Hirvonen, J. (1976). Necropsy findings in fatal hypothermia cases. *Forensic Sci*, *8*, 155-164.

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[40] Tsokos, M., Rothschild, M. A., Madea, B., Riβe, M. & Sperhake, J. P. (2006). Histological and immunohistochemical study of Wischnewsky spots in fatal hypothermia. *Am J Forensic Med Pathol, 27*, 70-74.

[41] Tanjoh, K., Utagawa, S., Kinoshita, K. & Hayashi, N. (2001). The effects of hypothermia on the oxygen metabolism. *ICU & CCU, 25*, 763-769.

[42] Roughton, F. J., Adair, G. S., Barcroft, J., Goldschmidt, G., Herkel, W., Hill, R. M., Keys, A. B. & Ray, G. B. (1936). The thermochemistry of the oxygen-haemoglobin reaction: Comparison of the heat as measured directly on purified haemoglobin with that calculated indirectly by the Van't Hoff Isochore. *Biochem J, 11*, 2117-2133.

[43] Karcioglu, O. (2017). Therapeutic hypothermia in trauma management: New tricks for an old dog? *Emerg Med Open J, 3*, 30-37. Doi: 10.17140/EMOJ-3-137.

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