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*Editors*

# Chocolate and Health

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Rodolfo Paoletti • Andrea Poli • Ario Conti • Francesco Visioli
(Editors)

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# Preface

Cocoa and chocolate are consumed throughout the world, as they provide instant enjoyment and pleasure. Chocolate is one of the most popular examples of foods consumed during comfort eating.

Due to the high polyphenol content of cocoa, coupled with its widespread presence in many food items, this food is now of particular interest from the nutritional and "pharmacological" viewpoints. Indeed, the number of publications concerning cocoa and chocolate is increasing steadily. As an example, more than 1,300 publications regarding "cocoa" or "chocolate" have been added to the PubMed database during the last 5 years; this is an increase of about 60% on the previous 5 years. Of such publications, an increasing proportion concerns the effects of polyphenols and, in particular, flavonoids on human biology and health. Finally, we witness an increasing number of patents covering industrial processes that aim at maintaining the highest possible amount of polyphenols in cocoa.

Cocoa and chocolate, on the other hand, are also correctly viewed as highly caloric foods and their use if often restricted by primary care physicians and dieticians, especially when weight loss is needed.

This book provides a state-of-the-art discussion on cocoa and chocolate, covering the multiple facets of their production, consumption and biological activities. The authors provide in-depth analyses of the manifold aspects of these foods. Notably, this book covers all aspects of the biological activities of cocoa, with particular reference to the cardiovascular system (which attracts the majority of research) and to patterns of consumption. The effects of cocoa and chocolate on the cardiovascular system are complex and comprise the control of risk factors (such as elevated blood pressure) or more basic effects such as stabilization of nitric oxide, a molecule whose activity is crucial in cardiovascular physiology. Notably, the effects of chocolate on plasma lipid levels (to which a specific chapter of this book is dedicated, Chapter 10) appear to be much less noticeable than what is usually perceived.

Moreover, it is now clear that cocoa and chocolate do not contain addictive substances in amounts high enough to cause cravings. Indeed, cravings are the result of an unhealthy relationship with the food, resulting from attributions displayed in newspaper and magazine headlines such as "chocolate is addictive", but the alleged craved-for chemicals are merely myths.

In terms of health benefits, cocoa indeed has the highest polyphenolic contents of all foods on a per-weight basis and markedly contributes to the total dietary intake of flavonoids. The main subclasses of flavonoids found in cocoa are flavanols, particularly the flavanol monomers catechin and epicatechin, and their oligomers, also known as procyanidins. Although the precise mechanisms responsible for their purported health benefits are unclear and likely to be manifold, flavonoids and flavanols have been shown to possess a range of cardiovascular-protective properties, including antioxidant and antiplatelet effects, immunoregulatory activity, and vasorelaxation (as outlined in this book).

In summary, this is to our knowledge the first comprehensive book on health effects of cocoa and chocolate, and readers will find an updated discussion on this topic completed by a detailed analysis of the various aspects of chocolate production.

Research in this area is rapidly progressing and other effects of cocoa on health are being discovered. Thus, we would welcome comments and feedback and we hope you will enjoy reading this book.

October 2011

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# Chocolate as Medicine: A Changing Framework of Evidence Throughout History

1

Philip K. Wilson

## 1.1 Introduction

In 1753, the noted nosologist, Carl Linnaeus, named it *Theobroma cacao*, food of the Gods. Two and a half centuries later, Joanne Harris emphasized this exotic's erotic sensations in her award winning fiction debut, *Chocolat*. For millennia, healers have touted its myriad medicinal, yet mystical, abilities. By the 1950s, chocolate, what had long been used as a drug, a food and as a source of currency, was being marketed merely as a pleasure-filled snack. Over the next half century, the craving to carve out chocolate's healthy, medicinal qualities resurged.

> *I haven't had this much good news since the early [19] 70s when I learned I had passed all of the math requisites for my college degree. First it was the study that found napping was good for us and now it's the news that cocoa may boost brain function and delay decline as we age. That's right, two of my favorite things which previously had gotten bad raps, have now been determined to be good for me* [1].

Few natural products have been purported to effectively treat such a wide variety of medical disorders as has chocolate, ranging from a "specific" to an aphrodisiac to a panacea. Many of these claims go as far back as Aztec medical practice. There, remedies concocted from cacao beans formed in pods of the "Chocolate Tree" were used to soothe stomach and intestinal complaints, control childhood diarrhea, reduce fevers, steady the faintheart-ed, expel phlegm by provoking cough, reduce the passage of blood in stool and to promote strength before military conquests as well as before "acts of venery". In later eras, chocolate remedies were used to combat emaciation, decrease "Female Complaints", delay hair growth, promote the expulsion of kidney stones, increase breast milk production, prolong longevity, both encourage and prohibit sleep, clean teeth and diminish one's timidity [2].

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R. Paoletti et al. (eds.), *Chocolate and Health*,

For centuries, chocolate's aphrodisiac aspect was purported.

> *'Twill make Old Women Young and Fresh*
> *Create New-Motions of the Flesh,*
> *And cause them to long for you know what,*
> *If they but Tast[e] of Chocolate* [3].

Dr Munday's *Treatise on Foods* noted a patient "in a miserable condition" who, after "supping of Chocolate ... [was] recovered in a short Time; but what is more extraordinary is, that his Wife in Complacency to her Husband, having also accustomed herself to sup Chocolate with him, bore afterwards several Children, though she was looked upon before not capable of having any" [4].

Seeking support for chocolate's aphrodisiac qualities, some turned first to the Bible and the Classics, then created "What If" history. For example, if the Biblical "Rachel had known [of chocolate], she would not have purchased mandrakes for Jacob. If the amorous and martial Turk should ever [have] taste[d] it, he would despise his Opium. If the Grecians and Arabians had ever tried it, they would have thrown away" their remedies in favor of "our rude Indian" decoction [5]. Elsewhere, some rather outlandish side effects of chocolate's otherwise healthy benefits were reported. According to the Marquise de Sévigné, the Marquise de Cöetlogon delivered a child as "black as an Indian". The reputed cause: she drank too much chocolate during her pregnancy [6]! More common was the side effect, as noted in the personal narratives of Thomas Gage and William Hughes, that regular chocolate drinkers just got a bit larger over time.

> *The "buttery parts" of the cacao tend to "fatten" people because "the 'hot ingredients' of medicinal chocolate serve as a type of pipe or conduit ... and make it pass by the liver, and the other parts till they arrive at the fleshy parts, where finding a substance which is like and comfortable to them, ... [they] convert themselves into the substance of the subject [whereby] they augment and fatten it"* [7].

Accounts of chocolate's reputed medical value typically recount the chronological discoveries of cacao seed (bean) products and the sequential improvements in cacao preparations. However, little attention has been directed to the various types of evidence used to support such claims. Among the earliest evidence for chocolate's use as a medical compound are the remaining iconographic works and fragments of Olmec, Maya, Zapotec, Mixtex and Aztec art. Additional records from these eras are provided in groups of writings preserved under such names as the Florentine and Tuleda Aztec Codices and the Dresden and Madrid Mayan Codices. In recent decades, new forms of evidence have been uncovered in the remnants of *Theobroma cacao* found in the pottery and crockery of the Mokaya of Mesoamerica dating back to 1900 BC [8].

Throughout much of history, the importance and relative weight of oral tradition has been paramount. Indeed, this was the means by which people in earlier times generally learned of chocolate's potential health benefits. Without diminishing the importance of oral traditions, this chapter focuses primarily upon documentary history drawn from surviving recorded evidence. Here, chocolate's medical use is explored by examining the types

of evidence generated in three distinct Euro-American eras: (1) The Early Modern Period, (2) Eighteenth through Early Twentieth Centuries, and (3) Modern Biomedical Worldviews. However, before turning to these eras, a brief reflection upon the "evidence" used to support general modern biomedical and health claims is warranted.

## 1.2 Valuing Medical "Evidence" in the Past and Present

In our era, the therapeutic efficacy of medical practices and remedies has been recast within the mold of Evidence-Based Medicine (EBM). Beginning in the early 1990s, the then newly established clinical discipline of EBM referred to the "conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients" [9]. Clinical expertise is combined with newly supported biomedical evidence obtained through systematic literature searches to ensure the delivery of the highest quality health care. EBM also incorporates a "thoughtful identification and compassionate use of individual patients' predicaments, rights and preferences in making clinical decisions about their care" [9]. Including the patient in the decision-making process conforms with the late Cornell University internist, Eric Cassell's notable directive that to effectively relieve suffering, physicians must be ever mindful of a patient's entire personhood [10].

Since the early 1990s, EBM has been adopted into medical school curricula, celebrated in medical manuals, deliberated in medical literature and featured at myriad medical conferences. The vast international consortium of clinicians and consumers known as the Cochrane Collaboration has provided systematic literature reviews as required by EBM methodology [11]. Despite its popularity, arguments have recently surfaced claiming that EBM is either "old hat" or "impossible to practice" [12]. At the heart of the matter lies the concern in acknowledging what counted as evidence in different eras of medicine's heritage.

Around the time when chocolate was first introduced into European culture, the terms "experience" and "evidence" were reexamined within scientific and medical contexts. Sir Francis Bacon, best known at the time for his financial prowess as Lord Chancellor under the reign of England's James I, is credited with providing a new framework for science: the experimental method. If the purpose of science was, as he argued, to give humans mastery over nature, thereby extending both human knowledge and power, then the laws of nature must be better understood. Such understanding, so Bacon proclaimed in *Novum Organum* (1620), was attainable only after shifting scientific thought from deductive reasoning towards an inductive approach coupled with experimentation.

Bacon's inductive method of interpreting nature, which others later applied to chocolate, involved the assembly of a "sufficient, ... accurate collection of instances", or evidence, gathered "with sagacity and recorded with Impartial plainness ... from which, after viewing them in all possible lights, to be sure that no contradictory ... [evidence] can be brought, some portion of useful truth", general law, or hypothesis will be established [13]. He decried that natural philosophers who relied solely upon the authority of the past, which for all university graduates of his day was still the ancient logic (or *Organon*) of Aristotle, failed to advance any new understanding of nature. Bacon advocated the experimental method as the most reliable manner to free science from the

"paralysing dependence of previous students of nature on the rough and ready conceptual equipment of everyday observation" [14].

"Experience" and "experiment", two synonymous expressions in the Romance languages, were used interchangeably in discussing the Baconian vision of evidence-based healthcare practice. For Baconian physicians, "ordered experience", founded upon methodological investigation, measurable criteria, and objectivity, counted as "evidence" whereas "ordinary experience" based solely upon chance observation and subjectivity, did not [15].

Bacon's suggestions for revolutionizing science were more formally embodied in the formation of London's Royal Society in 1660. This elite body, whose Fellows included the city's leading physicians, undertook the task of critically appraising the current state of knowledge. Their motto, *nullius in verba*, upon the word or authority of no one, stressed the Society's reliance upon experiment and personal experience over preconceived theorization. Moreover, the interactive environment within the Royal Society, explicitly according to Bacon's description of a utopian "college of experience", encouraged discussion and collaboration between investigators. This Society provided the first venue in which members with similar interests gathered to listen to reports of each other's experiences with various natural phenomena. The accounts in their publication, the *Philosophical Transactions*, were written from the viewpoint of the observer and, by convention, they contained details of the time, place, and participants or witnesses of a particular experience [16]. The elaborate narrative details in the reports were rhetorically constructed so as to "give the impression of verisimilitude", compelling the Fellows to accept the reported details as "matters of fact" [17].

Some areas of investigation readily adopted the experimental method as a means of gaining evidence. Other areas, including medical practice, continued to rely upon anecdotal evidence and individual case studies. Indeed, these forms of evidence substantiated much of the use of chocolate as medicine during the Early Modern Period.

## 1.3 Chocolate as Medicine: The Early Modern Period

Chocolate first became widely documented in the Old World during the 1600s. As part of this popularization, we find a number of monographs describing this new and somewhat mysterious substance. Antonio Colmenero de Ledesma's *Curioso Tradado de la Naturaleza y Calidad del Chocolate* (1631) widely spread the claims of this new substance through its translations into English, French, Latin and Italian. Among England's chief chocoholics was the physician, Henry Stubbe. His 1662 book, *The Indian Nectar; or, A Discourse Concerning Chocolata*, was specifically designed to help the English reading public overcome some common misconceptions regarding the strength and frequency of chocolate's use as a medicine. To support his claims, Stubbe relied upon case histories drawn from the lands of cacao's origin as the most solid form of evidence.

> *English soldiers "stationed in ... Jamaica lived [for many months on only] cacao nut paste mixed with sugar ... which they [drank having] dissolved [it] in water". Women of the New World were also reported as having eaten chocolate "so much ... that they scarcely consumed any solid meat yet did not exhibit a decline in strength"* [18].

Stubbe provided additional evidence from case studies of reputable New World physicians.

> *Chocolate is "one of the most wholesome and pretious [sic] drinks, that [has] been discovered to this day: because in the whole drink there is not one ingredient put in, which is either hurtful in it self, or by commixtion; but all are cordial, and very beneficial to our bodies, whether we are old, or young, great with child, or ... accustomed to a sedentary life"* [19].
>
> *Chocolate was "all that was necessary for breakfast, because after eating chocolate, one needed no further meat, bread or drink"* [20].

Further information regarding New World uses of chocolate is found in William Hughes' *The American Physician, or a Treatise of the Roots, Plants, Trees, Shrubs, Fruit, Herbs &c. Growing in the English Plantations in America; with a Discourse on the Cacao-Nut-Tree ... and All the ways of Making of Chocolate* (1672). Hughes penned his narrative after serving aboard ship to the West Indies where he became well acquainted with American herbs and their medical uses. In it, he noted that the inhabitants of Nicaragua, New Spain, Mexico, Cuba, and Jamaica so highly treasured the powers derived from the pods of the Chocolate Tree, that they took extreme measures to secure them within the "Shades of Plantain and Bonona[sic]-trees, against the Injuries of their fiery Sun ... ". He also noted anecdotally that Montezuma

> *is said to have treated Cortez and his Soldiers with it; and you cans carce read an American Traveller, but he will often tell you of the magnificent Collations of Chocolate, that the Indians offered him in his Passage and Journies [sic] through their Country* [21].

As a specific example of chocolate's medicinal properties, Hughes described how

> *Indians and Christians, in the American Plantations, have been observed to live several Months upon Cacoa Nuts alone, made into a Paste with Sugar, and so dissolved in Water; I myself have eat[en] great Quantities of these Kernels raw, without the least Inconvenience; and have heard that Mr [Robert] Boyle and Dr [Henry] Stubbe have let down into their Stomachs some Pounds of them raw without any Molestation; the Stomach seems rather to be satisfied than cloyed with them, which is an Argument they are soon dissolved and digested* [21].

Hughes informed his readers, drawing largely upon anecdotal information as well as upon what he termed "experimental Observations", that "curious Travellers and Physicians do agree" that chocolate "has a wonderful Faculty of quenching Thirst, allaying hectick Heats, [and] of nourishing and fattening the Body". He related how the English Dominican Friar, Thomas Gage who had spent time in the New World with Hernando Cortés, "acquaints us, that he drank Chocolate in the Indies, two or three Times every Day, for twelve Years together, and he scarce knew what any Disease was in all that Time", the only noticeable effect was that he grew "very fat". Others expressed disdain over the use

of pure chocolate, which they considered as "too oily and gross". Still, they admitted that "the Bitterness of the Nut makes Amends, carrying the other off by strengthening of the Bowels" [22].

Hughes offered his own personal narrative as evidence, informing readers that "he lived, at Sea, for some Months on nothing but Chocolate, yet neither his Strength nor Flesh were diminished". Indeed, like Gage, he "grew very fat in Jamaica, by Vertue of the Cacoa-nut". Accordingly, he claimed it to be of considerable help in counteracting "lean, weak, and consumptive Complexions". He also noted that it "may be proper for some breeding Women, and those Persons that are hypochondriacal and melancholy" [22].

Throughout Europe, case study evidence was among the most readily used rhetoric during the Early Modern Period to convince physicians and the public of chocolate's perceived benefits. Philippe Sylvestre Dufour provided such evidence in his popular and widely translated, *De l'Usage du Caphé, du Thé, et du Chocolat* (1671), as did Henry Mundy in his work for medical audiences, *Opéra Omnia Medico-Physica de Aëre Vitali, Esculentis et Potulentis cum Appendice de Parergis in Victu et Chocolatu, Thea, Caffea, Tobacco* (1685) and Marcus Mappus in *Dissertationes Medicae Tres de Receptis Hodie Etiam in Europa, Potus Calidi Generibus Thée, Café, Chocolata* (1695). Royal physician, Nicolas de Blégny offered case study evidence supporting chocolate's use in maintaining and restoring soldiers' health in his *Le Bon Usage du Thé, du Caffé, et du Chocolat pour la Préservation et pour la Guérison des Maladies* (1687).

Occasionally, cases were recorded from the view of the patient. For example, following festivities celebrating Charles II's Coronation, the diarist Samuel Pepys noted, "Waked in the morning with my head in a sad taking through the last night's drink, which I am very sorry for; so rose, and went out with Mr Creed to drink our morning draught, which he did give me in chocolate to settle my stomach" [23]. Like many of the earliest New World geographical and travel narratives, the few patient narratives of chocolate as medicine offered merely anecdotal evidence.

Reaching back to the earliest recordings of chocolate, evidentiary accounts beyond the mere anecdotal focus upon the importance of precise preparations. The Florentine Codex details the elaborate ritualistic preparations required to release cacao's therapeutic potential. Early travel narratives, such as Francisco Hernández's *Historia de las Plantas de la Nueva España* (1577), described how cacao's benefits were enhanced by mixing it with specific other ingredients local to a particular region. Stubbe, in *The Indian Nectar*, argued that all of the ingredients added to chocolate remedies must be precisely correlated with distinct individual constitutions. In addition to cacao itself, he noted,

> *the other Ingredients for making up Chocolate ... [must] be varied according to the Constitutions of those that are to drink it; in cold Constitutions, Jamaica Pepper, Cinnamon, Nutmegs, Cloves, & c. may be mixed with the Cacao-nut; some add Musk, Ambergrease, Citron, Lemonpeels, and odoriferous Aromatick Oils. In hot consumptive Tempers you may mix Almonds, Pistachos, ... and sometimes Steel and Rhuburb may be added for young green Ladies* [18].

In sum, Stubbe concluded,

> *[So] That you may know how to prepare your Chocolate, I will give you a short Direction – if you intend to make it up yourself, consult your own Constitution and Circumstances, and vary the Ingredients according to the Premises* [18].

In Early Modern Europe, an entire growing body of sources began to focus more upon an in-depth analysis of specific chocolate concoctions than upon either anecdotal or case study evidence. New herbals, pharmacopoeias, formularies and other works of *materia medica* drew upon many regions and cultures in their formulaic compilations of remedies. In such works, typically designed for professional use, the ingredients required for chocolate compounds were referenced alongside those of more long-standing use. One of the most widely translated of these new types of medical writing was Nicolas Lemery's *Traité Universel des Drogues Simples* (1698). In it, we learn that,

> *Chocolate is nourishing enough. It is strengthening, restorative, and apt to repair decay'd Strength, and make People strong. It helps Digestion, allays the sharp Humours that fall upon the lungs. It keeps down the Fumes of wine, promotes Venery, and resists the Malignity of the Humours. When Chocolate is taken to Excess, or that you use a great many sharp and pungent Drugs in the making of it, it heats much, and hinders several People to sleep* [24].

Guided by the medical wisdom of the four bodily humours, readers came to appreciate that,

> *Chocolate agrees, especially in cold Weather, with old People, with cold and phlegmatic Persons, and those that cannot easily digest their Food, because of the Weakness and Nicety of their Stomachs; but young People of a hot and bilious Constitution, whose Humours are already too much in Motion, ought to abstain from it, or use it very moderately* [24].

Acknowledgement is given to "the Americans" who "shew'd the Way of making it to the Christians", but readers of this French treatise were encouraged to consider that "the Chocolate made at Paris" was greatly "improved ... by the Compositions we use". The specific ingredients and formulaic steps of compounding remedies were offered, whereupon a product would be created that could be "eaten as is" or drank "after dissolving it in ... Common Water ... [or] Cows Milk, ... [or] Almond-Milk" or in the "juice ... of Plants" or mixed with "a little Bezoar stone ... to make it more Cordial". In specific reference to the compounding, Lemery noted that the name "chocolate" referred to the process of turning cacao into a drink. As an "Indian word", it is "compounded of *Choco*" meaning *sonus* or sound, and *Atte* or *Atle* meaning water, so named "because they commonly make use of Water to prepare Chocolate ... and make a little rustling with an Instrument called a Chocolate-stick [molinet], which is made use of to stir it" [25].

Lemery summarized chocolate's medicinal benefits, claiming that it would

*Help Digestion, recover decay'd Strength, and produce a great many the like Effects. It may be also good for phthisical [tubercular] People ... [particularly] because the Cacao-nut ... being full of oily and balsamic Principles, is ... very good for allaying and embarrassing the sharp Humours, which are predominant in those that are troubled with the Phthisick [sic], and for nourishing and recovering their solid Parts* [26].

Prescribers were warned to be mindful of dosages, noting that for just as chocolate

*produces good Effects, when used moderately, it also ... [produces] bad ones when taken to Excess, or mix't with too many sharp Drugs; for then it causes considerable Fermentations in the Humours, and heats much, and therefore is not good for bilious people. It also hinders People to Sleep, because its exalted Principles cause too great a Rarefaction in the Humours* [26].

Pierre Pomet, chief druggist to Louis XIV, in his *Histoire Générale des Drogues, Simples et Composées* (1694), also expounded at length upon compounding chocolate concoctions; the finest, he claimed, were available only in Paris [27]. London physician Edward Strother also described the various admixtures containing chocolate purely from a chemical viewpoint. Contemporary London practitioner, John Arbuthnot's account generally conformed with that of Strother, though he also hinted of chocolate's medicinal qualities. For example, the oil of chocolate "seems to be ... rich, alimentary, and anodyne". It is the oil, he emphasized, which gave it special power such that it "often helps Digestion and excites Appetite" [28].

Similar to their analysis of the benefits that particular components added to other compounded food and drug products, recipe books and pharmacopeias suggested a variety of ways in which essential ingredients must be combined with chocolate in order to achieve the desired effect. Typical additives to the cacao bean included cinnamon, saffron, nutmeg, Indian or Spanish Pepper, aniseed and vanilla to counteract the bitter tastes; flour made from cassava, maize or Indian corn, each of which acted as an emulsifier, then mixed together with egg yolk to bind it into a paste which was often dried into hard rolls or cakes or bricks. Almonds, hazelnuts and sugar were also frequently added. Such bricks, Dr Strother claimed, consisted of "particles truly nutritious and alimentary" which, due to the aromatics added, became "very nourishing, cordial and comfortable" [29].

## 1.4 Chocolate as Medicine: Eighteenth Through Early Twentieth Centuries

By the early 1700s, chocolate had become figuratively and literally linked with milk.

*Before chocolate was known in Europe, good old wine was called the milk of old men; but this title is now applied with greater reason to chocolate, since its use has become so common, that it has been perceived that chocolate is, with respect to them, what milk is to infants* [30].

London physician and Royal Society President Sir Hans Sloane specifically touted Milk Chocolate as the new restorative; an additive to his medical armamentarium gleaned from his 1687 voyage to Jamaica. Its benefits were primarily advertized for its "lightness on the stomach" and for its "great use in all Consumptive cases".

John Cadbury, and sons George and Richard, later purchased Sloane's Milk Chocolate. The Cadburys were Quakers who viewed chocolate as a nourishing, healthy alternative to alcohol and promoted it as a healthy "flesh forming substance". In order to enhance its popularity, the Cadbury Brothers promoted their Sloane recipe as a "health food" with rhetoric claiming that to call it a "medication would not be too strong a term" [31]. By diluting chocolate's singular nature with the addition of milk, its primary use as a medicine began to murkily blur with its use as a food.

In 1828, Coenraad Johannes Van Houten refined cacao into a more digestible form by extracting the natural fat (cocoa butter) from the bean, leaving only the powder. The powder was then mixed with potash to darken its color, lighten its flavor, and improve its solubility in water or milk. The powder produced by this "Dutching" process is what became known as cocoa. Van Houten's mixture soon became advertized as "The Food Prescribed by Doctors". Contemporary French manufacturers promoted their own milk chocolate remedies as being specifically beneficial for individuals with fragile stomachs as well as more generally for convalescents and children.

On occasion, earlier claims of chocolate's nutritive value reappeared. Cortés, for instance, had claimed that "a cup of this precious drink [chocolate] permits a man to walk all day without food". This claim paralleled Johann Wolfgang von Goethe's later notion that, "Whoever has drunk a cup of chocolate can endure a whole day's travel" [32]. Powerful though such claims were, their prevalence did not become prominent for many years.

The dual role of chocolate as both medicine and food carried over into the pharmacopeia literature as well. The 1834 *Dispensatory of the United States*, for example, touts the cocoa which was commonly being served "as a drink at the morning and evening meals" to also be "an excellent substitute for coffee in dyspeptic cases, being nutritive and digestible, without exercising any narcotic or other injurious influence". The same entry also promoted cocoa as "a good article of diet for convalescents" which "may sometimes be given advantageously as a mild nutritive drink in cases of disease" [33].

At this time, a new source of evidence for chocolate's health benefits became increasingly used: advertizing, or what in more recent eras has come to be called direct to consumer marketing. European as well as US advertisements throughout the 19th century established milk chocolate's enduring reputation as both medicine and food. In that way, entrepreneurial advertisers crossed multiple markets. Chocolate became the medicine handed out by confectioners and the food prescribed by physicians.

In 1764, Dr James Baker established the Baker Chocolate Company in Dorchester, Massachusetts. A century later, "Baker's Chocolate" began to be advertized as providing "an excellent diet for children, invalids, and persons in health; allay[ing], rather than induc[ing] the nervous excitement" that was a regular consequence "upon the use of tea or coffee". To further convince consumers, manufacturers noted that this product was "recommended by the most eminent physicians".

The Swiss inventor, Daniel Peter reached the end of an eight-year series of experiments in 1875 designed to establish a perfect combination of milk and chocolate. His experimental

product, milk chocolate, prepared from Henri Nestlè's condensed milk, cocoa, cocoa butter, and sugar, became quite popular. Challenging coffee's prior success, Peter's chocolate was "now a regular part of people's diet, but its nutritional value is higher [than that provided by coffee alone]". Conveying a strong sense of Swiss pride, Peter's chocolate ads were designed to persuade consumers that this "Original" milk chocolate was "a delicacy and a food in one luscious combination" which was "as distinct from ordinary eating chocolate as the Alps are from foot-hills".

Elevating chocolate's powers over that of the two other popular beverages was also an aim of Fannie Farmer's popular *The Boston Cooking School Cook Book* (1896), in which she argued that "cocoa and chocolate differ from tea and coffee inasmuch as they contain nutriment as well as stimulant". Many individuals were identified who "abstain from the use of tea and coffee [but who] find cocoa indispensible". Farmer continued, claiming that not only is chocolate "valuable for its own nutriment, but for the large amount of milk added to it. Cocoa may well be placed in the diet ... of a child after his third year", she claimed. Moreover, "invalids and those of weak digestion can take cocoa [with milk] where [pure] chocolate would prove too rich" [34].

The 19th-century Cocorico bar is advertized as "recommandé aux enfants & aux malades" as well as "constitute un ailment hygiénique par excellence". As with other contemporary chocolate products, it is recommended as "Délicieux pour malades & bien portants". Rothwell's Milk Chocolate was advertized as "a sweetmeat and a food". Cadbury ads urged their Dairy Milk Chocolate consumers to "Eat More Milk". Other ads presciently, though unknowingly, conveyed something of a double meaning, as in the cupid-clad ad for Versailles chocolate, Pascal, which meets "The heart's desire". Johnston's Chocolate ads of 1925 also recommend their R.S.V.P. brand for all "Affairs of the Heart".

The 19th century experienced a boom in improved methods for manufacturing larger quantities of chocolate, all the while maintaining its quality. Supporting this growth, chocolate's medicinal benefits became increasingly advertized in promotional literature. As such, these ads represent a shift in the source of evidence.

In an era of anti-germ campaigns of the early 1900s, a newly formed industrialist company in the United States began to promote the health and medical benefits of chocolate. Milton S. Hershey represented his milk chocolate as pure, natural, nutritional and wholesome. He advertized the nutritious qualities of chocolate against a background of green fields, cows and wholesome country milk. Milk, fortunately, was white, the color that the medical community had selected to represent clean, sterile and sanitary environments as part of their public health campaigns.

*The beautiful cows in the pasture fed,*
*Clean as could be from their tails to their head.*
*Making pure milk early and late*
*For making Hershey Cocoa and Chocolate.*

A further verse in this advertisement conveyed a similar idea:

*A child said to her mother dear,*
*Cocoa and Chocolate are healthful, I hear.*

*"Yes", said her mother, "if they're pure and fresh".*
*Said the child, "Well, that means Hershey's I guess"* [35].

Another booklet on chocolate advertisements displayed in a recent "The Kiss Story" exhibit at the Hershey Museum also focused on the healthy benefits of chocolate.

*The little story illustrated is interesting to the child, and there is also a message in this to all mothers who are interested in their children's health. Pure foods such as Hershey's Cocoa and Chocolate, manufactured under such ideal conditions, are used as a standard of purity. There is nothing more healthful or nourishing than good Cocoa. Its food value cannot be excelled for either young or old. Why injure the child's health with tea or coffee when Cocoa more than satisfies, builds up their tissues, or is in other words, 'A Food to Drink'* [36].

Specifying the nutrient content of chocolate products became more standard in mid-20th-century advertisements. Schrafft's claimed in 1931 that their chocolates provided "one of nature's shortest cuts to stimulation through food". Thus, "for your health's sake, keep a box handy when you work or play". This rhetoric of chocolate's utility was about to take a quick turn. By 1940, Nestlé proffered their chocolate as a "Fighting Food", featuring a comparative table of the energy provided by a lamb chop, milk, eggs, bread and their chocolate. The chocolate bar, they argued, has "come into its own on every fighting front of the war" since it was able to offer "maximum nourishment with minimum bulk". There is "more quick energy packed into the familiar chocolate bar than is contained in many recommended energy foods". As such, it became "one of the answers to the problem of keeping the soldier supplied with food in modern, high-speed warfare". Mars Bar ads in 1943 proclaimed that their "chunks of sheer delicious goodness" were "made with *chocolate* to sustain, *glucose* to energize, [and] *milk* to nourish" the troops.

Captain (later Colonel) Paul Logan envisioned chocolate's use as an emergency soldier ration. In 1937, he appointed Pennsylvania's Hershey Company to purposefully diminish the good taste of their product and create a 4 oz chocolate bar for troops to use only in emergency. Three of these individually foil wrapped 600 calorie "Logan bars" were distributed to GIs as emergency D Rations. As needed in emergency conditions, the caloric content of this D Ration could sustain a solider for a day. By 1943, Hershey produced a tastier "Tropical Bar" that was designed to withstand the more extreme heat of the Pacific Theatre of war [37].

Following WWII, another of chocolate's beneficial claims began to take center stage. Namely, chocolate was also being advertized as a means to allay mental stress. We find this in an enduring advertisement of Fry's Chocolate in which a young lad's face was shown sequentially moving through five sensations: desperation, pacification, expectation, acclamation, and finally realization, depicting anticipated craving-like experiences while longing for a Fry's chocolate bar. Concurrently, ads increasingly suggested chocolate cravings, particularly in women. Mental stress and chocolate craving were highlighted in an advertisement labeled "Martine and Her Problems – Chocolate: Whim or Necessity?" In this ad from a 1955 *Paris-Match* magazine, we learn the following:

*Often, Martine, you feel like eating a bar of chocolate ... but you don't, because you think you shouldn't – and so you are being unfair to yourself. You don't want chocolate; you need it! You've done the shopping and the housework, and still somehow found the energy to be the perfect hostess. You are exhausted, your body urgently requires energy in compensation, and you naturally feel the urge to eat chocolate, because it is a balanced food which instantaneously restores the essential elements that you have used up. You deserve it, so why feel guilty about tucking into a bar of chocolate* [38]*?*

## 1.5 Chocolate as Medicine: The Modern Era

Not all of chocolate's reputed medical benefits central to 20th century ads were new. However, some products containing ingredients derived from the cacao bean gained unprecedented popularity. Cocoa butter, for example, increasingly appeared in ads for ointments, suppositories, pessaries and pomades. It was also readily used as an emollient for skin massages as well as for healing chapped lips and, in nursing women, as a remedy to heal sore and cracked nipples. For individuals unable to digest cocoa fat, cocoa shell infusions were recommended as a restorative and analeptic tonic. Chocolate was also routinely used in a variety of laxatives, tonics, tablets and lozenges as an excipient to mask the rather harsh and unpleasant tastes of some of the other ingredients. Such flavorant properties were behind chocolate's use in Ex-Lax, Catstrophene, Bordon's Hemo and Bosco's Chocolate Syrup, which in 1951 was advertized from the viewpoints of both child and pediatrician. Post's Bran Chocolate, a "Delicious Health Confection", was promoted to parents as "Bran in Candy Form" for children in the mid 1920s.

By mid century, a new type of evidence was beginning to be used to support chocolate's medical benefits. These new claims conformed with the concurrent dramatic changes in 20th century biomedical representations of the human body. Early in the century, writings like that of the popular US medical authority Logan Clendening's *The Human Body* (1927) communicated aspects central to the biochemical makeup of the body for general reading audiences.

Though Sir Francis Bacon's recommendations from 1620 had long led scientific thinking, a reinvigorated experimental method emerged in medical practices during the second half of the 20th century. Within this new movement, quests for experimentally derived evidence were undertaken to support claims for the medical benefits of chocolate. Biomedical science began to experimentally assess chocolate's potential for alleviating medical disorders just as it did all pharmaceutical products. Randomized control trials of increasingly complex design based at multiple clinical sites were used to identify standards of normalcy and degrees of difference. By the close of the century, claims for chocolate's medical benefits were supported by a growing "science" of chocolate. Though anecdotal evidence still persisted to convince consumers, biomedical evidence was becoming much more prevalent in all types of literature and advertizing.

In the late 1900s, claims of chocolate's benefits focused upon its richness in carbohydrates and fat. Chocolate's natural flavonoid phenolics prevent the rancification of fat, thereby diminishing the need to add preservatives which might bring their own health risks. The plant-derived, saturated, stearic acid fats were not those guilty of increasing cholesterol levels. Cocoa butter within chocolate products was found to coat the teeth, thereby preventing tooth decay from chocolate's high sugar content. Tannins in cacao were noted to promote healthy teeth as they inhibited formation of dental plaque. More recently, investigations have centered around a particular flavonoid, epicatechin. Following chocolate consumption, epicatechin has been found to promote anti-oxidant activity, which decreases low-density lipoprotein (LDL) cholesterol activity, thereby delaying the onset or progression of atherosclerosis and arteriosclerosis. It may also increase high-density lipoprotein (HDL) cholesterol levels. Chocolate has been found to initiate anti-platelet activity, thereby reducing plaque formation and platelet clotting properties. Flavonoids are known to stimulate blood flow in brain, hands and legs due to regulation of nitric oxide synthesis. Dark (high cacao concentrated) chocolate also works to reduce blood pressure by promoting blood vessel dilation. As reported in a 1996 *Chocolatier* magazine editorial, "All of a sudden 70 percent cocoa solid chocolate bars ... [became] the rage. Two years ago you couldn't give them away" [39].

Similar to assessing any drug, possible adverse reactions must always be considered. In many areas, though some reactions have been repeatedly suggested, little conclusive biomedical evidence is available. Still, work is ongoing to identify chocolate's role in affecting headaches, bone density, kidney stones, allergic reactions, insulin sensitivity, acne and espophageal reflux. Concern over transmitting some of chocolate's active ingredients via breast milk is also being investigated.

For some time, high quality dark chocolate's psychoactive attributes have been linked to its high concentration of the stimulant theobromine. Late 20th century investigators have also explored chocolate's supposed aphrodisiac effects. When people become infatuated or fall in love, the levels of phenylethylamine (PEA) released from their brain increase. Chocolate was found to also promote this release, though in relatively small quantities. Chocolate appears to promote the neurotransmitter serotonin release as well, thereby producing calming, pleasurable feelings. Finally, an ananamide is also released following chocolate consumption, likely contributing to the euphoria which many claim that chocolate induces. All of these psychopharmacological alterations may contribute to chocolate's perceived aphrodisiac effects.

Questions also continue to rise over chocolate's reputed addictive nature. Despite anecdotal evidence that may say "Yes, Yes, Yes", little modern biomedical evidence supports such claims. Since depriving one of chocolate fails to produce scientifically significant signs of withdrawal, it is not technically classed as a physically addictive agent. Further, scientists have not shown a state of dependence regarding chocolate's use. Admittedly, chocolate may pharmacologically stimulate a motion for compulsive eating, but this may just as well be the result of a more generalized aesthetic craving for the sweetness and oily richness and complete orosensory experience that chocolate provides. Chocolate's rich natural complexity, a complexity that rivals any other food, makes the actual source of perceived cravings or "chocoholism" exceedingly difficult to ascertain.

One offshoot of early 20th century medical reform was the extensive research expended toward drug design and development. By the end of the century, drugs dominated the medical marketplace. Pharmaceutical industries have grown into Godfather-like figures commandeering physicians' polypharmacy practices. Though for many health care providers, a paradigm of polypharmacy is not a panacea [40]. The growing disenchantment with polypharmacy is strikingly clear in consumer health movement demands for more holistic, integrative health care [41]. Patients' skepticism over the benefits obtainable from traditionally prescribed remedies alone is evidenced by the increasing demand for more natural medicine.

What could be more natural than chocolate? As the nomenclature *Theobroma cacao* suggests, we have long viewed chocolate as a food of the Gods. Steadily, authorities have been reinforcing chocolate's potential medicinal benefits. According to Harvard Medical School's Norman K. Hollenberg, the "pharmaceutical industry has spent tens, probably hundreds of millions of dollars in search of a chemical that would reverse ... [or ward off vascular diseases]. And God gave us flavanol-rich cocoa which does that" [42].

"Death by Chocolate" might just be a dessert that we crave, but evidence of all forms seems to indicate that, regardless of how you look at it, chocolate is very good for the living. Even in popular fiction, J.K. Rowling depicted chocolate as Harry Potter's only life-saving first aid against the soul-consuming Dementors. Building upon the wisdom of Forrest Gump's quip that "Life was like a box of chocolates", various forms of evidence suggest that life may certainly benefit from at least an occasional box of chocolates.

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# Industrial Treatment of Cocoa in Chocolate Production: Health Implications

2

Herwig Bernaert, Ieme Blondeel, Leen Allegaert and Tobias Lohmueller

## 2.1 Ancient Use of Cocoa

Cocoa has a rich history of medicinal and ritual use. The medicinal use of cocoa originated among the Olmec, Maya and Aztec civilizations which bloomed in the geographical region that we now call Central America. According to the best estimates of archaeologists, the ancient Maya, are believed to have cultivated the cacao tree for the very first time around 1000 BC. The Aztecs regarded cocoa as a sacred plant, used it in a highly esteemed "drink of the gods" and valued cocoa beans as currency. It was Hernán Cortés, a Spanish conquistador, who brought cocoa to Europe at the beginning of the 16th century.

More than 100 medicinal uses for cocoa were described in manuscripts produced between the 16th and 20th century, with prescriptions to alleviate fever or to stimulate the nervous system, for example [1]. The use of cocoa or chocolate as a medicine has progressively disappeared, while the role of cocoa and dark chocolate in supporting health and wellbeing has been more and more explored in scientific nutritional research, in particular during the last two decades [2-13].

## 2.2 Cocoa Bean Goldmine

Multiple components in cocoa and chocolate can contribute to the complex interplay of nutrition and health. A fully grown cocoa bean is a goldmine of more than 200 substances that may potentially promote good health and wellbeing. Not all substances are present in a quantity which is meaningful to provoke a health effect. Phenyl ethylamine, serotonin or unsaturated acylethanolamines (structurally related to anandamide) are unlikely to have

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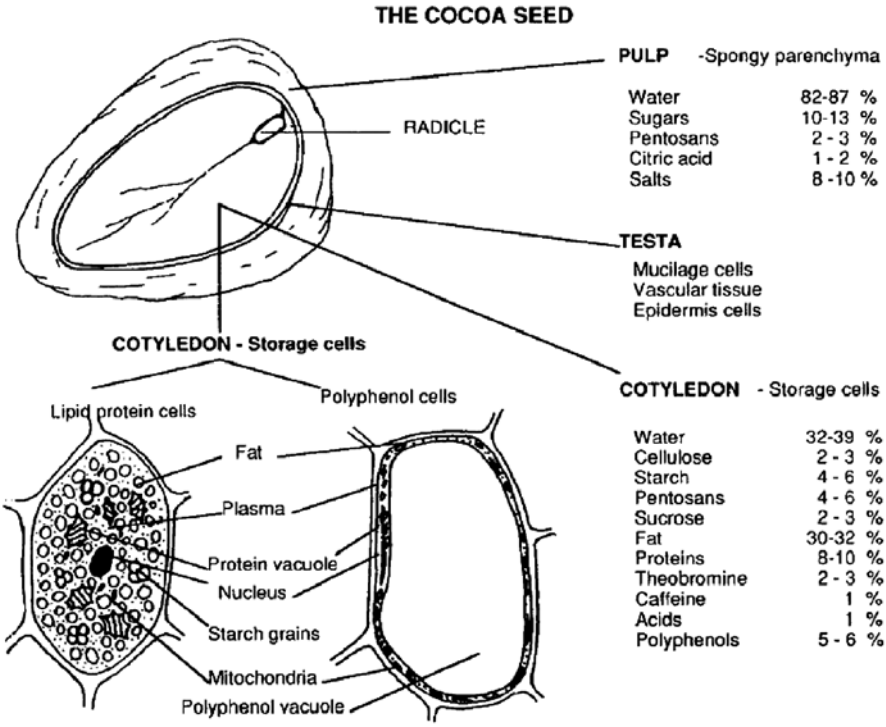


**Fig. 2.1** Localisation of the Polyphenols in the Polyphenolic Cells in the Cotyledons of the Cocoa Seed. From [15] with permission

an effect because of extremely low concentrations in the final chocolate product but the few publications about these substances have fed some myths about liking and craving for chocolate [14].

Some substances may have low bioavailability in humans, or may be broken down rapidly after intake. Of all constituents proposed to play a role in our liking for chocolate, caffeine is the most convincing, though a role for theobromine cannot be ruled out.

One particular group of substances in cocoa, which is present in a considerable amount and has been largely studied by different independent groups, are the polyphenols.

When having a closer look at the cocoa bean and its composition (Fig. 2.1) [15], the polyphenols are stored in the so-called polyphenolic cells, which make up about 10% of the cocoa seed. Polyphenolic cells consist of one single, large vacuole that takes almost all the space of this cell. Beside the polyphenolic storage cells, the cotyledons in the cocoa seed also contain protein-lipid storage cells in which, amongst other cell constituents, fat vacuoles can be found in clear abundance [16]. Accordingly, it is clear that the polyphenols are present in the non-fat cocoa fraction of the cocoa bean. However, an extra dark chocolate with a high cocoa percentage will not necessarily contain a high level of polyphenols, as there is a huge impact of processing from bean to final product on the final level of polyphenols.

## 2.3 Cocoa Flavanols

The structural features of polyphenols are the presence of one or more aromatic rings and at least two hydroxyl groups (Fig. 2.2) [17]. Polyphenols can be divided into at least 10 different classes depending on their basic structure, with some examples of polyphenol classes being flavonoids, phenolic acids, xanthones or lignins. Within the class of flavonoids, a further sub categorisation can be made into flavones, flavonols, flavanones, flavanols, etc.

For cocoa, the terms that are used to describe the particular polyphenolic compounds of interest are flavanols. Flavanols are a subclass of flavonoids which are, in turn, a class of polyphenols [2].

Flavanols can be further split into monomeric ((-)-epicatechin and (+)-catechin), dimeric (the most common in cocoa are B2 and B5, both made of two units of epicatechin with differing linkages) or polymeric combinations of these monomers (Fig. 2.2). For the polymers, chains of up to and over 10 units have been found in cocoa. The polymers are also called procyanidins [2–4].

Although flavanols are the most abundant flavonoids in the cocoa bean, anthocyanins, flavonols (quercetins) and flavones (apigenins, luteolin) have been identified in the seed as well, although in a much lower amount [18].

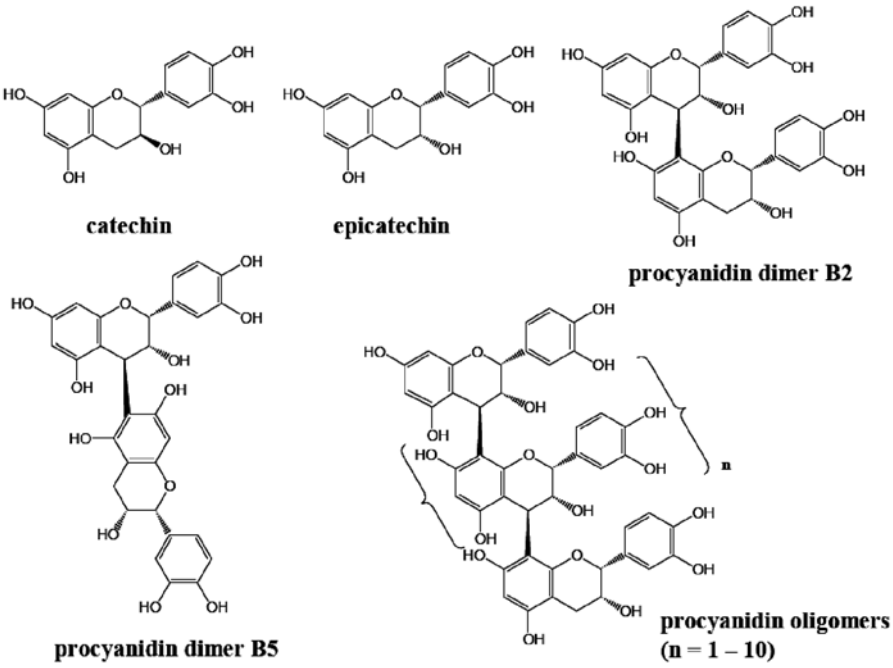


**Fig. 2.2** Major Flavanols Found in Theobroma Cocoa [17]

## 2.4 Cocoa and Health

Cocoa and dark chocolate have the highest flavanol content of all foods on a per-weight basis and can therefore be seen as a significant contributor to the total dietary intake of flavonoids [5–7, 19]. Dietary flavanols show promising potential for reducing cardiovascular disease risk via improvement in vascular function, a health area which has been most extensively researched during the last few years.

The available evidence from meta-analyses on the relation between cocoa or chocolate and blood pressure (BP) indicate a BP-lowering effect. The four meta-analyses that have been carried out over the recent years have progressively included an increasing number of studies and all come to the same conclusion that regular intake of cocoa or chocolate significantly lowers blood pressure [10, 11, 13, 20]. The most recent meta-analysis showed a considerable and clinically meaningful effect of cocoa flavanols on BP reduction with -3.16 mmHg for systolic BP and -2.02 mmHg for diastolic BP, which was highly statistically significant [11].

Chocolate and cocoa consumption also increase flow-mediated dilation (FMD), a biomarker of endothelial function. Daily consumption of dark chocolate has been shown to increase FMD by 4.0% acutely and by 1.4% chronically [21].

Other beneficial effects which have been ascribed to cocoa flavanol intake are related to platelet function, blood cholesterol levels, insulin action and inflammation.

Besides the cardioprotective domain, the outcomes from human studies indicate action from cocoa on both cerebral blood flow and cognitive performance, and even more appealing, skin health.

The cocoa bean is one of the richest sources of flavanols, but the art is to preserve these wholesome components as much as possible in the final consumable products. The negative impact of the manufacturing process of chocolate and cocoa powder products on the flavanol content should not be underestimated.

## 2.5 Final Products

Even though cocoa is well-known for its health properties, it is of course rarely consumed as such. The main reason for this is because cocoa is perceived as too bitter, mainly caused by the cocoa polyphenols and methylxanthines.

Cocoa is a preferred ingredient in many applications and consumed in big volumes (0.59 kg per capita worldwide in 2008/2009) [22]. The reasons for this huge turnaround are twofold. Firstly, cocoa is mainly used in combination with sugar to mask bitterness. Secondly, today's cocoa is processed extensively, so that unpleasant bitter notes are reduced as much as possible. A good example is alkalized cocoa powder, which seems to have the monopoly in chocolate beverages.

The average chocolate on today's market ideally contains relatively low amounts of cocoa and is conched extensively to flatten the taste. Furthermore, any characteristic cocoa flavor profile is most commonly reduced by blending cocoa from different origins.

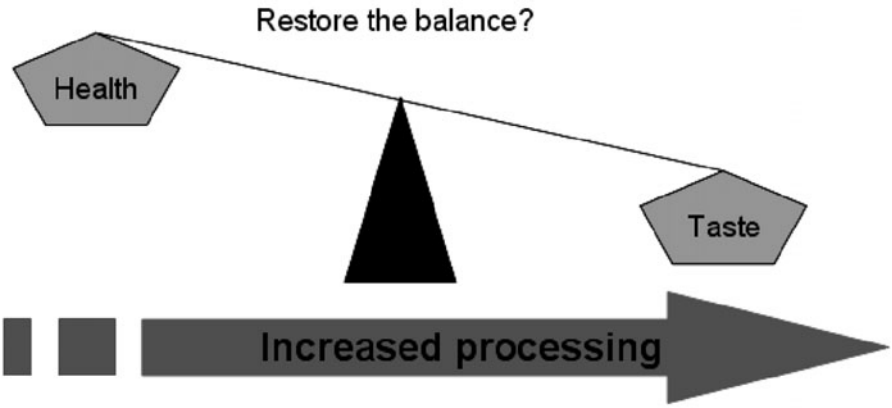


**Fig. 2.3** Visualisation of balance between taste and health in cocoa

A blend will render a reproducible and non-specific flavor, which doesn't contain any specific fruity, sour or bitter notes. It seems that consumers prefer a 'dull' chocolate over a specific origin flavor. By constantly 'improving' or 'adjusting' taste, the health effects of cocoa have been slowly forgotten (Fig. 2.3). Recently, there seems to be a trend to re-visit these health characteristics. Hence, some cocoa and chocolate manufacturers are processing under less harsh conditions. Trends such as 'raw chocolate' or 'origin chocolate' are now often found back in the industry, preserving the initial taste and/or healthy components.

Figure 2.4 is an illustration of today's top cocoa applications and volumes. In the crop seasons of 2009/2010 worldwide 3,596,000 tonnes of cocoa were harvested. The Ivory Coast supplied about 33% of this volume [22]. The global cocoa market in 2010 for cocoa powder was estimated at 808,679 tonnes. The global chocolate market in 2010 was about 7,204,700 tonnes [23].

The top 7 applications for cocoa powder in 2010 were dairy products and hot drinks (33.2%), bakery products (31.3%), ice cream (10.1%), dried processed food (9.2%), confectionery (5.5%), spreads (4.9%) and tobacco (4.5%) (Fig. 2.4) [23].

Meaning that around 69% of all cocoa is at least UHT treated, baked in an oven or even smoked. This might raise questions on the bioactivity or actual effectiveness on healthy cocoa components, especially if heat treatments are reducing such effects.

There are many applications containing chocolate, but the main application that is linked to health is still the plain chocolate tablet. When looking at the tablet market, there are major differences between different countries. Worldwide, milk chocolate is preferred over dark and dark over white, when it comes to tablet sales. Only in Canada, Iran and the Netherlands, is dark chocolate consumed more than milk chocolate (of the top 20 chocolate consuming countries, responsible for 80% of global consumption).

On average, when only looking at the top 20 countries that are responsible for 80% of the market, milk chocolate accounts for 48.1% , dark for 26.1% and white for 6.4% of the global market [23]. Barry Callebaut estimated that the market for dark chocolates containing 70% cocoa solids or more, is only about 1–3% .

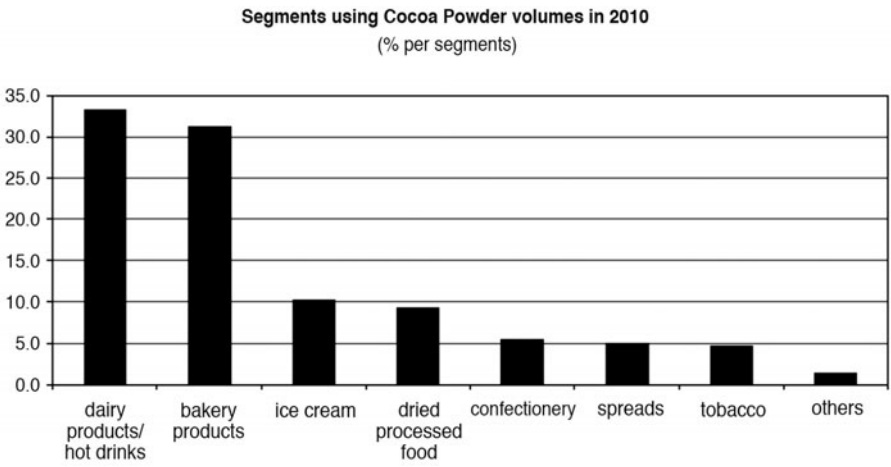


**Fig. 2.4** Cocoa powder use in 2010

Some applications have reduced healthy components because of the processing parameters needed to produce the final product. For example, baking of chocolate inclusions or UHT treatment of cocoa beverages.

For the remaining percentage of cocoa and chocolate products a good probability of retaining significant health components is therefore very limited. Taking into account the goldmine of health affecting components and especially cocoa flavanols, it can be concluded that the general health potential of cocoa is simply lost.

## 2.6 Impact of Processing

Flavanols have the highest potential to provide health effects, from the cocoa bean to the consumer. Flavanols are highly dependent on the production parameters and not as stable as components like theobromine. All results are based on internal research in Barry Callebaut, unless mentioned otherwise.

There are 4 main areas in the production process where big differences in health components can be observed: firstly, the origin and fermentation play an enormous role in the starting concentrations of health components; secondly, the production process from bean to cocoa liquor; thirdly, the production of cocoa powder or chocolate from this liquor; and fourthly the production of the final application, containing cocoa or chocolate.

All these areas within production and certainly those characterized by high temperature and moisture can alter or neutralize health components. Some components are increased or modified and enrich the product. Most components like flavanols in reality only reduce in amount and activity.

## 2.6.1 Cocoa Trees, Genotype, Origin, Fermentation and Bean Drying

### 2.6.1.1 Cocoa Tree

Cocoa grows on shadow trees called *Theobroma cacao* L. and 22 different genera are classified [24]. For chocolate production the most important subspecies are the Forastero, Criollo including the National-Cocoa, and the Trinitario. More than 1000 different genotypes, mainly via Forastero and Trinitario crossbreeding, can be found today in over 60 different countries between 20 degrees latitude of the equator. However, with recently developed genetic and molecular biologic methods, cocoa classification might change as several authors question for example, if the Forastero and Criollo are not more closely related than a subspecies classification allows [25, 26].

When classifying the different groups based on the taste property, the most fruity and flowery with a moderate low cocoa taste is the Criollo derived from Central America, followed by the Trinitario that was developed by cross breeding of Forestero and Criollo in the 18th century, and finally the Forastero from the Amazona region that shows the lowest fruitiness but strongest cocoa notes derived.

The cocoa trees generally reach a height between 8 and 20 m and become around 100 years old. However, in the commonly used agriculture practice the trees yield sufficient cocoa for only 30 years [27]. Based on the farming technique the cocoa yield differs strongly from 200 kg/ha to 3000 kg/ha depending if cocoa is grown as subsistence agriculture or from high-end farming plantations [28]. Most cocoa is grown in small/medium size farms using less than 20 ha land having a yield of 200–500 kg/ha and only 30% of the produced raw cocoa originates from high-end farming [28, 29].

### 2.6.1.2 Cocoa Seed Growth

Each cocoa tree can contain between 35,000 and 116,000 flowers per year whereas less than 5% are pollinated and less than 1% develop a mature fruit [29]. Depending on the genotype the cocoa pod shows different colours (e.g., green, yellow, red purple), surface and shape (e.g., even, warty, round, oval) and size from between 15 cm to 30 cm in length whereas the width of the pod is around 5–15 cm. A pod can contain 50 seeds that are attached to a placenta and surrounded by a pulp that contains around 10–13% sugar, 1% pectines and 1–2% citric acid [30]. The average cocoa bean composition on dry matter is 40–60% cocoa butter, 10–15% proteins, 6% starch and contains between 0.9–1.4% theobromine, 0.2% caffeine (Criollo cocoa can contain up to 1.3%) and 5–9% flavanols (Barry Callebaut internal information). The internal composition is influenced by the genotype, agronomic conditions and practice and is therefore dependent on origin. In order to obtain the desired chocolate taste the seeds need to be separated and only the pulp and the seeds are further processed.

### 2.6.1.3 Fermentation

The first production step for cocoa is the mucilaginous pulp fermentation that is essential for taste. It can be seen as the basis of the cocoa flavor development [31, 32]. During this process the energy rich but very bitter tasting cocoa seed changes into a seed that is rich in aromatic notes and stable for storage. The mucilaginous pulp of the cocoa

is fermented spontaneously with microorganisms such as yeast, acetic acid bacteria and lactic acid bacteria that metabolise the sugar and citric acids of the pulp into alcohols, lactic and acetic acid and $CO_2$ [33]. The fermentation itself can be split in two main phases; the first phase takes place under anaerobic conditions and is followed by an aerobic phase. The yeasts together with the lactic acid bacteria dominate the first phase and convert the sugar and citric acid in the pulp. The metabolic activity of yeast can be considered most important during the anaerobic condition as it metabolizes the sugar of the pulp into ethanol that is later oxidised by the acetic acid bacteria. During this phase the mucilaginous pulp is removed and drains away through pectin degradation by secreted enzymes.

In the second phase, the acetic acid bacteria are critical as they oxidise the alcohols by an exothermic reaction and increase the fermentation temperature significantly (up to 55°C) and form acetic acid that enters the seeds [34, 35]. Already at the first fermentation phase, the endogenous bioconversion (biochemical changes) of the seed starts by proteolysis of reserve proteins and increases by the acetic acid penetration during the second fermentation phase. The endogenous bioconversion activity during the first stage can be measured, monitoring γ-aminobutyric acid (GABA) that is formed by decarboxylation of glutamate whereas the highest activity of the protein degrading enzymes aspatylendoprotease and carboxypeptidase can be found during the aerobic fermentation [36–38]. During the whole fermentation, 50% of the polyphenols are lost either by oxidation (mainly due to the polyphenoploxidase) that can be visually observed (browning of the beans) or by polyphenol "bleeding" out of seeds [39, 40].

The required fermentation time in order to obtain the required taste differs between different groups: Criollo- and National-Cocoa are fermented for 2–3 days, whereas Forestero requires a fermentation time of around 6–7 days. Three main fermentation techniques are applied worldwide, the heap-, basked- and box-fermentation, over 50% of fermented cocoa derives from heap and box fermentation [41]. The heap fermentation consists of around 100–2,000 kg fresh seeds and a mixing of the heap is performed every 48 h. The box fermentation starts with a size of 60 × 60 × 60 cm but can go up to a fermentation volume of more than 1 $m^3$, whereas the basked fermentation has a fermentation volume of less than 100 kg of fresh cocoa beans.

#### 2.6.1.4 Cocoa Bean Drying

Cocoa drying reflects another very important processing step (5–7 days sun drying) as the desired flavor formation takes place due to residual cocoa self-enzymatic activity and the water removal to a value between 6 to 8% avoids mould growth (risk of mycotoxin). Furthermore the correct drying process removes volatile acids and avoids off-flavor formation [42, 43]. Origins that have problems based on rainfall in performing sun drying use mechanical drying systems. The best artificial drying systems use a gas burner. However, many artificial dryers work mainly with wood or petrol that can lead to off-flavors such as smoky, hammy, rubber or petrol notes. As rapid drying contributes to lack of desired flavors and bean properties, sun drying is the most desired drying technique for the chocolate industry [42].

### 2.6.2 Bean to Liquor

Different cocoa companies utilize slightly different processing flows or production parameters. The major differences are bean or nib roasting and the type of grinding equipment to transform nibs into liquor. The major influence on stability of health components, like flavanols, is however the roasting part and debacterization. This is because high temperatures are employed so the moisture content (at the start of roasting) in the bean is important. Chemical reactions, like oxidation, are therefore accelerated at this stage.

If no attention is given to the roasting temperature and time, the losses of flavanols can go up to 50% or more (Fig. 2.5). This is observed especially in regions like North America and Asia where cocoa beans are usually roasted at quite high temperatures. By keeping the roasting temperature just above 100°C, it is possible to reduce the flavanol content by only 10% . Payne et al. (2010) nicely documented the effect of roasting temperature on the flavanol monomers [40].

Roasting and debacterization can never be excluded in cocoa processing because of microbiological and taste reasons. However, simple monitoring of the temperature can add a lot to the health aspects of the final product. Moreover, it has been shown by Augusta et al. that different degrees of roasting play a very important role in racemisation of flavanol monomers. Resulting in the fact that some flavanols become more or less bio available and therefore more or less effective. The conclusion literally stated "it would be important to develop a standardized and controlled processing of cocoa beans, aimed at retaining the maximum extent of the beneficial compounds" (Augusta et al, 2009).

Apart from roasting, the production steps from bean to liquor are surprisingly mild to components such as flavanols. The main reason for this is that the cocoa is protected by the

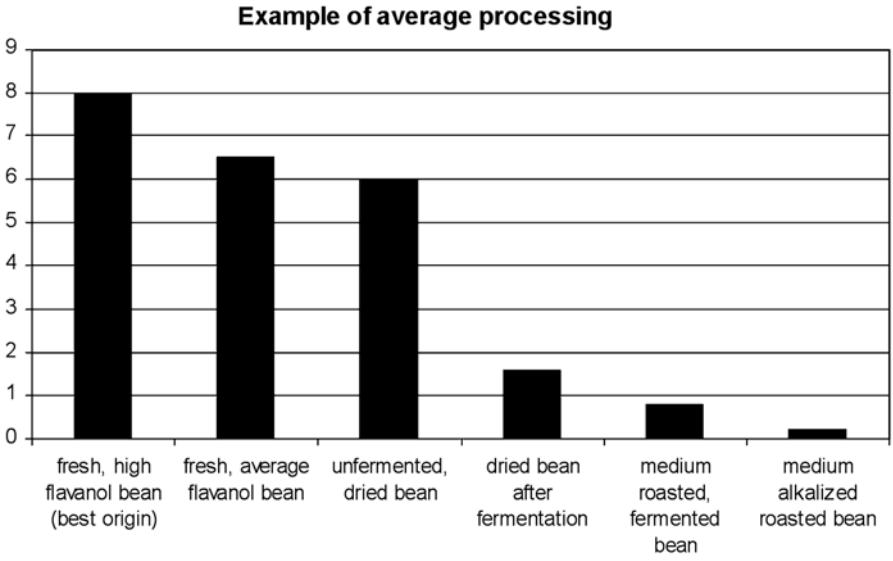


**Fig. 2.5** The potential flavanols and the loss of flavanols during conventional processing

cocoa fat (usually around 55% of the nib). This fat protects flavanols from oxidation and is not the best environment to induce chemical reactions, where water is usually required.

It has also been shown that during roasting, components like proteins and polyphenols are chemically linked to cell wall material as cellulose and pectins or simply consumed in chemical reactions like the Maillard reaction. It has been shown that water-soluble protein fractions are greatly reduced after roasting [44]. Not much is published in the literature, but it would not be surprising that high roasting causes a lower bioavailability of certain health components in cocoa.

### 2.6.3 Powder or Chocolate Production

Cocoa powder production from cocoa liquor is assumed to be a lot less harsh than producing liquor from cocoa beans. This assumption is incorrect. Cocoa liquor is pressed into cocoa cakes, usually containing between 10–12% and 22–24% of fat, by removing cocoa butter. After this pressing step, the cocoa cakes are ground into fine cocoa powder.

This grinding of the cakes into powder accounts again for losses from 10 to 30% of cocoa flavanols, which is perhaps unexpected. The reason is that during the grinding and pneumatic transport of this powder, a lot of heat is generated due to friction. Heat, oxygen and low (protective) amounts of fat, cause oxidation of the cocoa flavanols, for instance.

Most commercial cocoa powders in non chocolate applications are alkalized. During alkalization, water and potassium carbonate are added at elevated temperatures and sometimes elevated pressures, at the nib or cake stage in the process. Oxidation of flavanols is greatly increased by this process. The remaining amount of flavanols in cocoa powder depends on the extensiveness of the alkalization. It is possible to produce a natural cocoa powder on industrial lines, containing a consistent 10% of flavanols or even higher. These powders will however be lower in cocoa taste due to a lower fermentation degree. A cocoa powder with a good and recognisable taste development could contain still about 6% of flavanols. Generally alkalized powder on the market contains on average about 0.4% of cocoa flavanols, while a standard non-alkalized powder contains on average about 1.5% of cocoa flavanols. A strongly alkalized powder usually no longer contains detectable flavanols. Epicatechine levels especially demonstrate the effect of pH during alkalization [40].

The internal structure of cocoa changes a lot during alkalizing. Also here it was found that components like proteins are less extractable from cocoa when alkalized. This could have an effect as well towards bioavailability of certain components.

Production from cocoa liquor, or powder, into chocolate is a complex step in respect to health components. The chocolate mixture is refined and conched, before completing the recipe. After refining, chocolate is usually in powder form, where oxidization of flavanols can again take place (on average up to 10% loss). The conching, where chocolate is mixed under elevated temperatures, is responsible for many chemical reactions such as the Maillard reaction. Remarkably, during this step there are few to no flavanol losses. This can be explained by the fact that cocoa butter protects the flavanols once again.

There seems to be no loss in flavanols or Oxygen Radical Absorbance Capacity (ORAC) during storage of chocolate products or keeping chocolate liquid at 50°C for a longer time period (internal info Barry Callebaut) [45].

### 2.6.4 Stability of Cocoa Flavanols During Production of End Application

Even though chocolate is quite a stable matrix for health components such as flavanols, and cocoa powder is quite stable within the expected shelf life, the processing conditions into the final application are important to maintain the flavanol activity, for example. Chocolate beverages, for example, are most commonly UHT heat treated to ensure microbial safety. On average 40% of the remaining flavanols are oxidized and lose their activity during this UHT process.

Not only heat is important but the water activity should also be kept as low as possible. A beverage containing 10% of dry matter and a water based filling containing 78% dry matter were both pasteurized at 90°C for 9 minutes. Only the water content differed between both recipes. The beverage suffered a reduction of 69% in flavanol activity, while the water based filling only lost 3% of the active flavanols.

Along with water, the contact with air plays an important role in avoiding oxidation. A baking trial was done while producing a dense cookie and a "porous" cake. Both applications were produced containing either chocolate or cocoa powder. All four products were baked for 10 minutes at 180°C. There were no significant losses in flavanols in the dense cookie products, while in the cake application with cocoa powder, flavanol content was reduced by 24.5% (Fig. 2.6). The cake application containing chocolate pieces didn't show significant losses, proving the protective effect of cocoa butter.

As a conclusion on processing, it could be advisable to monitor all processing steps where increased water content, decreased fat content, contact with air and elevated temperatures take place. Of course, an important step is also the knowledge of where to

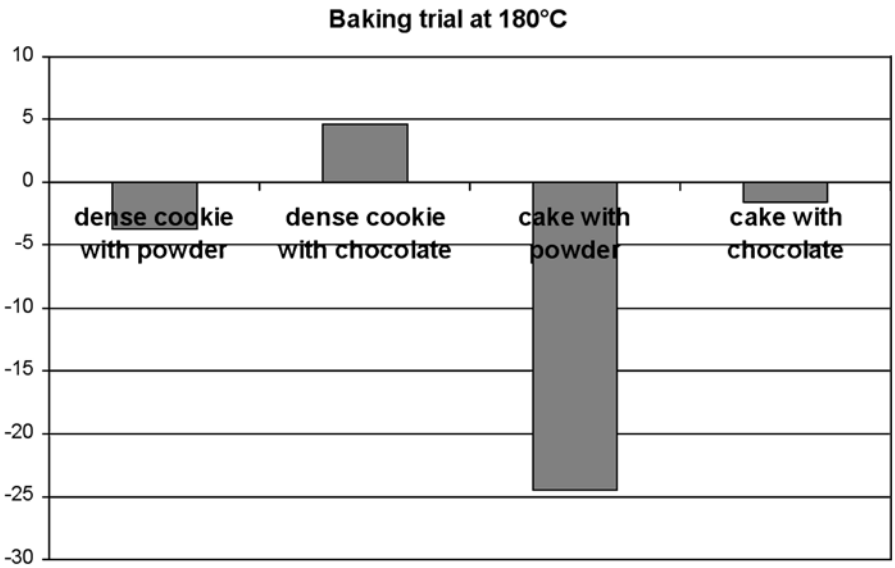


**Fig. 2.6** Stability of flavanols under high heat and water presence, showing the impact of oxygen

source the cocoa beans and how to ferment them, as this will define a low or high in flavanol end product. Monitoring mild processing without monitoring fermentation and sourcing is like trying to drive sportily in a 50-year old car.

For various health components, it is crucial to understand the chemical processes in each part of the processing chain, so that a reduction in health activity can be avoided.

## 2.7 Commercial Products vs Other Foods

There are few applications on the market claiming health from the cocoa bean. Most chocolate applications thrive on the fact that dark chocolate is generally perceived as healthy. Most chocolates containing a health claim are typically enriched with high antioxidant fruits or other ingredients.

Some applications however solely use cocoa as the origin of claimed health components. There are chocolates on the market that contain up to 3 times more cocoa flavanols than a regular chocolate by carefully selecting and processing the cocoa beans. The advantage is of course that one would need to consume 3 times less fat and sugar for a needed health effect.

If no cocoa or chocolate product can be found containing a health claim or labelling a high amount of flavanols, the next best thing is to look for high cocoa products, for example a chocolate with 70% or more cocoa solids. But most importantly for health benefits, products only using natural cocoa without the use of alkalized cocoa are best.

Comparing the health benefits of cocoa with other foodstuffs is very difficult. The types of flavanols in cocoa are never really the same as the type of "polyphenols" in other foodstuff. Cocoa flavanols are also reported to have health characteristics for skin, heart health and cognitive performances, and thus are certainly not restricted to only antioxidant effects. Most foodstuffs on the market considered healthy are however built on the fact that the product has a high antioxidant capacity. A universal method, which is maybe best used to compare, is an ORAC (Oxygen Radical Absorbance Capacity) measurement. An important remark is that ORAC readings only show the potential, as they do not prove bioavailability. Cocoa flavanols are proven highly bioavailable. Procyanidin monomers, dimers and trimers are considered to be bioavailable as they can be absorbed and are present in blood. On average about 30% of the cocoa procyanidins (flavanols) are monomers through trimers. Comparing ORAC measurements of standard cocoa products with well-known high antioxidant products shows the potential health benefits of cocoa (Figs. 2.7 and 2.8). Certainly it should be taken into account that most cocoa flavanols are lost due to normal, but harsh industrial processing. For example, it is possible to produce a cocoa powder containing about 2000 ORAC/g. This could easily lead to a nice chocolate beverage, containing more than 50 ORAC per ml. It is generally assumed that the daily ORAC intake should be around 4000 ORAC, which in this case means 80 ml (about a small cup).

It is proven that cocoa is a very interesting source of antioxidants and other healthy components. Taking into account the potential amount of cocoa flavanols in the final product, the issue is not reaching an effective amount of flavanols. The strong benefit of cocoa and

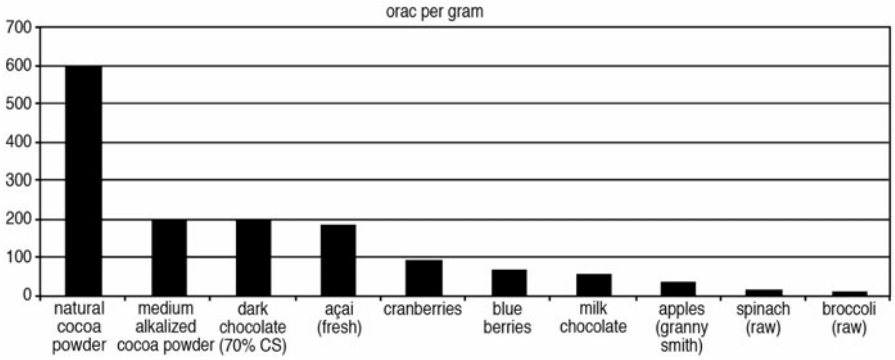


**Fig. 2.7** Comparison of cocoa to other well-known anti-oxidants (non spices), courtesy of Brunswick Labs

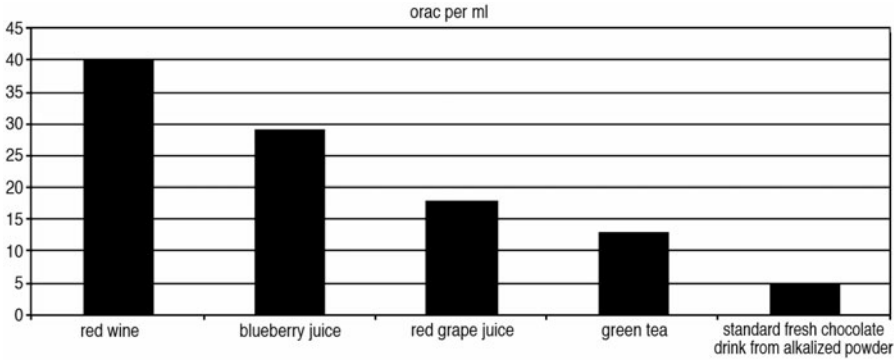


**Fig. 2.8** Comparison of well-known anti-oxidant drinks, courtesy of Brunswick Labs

chocolate is still its indulging taste. It is therefore desired to strive for a perfect balance between health and taste. From experience, a dark chocolate containing about 2% flavanols is really on the edge of being too bitter. Currently 70% cocoa chocolates only contain about 0.7% of flavanols because of heavy processing. The same chocolate would still be tasty with the flavanols content at least double.

## 2.8 Conclusions

Since the 16th of century, cocoa has been known as a medically relevant plant containing more than 200 active substances [1]. One of the most characterised health-related substances are the polyphenpols especially the subclass of flavanols, which can be seen as most specific to cocoa. The health potential of flavanols are closely linked to the end product concentration and therefore need to be maintained during chocolate processing in order to obtain

a “healthy” dark chocolate. Industry has monitored the flavanol losses over the whole chocolate process including the tree genotype, fermentation, drying and industrial process in order to be able to obtain a high flavanol concentration in the final dark chocolate. However, different brands and dark chocolates containing the same cocoa content show flavanol concentration differences of up to 3 times, showing that the practise to maintain flavanol concentration within the dark chocolate processing is still a challenge.

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# Biomarkers of Cocoa Consumption

# 3

Nasiruddin Khan and Nathalie M. Nicod

## 3.1 Biomarkers of Cocoa Consumption

Cocoa is a rich source of polyphenols; indeed cocoa beans contain approximately 6–8% polyphenols by dry weight [1].

Cocoa and its derived products are now well-known for their potentially positive health effects, which are mainly being attributed to flavan-3-ols, the most abundant flavonoids in cocoa. Flavan-3-ols are found as monomers (mainly as (-)-epicatechin) and as oligomeric (procyanidins $B_1$, $B_2$, and $C_1$) and polymeric forms (procyanidins) that account for 5–10% and ≥90% of total cocoa polyphenols, respectively [2, 3].

Before we can attribute any health effect to individual compounds or raw mixtures we need to unravel the bioavailability (absorption, distribution, metabolism, and elimination) of the molecules of interest. This, in turn, depends on the accurate estimation of polyphenol intake or dietary exposure.

In brief, flavanol monomers seem to be readily absorbed in the small intestine. As the result of the action of Phase II enzymes, (-)-epicatechin is converted into glucuronidated and sulfated metabolites as well as into methylated metabolites, which, in turn, may also be glucuronidated and sulfated. The absorption of dimeric procyanidins in humans appears to be limited [4, 5], while polymeric procyanidins are not well absorbed in their native form. These unabsorbed procyanidins reach the colon and are metabolized by the colonic microbiota, producing complex phenolic acids [6–8] that can be further metabolized in the liver and then excreted in urine.

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R. Paoletti et al. (eds.), *Chocolate and Health*,

## 3.2 Requisites and Limitations of Biomarkers of Intake

The relation between dietary intakes and physiological, i.e., in body fluids, concentrations of biomarkers is very complicated and several factors must be taken into account before assigning any biomarker a sensitive and accurate identity. For instance, complete knowledge about metabolic interactions of polyphenols in humans (considering environmental and physiological factors); time duration between consumption of polyphenol and their appearance in biological fluid; and required dose to enable the measurement of these parameters are indispensable.

Despite the available data from the US Department of Agriculture, listing some food compositions, an estimation of biomarkers of exposure in plasma and urine, e.g., phenolic microbial metabolites, would be of great help and importance especially in the case of proanthocyanidins. This example is paradigmatic of the difficulty in estimating their intakes, due to their structural complexity [9, 10].

As the relation between cocoa (and its metabolite) intake and the physiological concentrations of its major metabolites is very intricate, this chapter mainly focuses on the recently-studied biomarkers of cocoa polyphenol that can be used as a sensitive and accurate biomarker of exposure.

## 3.3 Biomarkers of Cocoa Consumption in Urine and Plasma

An important human clinical trial study was performed by Urpi-Sarda et al. [8], who involved 42 volunteers and designed it as a four-week randomized, controlled, crossover trial during which subjects received 40 g/day of cocoa powder with skimmed milk (total/day: 500 mL; cocoa intervention group) or 500 mL/day of skimmed milk (control group). The phenolic composition of the cocoa powder was: 1.15 mg/g of (-)-epicatechin and 0.26 mg/g of (+)- catechin, 13.4% dimers, including 0.91 mg/g of procyanidin B2, 0.94 mg/g of vanillin and flavonols, including 0.056 mg/g isoquercitrin, 0.005 mg/g quercetin, 0.003 mg/g quercetin-3-glucuronide and 0.02 mg/g quercetin-3-arabinoside. The total polyphenolic content of the cocoa powder was 12.38 mg catechin/g cocoa.

Targeted studies of phenolic metabolites derived from the colonic microbiota were performed and hydrolyzed urine and plasma samples were screened. The analysis of 24 h urine and fasting plasma using liquid chromatography mass spectrometry (LC/MS-MS) revealed the occurrence of glucuronide and sulfate derivatives of 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (DHPV; Table 3.1) and its methyl ester 5-(3′-methoxy,4′-hydroxyphenyl)-γ-valerolactone (MHPV), as possible indicators of the absorption of both monomeric flavanols and procyanidins present in cocoa. In addition, hydroxyphenylacetic acids (3,4-dihydroxyphenylacetic acid and 3-hydroxyphenylacetic acid) were also among the predominant urinary metabolites found after cocoa consumption. Notably, the quantification of these metabolites suggests their use as valid biomarkers of cocoa consumption [8].

Llorach and coworkers [11] studied human urine metabolome modifications after a single cocoa intake in a randomized, crossed, and controlled trial which included 10 subjects

**Table 3.1** Biomarkers of cocoa powder consumption (urinary metabolome). Modified from [11] with permission

| | Retention time | Detected mass | *w*c*[1] (contribution) 24 h | *p*(corr)[1] (confidence) 24 h | Metabolite putative identification | Theoretical mass |
|---|---|---|---|---|---|---|
| (1) Purine alkaloid metabolites | | | | | | |
| (A) | 0.90 | 199.0829 | 0.15 | 0.98 | AMMU | 199.0825 |
| | | 171.0882 | 0.13 | 0.98 | | 171.0876 |
| (B) | 1.25 | 199.0818 | 0.13 | 0.88 | AMMU | 199.0825 |
| | | 171.0872 | 0.13 | 0.98 | | 171.0876 |
| (C) | 1.83 | 183.0523 | 0.15 | 0.98 | 7-methyluric acid | 183.0512 |
| (D) | 2.18 | 167.0575 | 0.17 | 0.97 | 7-methylxanthine | 167.0563 |
| | | 124.0491 | 0.07 | 0.97 | | 124.0505 |
| (E) | 2.32 | 183.0525 | 0.06 | 0.69 | 3-methyluric acid | 183.0512 |
| (F) | 2.62 | 167.0586 | 0.19 | 0.96 | 3-methylxanthine | 167.0563 |
| (G) | 3.05 | 197.0675 | 0.08 | 0.93 | 3,7-dimethyluric acid | 197.0669 |
| (H) | 3.63 | 181.0720 | 0.18 | 0.96 | Theobromine | 181.0719 |
| (I) | 4.75 | 195.0875 | – | – | Caffeine | 195.0876 |
| (2) Polyphenol host metabolites | | | | | | |
| (A) | 4.15 | 169.0496 | – | – | Vanillic acid | 169.0495 |
| (B) | 4.23 | 226.0725 | – | – | Vanilloylglycine | 226.0709 |
| (C) | 6.93 | 371.0401 | – | – | Epicatechin-O-sulfate | 371.0431 |
| (D) | 7.10 | 305.1028 | – | – | O-Methyl-epicatechin | 305.1019 |
| (3) Polyphenolic colonic microbiota metabolism | | | | | | |
| (A) | 4.63 | 227.0936 | 0.02 | 0.64 | 4-hydroxy-5-(3,4-dihydroxyphenyl)-valeric acid | 227.0913 |
| (B) | 5.13 | 385.1134 | 0.04 | 0.77 | 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone glucuronide | 385.1129 |
| (D) | 5.47 | 399.1300 | 0.04 | 0.66 | 3′-methoxy-4′-hydroxyphenyl-valerolactone glucuronide | 399.1285 |
| (E) | 5.87 | 223.0981 | 0.03 | 0.80 | 3′methoxy-4′-hydroxyphenyl-valerolactone | 223.0964 |
| (F) | 6.12 | 289.0386 | – | – | 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone-sulfate | 289.0376 |
| (G) | 7.00 | 289.0404 | – | – | 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone-sulfate | 289.0376 |
| (4) Cocoa flavor and (5) Taste compounds | | | | | | |
| (A) | 3.02 | 151.1212 | – | – | 3,5-diethyl-2-methylpyrazine | 151.1229 |
| (B) | 3.60 | 137.0597 | – | – | Hydroxyacetophenone | 137.0597 |
| (6) Nicotinic acid metabolites | | | | | | |
| (A) | 0.67 | 140.0333 | – | – | Hydroxynicotinic acid | 140.0342 |
| (B) | 1.02 | 140.0705 | – | – | Trigonelline | 140.0706 |
| (6) Amino acids | | | | | | |
| (A) | 0.53 | 182.0805 | – | – | Tyrosine | 182.0811 |

*w*c*, Contribution; *p*(corr), Reliability correlation.

randomly consuming either a single dose of 40 g of cocoa powder with 250 mL of milk or 250 mL of water, or milk without cocoa. Urine samples were collected before the ingestion and at 0–6, 6–12, and 12–24 h after test-meal consumption. Samples were analyzed by HPLC-q-ToF, followed by multivariate data analysis. In this case, the phenolic composition of the cocoa powder (mean (SD)) was 23.1% monomers with 0.71 (0.09) mg/g of (-)-epicatechin and 0.21 (0.01) mg/g of (+)- catechin, 13.4% dimers, including 0.64 (0.06) mg/g of procyanidin B2, 63.6% 3–8mers and flavonols, including 33.87 μg/g isoquercitrin, 5.74 μg/g quercetin, 4.33 μg/g quercetin-3-glucuronide, and 36.32 μg/g quercetin-3-arabinoside. The total polyphenolic content of the cocoa powder was 11.51 (0.95) mg catechin/g.

The results indicated that cocoa powder intake has a pronounced effect on the urinary metabolome, after 24 h. Overall, 27 metabolites related to cocoa-phytochemicals, including alkaloid derivatives, polyphenol metabolites (both host and microbial metabolites), and processing-derived products such as diketopiperazines, were identified. The most important biomarkers of cocoa intake were divided into seven different groups (Table 3.1). Such biomarkers of cocoa ingestion were mainly derived from theobromine's metabolism: it is well-known that cocoa-based food products are good sources of purine alkaloids such as caffeine and theobromine [12].

Several studies had shown that metabolites such as epicatechin sulphate or *O*-methylepicatechin (Table 3.1) as well as vanillic acid (Table 3.1) could be used as valid biomarkers of cocoa or chocolate polyphenol consumption [7, 13].

A metabolomic study concerning catechin supplementation of rats fed a high-fat diet identified several metabolites of catechin, namely *O*-methyl catechin, catechin glucuronides, methylcatechin glucuronide, hydroxyphenylvalerolactone metabolites, hippuric acid and hydroxyhippuric acid. The latter is formed either in the tissue or by the microbiota in the colon and has been considered as an important biomarker of catechin consumption (Table 3.1) [14].

Some studies have revealed the presence of trigonelline and nicotinic acid (Table 3.1) in cocoa and coffee; their presence in human urine has also been recorded using several techniques. As an example, coffee beans contain large amounts of betaine *N*-methyl nicotinic acid, also known as trigonelline, and food chemical studies indicate that this compound is thermally converted to nicotinic acid and certain flavor compounds during roasting [15–17].

Hydroxynicotinic acid (Table 3.1) is formed by the enzymatic action of nicotinate with nicotinic acid hydroxylase or nicotinate hydroxylase. These enzymes participate in nicotinate and nicotinamide metabolism [18].

In a study performed by Xin-Qiang Zheng et al. [19], trigonelline has been found to accumulate in the leaves and fruits of *Coffee arabica* and *Theobroma cacao*. The endogenous levels of trigonelline varied from 14 to 124 nmol $g^{-1}$ fresh weight in the leaves and fruits of cacao plants [19].

A study by Lang et al. [20] using stable isotope dilution analysis (SIDA) and LC-MS/MS (MRM) for the quantitative determination of trigonelline, nicotinic acid, and nicotinamide in foods such as coffee, detected trigonelline (0.001 μmol/mL) as well as nicotinamide (0.001 μmol/mL) in plasma samples. Other quantitative data obtained from urine samples showed high trigonelline and nicotinic concentrations (0.53 and 0.203 μmol/mL, respectively) with low levels of nicotinamide (0.004 μmol/mL) [20].

Another study from the same group found that 8 h after coffee ingestion, a urinary excretion of 57.4 (6.9%) of trigonelline was recorded in male volunteers. Females excreted slightly lower amounts, namely 46.2 (7.4%) [21].

Ito et al. [22] studied the estimation of polyphenols recovery after ingestion of six different polyphenol-rich beverages, using HPLC coupled with electrospray ionisation mass-spectrometry (HPLC-electrospray-tandem MS) analysis of human urine. Polyphenol-rich beverages were made of 4 g instant coffee (equivalent to two cups of coffee), 0.3 g green tea extract (equivalent to one cup of tea), 10 g cocoa powder (equivalent to one cup of hot chocolate) or 18 g grape-skin extract, dissolved in 200 ml hot water. The phenolic profile of the cocoa powder revealed epicatechin (6.6 mg/g), catechin (2.8 mg/g), procyanidin dimers (4.2 mg/g), trimers (3.6 mg/g), tetramers (3.3 mg/g) and pentamers-to-decamers (8.4 mg/g of dry matter). The main polyphenols that were quantified were lignans (enterodiol and enterolactone), several phenolic acids (chlorogenic, caffeic, m-coumaric, gallic, and 4-O-methylgallic acids), phloretin, and various flavonoids such as catechin, epicatechin, quercetin, isorhamnetin, kaempferol, hesperetin, and naringenin. The urinary levels of excretion suggest that chlorogenic acid, gallic acid, epicatechin, naringenin, or hesperetin could be used as potentially specific biomarkers to evaluate the consumption of coffee, wine, tea or cocoa, and citrus juices, respectively [22].

Hydroxycinnamates, which include ferulic acid (FA) and other several components, are found in food as monomers, dimers, and bound forms esterified with hydroxy acids, mono/disaccharides, and polymers as well as amides (with amino acids and amines), particularly in coffee and cocoa [23]. FA metabolism, through hepatic β-oxidation, could conceivably account for the formation of urinary metabolites of FA such as dihydroferulic acid, vanillic acid, and vanilloylglycine (Table 3.1) in rats [24, 25]. A rapid gastric absorption of FA has been demonstrated in rats and humans [26, 27].

Cocoa and its products are rich in methylxanthines, namely caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (Table 3.1) [7, 28]. Caffeine and theobromine are purine alkaloids naturally present in chocolate and belong to group of chemical compounds referred to as methylxanthines [29]. Theobromine is found present in dark chocolate, in quantities six- to seven-times greater than caffeine [30]. As theobromine is present in high amounts in cocoa powder and dark chocolate, it is used as a marker for the absorption of cocoa powder and dark chocolate.

A quantification study performed by Ptolemy et al. [31], using liquid chromatography-tandem mass spectrometry to observe the biofluid (saliva, plasma, and urine) levels of theobromine and caffeine with pre-and post-intervention of cocoa consumption (376 mg of cocoa-derived theobromine), revealed significantly elevated levels of theobromine in all of the three biofluids, with highest concentrations in urine after 90 min post-dosing, followed by plasma and saliva. The elevated level of theobromine recorded in this study was thought to be a direct consequence of cocoa consumption. On the other hand, caffeine (50 mg of cocoa-derived caffeine) was also found in detectable amounts, but its concentration was lower than that of theobromine. Overall, the average concentrations of theobromine and caffeine ranged from 4.1 (saliva) to 9.3% (plasma) and 3.1 (urine) to 14.7% (saliva) respectively [31].

Thirty-two samples of chocolate products (local and imported), quantified and analyzed by using HPLC, showed high levels of caffeine and theobromine, ranging between 0.62 to 1.14 mg/g, and 0.026 to 0.153 mg/g, respectively [32].

The major metabolite of theobromine in human urine is 7-methylxanthine (34–48%), followed by 3-methylxanthine (20%) and 7-methyluric acid (7–12%), 6-amino-5-[*N*-methylformylamino]-1-methyluracil (6–9%), and 3,7-dimethuluric acid (1%) (Table 3.1) [12, 33].

In a recent study aimed at the identification of phenolic compounds in *Theobroma cacao* L., *N-trans*-caffeoyl-L-tyrosine and *N-trans-p*-coumaroyl-L-tyrosine have been demonstrated in cocoa beans and calli [34]. In another study done by Baba et al. [35], human subjects ingested 26 g of cocoa powder for 12 weeks. Oxidized and modified forms of lysine and tyrosine, such as $N^{\varepsilon}$-(hexanoyl)lysine, dityrosine, bromotyrosine, and dibromotyrosine, were detected in urine. Since these oxidative products were found to be stable in urine, they could also be used as biomarkers of oxidative stress [35].

The flavor components of cocoa and chocolate are compounds such as pyrazines, aldehydes (cocoa aroma, nut), esters (fruity aroma), and phenolic compounds (astringent property); many of these are formed following Maillard reactions [36].

Trialkylated pyrazines such as 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine were shown to be major flavor components of coffee [37], while 3,5-diethyl-2-methylpyrazine and hydroxyacetophenone are considered as odor compounds of cocoa powder (Table 3.1) [38]. Since these flavor and aroma components are highly cocoa specific they could be used as markers of cocoa powder intake.

## 3.4 Conclusions

Most of the scientific studies performed to date with cocoa and chocolate have focused on their effects on human health. The near totality of studies found healthful effects (as described in other chapters of this book) and suggests the use of cocoa within a balanced diet.

Apart from the intervention studies described in other chapters of this book, more epidemiological research and metabolomic data are needed to establish authentic, stable, and validated biomarkers of cocoa consumption. This will greatly facilitate and expand our knowledge and will eventually prove if cocoa and its derived products are to be included as an integral part of a healthful diet.

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# Botany and Pharmacognosy of the Cacao Tree

# 4

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## 4.1 The *Theobroma cacao* Discovery and History

Cacao trees originated in river valleys of South America and, by the seventh century AD, the Mayan Indians had brought them north into Mexico. Apart from the Mayans, many other Central American Indians including the Aztecs and the Toltecs seem to have at first domesticated and then cultivated cacao trees, and the word "chocolate" (the beverage) derives from xhocolatl (approximate spelling) or cacahuatl, both originating from the Aztec language. There are several mixtures of cacao described in ancient texts, for ceremonial, medicinal and culinary purposes. Some mixtures included maize, chili, vanilla (*Vanilla planifolia*), peanut butter and honey. Archaeological evidence of the use of cacao, while relatively sparse, has come from the recovery of whole cacao beans in Uaxactun, Guatemala and from the preservation of wood fragments of the cacao tree at the Belize sites (ex British Honduras). In addition, analysis of residues from ceramic vessels has found traces of theobromine and caffeine in early formative vessels from Puerto Escondido, Honduras (1100–900 BC) and in middle formative vessels from Colha, Belize (600–400 BC) [1, 2].

Christopher Columbus' first voyage to the Americas in 1492 launched an era of large-scale contact between the Old and the New World that resulted in this ecological revolution, hence the name "Columbian" Exchange [3], and in this context, the history of cacao began for the Europeans. By 1502, the year of the first European encounter with cocoa beans (Columbus' 4th voyage), cacao trees had already been domesticated for as long as two thousand years [4]. Despite the bitter taste of the drink produced from cacao beans, Columbus claimed the resulting concoction was a "divine drink which builds up resistance and fights fatigue. A cup of this precious drink permits a man to walk for a whole day without food" [4].

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According to Girolamo Benzoni (1519–1570), another Italian explorer of the New World, the Spaniards learned about the use of cacao from the populations of Mexico and Guatemala [5]. *Theobroma cacao* named by the Indigenous as "cacavate" or "cacauate" was described as a small evergreen tree, native to the deep tropical region that flourishes only in very warm and shady localities. The fruits ripen in the course of a year and are picked when ripe, the kernels are removed and laid on mats to dry. When they wish to use them for a beverage, they are roasted in an earthen pan over the fire, and ground between stones on a flat mortar or mealing stone, usually used for preparing bread [6]. Finally the paste is put into cups, made from calabashes produced by the *Lagenaria siceraria* tree that grows wild [7][1]; mixing it gradually with water, sometimes adding small quantities of aromatic spices, they drink it, though it seems "more suited for pigs than for men" [5]. Often certain ground aromatic spices are added to the cocoa, like cinnamon obtained from the inner bark of several trees of the Cinnamomum genus, the achiotte or achiote seeds (*Bixa orellana*, annatto), black pepper (*Piper nigrum*) and the long red pepper (*Capsicum annuum*) are first beaten with aniseed (*Pimpinella anisum*), and then the cacao, which must be beaten lightly, until it becomes a powder, while beating it must be mixed carefully. All these ingredients must be beaten separately, and then put into the vessel together with the cacao [6].

Both the Maya and the Aztecs used cocoa beans both as a ritual beverage but also as currency, and therefore drinking cocoa was literally "consuming money". Chocolate was considered a luxury only for the Aztec nobility as the commoners simply could not afford the luxury of eating money [5].

Conflicting reports make it difficult to determine exactly how chocolate was introduced into Europe, but it most certainly reached Spain before any other country. The traditional chocolate spices, vanilla, chilli, the colourant annatto and "ear flowers" *Cymbopetalum penduliflorum*, were all imported with the cacao seeds [8]. The Spanish initially found the bitter flavor of unsweetened "cacahuatl" unpalatable, and gradually introduced ingredients that made the drink more appealing to the European palate [8].

## 4.2 Botany of *Theobroma cacao* L.

The scientific name *Theobroma cacao* was given to this species by the Swedish botanist Carl Linnaeus in 1753, when it was published in his famous book *Species Plantarum*. The generic term *Theobroma* means "food" (from Greek: broma is food) "of the gods" (from Greek: Theo is God) and *cacao* is derived from the Nahuatl (Aztec language) word "xocolatl", from "xococ" (bitter) and "atl" (water).

The cacao *Theobroma cacao* L, *Malvaceae* family, formerly *Sterculiaceae* [9, 10] is a small evergreen tropical and subtropical tree that originates from the neotropical rainforests,

[1] *Lagenaria siceraria* or *Lagenaria vulgaris*, the calabash, bottle gourd, squash or long melon is a vine grown for its fruit, which can either be harvested young and used as a vegetable, or harvested mature, dried, and used as a bottle, utensil, or pipe. The calabash was one of the first cultivated plants in the world, grown not primarily for food but for use as a water container. The bottle gourd may have been brought from Africa to Asia, Europe and the Americas in the course of human migration [7].

primarily in the Amazon basin and the Guyana Plateau. The Cacao tree grows at elevations of around 200–400 m, in areas with 1,000–3,000 mm rainfall per year. It requires a humid climate with regular rainfall and good soil. *T. cacao* can only grow within 10 degrees north or 10 degrees south of the equator. The moist, shady, tropical rainforest region is its habitat. It is native to southern Mexico, Central America and tropical South America, in tropical humid forests on the lower eastern equatorial slopes of the Andes in South America. In its natural habitat, cocoa grows in the undergrowth of the evergreen tropical rainforest. It often grows in clumps along river banks, where the roots remain flooded for long periods of the year. It is an understory tree, growing beneath taller trees, such as the banana palm (*Musa* sp.), the coconut palm (*Cocos nucifera*) or leguminous tree crops, such as *Gliricidia sepium* (called "madre de cacao" in Spanish, meaning "mother of cacao"), *Inga* spp, and *Albizia* spp. These plants form a canopy for cacao trees, according to the direct observations of G. Benzoni in 1565 [5]. Today cacao trees are cultivated around the world between the tropic of Cancer and the tropic of Capricorn, mainly in African countries.

It is important to point out that the term "cacao" refers to the specific epithet of *Theobroma cacao*, the linnean binomial nomenclature of the tree. While the name "cocoa" is the drink produced from the cacoa seed. The "cocoa powder" is the ground fermented cacao seed after the removal of a definite portion of the fat.

The plant has two types of branches or stems: vertically growing (orthotropic), also called chupons, and laterally growing (plagiotropic), also called fan branches. A seedling grows orthotropically until it forms the first jorquette. On young trees, new chupons may sprout at any point on the original orthotropic trunk but usually appear just below the jorquette. Mature trees will occasionally form chupons on large plagiotropic branches. Chupons can also grow from the base of a mature tree and can often replace the main trunk. The cacao tree is a typical evergreen cauliflorous plant (not strictly), cross-pollinated and monoecious, and can reach up to 25 m in height in its wild state. Cauliflorous is a plant or tree producing flowers on the older branches or main stem covered by light brown bark. Its dark green leaves are shiny, leathery, simple (undivided blade), ovate to elliptic in shape and 20–35 cm long and 7–8 cm wide. The leaf surfaces are hairless or covered in scattered star-shaped hairs. The base of the leaf is rounded or heart-shaped, and the apex has a long drip-tip. Cacao flowers are small, yellowish white to pale pink, and grouped together in clusters arising directly from the trunk (cauliflory) (Fig. 4.1). Flowers are produced throughout the year. Each plant can produce between 50,000 to 100,000 flowers every year. The flowers are produced in clusters directly on the trunk and on older branches; they are small, 1–2 cm (1/2–1 in) diameter, with a pink calyx. While many of the world's flowers are pollinated by bees (Hymenoptera) or butterflies/moths (Lepidoptera), cacao flowers are pollinated by tiny flies, midges in the order Diptera. In the wild, only about 5% of flowers receive enough pollen to initiate fruit development, so today in modern cacao plantations, pollination is assisted artificially by man. When they are pollinated there is a dramatic change as the tiny flowers develop into massive cocoa pods. The flowers resemble orchids and they vary in size and color depending on the variety and region where the tree grows, just like the cacao fruits. It takes between 5 to 8 months for the flower to blossom into the fruit and become a pod. Both the fruit and the flowers are on the tree all year, which is

**Fig. 4.1** *Theobroma cacao* cauliflorous plant: it produces flowers on the older branches or main stem (www.ars-grin.gov)

an oddity in the world of fruit producing trees. The initial flowers appear to arise above leaf scars, in the axils of abscised leafs. It has been estimated that the flower development in the *T. cacao* tree takes between 21 to 30 days. Each flower is hypogynous and it has five separate petals, ten stamens (5 fertile and 5 infertile staminoids) and a superior ovary of five united carpels. The flower produces no nectar and has no discernible scent. The petals are narrow at the base but extend into cup-shaped pouches and are usually pink and white, the color indicative of a given genotype. The female part of flower consists of an ovary, which consists of five united carpels each having four to 12 locules, and one style that has several linear stigmatic lobes. The style is twice as long as the ovary and consists of five stigmas around an axis. The male part of the flower consists of stamens arranged in two whorls: the outer consists of five non-fertile staminoids and the inner of five fertile stamens. The stamens have two anthers that lie in the pouch of the corresponding petal [11].

When full bloom (anthesis) is reached, pollination takes place in the morning hours and if this does not occur within 24 hours, the flowers soon fall. The flower opens at about dawn, and the anthers dehisce just before sunrise. The stigma is usually pollinated 2 to 3 hours later, and it is receptive from sunrise to sunset of the day of opening. The stigma is receptive to pollen along its whole length, and not merely at the apex as in most flowers. If the flower is not pollinated, it usually sheds the following day. Pollination before noon is best. Although hermaphroditic, cacao flowers are self-incompatible: they cannot fertilize themselves. The insects that pollinate the cacao flowers live in the rainforest. They require a humid climate with some overhead shade and a wide range of species and decaying matter on the ground: the natural habitat of cacao. Cacao pollination is not completely understood, even in neotropical Asian

**Fig. 4.2** *Theobroma cacao* open mature fruits (courtesy of Renato Iguera, Settala, Milan)

and African regions with long cultivation histories [12]. While studies indicate that midges of the genus *Forcipomyia* can be crucial to cacao pollination, many additional arthropods visit cacao flowers, and may also play a role in pollinating them. For example, some investigators have found that trees occupied by ants have higher fruit production than unoccupied trees, indicating that ants and/or ant-tended homoptera (scale insects, mealy bugs and aphids) might provide pollination services. These conflicting indications highlight the need for further investigations into *T. cacao* pollination. In addition, other non-dipterous insects may play an important role in cacao pollination [13].

During the first 40 days after pollination, the tiny pods grow slowly, followed by a rapid phase of growth. During the next phase (85–140 days), the growth of the pods slows down and the growth of the cacao seeds commences with the accumulation of fat. This is followed by the ripening phase. It takes 5–6 months after pollination for the pod to reach its full maturity. The mature fruit, called a "cacao pod", is an indehiscent large ovoid berry, 15–30 cm long and 8–10 cm wide, ripening yellow to orange, and weighs about 500 g when ripe. The pods vary in size, shape and texture depending upon the variety of *Theobroma cacao*. The pod contains 20–60 seeds, usually called "beans", surrounded by sweet mucilage (Fig. 4.2). The sticky pulp inside the cacao pod is edible and it tastes like mango (*Mangifera indica*). Each seed contains the embryo and two big cotyledons, from whitish to deep purple in color. The seeds contain a significant amount of fat (40–50% as cocoa butter). Their most noted active constituent is theobromine, a compound similar to caffeine.

In the wild, ripe cacao fruits do not fall to the ground naturally as other fruits do so the seed dispersal can only be achieved by animals attracted to the mature fruits as a food.

The pulp of the cacao fruit is edible, it is yellowish, slippery and sweet and a bit less dense than an apple. It is described as lemony, although it has also been suggested that it tastes a bit like mango. Seeds are dispersed by monkeys and other small mammals that break the pod open in order to eat the pulp.

When the cacao tree is cultivated in intensive plantation, the fruits (pods) must be removed from the trees individually by hand. Farmers generally use machetes or large knives attached to poles to slice down the ripe pods, taking care not to damage nearby buds. Pods are left to mellow on the ground, then cracked open by hand, the beans are removed and the outer shell discarded.

The cacao tree is interesting in its biology, especially for the presence of endophytic fungi. Fungal endophytes are fungi that colonize and grow asymptomatically within the internal plant tissues without causing harm to their host, except when the host is under stress conditions. These fungi have been observed in many plants, but research has focused mainly on the association of temperate grasses and endophytes. In this symbiosis, endophytes help hosts to tolerate adverse abiotic and biotic factors highlighting their status as a new source of biological control agents to combat cacao pathogens. For this reason, the association is generally considered mutual, but the generality of such benefits is not clear for all host plants. Endophytes are abundant in the leaves, stems and fruits of *T.cacao* and belong to different strains. A total of 150 endophytic fungi have been isolated from stems of cacao; belonging mainly to the Ascomycetes group; the *Botryosphaeriaceae, Valsaceae* and *Nectriaceae* families are the most frequent. The *Fusarium* spp. fungus is the dominant genus and shows the highest diversity. The potential for endophytes to control cacao disease is supported by the observation that many endophytes antagonize other fungi that can cause diseases, e.g., the basidiomycete *Crinipellis perniciosa* (Stahel) Singer (witches' broom disease), the ascomycete *Moniliophtora roreri* (frosty pod rot or moniliasis disease), and *Phytophthora palmivora* (black pod rot) [14, 15].

The three major cultivars of *Theobroma cacao* are: the "Criollo group", which has an almost unique and homozygous genotype, it was among the first to be cultivated and was grown and used by the Maya; the "Forastero group", these trees are hardier and more productive; and the "Trinitario group", produced by crossing plants of the other two types, Criollo from Trinidad and the Brazilian Forastero. Due to the high interest from an agronomic and economic point of view, the Criollo variety genome was recently sequenced [16]. *Theobroma cacao* diploid tree has 2n=20 with about 28,000 genes, three quarters of which have now been sequenced. This tree, which has only ten pairs of chromosomes, can be easily propagated by both sexual and vegetative methods and can be transformed.

A high portion of cacao genome is homozygote, i.e., it possesses identical pairs of genes (or alleles) for a specific trait of DNA on both homologous chromosomes. This highly homozygous genotype can be considered the result of the many generations of self-fertilization that occurred during the domestication process. The DNA sequencing permitted the discovery of 84 genes that are potentially involved in lipid biosynthesis (cacao butter), 96 genes are involved in the flavonoid biosynthetic pathway, and 57 genes that encode terpene synthase, which catalyze terpenoid synthesis [16].

## 4.3 Cocoa and Health

Over the past 15 years there has been increased interest in the potential health-related benefits of antioxidants and phytochemical-rich dark chocolate and cocoa. More than 200 studies were reported on bioactive compounds, chemical compositions, and health benefits of cocoa and cocoa products. Many of the proposed health-protective activities associated with the consumption of cocoa and chocolate have been attributed to flavan-3-ols, including monomers. The major reported pharmacological activities of flavonoids include antioxidative effects, protection against cardiovascular disease and anticancer effects. Although still debated, a range of potential mechanisms through which flavanols and cocoa might exert their benefits on cardiovascular health have been proposed: activation of nitric oxide (NO) and antioxidant, anti-inflammatory, and anti-platelet effects, which in turn might improve endothelial function, lipid levels, blood pressure and insulin resistance. The are several excellent reviews on cocoa and cardiovascular health [17–19]. Preliminary in vitro investigations have suggested that cocoa flavanols and procyanidins may possess immunoregulatory effects and may help to modulate immune responses. A few studies have documented the use of polyphenols to increase blood flow and perfusion of the brain and experimental data suggests that flavanols may delay the onset of neurodegenerative diseases such as Alzheimer's disease through a number of different mechanisms. More recently, studies have begun to emphasize the bioactivities of flavonoids through modulation of several cellular signaling pathways. These pathways regulate cell apoptosis, proliferation, survival, and inflammatory responses.

In addition to flavonoids, foods derived from cocoa are rich in minerals, lipid, and other non-flavonoid-based phytochemicals [20–22]. For instance, cocoa and their products are rich in methylxanthines, namely caffeine, theobromine and theophylline and studies have demonstrated that methylxanthines can possess both positive and negative health effects. Cocoa is also rich in micronutrients and those such as copper or magnesium could contribute significantly towards human dietary intake.

Thus as cocoa contains a mixture of bioactive components, it is possible to postulate that there may be direct or indirect synergism between these components in delivering their health properties. Cooper et al. [23] suggested that if the biological effects are due to cocoa flavonoids rather than other components, the perfect control would be cocoa or cocoa products that contain "things other than flavonoids".

## 4.4 Cocoa and Chocolate

Cocoa trees (*Theobroma cacao L*) grow in a limited geographical zone approximately 10 degrees to the north and south of the equator, in particular Central America, West Indian islands, South America and Africa. There are three important varieties of cocoa: Forastero, Criollo and Trinitario. Forastero comprises 95% of the world production of cocoa, and it is the most widely used. Overall, the highest quality of cocoa comes from the native Forastero variety of Ecuador (known as Arriba) and from the Criollo variety. Cacao

(or cocoa) beans are technically not beans or legumes, but rather the seeds of the fruit of the *Theobroma cacao* tree. The pod shaped fruit is botanically classified as baccate-like (berry-like) and each pod produces approximately 35-50 seeds surrounded by a sweet pulp. The pod and the pulp surrounding the cacao seed in this case constitute the fruit of cacao. The processing of cacao seeds into chocolate or cocoa begins with the harvest of cacao pods, followed by the removal of the seeds from the pod and their subsequent fermentation, drying, and roasting. All of these are important steps in the development of the typical chocolate flavor and color. The roasted cocoa seeds are then usually ground into a suspension, called "cocoa or chocolate liquor", which contains cocoa butter and nonfat fine, brown particles. Cocoa powder is usually made by mechanically pressing the liquor to expel most of the cocoa butter, leaving a solid cake, which is then ground into the product that most people know as cocoa powder. Typical cocoa powders contain 10–12% residual cocoa butter. The cocoa powder is where the vast majority of the chocolate flavor and the polyphenol antioxidants reside. Chocolate and cocoa are two different terms and are not interchangeable. Cocoa is the non-fat component of cocoa liquor, which is used in chocolate making or as cocoa powder for cooking and drinks. Cocoa liquor contains approximately 55% cocoa butter and together these comprise cocoa solids, often referred to on chocolate packaging. Chocolate refers to the combination of cocoa, cocoa butter, sugar, etc. into a solid food product. Commercial chocolates usually range from 50 to 80% cacao. Cocoa nibs, chocolate liquor, and cocoa powder can be modified by a 180-year-old process of treatment with alkali, also known as Dutching. This process darkens the cocoa ingredients, changes the taste by reducing bitterness, and increases the dispersibility of cocoa powder for various applications such as beverages. Most of the alkali-treated powders are used in non-confectionery applications. Alkalized cocoa powder and alkalized liquor are not commonly used as major ingredients in the manufacture of chocolate confectionery, although there are several large brands of dark chocolate that use these ingredients.

## 4.5 Composition of Cocoa and Chocolate and Potential Health-related Benefits

### 4.5.1 Lipids

Cocoa butter accounts for 50% to 57% of the dry weight of cocoa and is responsible for the melting properties of chocolate. The predominant fatty acids in cocoa butter are saturated (stearic 35% and palmitic 25%) and monounsaturated (oleic 35%), with the remaining fat being primarily polyunsaturated linoleic (3%) [20, 22]. As a consequence of the high saturated fatty acid content of chocolate and cocoa, it is often viewed as a negative food with respect to the vascular system. However, stearic acid does not elevate blood cholesterol levels to the same extent as other saturated fatty acids. Possible explanations for this disparity may include chain length, inefficient absorption, metabolism kinetics, and hepatic desaturation of stearic into oleic acid. The inclusion of a moderate amount of chocolate containing stearic acid into the diet is not predicted to have adverse

effects on the lipid and lipoprotein profiles of individuals, as long as the total amount of fat and caloric intake is held constant. Consumption of a large amount of chocolate, which provides excess fat and calories to the diet beyond estimated maintenance needs, could contribute to obesity and negatively impact cardiovascular disease incidence. The long-term effects of stearic acid consumption on thrombogenetic factors are not well elucidated. Stearic acid is effective in promoting platelet aggregation and factor VII coagulant activity in vitro, whereas preliminary in vivo studies showed mixed results. The clinical studies reported on stearic acid to date are contradictory in that some report neutral effects on platelet activity and pro-coagulant factors, whereas others report negative effects.

### 4.5.2 Sterols

Plant sterols and stanols can contribute to improved blood lipid profiles by competitive inhibition of dietary cholesterol absorption in the gut. Very small amount of plant sterols including sistosterol and stigmasterol are present in cocoa butter. It is likely that the minor levels of sterols in finished chocolate have limited impact on cholesterol absorption; however this has not been investigated.

### 4.5.3 Fiber

The unprocessed cocoa bean has a seed coat, also termed bran, which accounts for 15% of the total bean weight. The bran is a good source of insoluble fiber (44%) and also has some soluble fiber (11%) that could contribute to lowering serum lipids. In comparison, cocoa powder contains less than 2% bran, and finished chocolate products have very little (Table 4.1) [24]. Thus, chocolate consumption does not contribute significantly to dietary fiber intake [22]. Recently Sanchez et al. [25] evaluated the effect produced by long-term intake of a soluble cocoa fiber product (SCFP) on the development of hypertension of spontaneously hypertensive rats. They demonstrated the antihypertensive and antioxidant properties of SCFP and suggested that the control of body weight and control of increased angiotensin II may be involved in the antihypertensive effect of this product.

### 4.5.4 Polyphenols in Cocoa Beans and Cocoa-based Products

Phenolic compounds or polyphenols constitute one of the most numerous and widely distributed groups of substances in the plant kingdom with more than 8000 phenolic structures currently known. They are products of the secondary metabolism of plants and arise biogenetically from two main primary synthetic pathways: the shikimate pathway and the acetate pathway. Both acetic acid and shikimic acid are derived from glucose metabolism [26]. Polyphenols can be divided into various classes depending on their basic structure: simple phenols, benzoquinones, phenolic acids, acetophenones, phenylacetic acids,

**Table 4.1** Chemical composition (partial) of various cocoa products for 100 g (USDA National Nutrient Database for Standard Reference) [24]

| Nutrient | Cocoa dry powder | Cocoa mix, powder (hot chocolate) | Milk chocolate beverage | Chocolate, dark 45-59% cacao solids | Chocolate, dark 60-69% cacao solids | Chocolate, dark 70-85% cacao solids |
|---|---|---|---|---|---|---|
| *Proximates* | | | | | | |
| Water | 3.00 g | 1.50 g | 82.45 g | 0.97 g | 1.25 g | 1.37 g |
| Protein | 19.60 g | 6.67 g | 3.52 g | 4.88 g | 6.12 g | 7.79 g |
| Total lipid | 13.70 g | 4.00 g | 2.34 g | 31.28 g | 38.31 g | 42.64 g |
| Fatty acids, total saturated | 8.07 g | 2.377 g | 1.431 g | 18.519 g | 22.031 g | 24.489 g |
| Fatty acids, total monounsaturated | 4.57 g | 1.325 g | 0.677 g | 9.540 g | 11.522 g | 12.781 g |
| Fatty acids, total polyunsaturated | 0.44 g | 0.114 g | 0.084 g | 1.092 g | 1.221 g | 1.257 g |
| Ash | 5.80 g | 4.10 g | 0.65 g | 1.70 g | 1.90 g | 2.32 g |
| Carbohydrate, by difference | 57.90 g | 83.73 g | 10.74 g | 61.17 g | 52.42 g | 45.90 g |
| Fiber, total dietary | 33.2 g | 3.7 g | 1.0 g | 7.0 g | 8.0 g | 10.9 g |
| Sugars total | 1.75 g | 65.55 g | 9,66 g | 47.90 g | 36.71 g | 23.99 g |
| *Minerals* | | | | | | |
| Calcium, Ca | 128 mg | 133 mg | 114 mg | 56 mg | 62 mg | 73 mg |
| Iron, Fe | 13.86 mg | 1.19 mg | 0.42 mg | 8.02 mg | 6.32 mg | 11.90 mg |
| Magnesium, Mg | 499 mg | 83 mg | 23 mg | 146 mg | 176 mg | 228 mg |
| Phosphorus, P | 734 mg | 315 mg | 105 mg | 206 mg | 260 mg | 308 mg |
| Potassium, K | 1524 mg | 712 mg | 197 mg | 559 mg | 567 mg | 715 mg |
| Sodium, Na | 21 mg | 504 mg | 44 mg | 24 mg | 10 mg | 20 mg |
| Zinc, Zn | 6.81 mg | 1.46 mg | 0.63 mg | 2.01 mg | 2.65 mg | 3.31 mg |
| Copper, Cu | 3.788 mg | 0.286 mg | 0.103 mg | 1.028 mg | 1.248 mg | 1.766 mg |
| Manganese, Mn | 3.837 mg | 0.269 mg | 0.013 mg | 1.419 mg | 1.325 mg | 1.948 mg |
| Selenium, Se | 14.3 mcg | 5.0 mcg | 2.7 mcg | 3.0 mcg | 8.4 mcg | 6.8 mcg |
| *Other* | | | | | | |
| Caffeine | 230 mg | 18 mg | 2 mg | 43 mg | 86 mg | 80 mg |
| Theobromine | 2057 mg | 323 mg | 68 mg | 493 mg | 632 mg | 802 mg |

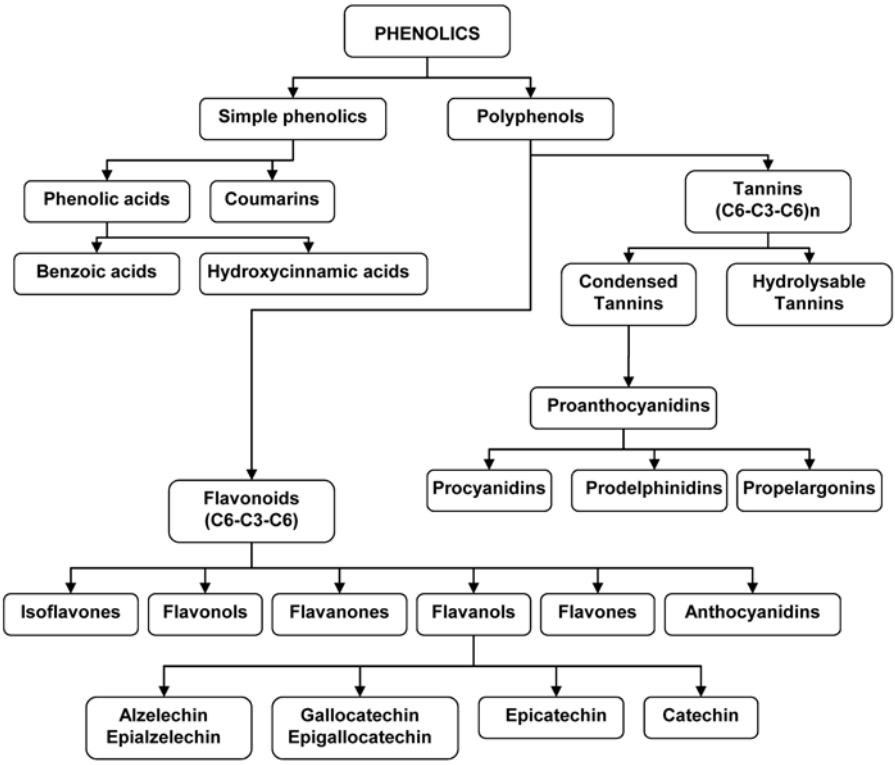


**Fig. 4.3** Classification scheme for polyphenols according to the number of phenol subunits and the hierarchy of common flavonoids monomers and polymers (adapted from [27])

hydroxycinnamic acids, phenylpropenes, coumarines, isocoumarines, chromones, naphtoquinones, xanthones, stilbenes, anthraquinones, flavonoids, lignans, neolignans and lignins. Polyphenols are sometimes referred to as "phenolics" due to the presence of at least one phenol substructure, a hydroxyl group on an aromatic ring - $C_6$. Figure 4.3 depicts a classification of polyphenols based on the number of phenolic subunits. Flavonoids, which constitute the most important single group may be further divided into various classes with more than 5000 compounds described. The absolute structure of these flavonoids can vary dramatically; however all plant flavonoids share a common 15-carbon structural backbone designated as $C_6$-$C_3$-$C_6$. Flavonoids share a common structure consisting of 2 aromatic rings (A and B); differences in the structure of the heterocyclic C ring result in distinct classes of flavonoids, including flavanols, flavanones, flavones, isoflavonols and anthocyanidins (Fig. 4.4). In nature, many flavonoids exist as glycosides which adds significantly to the complexity of characterization. Cocoa is a source of various polyphenolic compounds, including hydroxybenzoic acid (gallic, syringic, protocatethic, vanillic acids), hydroxycinnamic acids and analogues (caffeic, ferulic, p-coumaric, phloretic acids, clovamide, dideoxyclovamide), flavonols (quercetin), flavones (luteolin, apigenin), flavanones (naringenin), and flavan-3-ols ((+)-catechin), (-)-catechin, oligomers

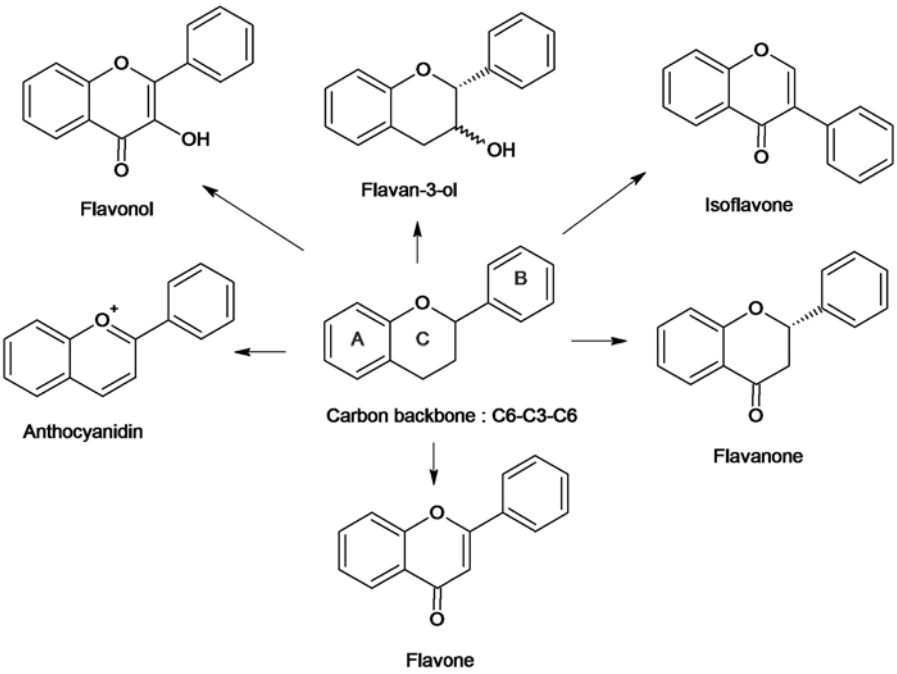


**Fig. 4.4** The generic 3 ring (A, B and C) carbon backbone structures (C6-C3-C6) of the major flavonoid subclasses found in food

and polymers/procyanidins) [20, 28]. The chemical structures of selected cocoa polyphenols are seen in Figure 4.5. Cocoa polyphenols mainly consist of the flavan-3-ols epicatechin, catechin and oligomeric and polymeric procyanidin build-up of (epi)catechin units. Kelm et al. [29] indicated that unfermented cocoa beans contain monomers up to 14 subunits (tetradecamer). Procyanidins consist of 2 or more units of flava-3-ols. B-type procyanidins are linked via a C4-C8 bond, but C4-C6 linkages also occur. A-type procyanidins bear an additional ether bond between C2 and C7. Procyanidins are, along with other substances, responsible for the oral sensation of astringency in ripening fruits (apples, peaches, grapes, berries), beverages (tea, wine), cocoa and chocolate. Wollgast and Anklam [30] published a review that included the methodology for analysis, quantification, isolation, purification, and structure elucidation of polyphenols in cocoa components. Polyphenols in cocoa and derived products were analyzed by several analytical methods: thin-layer chormatography (TLC), capillary electrophoresis (CE), high-performance liquid chromatography (HPLC) coupled to ultraviolet (UV), photo-diode array (DAD) or mass spectrometry (MS) and, more recently, tandem mass spectrometry (LC-MS-MS) [31] and nuclear magnetic resonance (NMR) [32].

The chemical structures of flavonols and procyanidins are important for their antioxidant activity as they possess both free radical trapping and chelation of redox-active metal properties. The measurement of plasma antioxidant concentration and

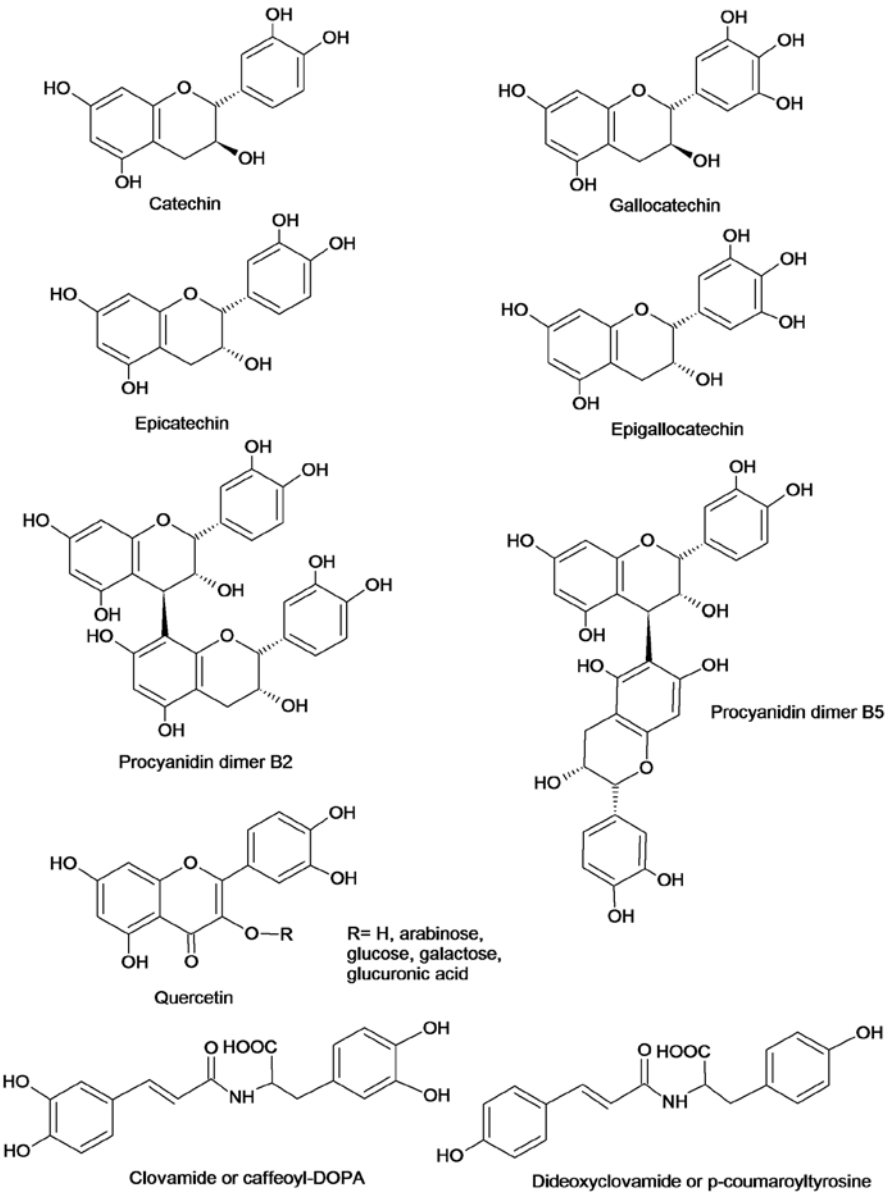


**Fig. 4.5** Chemical structure of selected cocoa polyphenols

oxidative stress levels are examples of determining antioxidant status. Adamson et al. [33] indicated that polyphenol content positively correlated with antioxidant properties as measured by oxygen radical absorbance capacity (ORAC). All polyphenols possess antioxidant properties in vitro but are not likely to exert the same properties in vivo and

in humans. In vivo studies indicated that epicatechin from cocoa could enhance the antioxidative activity in plasma. In theory, these antioxidant actions can result in a reduction of the steady state concentration of free radicals and other oxidants, diminishing the subsequent oxidation of target molecules such as lipids, proteins and nucleic acids. However, the plasma concentrations of flavanol and procyanidin observed after the consumption of foods rich in these compounds were relatively low. The actual concentrations that can be reached in plasma of humans subjected to realistic polyphenol consumption are in the nanomolar range [34, 35]. This low bioavailability leads to a kinetically unfavorable condition with respect to other compounds with similar free radical scavenger capabilities that are present in blood in significantly higher micromolar concentrations i.e., tocopherols and ascorbate. According to Galleano et al. [36] a function of flavanols as direct free radical scavengers is unlikely to be relevant, and could be limited to the blood and other tissues directly exposed after consumption, i.e., gastrointestinal tract. It has been suggested that other mechanisms, compatible with the physiological levels reached by flavanols, may explain the observed changes in cell or tissue oxidation levels after flavanol consumption. These mechanisms are beyond the ability of flavanols and other flavonoids to directly prevent free radical-mediated tissue damage [35]. Thus, the question remains of attributing beneficial effects to cocoa polyphenols vs cocoa as a whole. Other compounds in cocoa are known to be bioactive such as caffeine and theobromine.

It has been shown that chocolate is one of the most polyphenol-rich foods along with tea and wine. According to Lee et al. [37] cocoa contains a higher content of flavonoids per serving than tea or red wine; cocoa powder is one of the richest dietary sources of flavanols (on a weight basis) identified so far, exceeded only by a few food ingredients such as buckwheat hulls, sorghum and cinnamon [24]. (-)-Epicatechin has been reported as the major monomeric flavanol in cocoa, representing approximately 35% of the total phenolic content [30]. Catechin and epicatechin have been found at concentrations of 150–1580 mg/kg in chocolate and 2530–3170 mg/kg in cocoa liquor [38–42]. Procyanidins have been reported at concentrations from 2200 to 13,230 mg/kg in various cocoa liquors [28, 41]. Many phenols are found as glycosides in cocoa, mainly glucoside, galactoside, and arabinose. Miller et al. [43] showed that there is a strong degree of correlation between flavanols, with the possible exception of cathechin, and percentage of nonfat cocoa solids (NFCS) in chocolate- and cocoa-containing products, with cocoa powder being highest and chocolate syrup being lowest in these compounds. The level of flavanols is correlated with the total polyphenols measured in these products; the flavanols are also associated with the calculated percentage of cacao (proxy of cacao percentage) of the products studied, although the correlation coefficients are not as high as those found for percentage NFCS and for total polyphenols.

Quercetin identified in cocoa possesses higher free-radical scavenging properties than (+)-catechin and has been shown to be one of the most effective flavonoids for the preservation of endogenous α-tocopherol in LDL cholesterol. In addition, quercetin and its metabolites produce vasodilatation by means of endothelium-dependent and -independent mechanisms [40].

Two stilbenes were identified in a cocoa liquor from the Ivory Coast and in dark chocolate: trans-resveratrol and trans-piceid. Trans-resveratrol is known to exhibit interesting

anti-inflammatory, anticancer, cardioprotective, and estrogenic activities. In cocoa liquor, 0.4 mg/kg of trans-resveratrol and up to 2.6 mg/kg of its glucoside trans-piceid have been determined [44]. Hurst et al. [45] reported much higher levels in some cocoa-derived products: from 0.09 to 1.85 mg/kg and from 0.35 to 7.14 mg/kg for trans-resveratrol and trans-piceid, respectively. The highest values were obtained from cocoa powder. Resveratrol is currently one of the plant phytochemicals with a great potential to be used as a pharmacological drug in order to prevent or reduce the risk of some diseases. However, its role in human health as a dietary non-nutritional bioactive compound is not yet clear due to its low abundance in the diet and its low availability.

#### 4.5.4.1 Factors Affecting the Quantity and Quality of Polyphenols in Cocoa Beans and Cocoa-based Products

The amount of flavonoids and flavanols in cocoa and chocolate can vary widely as a result of a multitude of factors. Agronomic factors and genetics determine the flavonoid content of plants prior to harvest. After harvest, fermentation, roasting, alkaline treatment (Dutch processing) and baking have been shown to reduce both the level of total procyanidins and the level of low molecular weight flavanols. Cocoa from different varieties exhibited differences in polyphenol content by up to 4-fold; moreover, cocoa beans from different origins contains different amounts of catechin and epicatechin. For instance, cocoa beans from Ecuador possessed the highest amounts of catechin and epicatechin, followed by beans from Ghana and Trinidad. Fermentation is one of the steps involved in the production of cocoa beans. This step is crucial in determining the quality of the cocoa aroma. Much of the world's supply of cacao undergoes farm fermentation, but significant quantities of cacao are not fermented intentionally and are immediately dried. These beans are routinely available commercially from the states of Chiapas and Tabasco in southern Mexico, the Dominican Republic, Ecuador, the Amazonia region of Brazil, and Sulawesi in Indonesia. Typically these beans are either immediately dried with the pulp adhering to the bean or are water-washed to remove the pulp using an ancient, tradition process of washing, referred to locally in southern Mexico as "cacao lavado" (washed). Either unfermented or fermented beans are then dried to moistures ranging from 5 to 8% , typically in the sun or in wood-fired ovens. Beans dried this way can be shipped or stored and represent the cocoa beans of worldwide commerce.

Fermentation of cocoa normally takes between five to seven days; the production of aroma precursors during fermentation is important for producing the full aroma of chocolate. There are internal and external fermentation stages involved during cocoa fermentation. External fermentation primarily involves the catabolism of the sugar pulp by microorganisms, while internal fermentation encompasses the biochemical changes in the cotyledons of the beans. During fermentation, polyphenols diffuse from their storage cells and undergo oxidation to become condensed high molecular compounds, mostly insoluble tannins. Both non-enzymatic and enzymatic processes are involved and are catalysed by polyphenol oxidase. Epicatechin, catechin, procyanidin B2, procyanidin B5 and procyanidin C1 were the major compounds responsible for bitterness and astringency of roasted cocoa. However, the bitter taste and astringency were not just attributable to polyphenols, but were also contributed to by amino acids. During fermentation, between days two and three, epicatechin content was observed to decrease sharply, which could indicate that

it is either being used up for the formation of large tannins or lost in the fluids that drain away. After the fermentation, the cacao beans are deshelled and roasted at 100–150°C. Different degrees of roasting significantly increased the amount of (+)-catechin due to isomerization of (-)-epicatechin. Alkalinization (Dutching) is used to mellow the flavor of cocoa, however this process has been shown to destroy polyphenolic compounds and it is likely responsible for the significant differences in antioxidant activity. The extent of polyphenol destruction is proportional to the degree of alkalization and change in the water extractable pH of the resulting powder; about 40% of the natural level of flavanols is retained on average for lightly Dutched powders, and an average of 22% is retained in even moderately alkali processed cocoa powders. Miller et al. [43] observed that even for products that are labeled with "cocoa powder processed with alkali" there is a 20-fold difference between the lightest alkalized powder (24.56 mg/g) and the most heavily alkalized powder (1.33 mg/g). Several papers indicate that the level of epicatechin and catechin can vary independently from one another. It has been postulated that the ratio of epicatechin to catechin (epi/cat) could possibly be associated with the degree of cocoa processing. Recently Payne et al. [46] showed that the epicatechin content dropped substantially as beans were fermented (comparison of lavado, sun-dried, to fermented, sun dried), when beans are roasted to 120°C (comparison of fermented, sundried, to fermented, roasted), and when beans were Dutch-processed (comparison of natural powder to medium Dutch-processed powder). The epi/cat ratio showed that the highest ratios were found in unripe and ripe, unfermented dried beans (lavado) with a range of 32–35. Fermentation lowered the epi/cat ratio to about 20. Epi/cat ratios were further reduced to 0.90–1.40 by roasting. The lowest epi/cat ratios were found in Dutch-processed cocoa powders and range from 0.4 to slightly less than 1. The authors found that the loss of epicatechin begins before the cocoa pod is fully ripe. Once ripe the epicatechin content of beans is roughly the same regardless of whether beans were freeze-dried, sun-dried, or laboratory-dried. Roasting caused the epicatechin content of unfermented beans to drop 82% when roasted to a terminal temperature of 120°C and in medium fermented beans to 18% (roasting to 120°C) of reference beans.

Recently, various type of dark chocolate are available in the market with high flavonoid contents. These chocolates are produced by controlling bean selection, fermentation, and reduced heat and alkalization treatments. Furthermore, there are chocolate producers who produce chocolates from high-flavonoid beans from Ecuador and utilize special roasting methods that preserve flavonoids in the cocoa beans. By controlling the process involved in preparing the chocolates, a high-flavonoid chocolate can be produced that preserves up to 70% of the flavonoids present in the finished product. Most of the intervention studies used dark chocolate. This is due to the fact that dark chocolate contains more non-fat cocoa solid (cocoa powder) than other chocolates. The quantity of the flavonoids present in these products solely depends on the amount of non-fat cocoa solid content. On the other hand, white chocolate is prepared with cocoa butter and sugar without cocoa powder. Thus, dark milk and milk chocolates are expected to have flavonoids, while white chocolate will have none. Thus, the selection of the best quality cocoa and cocoa products could deliver the best antioxidant flavonoids. Current FDA regulations do not require that antioxidant capacity and/or polyphenol content be provided on food labels. Inclusion of this information has been suggested [23].

#### 4.5.4.2 Bioavailability and Metabolism of Cocoa Flavanols and Procyanidins in Humans

When discussing the biological activity of flavonoids in general, and flavanols in particular, there are four major factors to be considered: (a) bioavailability from food; (b) absorption and metabolism in the gastrointestinal tract; (c) tissue and cellular distribution after absorption; and (d) which are the chemical form(s) biologically available to the cell/tissue and their potential metabolism at cellular level. There are several excellent publications on bioavailability and metabolism of cocoa polyphenols [39, 47–49]. It is well recognized that orally administered flavonoids would undergo extensive presystemic first-pass metabolism. Substantial intestinal and hepatic glucuronidation have been found in a number of structurally diverse flavonoids. After ingestion, naturally occurring flavanols can undergo significant modifications that can result in a diverse family of bioactive molecules. Flavanols and procyanidins are relatively stable in stomach acid and during gastric transit. During digestion and transfer across the small intestine, and in the liver, flavanols are rapidly metabolized in phase I and phase II biotransformations to various *O*-sulfated, *O*-glucuronidated and *O*-methylated forms. Various UGT isozymes, expressed in the intestine and in the liver, have been identified to catalyze the glucuronidation of flavonoids. In humans consuming cocoa, plasma levels of nonmethylated epicatechins such as epicatechin-7-sulfate and methylated metabolites such as 3'-*O*-methylepicatechin have been reported to occur in micromolar concentrations within 1 h after intake. Metabolic studies have confirmed the presence of these conjugates in the plasma and urine of rodents and humans, as well as in the bile and brain of rats. It has been reported that colonic microflora can break the flavonoids flavan structure to form simple phenolics and ring-fission metabolites that may be physiologically relevant. In summary, non-metabolized flavanols or metabolites of flavanols can exert biological effects essentially depending on flavanol metabolism and presence in the target tissue. Certain monomers may be better absorbed than others. For example, in humans consuming a cocoa beverage containing equal amounts of epicatechin and catechin, epicatechin was identified to be the predominant plasma flavanol absorbed, with plasma catechin levels reaching less than 10% of epicatechin concentrations. The differences in plasma flavanol concentrations, could partly be due to procyanidin degradation: dimers has been shown to form epicatechin and methylated epicatechin under certain conditions, although the physiological relevance of such degradation remains to be confirmed. Although it is uncertain whether the trimers and higher polymers of the flavanols are readily absorbed, it has been suggested that over prolonged exposure, bacterial breakdown and colonic absorption of smaller molecules may occur. Both absorption and metabolism of cocoa-derived catechins appear to be influenced by chocolate matrix. Several studies have been performed regarding the influence of milk protein on the bioavailability of epicatechin from cocoa beverages and chocolate. Serafini et al. [50] reported that milk resulted in a reduced AUC for epicatechin relative to control in chocolate confections, while others reported no statistical difference between the AUC of epicatechin from cocoa beverages consumed with water or milk. Recently, Mullen et al. [51] reported that milk decreases urinary excretion but not plasma pharmacokinetics of cocoa flavan-3-ol-metabolites. Ortega et al. [52] observed that the fat content of certain cocoa samples enhances the digestibility of some phenolic compounds, especially

procyanidins during duodenal digestion. Recently, Neilson et al. [53] suggested that chocolate confections containing high levels of sucrose may enhance plasma levels of the predominant catechin and epicatechin metabolites as compared to milk chocolate confections, while confections containing moderate levels of sucrose and no milk deliver intermediate plasma levels of these compounds. However, the physical state of the product may significantly modulate this effect.

### 4.5.5 Minerals

Cocoa is an extremely rich source of many essential minerals. As in any other plant food, the mineral content of cocoa reflects the soil in which it was grown, and reported values are quite variable. Another possible source of variability in the data may be contamination with copper and zinc from the grinding tools used to produce the cocoa masses. Table 4.1 provides the values for several minerals in cocoa products [24]. Cocoa has the potential to provide significant amount of minerals in the human diet, in particular copper, iron, magnesium, calcium, potassium and zinc. However, little is known about bioavailability of minerals from cocoa and chocolate. The amount of mineral retained from the cocoa bean depends on the amount of cocoa bean solids in chocolate; therefore dark chocolate typically has higher amounts of minerals than milk chocolate. It must be noted that oxalic and phytic acids, polyphenols (particularly procyanidins) and fiber can interfere with mineral absorption.

Cocoa and cocoa products contain relatively higher amounts of magnesium compared to black tea, red wine, and apples [22]. Magnesium is involved in catalyzing a multitude biological reactions, including protein synthesis, transmission of nerve impulse, muscle relaxation, energy production, and bone and teeth adsorption. The magnesium content of cocoa is very high: approximately 500 mg per 100 g. In rodent models, the amount of magnesium provided by cocoa can be sufficient to prevent signs of magnesium deficiency in animals fed low magnesium diets. With regard to platelet function, magnesium reduced ADP-induced platelets and has been reported to decrease collagen-induced platelet activation, possibly through changes in membrane fluidity and reduced thromboxane formation.

According to the USDA nutrient database [24], cocoa powder (44 g) could provide 11% selenium followed by milk and white chocolate (4%), dark chocolate (3%), sweet chocolate (2%) and cocoa drink (1%). The selenium content of cocoa is 14 mcg per 100g. Selenium is an essential micronutrient as a cofactor in the formation of glutathione peroxidases, thioredoxin reductase, iodothyronine deiodinases, selenophosphate synthetase, selenoprotein P and other selenoproteins. Specifically, it works by detoxification of hydrogen peroxide and organic peroxides. Based on the significant contribution of selenium to antioxidant enzymes, it is important to consider the contribution of selenium in cocoa and cocoa products.

Copper, like most trace minerals, is involved in multiple enzymatic reactions including the synthesis of collagen and neurotransmitters. Cocoa is a very rich source of copper, with 3.8 mg per 100 g. Deficiency of copper during early development is known to result in cardiovascular abnormalities, and low dietary copper intakes have been postulated to contribute to the occurrence of vascular disease later in life.

The iron content of cocoa is 13.9 mg per 100 g, which is higher than the level found in beef or chicken liver. Of course, the form in which iron is present in food is a major factor of bioavailability. Approximately 20–30% of heme Fe (found only in meats) is absorbed, whereas absorption of nonheme Fe is in the range of 5–10% . Phytates and polyphenols, both of which are present in cocoa, can decrease iron absorption while ascorbic acid can enhance absorption of nonheme iron.

Potassium is essential for maintaining cellular osmolality and membrane potentials, thus playing a role in vascular tone and other biochemical pathways related to cardiovascular health. Large epidemiologic studies have indicated an inverse association between potassium intake, blood pressure, and stroke-related mortality. This association has been reinforced based on results of clinical trials. The potassium content of cocoa is 1524 mg/100 g, from 560 to 715 mg/100 g in dark chocolate (from 45% to 85% cacao solid), 712 mg/100g in a cocoa mix (hot chocolate) but only 197 mg in a milk chocolate beverage.

Calcium is a mineral required in macronutrient amounts for numerous important functions, including formation of bones and teeth, muscle contraction, and transmission of nerve impulses. Cocoa does not contain large amounts of calcium but a serving of milk chocolate provides about 84 mg per serving. The susceptibility of calcium to form complexes with oxalates and phytates interferes with its absorption. The main problem with oxalic acid in chocolate appears to be an increased risk for calcium oxalate stone formation in people predisposed to lithiasis. One-time consumption of 100 g of dark chocolate by healthy subjects increased calciuria and oxaluria, compared with a control group fed a sucrose load. A study of dietary risk for urinary stone formation also found that cocoa consumption promoted oxaluria and calciuria [54]. These conditions favor formation of calcium oxalate calculi in susceptible persons.

### 4.5.6 Methylxanthines

Apart from polyphenols, cocoa is also rich in methylxanthines, namely caffeine, theobromine and theophylline. Theobromine is the dominant purine alkaloid present in cocoa beans. Theobromine is also a major alkaloid in young pericarp and is present almost exclusively in the cotyledons of the beans. Caffeine and 3-methylxanthine are the major alkaloids in mature pericarp. The biosynthesis pathways of caffeine and related purine alkaloids has been reviewed recently by Ashihara et al. [55]. The content of theobromine and caffeine for various cocoa and cocoa-related products is presented in Table 4.1. Dark chocolate is high in theobromine and caffeine due to the addition of more cocoa solids than that of milk and white chocolates. Caffeine levels are relatively low in cocoa, compared to those found in coffee and tea. Typically, the cocoa beverage used in most studies contains less caffeine than found in a cup of decaffeinated coffee. Most of the studies underline the effects of polyphenols on the studied subjects, but the question of whether the presence of methylxanthines enhances or reduces the health benefits of the cocoa flavonoids remains unanswered, as the evidence in favor or against is often contradictory. Furthermore, the possible synergistic interactions between flavonoids and methylxanthines are also unclear and need further study. There is at least one report in the literature showing that removal of theobromine and caffeine enhances

in vitro antioxidative activity of cocoa extracts, suggesting that these methylxanthines inhibit this activity [56]. Both caffeine and theobromine have been found to result in reproductive toxicities in experimental animals, particularly males, among other toxic effects. Theobromine can lead to vacuolation within the Sertoli cells, altered spermatid shape, and failure in the release of late spermatids in male rats. Moreover, high-dose cocoa extract containing theobromine could alter testis structure to a greater extent than that of pure theobromine. In pregnant rats, caffeine from coffee or cocoa beverages is freely absorbed through the placenta and eventually leads to fetal growth retardation. Caffeine supplementation enhanced net hepatic glucose uptake through increased glucose-6-phosphate production in the liver.

### 4.5.7 Peptides

Cocoa beans contain four types of proteins, namely albumins, globulins, prolamin and glutelin. Albumin constitutes the major protein fraction. Albumin and globulin fractions accounted for 52% and 43% of total bean protein, respectively. There are few studies on the contribution of cocoa peptides towards health. Bioactive peptides were reported to possess antihypertensive, antithrombotic, antiobesity, hypocholesterolemic and hypotriglyceridemic effects. The antioxidant activity was attributable to histidine, tyrosine, methionine and cysteine. Of these, histidine possesses strong radical scavenging activity due to the decomposition of its imidazole ring. In addition, hydrophobicity of peptides also appears to be an important factor in their antioxidant activity, due to the increased interaction with hydrophobic targets (e.g., fatty acids). However, the health effects of peptides in humans and the optimal plasma levels remain to be elucidated.

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# Chocolate and Health: A Brief Review of the Evidence

5

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## 5.1 Introduction

Interest in the biological activities of cocoa polyphenols is steadily increasing. In fact, the high polyphenol content of cocoa, coupled with its widespread presence in many food items, render this food of particular interest from the nutritional and "pharmacological" viewpoints. Indeed, the number of publications concerning cocoa and chocolate is increasing steadily (Fig. 5.1). Of such publications, an increasing proportion concerns the effects of polyphenols and, in particular, flavonoids on human biology and

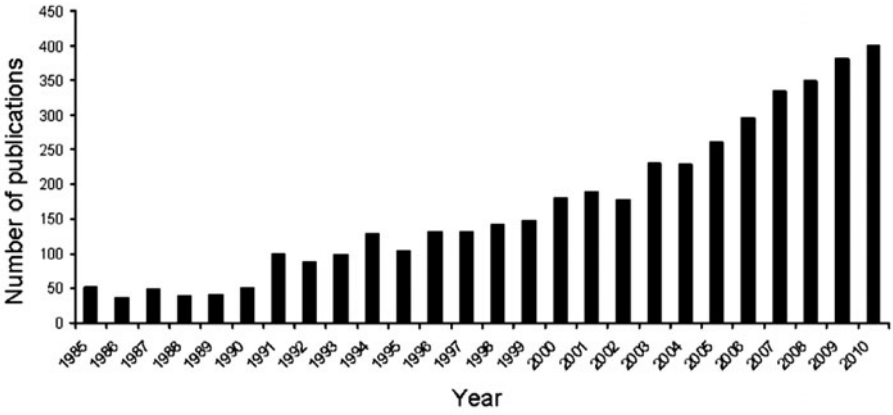


**Fig. 5.1** Number of publications in biomedical journals (1985-2010) featuring the keyword "chocolate". Database Web of science = SCI-EXPANDED. Topic = (caco* OR cocoa* OR chocolate*) AND (flavonoid*). Year Published = (1985-2010)

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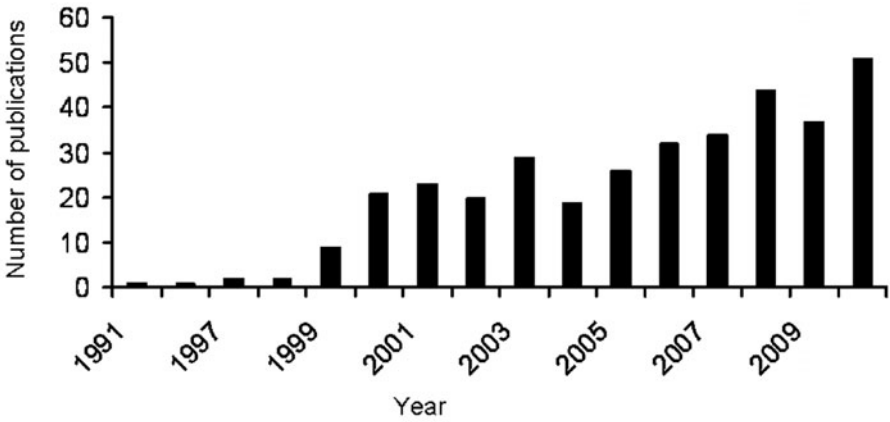



**Fig. 5.2** Number of publications 1991-2010 featuring the keywords "cocoa and flavonoids". Database Web of science = SCI-EXPANDED. Topic = (caco* OR cocoa* OR chocolate*) AND (flavonoid*). Year Published = (1985-2010)

health (Fig. 5.2). As outlined in detail in other chapters of this book, research is unraveling new and diversified healthful actions of "minor constituents" of cocoa. This is also reflected by the increasing number of patents covering industrial processes that aim at maintaining the highest possible amounts of polyphenols in cocoa. In fact, the current manufacturing process destroys roughly 95% of the polyphenols originally present in the cocoa bean [1].

We will provide an overarching vision of the current state of the investigation on this topic: details on the specific actions of cocoa polyphenols are provided in the appropriate chapters.

Cocoa has, indeed, the highest polyphenolic contents of all foods on a per-weight basis and markedly contributes to the total dietary intake of flavonoids [2, 3]. The main subclasses of flavonoids found in cocoa are flavanols, particularly the flavanol monomers catechin and epicatechin, and their oligomers, also known as procyanidins (Chapter 4) [4, 5]. Although the precise mechanisms responsible for their purported health benefits are unclear and likely to be manifold, flavonoids and flavanols have been shown to possess a range of cardiovascular-protective properties, including antioxidant and antiplatelet effects, immunoregulatory activity, and vasorelaxation (Chapters 6, 7, 8, 9 and 10).

## 5.2 The Potential Role of Cocoa Polyphenols in Human Health

Of the several compounds found in the cocoa bean that might be beneficial for the human body, e.g., proteins, fiber, minerals, etc., the main focus of research revolves around polyphenols. Of note, bioavailability of cocoa polyphenols is quite low, but depends on the matrix in which the cocoa polyphenols are delivered. Further, current efforts to increase bioavailability might lead to the formulation of cocoa-based products that, in theory, should confer increased healthy benefits. For the time being, based on the growing

body of scientific evidence pointing to the benefits of a diet rich in polyphenols, including those from cocoa, many commercial chocolate brands are starting to promote their products on the strength of the high phenolic content of cocoa. As an example, special processes have been developed which preserve up to 80% of the natural polyphenol content of raw cocoa without the use of extracts, additives or other chemical substances (Chapter 2). One of the downsides of this technology is that the resulting product is often very bitter and disliked by the consumer. To compensate for this, several approaches are being studied, including the addition of calorie-free sweeteners and/or soluble fiber. In brief, the industrial result of the growing number of studies that point to healthful effects of cocoa flavonoids is the formulation of novel chocolates with modulatory effects on human physiology.

### 5.2.1 Cocoa and Vascular Function

The effects of cocoa on blood pressure were first observed in the Kuna tribe, who consume high amounts of cocoa polyphenols (approximately 900 mg/day) and reside in the Las Bals islands of Panama. The inhabitants of this archipelago have lower blood pressure than their genetically-identical compatriots who inhabit the mainland. Therefore, a role for cocoa in lowering blood pressure has been proposed and controlled experiments have been carried out, as reviewed hereafter.

Nitric oxide (NO) bioavailability represents a main contributor to endothelial dysfunction, which, in turn, has been suggested to be the earliest triggering event in atherogenesis [6, 7]. Accordingly, impaired endothelium-dependent vasorelaxation has been described in human conditions characterized by an increased risk for developing atherosclerosis and its clinical sequelae, i.e., essential hypertension, hypercholesterolemia, type 2 diabetes, obesity and aging [8]. Thus, the hypothesis was formulated that polyphenols and flavonoids, particularly the subclass of flavanols, might improve NO-dependent peripheral glucose uptake, i.e., insulin sensitivity, in both normal and insulin resistant conditions. This hypothesis has been tested with several polyphenol-rich foods and beverages, such as tea. As far as cocoa is concerned, it was shown that flavanol-rich dark chocolate decreases blood pressure and improves insulin sensitivity and NO-dependent flow-mediated dilation in healthy subjects [9], essential hypertensives with normal glucose tolerance [10], and glucose intolerant hypertensives (Chapter 9). In addition, data from in vivo experiments report an impaired NO-mediated endothelial function during acute hyperglycemia [11]. Some experiments in healthy volunteers explored the acute effects of an oral glucose tolerance test alone and after three days of either flavanol-rich or flavanol-poor chocolate administration on vascular health. The results show an amelioration of vascular function that follows cocoa intake, suggesting that regular consumption of cocoa-based products (within a balanced and equicaloric diet) might convey beneficial effects in endothelial dysfunction patients.

In addition to vascular function, Grassi et al. [9, 10] investigated the effects of flavanol-rich dark chocolate on insulin resistance. The authors, as fully described in Chapter 9, demonstrated a significant amelioration of the index of insulin resistance using the homeostasis model assessment of insulin resistance (HOMA). In contrast, flavanol-rich dark

chocolate administration did not influence the two indexes of insulin sensitivity, namely quantitative insulin sensitivity check (QUICKI) and insulin sensitivity (ISI). This is probably due to the difference in study periods, i.e., three days vs fifteen days [9, 10], and the small number of healthy subjects that have been evaluated. Taken together, these findings suggest that cocoa flavanols may contribute to protection of the vasculature by decreasing stress-induced hyperglycemia and improving the global cardiovascular risk profile. Mechanistically, a study conducted in C57BL/KsJ-db/db obese diabetic mice indicated that cocoa dose-dependently prevented hyperglycemia [12], thereby supporting the evidence that cocoa flavanols might not only counteract blood pressure elevation (as suggested by the epidemiological studies performed on the Kuna Indians) but also positively influence glucose homeostasis.

In terms of pure arterial dysfunction, it must be underscored that this phenomenon is exacerbated (and maybe triggered) by oxidative stress, as shown by restoration of arterial dilatation in hypercholesterolemic children treated with antioxidants, for example [13]. Consistent with this finding is the recent demonstration that enhanced oxidative stress is an early phenomenon occurring in children with hypercholesterolemia [14]. In addition to some clinical observations such as the ones reported above, several in vitro and in vivo data support a role for oxidative stress in impairing arterial dilatation. NADPH oxidase is the most important cellular producer of the superoxide anion and animals with NADPH oxidase up-regulation exhibit lower arterial dilatation [15, 16]. Also, patients with neurovascular hypertension and systemic signs of enhanced oxidative stress have impaired arterial dilatation, which is restored by lowering oxidative stress with antioxidants [17]. Interventional studies with cocoa in humans demonstrated a significant improvement in arterial dilatation, an effect that was attributed to the polyphenol ability to enhance the bioactivity of NO [18, 19]. As mentioned, the vasodilatory status of the endothelium is largely dependent on the production of NO, a free radical that can be rapidly inactivated by oxygen free radicals such as a superoxide anion. Polyphenols could counteract such effects by either enhancing NO production (antioxidant-independent activities) or reducing its inactivation by free radicals (antioxidant-dependent activities). Using platelets to investigate the hypothesized mechanism, the Pignatelli and Violi group observed that polyphenols are able on one hand to reduce the production of superoxide anion and on the other hand to increase platelet NO with an inverse correlation between these two effects (Chapter 6). Mechanistically, these effects have been attributed to the ability of polyphenols to inhibit the activation of NADPH oxidase [20, 21]. Notably, polyphenols are synergic in terms of antioxidant effects [21]. Bioavailability studies report that blood concentrations of single polyphenols are $<1\ \mu M$; hence, a synergistic combination of their effects might at least partly explain the documented effects in vivo.

In humans, by using noninvasive ultrasound measurements of flow mediated vasodilation, Hermann et al. [22] showed that dark chocolate, but not white chocolate improves endothelial function and platelet function in young healthy smokers, a group chosen because they have impaired endothelial function and platelet hyper-reactivity. In summary, cocoa and its polyphenols are able to positively modulate vascular function, possibly via concomitantly increasing NO production and limiting its inactivation by free radicals. Although we should keep in mind that vasculo-impaired patients are often overweight and/or

insulin-resistant, and therefore, need to follow hypocaloric and sugar-restricted diets, the inclusion of cocoa products, namely those rich in flavanols, might be a valuable tool to accompany and support pharmacological therapy.

### 5.2.2 Mechanisms of Action: Cocoa Polyphenols as Antioxidants

It should be underscored that randomized trials of antioxidant supplements, namely vitamins such as vitamin E and beta-carotene, have mostly yielded negative results [23]. Conversely, a wide body of epidemiological studies demonstrate that whole foods, and their polyphenols, are beneficial to health [24]. Most attention has been paid to the antioxidant activities of polyphenols, mainly because of biochemical studies that show how oxidative stress increases the risk of degenerative diseases. Also, most polyphenols and, in particular, ortho-diphenols are strong antioxidants in vitro. Accordingly, several studies have measured the in vitro antioxidant activity of foods and beverages as components of the diet. Indeed, the total polyphenols in fruits, vegetables, beverages, nuts, spices, oils, and chocolate have been determined using a sample preparation technique which hydrolyzes sugar-bound polyphenols. Chocolate provides 4% of the total daily antioxidants in the USA, and recently published data compared both chocolate bars and chocolate drinks. Data for commercial chocolate bars from the US and Europe, especially those with the percentage of cacao listed in the label, are also available. However, we are just now beginning to understand that the mechanism of action of polyphenols is not always an antioxidant one and most likely involves the metabolites rather than the original polyphenols [25, 26]. For example, polyphenols produce benefits in endothelial function, platelet activation, blood pressure and flow, and gene expression [25, 26].

While most of the suggestive evidence comes from in vitro studies, in vivo (animals, humans) trials are needed to clearly establish causation and to prove the biological activities of cocoa beyond any doubt. Accordingly, Vinson and co-workers were the first to investigate the effect of cocoa powder in an atherosclerotic hamster model [27]. Cocoa, added to the diet at a human dose equivalent to two normal dark chocolate bars per day inhibited the development of atherosclerosis by 38% . In addition, cocoa decreased LDL, increased HDL, and decreased lipoprotein oxidizability, hence positively modulating the plasma lipid profile and one surrogate marker of cardiovascular disease. Two human studies were also carried out by the Vinson group and the Penn State University [27, 28]. This was a single dose study, in which dark chocolate and cocoa powder were given to fasting humans to investigate the post-prandial protection of LDL+VLDL from oxidation. The authors reported inhibitory effects of cocoa, while the fat and sugar placebo produced an increased oxidation of LDL+VLDL, i.e., increased postprandial oxidative stress. Based on the Vinson and other studies, including long-term supplementations, cocoa is able to significantly increase HDL, increase plasma antioxidant capacity, and decrease the oxidizability of LDL [29, 30]. The mechanisms by which cocoa exerts these potentially anti-atherosclerotic activities are yet to be fully elucidated and are shared by other polyphenol-rich foods. Indeed, there is growing evidence that polyphenols are able to increase HDL concentrations, but the precise nature of this effect remains obscure. It

is noteworthy, as mentioned, that cocoa has one of the highest content of flavonoids among all foods. Therefore, its contribution to the overall lipid-modulating effect of the diet is likely to be relevant.

The antioxidant effects of cocoa flavonoids in humans have been extensively investigated in the past few years, mostly by the Serafini group. As an example, the group showed that the acute ingestion of 100 g of dark chocolate by healthy subjects was able to improve plasma total antioxidant capacity (TAC) and (-)-epicatechin plasma levels [31]. However, these effects were markedly reduced when dark chocolate was consumed with milk or when milk instead of dark chocolate was eaten [31]. An explanation is that proteins of the food matrix in which cocoa is eaten, in this case milk, might bind flavonoids and reduce their bioavailability and therefore, their potential antioxidant properties in vivo. It must be underlined that the effects of milk on polyphenol bioavailability and thus, biological activities are still controversial. As an example, evidence showed that the improvement of flow-mediated dilation (FMD, a marker of vascular function), induced by the ingestion of 500 mL of black tea infusion was completely abolished by addition of milk to tea [32]. Conversely, Schroeter et al. [33] showed that consumption of a single dose of liquid chocolate mixed with milk did not abolish its antioxidant effect. The very low amount of milk added to chocolate (3%) might explain these findings. In summary, the addition of milk to cocoa foods and beverages might decrease absorption of polyphenols and limit the health effects of cocoa. However, this hypothesis requires further, solid confirmation.

To establish causality, one needs to correlate the increase in plasma TAC that follows ingestion with the absorption of cocoa polyphenols. To this end, 80 g of procyanidin-rich chocolate was given to humans and the rapid absorption of (-)-epicatechin was demonstrated, peaking in plasma after two hours [34]. In association with the increase in plasma (-)-epicatechin levels, a parallel increase in plasma TAC and a decrease in the concentration of plasma oxidation products was shown. However, when three different doses (27, 53, and 80 g) of the same chocolate were given to the subjects, no significant increase of TAC or decrease of lipid peroxidation levels were observed, despite the dose-response increase in plasma (-)-epicatechin levels [35].

In terms of circulating markers of lipid peroxidation, Wiswedel et al. [36] showed that acute ingestion of a cocoa drink by healthy volunteers was able to lower plasma levels of $F_2$-isoprostanes, a validated biomarker of lipid peroxidation. Conversely, Mursu et al. [30] showed a lack of effect on plasma isoprostane levels following daily ingestion of 75 g/day of dark chocolate for three weeks.

To summarize, cocoa is rich in polyphenolic molecules that exhibit powerful antioxidant activities in vitro. Whether these activities can be reproduced in vivo, i.e., after ingestion, remains to be fully established, mostly because of the low bioavailability of these compounds and the lack of appropriate biomarkers of oxidative stress and antioxidant actions.

### 5.2.3 Cocoa and Platelet Reactivity

Pro-thrombotic profiles are associated with increased cardiovascular risk. Following initial findings that suggested inhibitory actions of cocoa flavonols on platelet aggregation,

an ex vivo, double-blind and randomized study was undertaken to determine whether the in vitro effects of flavanols can be reproduced following consumption of cocoa beverages [37]. Twelve healthy volunteers each consumed cocoa beverages that contained four different amounts (80 mg, 300 mg, 600 mg, or 900 mg) of total flavanols. The different beverages were tested after washout periods of at least 10 days. The beverages containing 600 mg and 900 mg of added flavanols significantly inhibited platelet aggregation induced by collagen. Similar results were obtained for platelet-monocyte conjugation and platelet-neutrophil conjugation and for the other measures of platelet activation. Overall inhibition of the measured parameters was greatest 4 hours after consumption of the beverage that contained 600 mg of added flavanols, although some inhibition was also evident at 2 and 6 hours, suggesting a causal link between absorption of cocoa flavonoids and inhibition of platelet reactivity. In fact, the timeframe over which anti-platelet activities were observed is superimposable with that of flavonoid absorption and metabolism.

Cocoa flavanols and their metabolites inhibit various measures of platelet activity both in vitro and ex vivo (after consumption of cocoa beverages). Other constituents may also be involved in the anti-inflammatory actions of cocoa [38], thus adding to its overall anti-thrombotic and anti-inflammatory effect.

### 5.2.4 Healthful Effects of Cocoa Beyond the Cardiovascular System

While cocoa and its components are mostly being evaluated in the area of cardiovascular disease, other fields are being explored. As an example, Heinrich et al. evaluated the effects of cocoa on skin health [39, 40]. In particular, a polyphenol-rich cocoa drink was given for up to 12 weeks to volunteers. This supplementation significantly decreased UV-induced erythema from a solar simulator and led to increases in blood flow of cutaneous and subcutaneous tissues. The improved microcirculation was associated with an increase in skin density and hydration. As expected, these effects were not recorded after the administration of a low-flavanol cocoa drink. Finally, the flavanol-rich cocoa drink ameliorated the appearance of the skin, as measured by a significant decrease of skin roughness and scaling at the end of the supplementation period [39]. Expanding on microcirculation, flavanol-rich cocoa consumption acutely increases dermal blood flow and oxygen saturation, as measured by laser Doppler techniques.

In summary, cocoa flavanols increase endogenous photoprotection (through mechanisms that deserve further investigation), improve dermal blood circulation, and improve skin surface and hydration variables [40], resulting in an overall enhancement of skin appearance and texture.

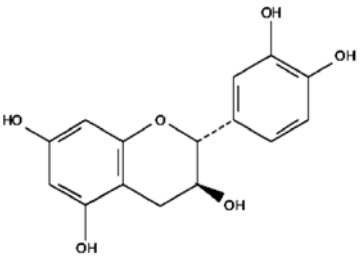
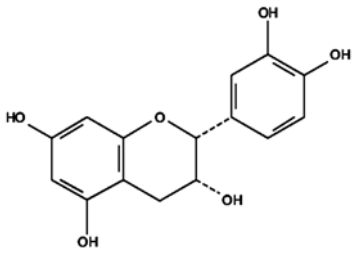
A role for cocoa and chocolate in mental health has also been suggested by an observational study carried out in the Kuna Indians of Panama, where the investigators recorded a significantly lower (9.66 vs 12.17 cases/100,000) incidence of brain disorders (excluding stroke) in the Kunas compared with their mainland counterparts. After correction for several confounding factors, these data suggest that cocoa flavanols exert neuroprotective effects [41].

Still in the area of central nervous system and neurological disorders, functional magnetic resonance imaging based on blood oxygenation level-dependent (BOLD) contrast was employed to investigate the effect of cocoa flavanols on the human brain. In particular, BOLD responses to a cognitive task were measured in 16 healthy young subjects [42]. The results show an increase in the BOLD signal intensity in response to a cognitive task following ingestion of flavanol-rich cocoa. Other physiological parameters such as behavioral reaction times, switch cost, and heart rate were not modified by consumption of cocoa flavanols. In terms of mechanisms of action, the central effects of cocoa flavanols can be tentatively explained by altered neuronal activity or increased vascular responsiveness. The latter mimics what has already been observed in the skin and is also suggested by the observed increase in cerebral blood flow to grey matter [42].

## 5.3 Conclusions

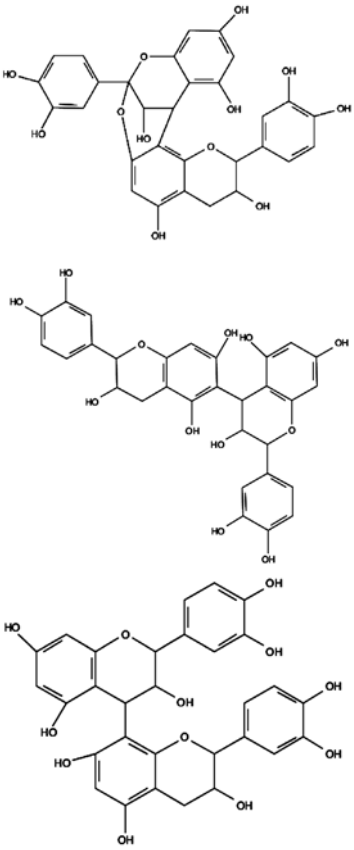
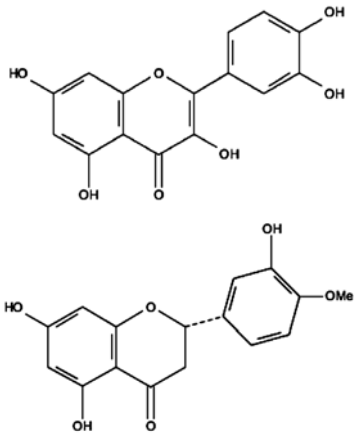
In conclusion, cocoa and dark chocolate contain bioactive ingredients that are being thoroughly investigated and whose activities are exemplified in this book (also, Table 5.1).

**Table 5.1** Molecular targets and effects of cocoa minor components (modified from [43])

| Compound | Structure | Target |
|---|---|---|
| Catechin |  | ↓ α-glucosidase (catalyzes hydrolysis of polysaccharides) |
| Epicatechin |  | ↓ Xanthine oxidase (catalyzes the oxidation of hypoxanthine to xanthine, generates ROS)<br>↓ NADPH oxidase (Scavenges NO)<br>↑ cGMP (Activates intracellular protein kinases)<br>↑ endothelial NO• synthase (eNOS) (Vasodilating factor)<br>↑ extracellular signal regulated kinase and Cyclic AMP response element binding protein (CREB) activation in cortical neurons (Involved in long-term memory formation)<br>↓ plasma levels of endothelin-1 (constricts blood vessels; raises blood pressure) |

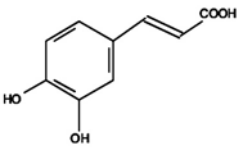
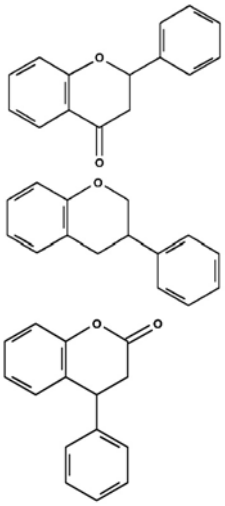
(*cont.* →)

**Table 5.1** (continued)

| Compound | Structure | Target |
|---|---|---|
| Procyanidins: Molecular structure of the dimeric A type<br><br>procyanidin, dimeric 4-6<br>procyanidin B, dimeric 4-8<br>procyanidin B |  | ↓ lipoxygenases (Arachidonic acid metabolism and the biosynthesis of leukotrienes [inflammatory pathways])<br>↑ IL-2 transcription (Cytokine immune system signaling molecule) |
| Quercetin, hesperetin, and caffeic acid |  | ↓ 5-*S*-cysteinyl-dopamine and its oxidation product, dihydrobenzothiazine (Endogenous neurotoxin) |

(*cont.* →)

**Table 5.1** (continued)

| Compound | Structure | Target |
|---|---|---|
| |  | |
| Cocoa polyphenols (unspecified) Molecular structure of the flavone, isoflavan and neoflavonoid backbone |  | ↑ prostacyclin (vasodilating factor)<br>↑ endothelium-derived hyperpolarizing factor (EDHF) (vasodilating factor)<br>↓ endothelin-1 (angiogenic factor)<br>↓ vascular endothelial growth factor (VEGF) (angiogenic factor)<br>↓ NF–ϰB activity (DNA transcription; factor in inflammatory processes)<br>↓ angiotensin-converting enzyme (regulates renin-angiotensin system)<br>↓ 8-isoprostane (biomarker of oxidative stress)<br>↓ monocyte chemoattractant protein-1 (recruitment of monocytes and lymphocytes to sites of cellular immune reactions)<br>↓ TNF-α in lipopolysaccharide-stimulated macrophages (Cytokine involved in systemic inflammation)<br>↑ IL-1 secretion from stimulated human peripheral blood mononuclear cells (Cytokine, involved in regulation of immune responses)<br>↑ IL-6 secretion from stimulated human peripheral blood mononuclear cells (pro-inflammatory and anti-inflammatory cytokine)<br>↑ IL-10 secretion from stimulated human peripheral blood mononuclear cells (anti-inflammatory cytokine)<br>↑ IL-4 release (acquired immune response)<br>↓ expression of CD40 on monocyte surfaces (adhesion molecule)<br>↓ expression of VLA-4 on monocyte surfaces (adhesion molecule)<br>↓ expression of CD36 on monocyte surfaces (adhesion molecule)<br>↓ circulating levels of P-selectin (inflammatory marker)<br>↓ circulating levels of ICAM-1 (inflammatory marker)<br>↓ phosphoinositide 3-kinase (upregulates vascular endothelial growth factor (VEGF), mediated through TNF-α)<br>↓ mitogen-activated protein kinase kinase-1 (upregulates vascular endothelial growth factor (VEGF), mediated through TNF-α)<br>serine/threonine kinase (involved in neuronal survival) |

Based on the results of the "cocoa and health" studies, the cocoa industry is aiming at producing chocolates and cocoa products with better nutritional profiles. Despite the current hype, however, some caution should be exerted: chocolate is a calorie-rich voluptuous food item and its consumption is to be positioned within a balanced and isocaloric diet. In addition, the psychological aspects of chocolate consumption are to be taken into account, especially in population subgroups that might be more vulnerable to excessive caloric intake, e.g., children and depressed subjects. With this in mind, the inclusion of adequate quantities of cocoa and chocolate in a balanced diet might, indeed, convey positive consequences on human health.

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# Polyphenolic Antioxidants and Health

# 6

Lorenzo Loffredo and Francesco Violi

## 6.1 Introduction

Oxidative stress is believed to play a pivotal role in several physiologic (aerobic metabolism, immunologic responses, cellular signaling, regulation of gene expression, cell differentiation) [1] and pathologic (atherosclerosis, tumorigenesis, neurodegenerative diseases, etc.) processes. Antioxidants react with oxygen free radicals protecting the cells from oxidative stress damage. Polyphenols are the most widely known antioxidant nutrients. Polyphenols are a class of natural, synthetic, and semisynthetic substances characterized by the presence of large multiples of phenol units. The term polyphenols was proposed in 1962 by the phytochemists White, Bate-Smith, Swain and Haslam [2]. They defined polyphenols as "water-soluble phenolic compounds having molecular weights between 500 and 3000 (Da). Besides giving the usual phenolic reactions, they have special properties such as the ability to precipitate alkaloids, gelatin and other proteins from solution" [2]. Polyphenols, in the form of flavonoids, are broadly classified into anthocyanidins (e.g., cyanidin, delphinidin, malvidin), flavanols (e.g., catechin, epicatechin), flavonols (e.g., quercetin, fisetin), and flavones (e.g., luteolin) [3]. Natural polyphenols are prevalent in cocoa, fruits, vegetables, wine and tea. Flavonoids account for two thirds of the total polypenolic daily intake (approximately 1 g) [4]. After oral ingestion, flavonols undergo a biotransformation from the gut microflora generating a large variety of metabolites [4, 5]; the maximum plasma concentration of flavonols rarely exceeds 1 μM [4, 5].

In recent decades, a growing interest in polyphenols has resulted from prospective and epidemiological studies that showed the beneficial effects of these substances on health. This chapter will analyze these aspects focusing attention on the future opportunities to use polyphenols in the prevention and care of human diseases.

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## 6.2 Polyphenols and Cardiovascular Protection

Atherosclerotic complications (myocardial infarction and stroke) represent the highest cause of death in the Western world. Even though the development of new pharmaceutical therapies continues, the prevalence of cardiovascular disease is growing [6]. Polyphenols exert cardiovascular protection that could be useful in the future to reduce atherosclerosis and its complications.

### 6.2.1 Evidence from Epidemiological Studies

Epidemiological studies demonstrate that a diet rich in polyphenols reduces cardiovascular events in the general population and in patients at risk of cardiovascular disease [7–12]. The clinical effects included a reduction of cardiovascular mortality, myocardial infarction and stroke [7–12]. This cardio-protective phenomenon was first observed two decades ago in countries like France and Italy, two major European wine-producers, and is commonly referred to as the "French Paradox" [13]. Most of the beneficial effects of wine on cardiovascular disease have been attributed to the presence of resveratrol and other polyphenols [13, 14]. In one of the largest reports on this argument, Grønbaek et al. [15] showed that moderate daily drinkers (3–5 glasses per day) had a lower risk of cardiovascular death compared with nondrinkers.

Prospective cohort studies have provided evidence for a protective effect of polyphenols, such as catechin and epicatechin [8, 9, 11, 12]. The Zutphen study [8] showed that polyphenolic intake was inversely associated with ischemic heart disease mortality by comparing the highest vs the lowest tertile of daily catechin intake. Furthermore, the Iowa Women's Health Study [11] found an inverse relationship between coronary heart disease mortality and catechin/epicatechin intake in postmenopausal women.

Further studies have evaluated the effect of other substances rich in polyphenols such as cocoa. The European Prospective Investigation into Cancer and Nutrition [10] analyzed the effect of chocolate on cardiovascular mortality. After an 8 year follow-up, this study reported a lower rate of myocardial infarction and stroke in the quartile of subjects with the highest chocolate consumption (7.5 g/day). Another prospective study, the Stockholm Heart Epidemiology Program [16], reported (after an initial acute myocardial infarction) a reduction for cardiac mortality in the group at higher chocolate intake in nondiabetic patients. However, the lack of interventional randomized trials limits any conclusion. Therefore the effect of polyphenols on cardiovascular protection needs further evaluation in the future.

### 6.2.2 Effect of Polyphenols on Cardiovascular Risk Factors

The beneficial properties of polyphenols on the cardiovascular system may be due to their capability to exert antihypertensive effects [9], reduce cholesterol levels [17] and improve insulin sensitivity [17].

Consumption of polyphenol-rich foods such as cocoa [9, 18] and berries [19] reduces blood pressure. Interestingly, Taubert et al. showed a decrease in systolic and diastolic blood pressure after dark chocolate intake; the hypotensive effect was associated with an increase in circulating levels of S-nitrosoglutathione [18], a bioactive nitric oxide species. This suggests that polyphenols increase nitric oxide levels, a molecule directly involved in modulating blood pressure and vascular tone [20].

A meta-analysis of randomized controlled trials confirmed the hypotensive action of polyphenols contained in cocoa [21]; however, considering the small sample size of these trials and the great differences in the content of flavonols in chocolate, large interventional studies are needed to better evaluate the real anti-hypertensive efficacy.

Another important action of polyphenols is on lipids. Even though the studies of polyphenols on blood lipids are not conclusive, they demonstrate an increase in HDL cholesterol [17], and a reduction of triglycerides [22] as well as LDL cholesterol [23]. Moreover, cocoa is able to inhibit LDL cholesterol oxidation, potentially exerting an anti-atherosclerotic function [24].

Recent studies suggested that polyphenols improve insulin sensitivity in humans with cardiovascular risk factors such as diabetes [25] and hypertension [26]. This mechanism could be mediated by oxidative stress reduction as oxidative stress may reduce the activity and/or sensitivity of insulin [25].

### 6.2.3 Effect of Polyphenols on Oxidative Stress, Endothelial Function and Platelet Aggregation

The formation of an atherosclerotic plaque is the pathogenic mechanism that leads to cardiovascular disease. Oxidative stress is believed to play a crucial role in initiation and progression of atherosclerosis [27]. Oxidative stress occurs when an imbalance develops between the production of Reactive Oxygen Species (ROS) and the efficacy of the cell's antioxidant defense. This leads to an altered redox status, which contributes to atherosclerosis formation [28].

Polyphenols exert their antioxidant activity not only scavenging ROS but also inhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, the major cellular ROS source (Fig. 6.1) [29].

NADPH oxidase is a key enzyme for the cellular ''respiratory burst'', the phagocytic process that converts molecular oxygen to the oxygen free-radical superoxide ($O_2^-$). NADPH oxidase and its oxygen derivatives play a pivotal role in the processes involved in killing infections such as bacteria and fungi. Furthermore, NADPH oxidase is involved in several other human pathologies such as atherosclerosis, cancer, Alzheimer's disease or primary immune-deficiency diseases like chronic granulomatous disease [1]. The prototypical NADPH oxidase is that found in neutrophils, which has five subunits: three located in the cytosol ($p47^{phox}$, $p67^{phox}$, $p40^{phox}$) and two located in the membrane ($p22^{phox}$, and the catalytic subunit $gp91^{phox}$) (Fig. 6.1). Originally NADPH oxidases were considered as enzymes expressed only in phagocytic cells; subsequent studies indicate that there is an entire family of NADPH oxidases based on the discovery of $gp91^{phox}$ homologues [1] now designated as Nox. This group comprises

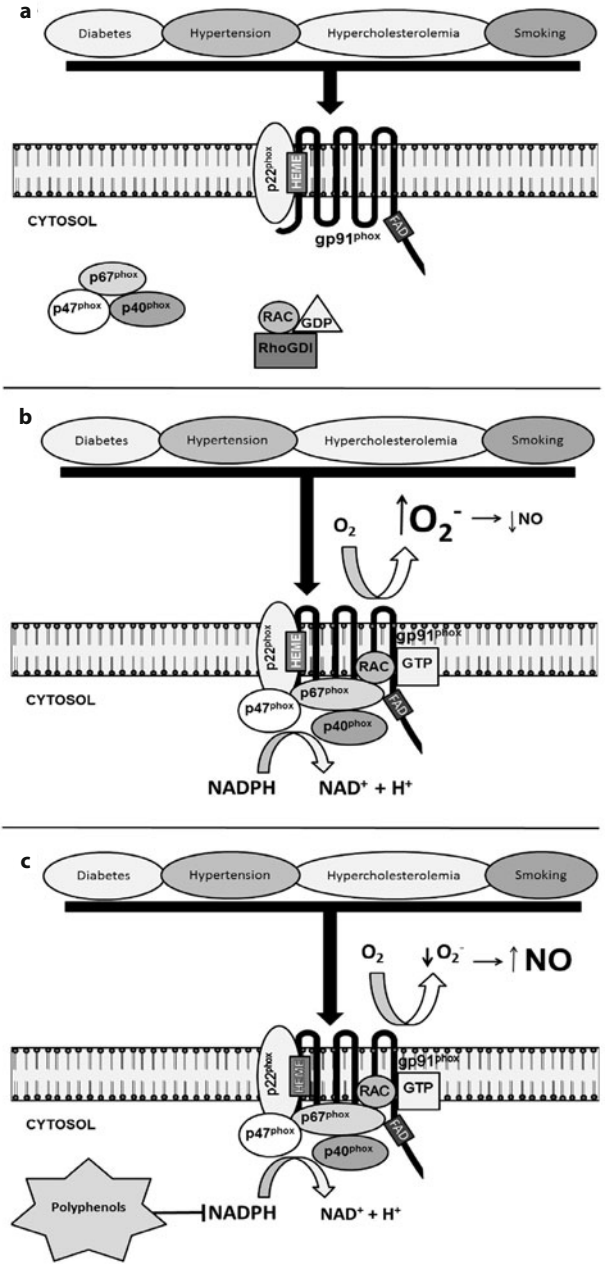


**Fig. 6.1** Classic cardiovascular risk factors (hypertension, dyslipidemia, diabetes, smoking) directly activate NADPH oxidase (**a**). Upon activation, there is a translocation of the cytosolic regulatory subunits (p47phox, p40phox, p67phox, and G proteins RAC1 or RAC2) to the membrane (**a**) and association with cytochrome b558; the active enzyme complex transports electrons from cytoplasmic NADPH to the extracellular space to generate superoxide (O2-) (**b**). Polyphenols reduce oxidative stress by NADPH oxidase inhibition (**c**)

seven members: Nox1, Nox2 (formerly termed "gp91$^{phox}$"), Nox3, Nox4, Nox5, Duox1, and Duox2 [1]. Of the various Nox isoforms, Nox2 is expressed in white blood cells [1] and Nox1, Nox2 and Nox4 are the most important Nox members found in vascular cells [1]. Various factors such as shear stress, hypertension, diabetes and hypercholesterolemia may activate NADPH oxidase, generating ROS (Fig. 6.1) [29]; this activation requires the translocation of several cytosolic regulatory subunits (p47 $^{phox}$, p40 $^{phox}$, p67 $^{phox}$, and G proteins RAC1 or RAC2) to the membrane (Fig. 6.1) and association with cytochrome b558. The active enzyme complex transports electrons from cytoplasmic NADPH to extracellular or phagosomal oxygen to generate superoxide ($O_2^-$) (Fig. 6.1).

Polyphenols (quercetin and catechin) exert their antioxidant action inhibiting the phosphorylation of protein kinase C (PKC) [30], a key enzyme for NADPH oxidase activation. As a consequence of NADPH oxidase inhibition, polyphenols reduce oxidative stress, ameliorating endothelial function, inhibiting platelet aggregation and modulating pivotal processes of inflammation, neurotransmission and cytotoxicity. However, the mechanisms through which polyphenols exert their vascular protective action are not yet fully understood.

Several studies have shown the acute beneficial effect of food rich in polyphenolic content (cocoa, dark chocolate, tea, grape juice and red wine) on endothelial function [31].

An imbalance between superoxide and nitric oxide production could be responsible for endothelial dysfunction that represents the "primum movens" of the atherosclerotic process [32]. Several ROS-generating enzymes, including myeloperoxidase, xanthine oxidase, and NADPH oxidase, may be implicated in arterial dysfunction [33]. Experimental studies performed in animal models suggest a pivotal role of NADPH oxidase in modulating arterial tone [34–36]. Recently, we demonstrated in humans that vascular tone is modulated by NADPH oxidase-generating ROS with a mechanism involving nitric oxide [37]. A previous study hypothesized that polyphenols contained in cocoa improve endothelial dysfunction by reducing oxidative stress and increasing NO generation [38], an effect that could be mediated by NADPH oxidase down-regulation.

Another important role in atherothrombosis is played by platelets [39]. The release of inflammatory mediators from activated platelets at an initial site of thrombosis leads to plaque rupture and to acute vascular ischemic syndromes [39]. Large epidemiological studies suggest that polyphenols could contribute to the reduction of cardiovascular events by inhibiting platelet function [40]. Platelet incubation with polyphenols (quercetin and catechin) reduces their recruitment via inhibition of PKC-dependent NADPH oxidase activation and, conversely, enhances NO generation [30]. NADPH inhibition by polyphenols was also demonstrated in an in vivo study after wine consumption; interestingly, after red wine consumption (compared to white wine) there was a more marked reduction of oxidative stress levels, as assessed by urinary isoprostanes excretion. The explanation of this phenomenon could be due to the higher concentration of polyphenols contained in red wine compared to white wine [30].

However, long-term studies are needed to evaluate if polyphenolic intake is associated with an inhibition of platelet function in humans.

## 6.3 Polyphenols and Cancer

Cancer is a leading cause of death worldwide and accounted for 7.6 million deaths in 2008 [41] according to the World Health Organization. Several studies have investigated the relationship between the potential health benefits of polyphenols and cancer, in order to develop new lines of therapeutic interventions [42]. Polyphenols are able to modulate oxidative stress and chronic inflammation, which are recognized as two important contributors to the etiology of some cancers [42]. The probable role of polyphenols as an anti-tumorigenic agent includes several processes such as inhibition of gene expression, inhibition of angiogenesis, inhibition of metastasis and suppression of cell proliferation [42]. These anti-tumorigenic effects have been studied using in vitro models of disease including proliferation of prostate cancer, cervical cancer and epidermal carcinomas. The promising results coming from in vitro studies must be confirmed in humans, where several variables (such as the bioavailability of polyphenols after the transit in the gastrointestinal tract, presence of concomitant pathologies or drugs consumed) could change the positive effect observed.

Thus, epidemiological studies analyzed the effect of polyphenols contained in green tea, but the results are inconclusive; some studies showed an inverse association between tea consumption and cancer risk, suggesting a cancer-preventive effect of tea, whereas other studies showed no effect [42, 43]. Future interventional studies must demonstrate the real efficacy of polyphenols in prevention and care of cancer in human.

## 6.4 Polyphenols and Neuroprotection

Neurodegenerative diseases include a group of heterogeneous pathologies (amyotrophic lateral sclerosis, Parkinson's and Alzheimer's diseases) characterized by the destruction of neural cells [44]. Despite the differences in clinical symptoms, localization and prognosis, these diseases are unified by a common factor: enhanced oxidative stress [44]. There is emerging experimental evidence that ROS (derived from NADPH oxidase NOX2) are important in initiation of apoptotic pathways and in mediating the oxidative and inflammatory responses in neurodegenerative diseases [44]. NADPH oxidase inhibition could represent a future target to treat neurodegenerative disease; thus, a recent study in mice demonstrated that resveratrol protects neurons against lipopolysaccharide-induced neurotoxicity through NADPH oxidase inhibition [45]. Furthermore, polyphenols can exert neuro-protection by activating or inhibiting various other signaling pathways (such as via the regulatory proteins NF-$\varkappa$B, SIRT1, MAP kinases and the heat shock proteins) involved in apoptosis and senescence of neural cells [42].

Other experimental and epidemiological studies have demonstrated that polyphenols, particularly from green tea and blueberries, improve age-related cognitive decline and are neuro-protective in models of Parkinson's disease, Alzheimer's disease and cerebral ischemia/reperfusion injuries [46]. The main polyphenolic component of green tea is epigallocatechin gallate. This polyphenol was able to suppress the disease progression of amyotrophic lateral sclerosis in mice [47].

Despite the fact that these are promising results, there are no data with interventional studies using polyphenols in humans. Future studies must evaluate their efficacy for prevention and care in neurodegenerative diseases.

## 6.5 Conclusions

Experimental and epidemiological studies have demonstrated that polyphenols exert beneficial actions on human health. These findings are biologically plausible because polyphenols possess an antioxidant property via NADPH oxidase inhibition, an enzyme directly implicated in several human diseases (atherosclerosis, cancer, amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases). However, there isn't strong evidence yet to justify the use of polyphenols in humans for the prevention and care of diseases; this uncertainty is due to the lack of interventional and prospective trials that are needed to evaluate the clear benefits of polyphenols on human health in the future.

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# Acute Vascular Effects of Chocolate in Healthy Human Volunteers

7

Manuel Rusconi, Maria Grazia Rossi, Tiziano Moccetti and Ario Conti

## 7.1 Introduction

We have all experienced a sense of impending illness prior to its actual onset. Indeed, a lot of us have felt that either before or during sickness our emotions may have influenced the course of the disease. Such anecdotes have existed since ancient time and in the last decade have been the initial foundation for a new and rapidly developing area of medical research. Central to these sensations, as well as the new field of neuroendocrine-immune interactions, is the realization that our brain and endocrine system can influence our immunity and that the immune system serves as a sensory organ, which ultimately signals the brain. In recent decades, conceptual shifts in biological sciences have provided new evidence to support intuitive beliefs regarding the connection between the mind-body unit, external and/or internal stimuli such as viruses and bacteria, and primordial environmental stimuli such as light-dark cycle, moon cycle, tides, magnetic forces and humidity. Moreover, many new factors such as climate changes, air pollution, the rise in world population, particularly in developing countries, the rise of poverty in developed countries, and their social and environmental effects are becoming increasingly sophisticated. Consequently, the role of human management of the ecosystem, has been reconsidered by each and every one of us, scientists, politicians, and the lay public. On the other hand, methodological communication was not a major problem during the early days of medicine. Treatment modalities were based on a gift from mother nature: plants and extracts thereof. Herbal medicines, which have to be integrated in this vision, have long been an accepted treatment of various diseases throughout the world. One key to eventually understand and use this complex communication network, which entails external and/or internal human environments

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and psycho-immune-nervous-endocrine systems for the treatment of human diseases, would seem to be complete knowledge of cellular and molecular processes. However, to help this network to maintain a good balance between all small and big components, it might be possible to contribute to it with natural products. Before going on with this approach and therapeutic philosophy, it is necessary to be aware of the big difference of action between the synthetic product represented by one molecule and the naturally-derived product represented by hundreds of molecules [1, 2].

## 7.2 Cocoa, Chocolate and Derivatives: Between Ethnobotanic and History

In recent decades, the international scientific community has become aware of the therapeutic potentiality of cocoa and/or cocoa derivatives and by-products. A scientific paper review has recently been published [3] reporting a short history of cocoa and chocolate, the implications on public health and their importance for the international market. The characteristics of cocoa seeds, beans and different cultivar groups of cacao beans used to make cocoa and chocolate, as well as the chemical content of cacao, chocolate and/or derivatives are well known [4, 5].

Going back in history, chromatographic analyses of residues extracted from pottery vessels show that cacao beverages were made before 1000 BC, extending the confirmed use of cacao in Mesoamerica back to at least 500 years earlier. Cocoa came to Europe in the 16th century and in 1737, Linnaeus named the cocoa tree Theobroma (Food of God) [3]. In 1590, the Florentine Codex suggested a remedy made out of cocoa beans, maize and the herb *tlacoxochitl* (*Calliandra anomala*) to alleviate fever, shortness of breath and heart conditions. Manuscripts produced in Europe and New Spain from the 16th to early 20th century revealed more than 100 medicinal uses for cocoa and/or chocolate [3]. Cocoa beverages were manufactured as a complex mixture of cocoa and spices diluted with water, milk, beer or wine and sweetened with honey. In Europe, chocolate was sold by travelling salesmen and the art of making chocolate was learned in Italy. There are three main cultivar groups of cacao beans used to make cocoa and chocolate: Criollo, Forastero and Trinitario. The Criollo cocoa tree used by the Mayas, from which only 5–10% of chocolate is made, is highly prized and rare, less bitter and more aromatic than other beans. Latin America and Asia are the two continents with the highest production. Forastero trees, which include several sub varieties, are significantly harder than Criollo trees and produce cheaper cocoa beans, in fact they are used for 80% of world chocolate production. The Arriba variety is considered the best one. Countries producing quality Forastero are Africa, Brazil and Equador. The last cultivar is represented by Trinitario, a hybrid of Criollo and Forastero, used in about 10–15% of chocolate production [3] and produced where the growing conditions are satisfactory. The cocoa beverage is a product that everybody enjoyed over the centuries and it became a popular drink.

A common worldwide saying is that “Switzerland is the country of clocks and chocolate”: is that true? When it comes to chocolate, the saying is not completely wrong but it should be seen in the right perspective. The pioneers of Swiss chocolate helped to

develop and ameliorate the quality of chocolate. François Luis Cailler (1796–1852) in Corsier, State of Vaud, built the first factory with specific machinery useful for manufacturing chocolate. Later, Daniel Peter (1836–1919) invented milk chocolate, while Rudolph Lindt (1855–1909) invented plain chocolate. Moreover, Henri Nestlè (1814–1890) invented a special product defined as "child's flour", a milk-based product. Nestlè represents the world's first big entrepreneur. Beginning in the last century (1904) his company started reselling chocolate produced by a third party and began its world expansion.

The clinical study reported here was planned in Olivone by Ario Conti, born and bred in Blenio, and executed together with Manuel Rusconi, collaborator of the Alpine Institute of Chemistry and Toxicology. This is due to a chocolate factory that was born in Blenio Valley at the beginning of the last century (1906), in the same time as Nestlè. A special chocolate was there produced till 1968: then fierce competition with other chocolate firms in Switzerland and abroad, concluded the life of this Blenio factory.

## 7.3 The Chemistry of Cocoa and Chocolate

*Theobroma cacao* L. is a small but economically important tree. It is an evergreen, 4–8 m tall, of the *Sterculiaceae* family, native of the tropical region of the Americas. Each seed contains a significant amount of fat (40–50% as cocoa butter) and polyphenols, which make up about 10% of the whole bean's dry weight. Fermentation and drying of cocoa beans are crucial steps for the development of precursors for chocolate flavour and the integrity of molecules. The colour of the bean changes from purple to brown in well-fermented beans [3].

In the last century (1937–1950), with the discovery of new technologies based on analytical chemistry chromatography, as well as liquid and gas chromatography associated with mass spectrometry, it has become easier to run specific analyses of natural products, i.e., cocoa and chocolate. Around 380 chemical compounds have been classified into two groups: non polyphenol and polyphenol compounds [3]. Among the first group are alkaloids, ascorbic acid, caffeine, cholesterol, coumarin, ethylamine, fat, fatty acids, fructose, galactose, galacturonic acid, galanin, isobutylamine, methylamine, neuropeptide Y, nicotinamide, normetanephrine, octopamine, phenylalanine, saccharose, serotonin, synefrine, theobromine, tryptamine, tryptophan, tyramine, vitamin A, vitamin B12, vitamin E and vitamin K [3]. However, the most relevant are polyphenols. The polyphenol content of unfermented cacao beans is high (12–18% of dry weight), although it varies considerably depending on the country of origin. Of the total polyphenols in fresh cacao beans, at least 60% are in the form of procyanidins (formerly called leucocyanidins), mostly homodimers and homotrimers of (-)-epicatechin (EC) or heterodimers of EC and (+)-catechin. The precise chemical structures of several of these procyanidins have been identified. The presence of oligomers of up to ten subunits was recently confirmed in fresh cacao beans and reported for the first time in dark chocolate. The most abundant flavonoid monomers in fresh beans are EC and (+)-catechin, and they remain the predominant flavonoids in cacao liquor and cacao powder, even though the EC content can drop dramatically (by as much as 90%)

during fermentation. Polyphenols in cacao beans are stored in the pigment cells of the cotyledons. Depending on the amount of anthocyanins, the pigment cells, which are also called polyphenol-storage cells, range in color from white to deep purple. Three groups of polyphenols can be distinguished: catechins or flavan-3-ols (approximately 37%), anthocyanins (approximately 4%) and proanthocyanidins (approximately 58%). The main catechin is (-)-epicatechin being up to 35% of polyphenol content. Phenolic compounds make up 12–18% of the total weight of dried cacao nibs (i.e., roasted cacao beans). Approximately 35% of the total content of polyphenols in non-fermented cacao nibs belonging to the Forastero variety is epicatechin. Epicatechin content in non-fermented cacao nibs of different varieties ranges between 34.65 and 43.27 mg/g (defatted sample). This amount decreases during the process of fermentation and drying. Depending on the place of production, epicatechin content may change between 2.66 and 16.5 mg/g of the defatted sample, while the amount of catechin increases. An exhaustive list of analyses has been reported [3].

## 7.4 Cocoa, Chocolate and Derivatives: Clinical Studies

In 1996, the first human clinical study with chocolate was run by Kondo et al. who found that 35 grams of delipidated cacao decreased LDL oxidation between 2 and 4 hrs after ingestion [6]. Since then, cacao and chocolate products have generated significant interest for the pharmaceutical and nutraceutical industry, due to their association with various health-protective and therapeutic activities, and at least 45 human studies involving the use of cacao in different forms have been performed [3, 6–11].

## 7.5 Cocoa, Chocolate and Derivatives: Effect on Biological Systems

The first available scientific data on the relationship between cacao, reactive oxygen species (ROS) and cytokine production show that cacao liquor polyphenols inhibit ROS and reduce the expression of interleukin 4 (IL-4) mRNA in human lymphocytes [12]. Excluding some very interesting studies [11–17], there is a paucity of information regarding the potential immunoregulatory effects of cacao and chocolate on human peripheral blood mononuclear cells (PBMCs) and polymorphonuclear cells (PMNs). On the other hand, recent findings suggest immunomodulatory functions like regulation of cytokine production in vivo [18–20]. Chronic and acute inflammation underlies the molecular basis of many pathologies: cacao products with flavonoids, i.e., flavonols, as demonstrated by in vitro and ex vivo studies might represent an interesting dietary support to condition and modulate the inflammatory pathway. Indeed, pro-inflammatory cytokines [13–16], activation of platelets [13], nitric oxide-mediated mechanisms and plasma leukotriene/prostacyclin ratio, a measure of the proinflammatory/anti-inflammatory eicosanoid balance; speak in favor of an anti-inflammatory role for cacao [21]. In more detail, the activity of cocoa molecules seems to act on the Th1/Th2 balance by increasing IL-4 secretion and therefore, Th2 response [22]. Degree of flavonoid polymerization might involve Th2 cytokines:

short-chain cacao procyanidins increase IL-4 and IL-5, while long-chain ones reduce them [15, 23]. The mechanism by which cacao exerts its opposing effects on Th1/Th2 cytokines remains to be established [24, 25]. Other effects, such as cacao macrophage and lymphocyte down regulation in vitro have been reported. In vivo effects of cocoa rich compounds include beneficial effects on blood pressure, insulin resistance, vascular damage, and oxidative stress [26]. Given their powerful antioxidant activity, flavonoids seem to be the perfect candidates for immune regulation; nonetheless, studies showing opposite effects among different cacao flavonoid fractions suggest that other compounds may contribute to cacao's immune effects. In any case, as the profile of flavonoids absorbed in vivo differs from the one present in crude cacao extract, the physiological relevance of these data is limited [11].

## 7.6 A 28 Day Study of the Effects of Black and White Chocolate on the Neuroimmune System of Healthy Human Volunteers

A 28 day treatment with and/or without chocolate was planned to lay the basis for further specific clinical studies (Fig. 7.1). The aim of the project was to understand whether a relatively long-term chocolate treatment might have some effects on psychoneuroimmunological parameters in healthy adult human volunteers. Since it was not possible to characterize 380 molecules, a chocolate with a high polyphenol, theobromine and epicatechin content (dark) or without these (white) was used. All results obtained are in accordance with data reported in the literature by Tomas-Barberan et al. [27].

Both dark and white chocolate presented the following characteristics: energetic values (Kcal/100 g: 550 dark, 561 white) and proteins (g/100: 8 dark, 6 white) were similar

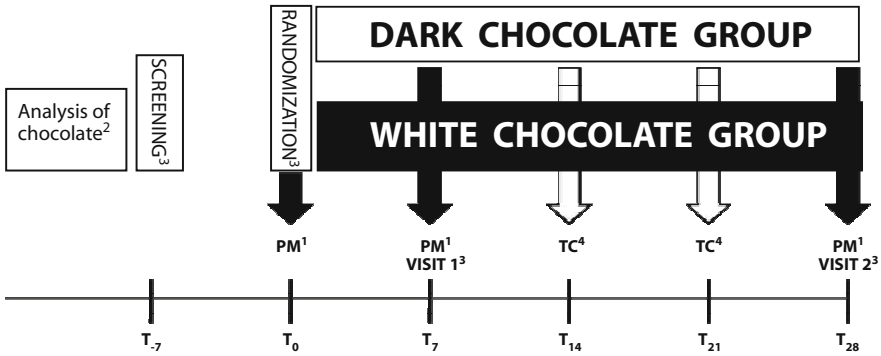


**Fig. 7.1** Acute vascular effect of dark and white chocolate on healthy human volunteers: study plan (see text for detail)

between dark and white, while there was a notable difference for carbohydrates (g/100g: 13 dark, 57 white) and fats (g/100g: 63 dark, 34 white). The most relevant difference between dark and white chocolate was reported for total polyphenols (25.03 mgGAE/g), theobromin (9.25 mg/g) and epicatechin (0.930 mg/g) in dark chocolate, 78% versus not detected values in white chocolate. The 28 day study design involved 21 healthy adult men aged between 25 and 30, investigator blinded and a parallel trial group in accordance with ethical guidelines. Chocolate doses of 25 g were wrapped in aluminum foil and provided in nontransparent bags that transferred no information about the content. After a cacao-free run-in period of at least 4 days and an overnight fast of ≥10 hours, participants were allocated to receive 75 g/day of commercially available polyphenol-rich dark chocolate or a matching dose per day of white chocolate, containing similar energy, over 4 weeks. Participants were instructed to ingest the total daily amount of chocolate in three doses of 25 grams. Each dose was eaten at three different moments: at 4:00, 6:00 and 8:00 p.m. All plasma parameters were assessed while each participant was in the 12-hour fasting state between 6 and 10 a.m. Peripheral venous blood was taken after the run-in period ($T_0$), one ($T_7$) and four ($T_{28}$) weeks of treatment. Adverse events were monitored every week via interview, on $T_7$ and $T_{28}$ throughout physical examinations and laboratory testing, and on $T_{14}$ and $T_{21}$ via telephonic interview. All personal data, for each participant in the study, were sent to their respective physician.

The aim of this study was to evaluate whether the immune system cell subpopulation and derived cytokines in venous peripheral blood are affected upon chocolate intake. More than 40 basic clinical parameters, T and B lymphocyte cell markers (whether different T cells were subsets activated or not), neurotransmitters such as serotonin (5-HT), 5-hydroxytryptophan (5-HTOL), 5-hydroxyindolacetic acid (5-HIAA), dopamine (DA), homovanillic acid (HVA), adrenalin (A) and norepinephrine (NA) were measured. The functionality of some peripheral blood cells in vitro has been verified by assessing three specific cytokines, i.e., interleukin 10 (IL-10), interleukin 8 (IL-8) and tumor necrosis factor α (TNF- α). This 28 day clinical study aimed at assessing the effect of dark and white chocolate on healthy human volunteers, has allowed us to corroborate some previously published evidence by other authoritative scientists. On the other hand, we have been surprised by some new effects on neuroimmune parameters. No adverse events and/or side effects occurred in the treatment groups, for dark or white chocolate. Fifty percent of the participants allocated to the chocolate treatment group found it hard to eat 75 g of chocolate every day for 4 weeks. Supplementation with dark or white chocolate was well tolerated. Baseline data at time $T_0$ for the treated groups did not differ, either in terms of anthropometric and hemodynamic measures or systolic and diastolic blood pressure. Finally, 21 participants were randomized, with 19 completing the study having attended all monitoring sessions. None of the data were lost. According to the participants' self-reports, the weekly monitoring of the returned empty bags and the personal interview during each week, all chocolate portions were eaten and no other cacao products were consumed. Moreover, all chocolate portions were consumed at the requested time with a maximum of one hour difference. Participants showed no significant difference at $T_0$ of the reported habitual frequency of food intake and physical activity. During treatment and from the participants' food diaries, no important changes in nutritional composition of diets from the run-in phase to the end of the intervention was observed. Due to the energy, nutrient, and electrolyte contribution of the daily dark or white chocolate doses

to the total diet, body weight and clinical parameters did not change with clinical relevance during the study. Also, the body mass index (calculated as weight in kilograms divided by height in square meters) did not show any modification and remained between 18.5 and 25. Basic clinical parameters monitored along the 28 days remained in the normal range. The cytofluorimetric analysis to determine any possible changes in lymphocyte subset populations, for example, B lymphocytes, T helper and T suppressor lymphocytes (activated or not), showed no statistical difference between the dark and white chocolate treated groups. However, the main question at this point was to know whether black and/or white chocolate in healthy human volunteers showed any activity on other parameters. First of all, dark and white chocolate influences blood pressure (Fig. 7.2), with only systolic blood pressure decreased in both treatment groups. This result is only statistically significant in the dark chocolate group and all data confirmed those reported in other studies [28–30].

Other interesting results have been obtained on parameters connected with the immunologic system, i.e., C-reactive protein (CRP), the first marker of inflammation, interleukin 10 (IL-10) and tumor necrosis factors α (TNF-α).

Whilst not statistically significant, CRP concentration showed a decreasing trend in the dark chocolate group while no difference was noticed in the white chocolate one.

Interleukin 10 (IL-10) is a regulator of lymphoid and myeloid cell function produced by B and T cells, activated mast cells, macrophages and keratinocytes. Due to the ability of IL-10 to block cytokine synthesis and several accessory cell functions of macrophages, this cytokine is a potent suppressor of the effector functions of macrophages, T-cells, and natural killer cells. In addition, IL-10 participates in regulating proliferation and differentiation of B cells, mast cells and thymocytes. Moreover, IL-10 was recently identified as the cytokine produced by the T helper subpopulation cells (Th2) that inhibit the synthesis of immunostimulatory cytokines by the Th1 cells. IL-10 exhibits inhibitory effects on monocytes including down-regulation of MHC class II antigen expression and suppression of

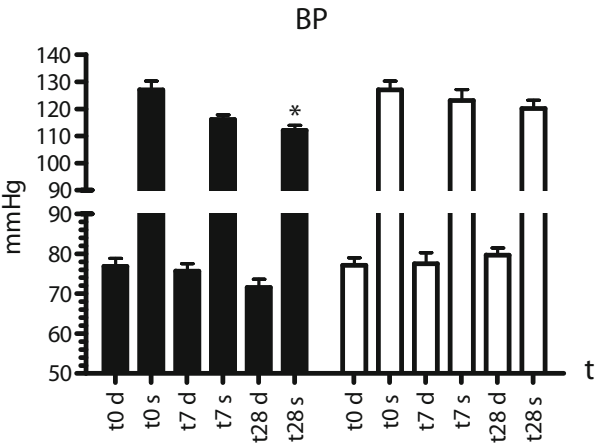


**Fig. 7.2** Effect of dark and white chocolate on diastolic and systolic blood pressure. Systolic pressure decreased in both groups during treatment but only in the dark chocolate treatment group the decrease were statistically significant

IL-1α and β, IL-6, IL-8, GM-CSF, G-CSF and TNFα production, synergizes with IL-2 and IL-4 to promote the proliferation of thymocytes and synergizes with IL-3 and IL-4 to enhance the survival of mast cells. IL-10 production can be induced by mitogenic lectins and LPS while IL-4 and IFNγ inhibit this production. The suppressive effects of IL-10 on monocytes and Th1 cytokine synthesis suggests that IL-10 may have utility as a general suppressor of immune function. The targets for such immunosuppressive drugs include infectious disease, transplantation, induction of tolerance and possibly cancer. IL-10 is currently in preclinical studies to evaluate its potential in various disease states [31].

No statistically significant differences in IL-10 concentration in cultured resting and PHA activated PBMCs were observed in the dark chocolate group, while a statistically significant ($p<0.01$) increase in IL-10 production by PHA activated PBMCs from week one to four of treatment was assessed in the white chocolate group (Fig. 7.3).

Tumor necrosis factor α (TNF-α), produced by monocytes and macrophages through mediation by lymphokines and endotoxins and secreted upon stimulation by IFNγ is a multipotent modulator of immune response [32, 33]. TNFα responds to stimuli such as infectious agents or tissue injury by activating neutrophils, altering properties of vascular endothelial cells, regulating metabolic activities of other tissues, as well as exhibiting tumoricidal activity by inducing localized blood clotting [34, 35]. Activation of B-cells by the Epstein Barr virus can be inhibited by TNFα [36]. Moreover, TNFα may play a role in the pathogenesis of many disease states such as inflammatory disease of joints and other tissues, septicemia and meningococcal disease [37], parasitic infections [38], myocarditis [39], AIDS [41–43]. No statistically significant modulation by 28 days of chocolate administration is observed:

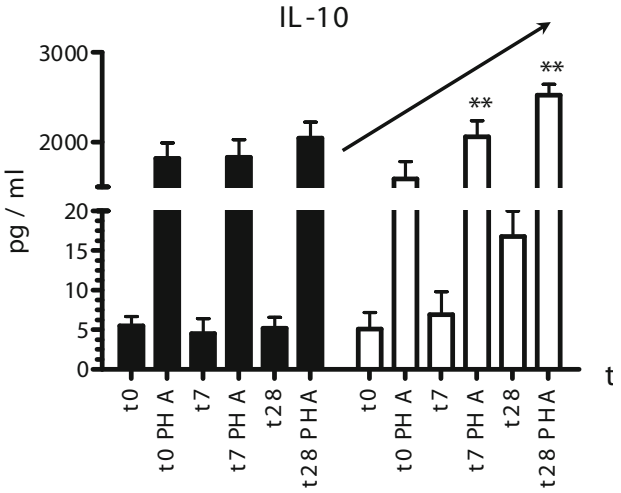


**Fig. 7.3** Effect of dark and white chocolate treatment on in vitro production of IL-10 by cultured resting and Phytohematoagglutin PHA-activated peripheral blood mononuclear cells (PBMCs) collected from human venous blood. No significant differences in IL-10 concentration in cultured resting and PHA-activated PBMCs were observed in the dark chocolate treatment group, while IL-10 production increase in PHA-activated PBMCs collected from the white chocolate treatment group after 1 and 4 weeks of treatment ($p < 0.01$)

although, a decrease in the production of this proinflammatory cytokine, TNFα, by cultured resting and activated PBMCs was observed in both groups (Fig. 7.4).

The last group of molecules assessed by this study is connected with indolamines and catecholamines, where 5-HT (serotonin) and 5-HIAA increased during treatment in both the dark chocolate and white chocolate groups. Serotonin increase is statistically significant on $T_{28}$ in the WCG whereas its metabolite 5-HIAA increased significantly in the DCG (Fig. 7.5).

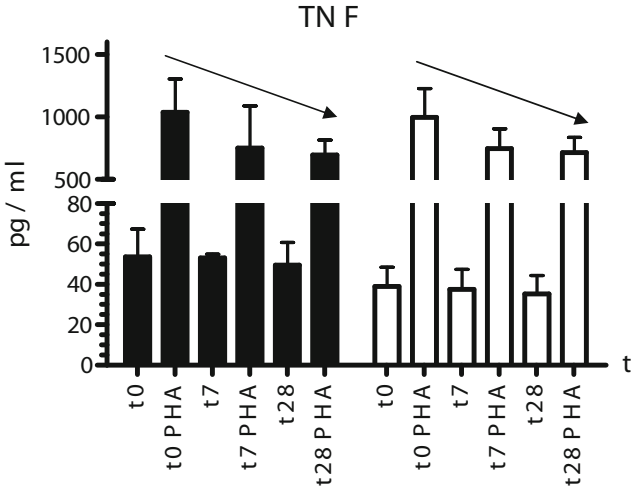


**Fig. 7.4** Production of TNF-α by cultured resting and PHA-activated PBMCs. No apparently significant modulation has been reported although it has been observed a trend to TNF-α decrease in both dark and white chocolate treatment groups

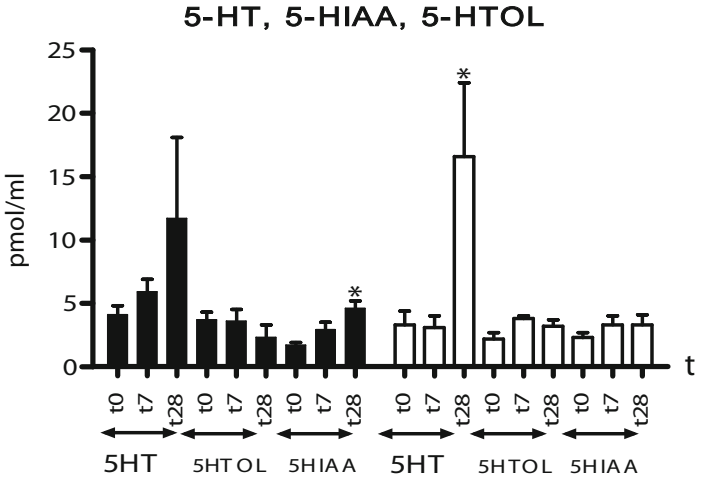


**Fig. 7.5** Plasmatic concentration of serotonin (5-HT) and 5 hydroxyindolacetic acid (5-HIAA). 5-HT increased in both treated groups after 28 day treatment where the increase is statistically significant only in the white chocolate group ($p < 0.01$). The metabolite 5-HIAA increase only in the dark chocolate treated group after 28 day treatment ($p < 0.01$)

The amount of catecholamines in the extracellular space is a function of the balance between their vesicular release and their reuptake by the monoamine transporter system.

A significant increase of dopamine plasma concentration was detected in the white chocolate group whereas there was a very small increase in the dark chocolate group (Fig. 7.6).

The decrease of adrenalin (A) is statistically significant at $T_{28}$ vs $T_0$ in the dark chocolate group. Noradrenalin (NA) increased at $T_7$ (+51.3% vs $T_0$), returning to baseline values at $T_{28}$. On the other hand, in the white chocolate group A increased 2.7% at $T_7$ and decreased by 32% at $T_{28}$ vs baseline, however, differences are not statistically significant. Noradrenalin increase in the white group is almost linear showing an increase of 34% on $T_{28}$. However, again the differences are not significant (Fig. 7.7).

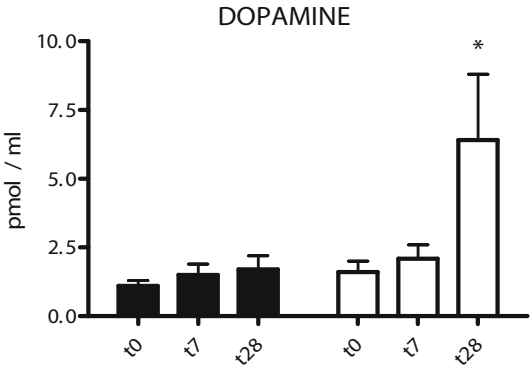


**Fig. 7.6** Effect of dark and white chocolate treatment on dopamine plasmatic concentration where a significant increase is detected in the treated white chocolate group ($p < 0.01$), whereas no changes in dopamine concentration have been reported in the dark chocolate group

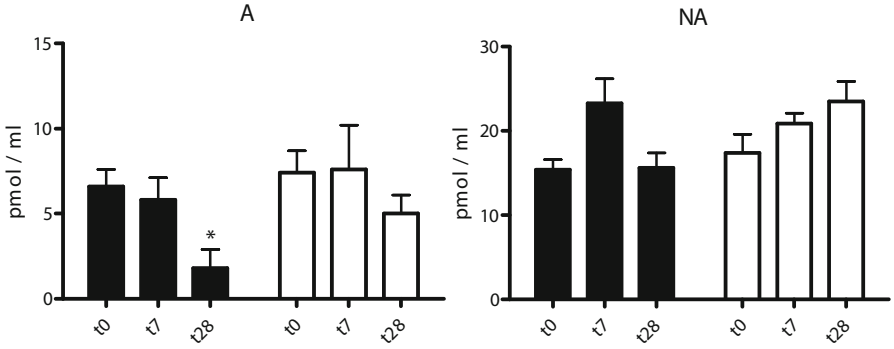


**Fig. 7.7** Change in plasmatic concentration of adrenalin and noradrenalin. The decrease of adrenalin in the dark chocolate group is statistically significant after 28 day treatment, while noradrenalin, in the same group, increased significantly after 7 day treatment and return to basal level after 28 days. In the white chocolate group no differences have been observed on adrenalin production while a clear trend to increase has been noted on noradrenalin

## 7.7 Conclusions and Perspectives

This clinical study is one of the first reports demonstrating a neuroimmunomodulatory role of chocolate in healthy human volunteers and, surprisingly, not only does dark chocolate seem to have special effects but white chocolate does too. In fact, the latter increases (29.6% vs baseline) IL-10 production from PHA activated PBMCs, and causes a decrease of the monocyte population. This may indicate the suppressive effects of IL-10 on monocytes. At $T_{28}$ both IL-10 and monocytes are increased. It is not easy to explain this data. In fact, other active compounds like biogenic amines present in cacao and chocolate, may also affect the stimulation capacity of PBMCs. Moreover, this study confirms the systolic blood pressure lowering effect of dark chocolate with a high concentration of polyphenols in subjects with recently diagnosed and untreated stage one mild isolated systolic hypertension and adults with untreated upper-range pre-hypertension or stage one hypertension without concomitant risk factors [10, 30]. On the other hand this data are to date controversial and need to be confirmed in other models [43, 44]. Although, taken together this indicates that cacao and dark chocolate may play a role in normalizing elevated blood pressure. In people who have normal blood pressure a dietary supplement of chocolate may help to maintain pressure in the normal range. How is such an interesting action of chocolate explained? The decrease of BP might be related to a decrease of adrenalin production, which plays a role in limiting the production of the angiotensin II precursor, renin, which in turn is responsible of the BP increase. Moreover, chocolate contains moderate concentrations of potassium (dark chocolate has approximately 263 mg and white chocolate about 287 mg) [45]. It is extensively recognized that reduction of BP correlates with increased intake of potassium, probably because potassium increases the excretion of sodium and for its activity on blood vessels. Nevertheless, certain cacao flavanol fractions are able to induce synthesis of prostacyclin and relaxation of isolated aortic rings with increased nitric oxide synthesis. Finally, the biological activity attributed mainly to flavonoids seems to be most effective on the endothelium and, in turn, on BP decrease. In fact, the decrease of BP is observed in the dark chocolate group only. Moreover, the white chocolate group did not take polyphenols. Epicatechin, catechin and dimeric flavanols might exhibit a dose-dependent accumulation within the nuclei and this might also be possible for metabolites. To get any presumably antioxidant effect, the first dose of flavanol-rich cacao is not sufficient to reach the maximum effect. The response becomes interesting after many doses have been administered and a new higher plateau will be reached: data indicate that 5 to 7 days are required to reach a new steady state [44, 47]. In fact, both Engler et al. [43] and Fisher et al. [44], were unable to document any blood pressure change in young, healthy, normotensive persons after flavonoid-rich dark chocolate consumption. The reason is more likely to be linked with the period of treatment (5 days). Results obtained with the present long period study seem to confirm this hypothesis. Moreover, the most abundant flavonoid compounds may not necessarily lead to the highest concentrations of biologically active metabolites in target tissues nor may they be the most biologically active in relation to specific health outcomes [46]. Moreover, a possibly related residual vascular response is evident 15 hours after the last dose, at a time when pharmacokinetic studies indicate that the flavanols and their known metabolites have largely disappeared from the circulation. Thus, one possible mechanistic sequence would involve activation of nitric oxide

synthase at gene level, as a first step. As a second possibility the responsible agent may be a metabolite of the flavanols that gradually accumulates [47]. However, metabolites of dietary phenolics, which appear in the circulatory system in very low concentrations (nmol/L to low mmol/L), might exert modulatory effects in cells through actions on components of the intracellular signalling cascades. Such events are important for cellular growth, proliferation and apoptosis. In addition, the intracellular concentrations required to affect cell signalling pathways are considerably lower than those required to impact on antioxidant capacity.

Our clinical study seems to confirm the preliminary in vitro data showing that plasma concentration of 5-HT (serotonin) and 5-HIAA increased during dark and white chocolate treatments [17]. Interestingly and surprisingly, our data seems not to be correlated with the non-fat cacao percentage, i.e., polyphenols, epicathechin and theobromin which are not present in white chocolate. Chocolate craving was reported to be a form of self-medication in atypical depression, in seasonal affective disorder, as well as an interesting impact on brain neurotransmitters with antidepressant benefits [48, 49]. Accordingly, several psychoactive constituents including anandamide, caffeine or phenylethylamine, also with neural and immunologic activity have been identified in cacao. Moreover, at least in the gastrointestinal tract, the existence of all compounds present in cacao at effectual concentrations can be assumed where they could also increase the availability of tryptophan and production of serotonin [17]. A great proportion of serotonin is synthesized and stored in the gastrointestinal tract where it plays a role in paracrine secretion and motility. On this basis, ingestion of cacao products like polyphenols, might play an important role in tryptophan metabolism and serotonin availability. Furthermore, antioxidant capacity of cacao products may locally shift the redox equilibrium in the gastrointestinal tract, which could be of benefit for the intestine and the whole organism and might improve quality of life [17]. An increase in plasma serotonin concentration has been observed in the white chocolate control group. This unexpected increase could be linked with an enhanced consumption of carbohydrates and fats, which in turn, may increase serotonin synthesis and modulate neurochemical imbalance. On the other hand, some components present in chocolate, diminished tryptophan degradation, most probably by the inhibition of indoleamine 2,3-dioxygenase (IDO) activity. Consequently, this fact might increase the production of serotonin (5-HT). Serotonin (5-HT) and 5-Hydroxyindoleacetic acid (5-HIAA) increased in both groups, but reached smaller values of plasma concentration in the dark chocolate one. It might be believed that some active compound, and/or combination of two or more different molecules present in both dark and white chocolate, could be responsible for the observed effects.

This clinical study on the effect of chocolate shows that C-reactive protein, a specific marker of inflammation, remains within the normal range in both groups, thus confirming previous data in other clinical studies [30]. On the other hand, a decrease of CRP concentration after 30 days intervention with flavanol rich cacao containing 321 mg flavanols per dose/day on 41 medicated diabetic patients was reported [50]. Popular medicine indicates chocolate and cacao as a craved food that may create dependence. Interestingly, within this clinical study it was observed that many volunteers at the end of the 28 day protocol, manifested a moderate aversion to chocolate, in particular for the dark one. Discussion about craving is a little bit complex because chocolate is a complex of cocoa molecules including sugars and psychoactive molecules [51–53]. As chocolate craving has some features

of addiction, attempts have been made to identify any psychoactive ingredient. Several candidates have been identified (the biogenic stimulant amines caffeine, theobromine, tyramine and phenylethylamine), but their concentrations are considered too low to have a significant psychoactive effect and they are also present in higher concentrations in non-craved foods [53]. Comparisons of subjects ingesting milk chocolate, dark chocolate, white chocolate and cacao powder (powdered cacao mass with some cacao butter extract) have demonstrated that milk chocolate is the preferred one. If psychoactive substances were involved, then cacao powder should equally satisfy craving and dark chocolate should be the most preferred [54]. Chocolate contains two analogues of anandamide, which are similar to the cannabinoid responsible for euphoria caused by cannabis [55]. Our project was mainly aimed at evaluating whether intake of chocolate by human volunteers might influence some neuroimmunological parameters and represents one of the few studies focused to achieve data on the effects of long term administration of chocolate on the neuroimmune system of healthy volunteers. The clinical study was conducted on healthy human volunteers leading a normal lifestyle. Our basic results in addition to the paucity published in the last decade, might constitute a sufficient rational for a more complete and multidisciplinary study on selected human volunteers suffering from specific pathologies. With this preliminary clinical study, many other relevant conclusions might be formulated and will need to be confirmed in larger multicenter studies. No changes in body mass index or other adverse effects were detected. In fact all clinical parameters tested during the study protocol, remained within the normal range. We could nevertheless observe an increase of the total leucocytes and eosinophil subpopulation in the dark chocolate group. Data remained within the normal clinical range, a slight allergic response to the dark chocolate can consequently be assumed. Possibly increased eosinophil population is related to the Th2 immune response. An increase in monocyte and basophil plasma concentration was detected in the white chocolate group. Also in this case, all values remained within the normal range and this clinical evidence could be in association with an increased consumption of triglycerides and fats [56]. Finally, our study has made us think of two different in vivo paths of action for cacao and chocolate. The antioxidant effect seems to be linked to the blood pressure lowering effect and positively related to the content of particular polyphenols such as the flavanols (catechin, epicatechin and polymeric procyanidins). However, concentration of flavanol in cacao and cacao-derived products like chocolate, depends on the geographical origin of the beans and their different manufacturing steps such roasting and fermentation which in turn, can decrease the polyphenol content by more than 80% .

The presence of these molecules is linked to the non-fat cacao mass. Cacao powder and some dark chocolates contain very high concentration of polyphenols, and their effect on the endothelium is now recognized. In contrast, white chocolate doesn't contain these types of antioxidant molecules and consequently, no decrease in BP were found after white chocolate consumption. White chocolate contains much more cacao butter, fat, fatty acids, sterols and sugar than dark chocolate. In addition, some lipophilic substances such as tryptophan, serotonin, dopamine and anandamide, possibly act synergistically to elicit a human immunomodulating effect in vivo and ex vivo. These substances could be present in the cacao fat fraction, also in dark chocolate but at lower concentrations than in the white one. This might be the reason why a similar but less potent effect on cytokine production in the dark chocolate group in both resting and activated PBMCs was observed

in the present study. Moreover, β-sitosterol and stigmasterol have been shown to be safe and effective in lowering circulating cholesterol levels [57]. Recent studies have linked the consumption of plant sterol and stanol esters with a reduction of cardiovascular disease (CVD) risk in those who do not adopt a Mediterranean diet [5]. As largely reported in related literature, the cacao extract used in vitro, in vivo and ex vivo, is depleted from the fat component (defatting cacao and chocolate is a crucial step in obtaining a flavanol extract). As a consequence lipophilic substances, which are also not easily water-soluble, are not present in the extract. For this reason the immunomodulating effect of this extract could be related to the presence of a very high concentration of antioxidant substances that are not the same of those present in chocolate. Moreover, the concentration used for cell treatment in vitro is difficult, if ever possible, to reach in vivo.

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# Vascular and Platelet Effects of Cocoa

# 8

Isabella Sudano, Andreas J. Flammer, Georg Noll and Roberto Corti

## 8.1 Introduction

Epidemiological studies demonstrate that regular dietary intake of plant-derived foods and beverages reduces the risk of coronary heart disease [1–4] and stroke [5], and is inversely associated with the risk of cardiovascular disease in general [2, 4].

A prospective study with a 16 year follow-up of 34,489 postmenopausal women who were free of cardiovascular disease in the Iowa Women's Health Study found that food rich in flavonoids were associated with a decreased risk of death due to coronary heart disease. The researchers also observed a borderline significant inverse association between chocolate intake and cardiovascular mortality after multivariate adjustment [6]. Therefore, habitual cocoa intake per se might reduce cardiovascular risk considerably [7]. In a prospective cross-sectional analysis cocoa intake was inversely related to cardiovascular and all-cause mortality [7]. The Zutphen Elderly Study involving 470 elderly men free of chronic disease highlighted the protective effects of cocoa intake. After adjustment for age, body mass index, lifestyle factors, drug use, food and caloric intake, the risk of cardiovascular mortality for men in the highest tertile of cocoa intake was reduced by 50% , compared to the lowest tertile ($p = 0.004$). The adjusted relative risk for all-cause mortality was 0.53 (95% CI, 0.39–0.72, $p = <0.001$). Moreover, a retrospective analysis of the Potsdam arm of the European Prospective Investigation into Cancer and Nutrition recently showed a high consumption of cocoa was associated with less prevalence of stroke and myocardial infarction [8].

In the context of human nutrition, green and black teas, wine and grape juices, orange, berries and especially cocoa represent noteworthy sources of antioxidants (Table 8.1) [9, 10].

The main flavanols contained in cocoa and in fruits in general are the monomers epicatechin and catechin [11, 12] and their dimers, oligomers and polymers, the so-called procyanidins [13, 14].

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**Table 8.1** Catechin/epicatechin concentrations found in foods. Modified from [10]

| Source | Flavanol content per mg/kg or mg/L |
|---|---|
| Chocolate | 460-610 |
| Beans | 350-550 |
| Apricot | 100-250 |
| Cherry | 50-220 |
| Peach | 50-140 |
| Blackberry | 130 |
| Apple | 20-120 |
| Green tea | 100-800 |
| Black tea | 60-500 |
| Red wine | 80-300 |
| Cider | 40 |

Procyanidins are also known as condensed tannins which, through the formation of complexes with salivary proteins, are responsible for the bitterness of cacao [15].

Importantly, during the conventional chocolate manufacturing process, from the fresh cocoa seeds (Fig. 8.1) to the finished product, the concentration of flavanols and procyanidins markedly decreases [16]. In particular, food processing methods such as fermentation and roasting have a detrimental impact on the final flavanol content of foods [10].

After cocoa ingestion we may observe a dose-dependent increase in plasma concentration of cathechins that reach the highest peak usually after 2–3 h [17, 18] and are still measurable after 8 hours [19].

Cocoa and the flavanols might increase nitric oxide (NO) bioavailability, activate nitric oxide synthase (NOS), and exert antioxidative, anti-inflammatory and anti-platelet effects which in turn might improve vascular function, reduced blood pressure and therefore, explain the positive impact on clinical outcome proposed by epidemiological studies [10].

This review will focus on the effect of cocoa on vascular and platelet function.

## 8.2 Cocoa and Vascular Function

### 8.2.1 Endothelial Function and Nitric Oxide

The vascular endothelium plays a fundamental role in modulating vascular tone and structure. Physiological production of vascular relaxing factors, including NO, prostacyclin and hyperpolarizing relaxing factor protect the vessel wall by antagonizing the initial pathological steps of atherosclerosis and thrombosis (Fig. 8.1). Cardiovascular risk factors and disease are associated with endothelial dysfunction or damage [20, 21].

The presence of endothelial dysfunction in the forearm circulation correlates with coronary vascular dysfunction and is predictive of future coronary events [22–24].

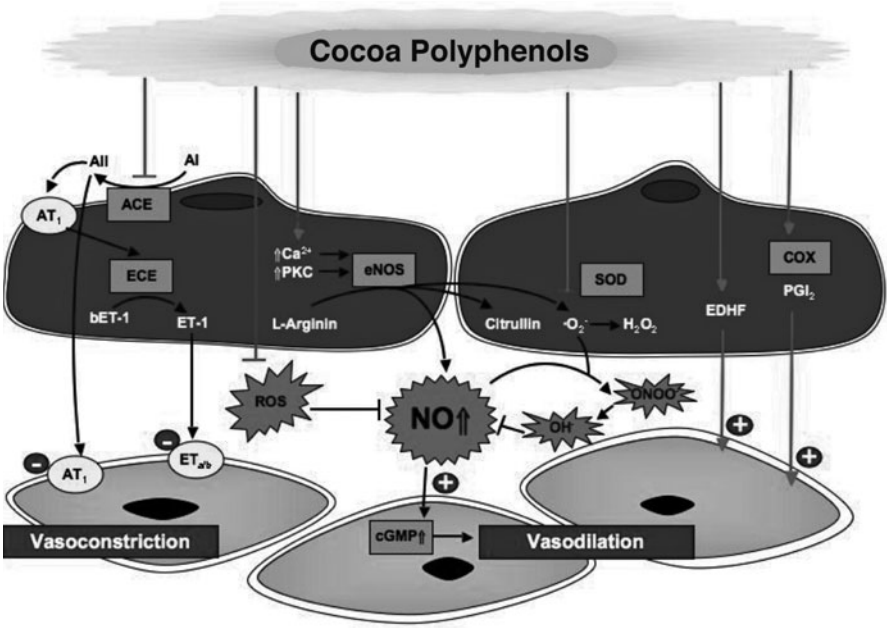


**Fig. 8.1** Endothelium-dependent effect of cocoa polyphenols. AII indicates angiotensin II; AI, angiotensin I; PKC, protein kinase C; SOD, superoxide dismutase; PGI2, prostacyclin; ACE, angiotensin-converting enzyme; ECE, endothelin-converting enzyme; AT1, angiotensin receptor; ET-1, endothelin 1; bET-1, big endothelin 1; ETa/b, endothelin receptor a and b; cGMP, cyclic guanosine monophosphate; and ROS, reactive oxygen species. Modified from [10]

Short-and long-term consumption of flavonoids/flavonoid-rich foods was shown to be associated with an improvement in endothelial function [25].

A recent metanalysis summarized the effect of tea on endothelial function as measured by flow-mediated dilation of the brachial artery in healthy subjects, patients with dyslipidemia, renal transplant recipients, chronic kidney disease patients and patients with coronary artery disease [26]. The author concluded that moderate consumption of tea substantially enhances endothelial-dependent vasodilatation [26].

Moreover, red wine, grape juice, dealcoholized red wine and extract from grape seeds improved endothelial function and increased NO production in animal models and in humans [27, 28] and the daily consumption of 500 ml orange juice for 4 weeks increased flow-mediated vasodilatation in healthy volunteers [29].

In line with these findings, cocoa induces NO-dependent vasodilatation in the rat aorta [30] and improves endothelial function in healthy humans and in patients with cardiovascular risk factors or disease (Table 8.2).

In patients with cardiovascular risk factors including smoking, a cocoa drink high in flavonol content (176–185 mg) rapidly enhances the circulating pool of bioactive NO by above a third and in turn flow-mediated vasodilation [31, 32]. Moreover, infusion of L-NMMA, an inhibitor of NO-synthesis, reverses the increase in NO and the augmentation in endothelial function associated with cocoa intake, while infusion of

**Table 8.2** Studies investigating cocoa and endothelial function. Modified from [10]

| Author | Year | No. | Animals/patients | Duration | Intervention | Outcome |
|---|---|---|---|---|---|---|
| Heiss [31] | 2003 | 26 | Patients with at least 1 cardiovascular risk factor | 2 hours (crossover) | Flavanol rich cocoa drink (100 ml) | Improvement of FMD and increased levels of nitrosated and nitrosylated species |
| Fisher [61] | 2003 | 27 | Healthy people | 5 days | Flavanol rich cocoa (821 mg/d) | Peripheral vasodilatation, improved vasodilator response to ischemia assessed by pulse wave amplitude on the finger |
| Engler [62] | 2004 | 21 | Healthy subjects | 2 weeks | High flavonoid chocolate (213 mg procyanidins, 46 mg epicatechin) vs low flavonoid chocolate | Improvement of FMD of the brachial artery, increased epicatechin concentrations |
| Grassi [36] | 2005 | 20 | Untreated essential hypertension | 15 days (crossover) | 100 g dark chocolate (21.91 mg catechin, 65.97 mg epicatechins) vs flavanol free white chocolate | Increased FMD of the brachial artery. Decrease in blood-pressure and LDL cholesterol, increase of insulin sensitivity |
| Heiss [32] | 2005 | 11 | Smokers | 2 hours (crossover) | 100 ml cocoa drink with high (17–18 mg) or low (<11 mg) flavanol content | Increase of FMD and circulating NO pool. Increase of flavanol metabolites |
| Hermann [34] | 2006 | 20 | Healthy smokers | 2 hours | 40 g commercially available dark chocolate vs white chocolate | Increase in FMD of the brachial artery. Improvement of anti-oxidant status and improvement of platelet function |

(*cont.* →)

**Table 8.2** (continued)

| Author | Year | No. | Animals/patients | Duration | Intervention | Outcome |
|---|---|---|---|---|---|---|
| Schroeter [63] | 2006 | 16 | Healthy subjects, isolated rabbit rings | 2 hours | Drink with high flavonoid content | Improvement of FMD paralleled the appearance of flavanoles in plasma. Concentrations in plasma enough to mediate ex vivo vasodilatation. Pure epicatecins mimics vascular effects of cocoa. High flavanol diet is associated with high urinary excretion of NO metabolites |
| Flammer [40] | 2007 | 22 | Heart transplant recipients | 2 hours | 40 g commercially available dark chocolate vs flavonoid free placebo chocolate | Inducing coronary vasodilation, improvement in coronary endothelial function and improvement of platelet function |
| Balzer [37] | 2008 | 41 | Diabetics | 30 days | flavanol-rich cocoa (321 mg flavanols ×3) or a nutrient-matched control (25 mg flavanols ×3) | Improvement in brachial FMD |
| Shiina [64] | 2009 | 39 | Healthy | 2 weeks | 45 g commercially available dark chocolate vs white chocolate | Improvement in coronary circulation as measured by coronary velocity flow reserve |
| Davison [38] | 2008 | 49 | Obese and overweight patients | 12 weeks | Dietary high (902 mg) vs low (36 mg) flavanol intake | Improvement in brachial FMD |
| Heiss [39] | 2010 | 16 | CAD | 30 days | Dietary high (375 mg ×2) vs low (9 mg ×2) flavanol intake | Improvement in FMD and mobilization of endothelial progenitor cells |

*CAD*, coronary artery disease; *LDL*, low density lipoprotein; *NO*, nitric oxide; *FMD*, flow mediated dilatation.

ascorbic acid had no effect [32]. Interestingly, drinking a flavonoid enriched cocoa beverage (containing 450 mg flavanols) results in regional changes of cerebral blood flow and an overall increased blood flow to grey matter for up to 3 hours as assessed by functional MRI, suggesting that cocoa flavanols may potentially prevent dementia or stroke [33].

Commercially available dark chocolate (74% cocoa), but not white chocolate, improves flow-mediated vasodilatation by 80% in young healthy smokers. This effect was already seen two hours after chocolate ingestion and lasted for up to 8 hours. Because the plasma antioxidant status was significantly improved as early as 2 hours after ingestion, it is likely that not only an induction of endothelial NOS and in turn elevated NO levels, but also a reduction in oxidative stress and in turn a reduced breakdown of NO by reactive oxidant species, contributes to the enhanced endothelial function under these conditions [34]. Indeed, antioxidants may prevent NO transformation into peroxynitrite and in turn protect against vasoconstriction and vascular damage [35]. Oxidative stress and reduced antioxidant defenses play a crucial role in the pathogenesis of atherosclerosis, in particular in transplant vasculopathy.

The improvement in peripheral endothelial function observed in smokers was confirmed in patients with arterial hypertension [36], diabetes mellitus [37], overweight and obesity [38] and coronary artery disease [39].

Looking at the coronary circulation, our research group demonstrated that flavonoid-rich dark chocolate improves coronary vasomotion in cardiac transplant recipients. Most interestingly two hours after consumption of 40 g of dark chocolate there was induced coronary vasodilatation, improved coronary vascular function and decreased platelet adhesion. These beneficial effects were again paralleled by a reduction of serum oxidative stress, and were positively related to serum epicatechin concentrations [40].

Not only endothelial function is improved after consumption of cocoa or chocolate. Vlachopoulos and colleagues showed that chocolate acutely decreases the augmentation index of the central (aortic) pressure waveform suggesting dilation of small and medium-sized peripheral arteries and arterioles [41].

Moreover, an observational study in 198 healthy subjects showed that habitual cocoa consumption is associated with decreased aortic stiffness and wave reflections and with improved central hemodynamics in healthy subjects [42].

Many mechanisms are proposed to explain the improvement in vascular function induced by cocoa, the antioxidative effect of the flavonols and procyanidins contained in cocoa may reduce the production of oxygen free-radicals and therefore improve NO bioavailability (Fig. 8.2).

It has to be noted, however, that the antioxidative effect of cocoa is controversial as macro- and micronutrients in addition to flavanols [43], as well as the increased uric-acid levels resulting from fructose metabolism [44], could affect the total antioxidative capacity of plasma.

Moreover, Ramirez-Sanchez and coauthors showed epicatechin is capable of inducing the synthesis of NO via eNOS activation in human coronary artery endothelial cells [45].

A further study by the same research group demonstrated that epicatechin-induced NO production in human endothelial cells can be obtained through both $Ca^{2+}$-dependent and $Ca^{2+}$-independent eNOS phosphorylation [46], suggesting that epicathechin may act to

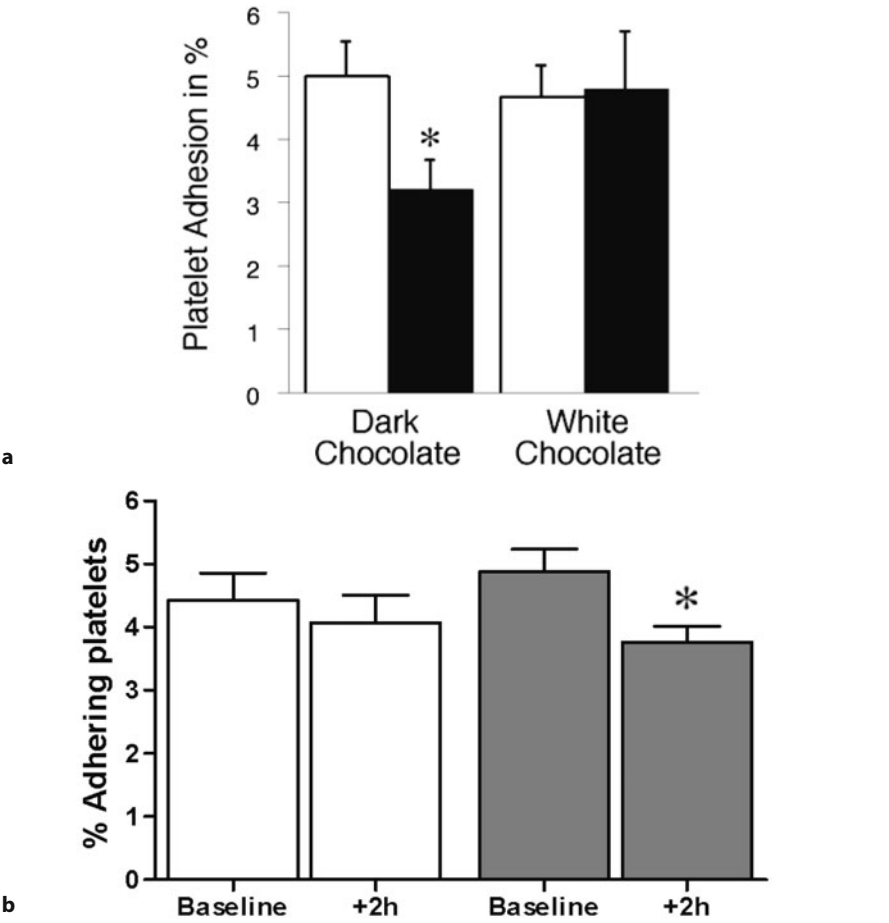


**Fig. 8.2** Effect of flavanol-rich cocoa on platelet adhesion in smokers (**a**) and heart-transplanted patients (**b**). *Denotes $p < 0.05$ vs baseline. In panel **b** the whie boxes represent platelet adhesion before and after placebo, while the grey boxes represent platelet adhesion before and after dark chocolate. Modified from [34] (**a**) and [40] (**b**)

retain vascular function in diseases where NO production is limited. These actions on eNOS may also serve to partly explain the cardioprotective effects of epicathechins [46].

However, further studies are still needed to clarify the mechanisms underlying the vascular improvement due to cocoa consumption.

## 8.3 Platelet Function

Platelet dysfunction is an important player in establishment and development of atherothrombosis [47–49].

Regular consumption of fruits and vegetables have been shown to protect against thrombosis possibly due to their high flavanol content [50]. Several in vitro and in vivo studies also demonstrated platelet inhibitory properties of cocoa and chocolate [51–55].

A recent review article [56] summarizes the effect of food and beverages rich in antioxidants on platelet function concluding that while for berries, tea, wine and other products the data are quite inconsistent, cocoa-related products consistently showed inhibition of platelet activation and aggregation when consumed in moderate amounts, either on an acute or chronic basis [56].

Within hours of ingestion, cocoa reduces ADP/collagen-activated, platelet-related primary hemostasis [51]. These effects were at least partly explained by a reduction in the ADP induced expression of the activated conformation of GpIIb/IIIa surface proteins [51]. Furthermore, ex vivo catechin and epicatechin reduce GpIIb/IIIa expression, similarly but to a lesser extent than that of low-dose aspirin, thereby exerting antiplatelet effects [53]. In healthy volunteers consuming 100 g of dark chocolate, a significant reduction in platelet aggregation was found but not in those consuming white or milk chocolate [57].

Moreover, in healthy volunteers, chocolate consumption in the past 24 hours was significantly and independently associated with a decrease in platelet function in both an ex vivo test of aggregation (PFA closure time) and an in vivo test of platelet activation (thromboxane production measurement by urinary 11-dehydro thromboxane B2), even after controlling for major potential confounding variables, including other possible platelet-modifying foods [58].

Cocoa not only decreases platelet aggregation, but also adhesion. In young healthy smokers [34], and in heart-transplant patients [40] dark chocolate reduces platelet adhesion as assessed in a shear stress dependent platelet test (Fig. 8.2).

This reduction in platelet activation can be in part explained by the flavanols and procyandins present in cocoa.

The exact mechanism(s) by which these substances may lead to an inhibition of platelet activity is not yet well understood, although several possible candidates have been proposed, including flavanol-induced changes in membrane fluidity, ligand-receptor affinity, intracellular signaling pathways, modulation of eicosanoid metabolism, antioxidant effect and increase in NO bioavailability [51].

Moreover, other components of cocoa and chocolate such as stearic acid may play a role in reducing platelet activation in humans [59, 60].

## 8.4 Conclusions

For many centuries, cocoa has been loved for its good taste and praised for its beneficial effects on health. In the last ten years many research studies have confirmed that cocoa does indeed exert beneficial effects on vascular and platelet function, probably primarily mediated by its polyphenols, a heterogeneous group of molecules mainly found in fruits and vegetables. The beneficial effects of cacao are most likely due to a decrease in oxidative stress, induction of NOS and in turn an increased bioavailability of NO.

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# Cocoa, Chocolate and Hypertension

# 9

Davide Grassi and Claudio Ferri

## 9.1 Introduction

Cardiovascular disease is the main cause of mortality both in developed and developing countries, killing an estimated 17 million people each year [1, 2]. In particular, hypertension is one of the most important cardiovascular disease risk factors, accounting for nearly two-thirds of all strokes and half of all ischemic heart disease [1–3]. It is also a major risk factor for dementia, chronic kidney disease and heart failure [2, 3]. Hypertension affects approximately 67 million US adults, and another 85 million have pre-hypertension [4, 5]. The most recent estimates suggest that 7.6 million premature deaths globally (13.5% of total global mortality) and 92 million disability-adjusted life years (6.0% of the global total) are attributable to hypertension [6]. Moreover, in developed countries, 90% of adults aged 55–65 years and with normal blood pressure will present with hypertension during their lifetime [7]. Conversely, lowering of an abnormally high blood pressure is associated with significant reductions in cardiovascular morbidity and mortality. However, blood pressure control requires extensive healthcare resources and, in clinical practice, most hypertensive patients are either undiagnosed, untreated or sub-optimally treated [8, 9]. Thus, considering the graded and continuous nature of the relation between blood pressure and vascular risk, international guidelines for the diagnosis and treatment of arterial hypertension [8, 9] introduced the concepts of "high-normal blood pressure" and "pre-hypertension." The respective committees recommended the identification of these individuals as they are at increased risk for progression to hypertension and subsequently other cardiovascular diseases [8, 9]. Indeed, both conditions are very prevalent (especially in obese young people), are often associated with other cardiovascular risk factors and result in a 3.0-fold greater likelihood of progression to hypertension and a 1.4- to 2.0-fold greater risk of cardiovascular events [10]. Accordingly, preventing hypertension through population interventions is a valid and attractive cost-effective strategy [6–8]. Lifestyle changes

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(physical exercise and a healthy diet) are commonly recommended to reduce the risk of hypertension. Concordantly, lifestyle modifications represent the only management option recommended for most pre-hypertensives [8, 9]. Therefore, lifestyle changes should be instituted by all patients, including low-risk individuals with high-normal blood pressure and patients who require drug treatment for hypertension [8, 9]. The Dietary Approaches to Stop Hypertension (DASH) diet emphasizes the importance of increasing the consumption of fruit, vegetables and low-fat dairy products [11, 12]. The Mediterranean diet [12, 13] is a polyphenol/antioxidant-rich diet associated with greater longevity, the maintenance of health and improvement of the quality of life [12–14]. Adherence to the Mediterranean diet has been shown to reduce cardiovascular morbidity and mortality in high-risk patients, with favorable effects on hypertension, lipoprotein levels, endothelial vasodilatation, insulin resistance, metabolic syndrome and antioxidant capacity [12–14].

The beneficial effects of fruits and vegetables have been largely ascribed to their content of flavonoids. These compounds are synthesized in many edible plants and are retained when the plants are processed to foods. Cocoa and chocolate, black and green tea, and wine and grapes are the most important sources of flavonoids in the human diet. A significant number of studies have been developed in humans, analyzing the effect of foods and beverages rich in flavonoids on the presence and progression of risk factors associated with cardiovascular disease [12–16]. Further research on the components of plant foods that may play a role in reducing hypertension will enable more targeted public health recommendations, thus allowing consumers to make more informed nutritional choices.

## 9.2 Cocoa and Flavonoids

Dietary flavonoids are a group of bioactive polyphenolic compounds that occur in plant-derived foods, including, as noted above, cocoa and chocolate. They are also naturally present in significant amounts in many foods consumed daily: fruit, vegetables, grains, herbs, and various beverages (tea, wine and juices) [13, 15].

Polyphenols constitute one of the most numerous and widely distributed groups of phytochemicals in the plant kingdom, with more than 8000 known phenolic structures [13, 15]. The common structure of flavonoids consists of two phenyl rings (A and C) joined with three carbons to form a closed pyran ring structure (B ring) [15]. Based on their structural differences, flavonoids are further subdivided into six subgroups, namely, flavanones, flavones, flavonols, flavan-3-ols, anthocyanins and isoflavones [15]. Differences in the chemical structures of these subclasses influence both their biological efficacy and their bioavailability [15, 16].

Cocoa is particularly rich in flavonoids, with the total flavonoid content of cocoa beans estimated at 6–8% by weight of the dry bean [15, 16]. In addition, important amounts of flavan-3-ols or flavanols are also found in cocoa and cocoa-containing foods/beverages. Flavan-3-ols are distinguished by the presence of a hydroxyl group at position 3 (C ring) and represent the most complex subfamily of flavonoids, ranging from simple monomers to the oligomeric and polymeric forms, called procyanidins [15–17]. The qualitative and quantitative content of flavanols and procyanidins varies among the different flavonoid

rich-foods [15, 16]. Accordingly, as evaluated by high-performance liquid chromatography/mass spectrometry analysis, cocoa flavanols are mostly non-esterified monomers of flavanols, such as (−)-epicatechin and (+)-catechin, and B-type procyanidins that are oligomers of (-)-epicatechin [15–17]. It is therefore important to specifically distinguish between the natural product cocoa and the processed product chocolate, which refers to the combination of cocoa, sugar, and in most cases additional ingredients into a solid food product [17–19]. Attention must also be given to the flavanols present in the finished cocoa products. Indeed, the flavanol content is largely dependent on the cultivar type, post-harvest handling, and processing of the flavanol-containing ingredients. In fact, flavanols that are abundant in the raw cocoa bean are progressively destroyed during the various phases of conventional/industrial chocolate-making processes. Following the fermentation and drying of natural cocoa beans and their subsequent processing by alkalization, roasting, liquor extraction and conching, up to 85% of the original flavanol content can be lost, as from the original content typically just 0.5% of the original cocoa bean remains in the final product [15, 17–19]. Nevertheless, it has been demonstrated that epicetechins reach detectable values in human plasma after the dietary administration of chocolate or of cocoa-derived products. Thus, even after the modifications due to manufacturing and to the metabolic processes that take place following digestion, the flavanols present in the raw material, i.e., cocoa beans, are able to reach the tissues [15, 20].

## 9.3 Cocoa and Blood Pressure: Evidence from Epidemiology

Recent studies have demonstrated a potential and to some extent unexpected role of cocoa in promoting health [15, 16, 18, 19]. Indeed, a large body of evidence supports the dietary intake of flavonoids, with the specific class of flavanols from cocoa postulated to exert beneficial vascular effects, reduce the risk of cardiovascular morbidity and mortality and contribute to the prevention of other chronic diseases [15, 16, 18, 19]. Several mechanisms have been proposed to explain the protection conferred by cocoa flavonoids against cardiovascular disease, including: antioxidant, anti-platelet and anti-inflammatory effects, increasing HDL, decreasing blood pressure and improving endothelial function [15, 16, 18, 19].

A key study regarding the effects of cocoa on blood pressure derives from Kuna Indians living in the San Blas Islands off the coast of Panama. In particular, Hollenberg et al. [21] reported that the Kuna drink several servings of unprocessed cocoa daily, and they are the only known population with regular daily sodium intake but nonetheless free from age-related increments in blood pressure. They also have a very low incidence of hypertension (2.2% , as determined by the minimal criteria of a mean systolic and diastolic blood pressure of 140 and < 85 mmHg, respectively) and cardiovascular disease [21]. However, these benefits disappear in assimilated Kuna Indians living on the mainland of Panama, who consume cocoa purchased from stores and consequently drink either lower amounts of flavanol-rich cocoa beverages or no flavanols at all [21]. In this population, the overall prevalence of hypertension was 10.7% and 45.1% in those > 60 years of age. Furthermore, in Kuna Nega, a Panama City suburb designed to maintain a traditional Kuna lifestyle but with access to the city, the findings were intermediate. These observations

suggested that migration by the Kuna has favored cultural changes that include a decrease in cocoa consumption and thus of the protective effects on blood pressure. Accordingly, flavanol-rich cocoa might be causally linked to the low prevalence of hypertension in island-dwelling Kuna Indians [21].

A possible blood-pressure-lowering effect of chocolate was also indicated based on a sub-study of the population residing in Zutphen (The Netherlands), the Zutphen Elderly Study [22]. In this cohort of 470 elderly men free of chronic diseases, blood pressure was measured at baseline and 5 years later, and causes of death were evaluated during 15 years of follow-up. Even after multivariate adjustment, the men in the highest tertile of cocoa intake (> 2.30 g/day) had a mean systolic blood pressure 3.7 mmHg lower (95% confidence interval (CI), -7.1 to -0.3 mmHg; $p = 0.03$ for trend) and a mean diastolic blood pressure 2.1 mmHg lower (95% CI, -4.0 to -0.2 mmHg; $p = 0.03$ for trend) than men in the lowest tertile (< 0.36 g/day). Compared with the latter group, the adjusted relative risk for men in the highest tertile was 0.50 (95% CI, 0.32-0.78; $p = 0.004$ for trend) for cardiovascular mortality and 0.53 (95% CI, 0.39–0.72; $p < 0.001$) for all-cause mortality [22]. Thus, data from this study suggested that cocoa intake is inversely associated with blood pressure levels and cardiovascular and all-cause mortality [22]. In accordance with this finding, a recent study by Buijsse et al. [23] evaluated a large cohort of 19,357 middle-aged German men and women without cardiovascular disease at inclusion. After a mean follow-up of approximately 8 years, the authors determined that the relative risk of the combined outcome of myocardial infarction (MI) and stroke for the quartile with the highest (7.5 g/day) versus the lowest (1.7 g/day) chocolate consumption was 0.61 (95% CI 0.44–0.87; $p$ linear trend = 0.014). In addition, chocolate consumption was linearly related to a lower systolic and diastolic blood pressure. After adjustment for age and sex, lifestyle variables, indicators of socio-economic status, dietary factors and the prevalence of diabetes, the difference between the top and bottom quartiles was 1.0 mmHg for systolic blood pressure (95% CI, 21.6–20.4 mmHg; $p$ linear trend = 0.0008) and 0.9 mmHg for diastolic blood pressure (95% CI, 21.3–20.5 mmHg; $p$ linear trend < 0.0001) [23]. The findings were essentially similar after participants with prevalent diabetes at baseline were excluded. Baseline blood pressure explained 12% of the inverse relationship between chocolate and the combined outcome of MI and stroke. These estimates were 16% for MI and 10% for stroke. Surprisingly, although there is a well-known association between high vegetable intake and cardiovascular benefits, the subgroup with the lowest risk was the group with the lowest vegetable intake who also had the highest chocolate intake [23]. This observation was confirmed in a prospective study [24] evaluating the association between habitual flavonoid intake and incident hypertension in men and women. In 87,242 women from the Nurses' Health Study (NHS) II, 46,672 women from the NHS I, and 23,043 men from the Health Professionals Follow-Up Study (HPFS), the mean amounts of flavan-*3*-ols ranged from 50.1 to 61.7 mg flavan-*3*-ols/day (interquartile range (IQR): 12.0–72.0 mg flavan-*3*-ols/day) across cohorts, whereas mean anthocyanin intake ranged from 12.5 to 15.2 mg anthocyanin/day (IQR: 4.6–19.3 mg anthocyanin/day). During 14 years of follow-up, 29,018 cases of hypertension in the women and 5,629 cases in the men were reported. After pooled analyses, a high anthocyanin intake was associated with an 8% lower risk of hypertension (quintile 5 compared with quintile 1, relative risk: 0.92; 95% CI 0.86–0.98; $p$ for trend < 0.03). The magnitude of the association was greater (12%)

in participants < 60 years of age (quintile 5 compared with quintile 1, relative risk: 0.88; 95% CI 0.84–0.93; $p$ for trend < 0.001; $p$ for age interaction = 0.02). Regarding the flavan-*3*-ol subclass, in analyses restricted to participants < 60 years of age, the rates of hypertension were lower in participants in the highest versus the lowest quintiles of catechin (7% ; 95% CI 3% –12% ; $p$ = 0.002) and epicatechin (5% ; 95% CI 0% –9% ; $p$ = 0.05) intake. In all participants, there was evidence of a modification effect of the epicatechin and hypertension association ($p$ for sex interaction = 0.03); in women, the relative risk was 0.95 (95% CI 0.92–0.99; $p$ = 0.015).

Contrasting with the above, the Seguimiento Universidad de Navarra Study [25] did not find an association between chocolate consumption and the incidence of hypertension in a cohort of healthy university graduates. The authors postulated that differences with respect to previous studies were the result of cocoa- and flavanol-poor chocolate intake by the study population. They also reported that chocolate consumption in the studied population was significantly associated ($p$ < 0.001, adjusted for age and sex) with snacking (individuals did not consume chocolate in isolation but in an indulgent dietary pattern with high-energy food intake) [25].

## 9.4 Cocoa and Blood Pressure: Experimental and Clinical Studies

Besides epidemiological evidence, in vitro as well as randomized interventional studies have demonstrated that flavonoid-rich cocoa products such as dark chocolate and cocoa beverages have blood-pressure-lowering properties. A recent experimental study [26] showed that a single oral administration of natural flavonoid-enriched cocoa powder (procyanidins = 128.9 mg/g, especially monomers, dimers and trimers = 54.1 mg/g, and mainly (-)-epicatechin = 19.36 mg/g) at different doses (50, 100, 300 and 600 mg/kg) decreased blood pressure in spontaneously hypertensive rats (SHR) but not in normotensive Wistar-Kyoto rats. The maximum effect in decreasing systolic blood pressure was achieved with 300 mg cocoa powder/kg. Interestingly, the antihypertensive effect was similar to that achieved with captopril (50 mg/kg), a recognized antihypertensive agent that inhibits angiotensin-converting enzyme (ACE). Although the experimental data are, as yet, limited, these observations support the use of flavanol-enriched cocoa powder as a functional ingredient with antihypertensive effects [26]. Accordingly, Sanchez et al. [27] studied the long-term intake of a soluble cocoa fiber product with respect to the development of hypertension in SHR. Active treatment reduced the development of hypertension; conversely, withdrawal of the product promoted a blood pressure increase [27]. In several clinical intervention trials, cocoa and chocolate were consumed by different groups of subjects: normotensive (young, old, overweight, hypercholesterolemic), pre-hypertensive, and hypertensive, with and without impaired glucose tolerance. Most of these trials reported an antihypertensive effect after cocoa/chocolate consumption [28]. Of note, we observed that 15 days of consumption of flavanol-rich but not flavanol-free chocolate was able to significantly lower both systolic and diastolic blood pressure in healthy subjects [29] and in hypertensive patients without (after dark chocolate: 24 h systolic blood pressure -11.9±7.7 mmHg, $p$ < 0.0001; 24 h diastolic blood pressure -8.5±5.0 mmHg, $p$ < 0.0001) [30] and

with [31] glucose intolerance. In hypertensive glucose intolerant patients [31], we observed that monitored and clinical systolic and diastolic blood pressure significantly decreased ($p < 0.0001$) after flavanol-rich (systolic blood pressure: -3.82 ± 2.40 mmHg; diastolic blood pressure: -3.92 ± 1.98 mmHg; 24 h systolic blood pressure: -4.52 ± 3.94 mmHg; 24 h diastolic blood pressure: -4.17±3.29 mmHg) but not after flavanol-free chocolate ingestion [31]. These findings are supported by the work of Taubert et al. [32], who provided additional evidence on the potential blood-pressure-lowering ability of cocoa ingestion by comparing the long-term effect of dark versus white chocolate consumption in patients with pre-hypertension or stage I hypertension [32]. In this interventional study, the daily administration for 18 weeks of 6.3 g (30 kcal) of dark chocolate was able to decrease mean systolic blood pressure by 2.9±1.6 mmHg and diastolic blood pressure by 1.9±1.0 mmHg. In addition, the prevalence of hypertension declined from 86% to 68% [32]. Similarly, Faridi et al. [33] reported that, compared with placebo, the consumption of dark chocolate and sugar-free cocoa significantly decreased blood pressure (dark chocolate: systolic blood pressure: - 3.2 ± 5.8 mmHg versus 2.7 ± 6.6 mmHg; $p < 0.001$; and diastolic blood pressure: - 1.4 ± 3.9 mmHg versus 2.7 ± 6.4 mmHg; $p = 0.01$; sugar-free cocoa: systolic blood pressure: -2.1 ± 7.0 mmHg versus 3.2 ± 5.6 mmHg; $p < 0.001$; and diastolic blood pressure: -1.2 ± 8.7 mmHg versus 2.8 ± 5.6 mm Hg; $p = 0.014$).

Another study [34], in which the dose-related effect of cocoa flavanols on 24 h mean arterial blood pressure in untreated mild hypertensive patients was evaluated, showed significant reductions in 24 h systolic blood pressure (-5.3± 5.1 mmHg; $p = 0.001$), diastolic blood pressure (-3± 3.2 mmHg; $p = 0.002$) and mean arterial blood pressure (- 3.8± 3.2 mmHg; $p = 0.0004$) after the consumption of cocoa with the highest dose of flavanols (1052 mg). By contrast, no significant blood pressure variations were achieved with any other flavanol dose. The same research group [35] observed that high-flavanol but not low-flavanol cocoa beverage administration was able to attenuate the blood pressure response to exercise (diastolic blood pressure increase was 68% lower; $p = 0.03$ and mean blood pressure 14% lower; $p = 0.05$).

Saftlas et al. [36] examined whether regular chocolate intake during pregnancy was associated with reduced risks of preeclampsia and gestational hypertension. The authors reported that chocolate intake was more frequent among normotensive (80.7%) than among pre-eclamptic (62.5%) or gestational hypertensive women (75.8%). Additionally, chocolate consumption was associated with a reduced odds ratio (OR) of pre-eclampsia (first trimester: OR, 0.55; 95% CI, 0.32–0.95; third trimester: OR, 0.56; 95% CI 0.32–0.97) and only in the first trimester was it associated with a reduced odds of gestational hypertension (OR, 0.65; 95% CI 0.45–0.87). An early meta-analysis [37] of five randomized controlled studies of cocoa administration (173 subjects, median duration of 2 weeks) suggested that after cocoa-rich product intake, pooled mean systolic blood pressure and diastolic blood pressure were 4.7 mmHg (95% CI -7.6 to -1.8 mmHg; $p = 0.002$) and 2.8 mmHg (95% CI -4.8 to -0.8 mmHg; $p = 0.006$) lower, respectively, than the corresponding values of the cocoa-free controls.

Concordantly, an additional meta-analysis by Hooper et al. [38], systematically reviewing the effectiveness of different flavonoid subclasses and flavonoid-rich food sources on cardiovascular disease and risk factors, reported that chocolate and cocoa intake significantly reduced systolic blood pressure (-5.88 mmHg; 95% CI -9.55 to -2.21; 5 studies) and

diastolic blood pressure (-3.30 mmHg; 95% CI -5.77 to -0.83; 4 studies). The effects were greater in studies with higher doses and of shorter duration. By contrast, the chronic intake of other flavanols (≥ 3 included studies) did not show any effect on blood pressure. Accordingly, a meta-analysis [39] of 10 randomized controlled trials (treatment duration from 2 to 18 weeks with 297 subjects) assessing the antihypertensive effects of flavanol-rich cocoa products showed a decrease of 4.5 mmHg (95% CI -5.9 to -3.2, $p < 0.001$) for mean systolic blood pressure and of 2.5 mmHg (95% CI -3.9 to -1.2, $p < 0.001$) for diastolic blood pressure. Moreover, in a pooled meta-analysis of all trials (13 assessed studies), Ried et al. [40] determined a significant blood-pressure-reducing effect of cocoa-chocolate compared with the control (mean blood pressure change ± standard error: systolic blood pressure: -3.2 ± 1.9 mmHg, $p = 0.001$; diastolic blood pressure: -2.0 ± 1.3 mmHg, $p = 0.003$). However, meta-analysis of the subgroups evidenced a significant antihypertensive effect only for the hypertensive or pre-hypertensive subgroups (systolic blood pressure: -5.0 ± 3.0 mmHg; $p = 0.0009$; diastolic blood pressure: -2.7 ±2.2 mmHg, $p = 0.01$), while blood pressure was not significantly reduced in the normotensive subgroups (systolic blood pressure: -1.6 ± 2.3 mmHg, p = 0.17; diastolic blood pressure: -1.3 ± 1.6 mmHg, $p = 0.12$). Nine of those trials used chocolate containing 50% to 70% cocoa compared with white chocolate or other cocoa-free controls, while six trials compared high- with low-flavanol cocoa products. Daily flavanol doses ranged from 30 mg to 1000 mg in the active treatment groups, and the interventions ran for 2 to 18 weeks. In addition, meta-regression analysis showed study design and type of control to be of borderline significance but possibly served as indirect predictors for blood pressure outcome [40]. In contrast to previous meta-analyses, the subgroup analyses by Ried et al. [40] suggested a difference in outcome depending on baseline blood pressure (hypertensive versus normotensive). Moreover, it seems important to remark that the relatively modest but significant blood-pressure-lowering effect of cocoa observed in the hypertensive subgroup should be considered of clinical relevance. A reduction of 5 mmHg in systolic blood pressure has been shown to reduce the risk of cardiovascular events by about 20% over 5 years [8, 9, 41]. Therefore, the effect of cocoa in hypertensive patients is comparable to other lifestyle modifications, such as moderate physical activity (30 min/day), which has been reported to decrease systolic blood pressure by 4–9 mmHg [8, 9].

Thus, all the above data indicate that the ingestion of cocoa-rich and therefore flavonoid-rich chocolate promotes a reduction in blood pressure. However, not all the results are in agreement and in some cases are even conflicting. There is significant statistical heterogeneity across the various studies. Considering the small number of subjects studied, the different quality assessment of the trials, the blood pressure measurement methodologies (number, accuracy, devices, etc.), and the variable dose of flavanols and/or chocolate used, a large, well-controlled, interventional study is warranted [37–40, 42]. Furthermore, meta-regression analyses [40] concluded that study design (parallel versus crossover) and type of control (flavanol-free versus low-flavanol) could be significant predictors of blood pressure outcome. The results of trials using flavanol-free controls, including white or milk chocolate, could be considered a potential bias for unblinded participants and might lead to overestimation of the effect of the active treatment [42]. Nevertheless, a placebo effect was found to be an unlikely explanation for the blood pressure effects of flavanol-rich dark chocolate administered in randomized, open-label crossover studies [42].

## 9.5 Cocoa and Blood Pressure: Pathophysiological Aspects and Mechanisms of Action

The flavanol-dependent increase in nitric oxide (NO) bioavailability is thought to be the main mechanism underlying the observed reduction in blood pressure, with NO known to play a pivotal role in the regulation of blood pressure and endothelial function [43], including after flavanol-rich chocolate ingestion [15, 28–31]. However, the reported evidence suggests that flavonoids from food also act to reduce blood pressure levels by modulating the renin-angiotensin-aldosterone system [15, 44]. Accordingly, Actis-Goretta et al. [44] demonstrated that incubation of purified ACE in the presence of flavanol-rich foods resulted in a volume-dependent inhibition of enzyme activity and that both high-procyanidin chocolate and chocolate ranked within the lowest $IV_{50}$ values. The $IV_{50}$ values for each food correlated with its phenolic content ($R^2 : 0.73, p < 0.003$) and its flavanol content ($R^2 : 0.85, p < 0.001$). To evaluate ACE activity inhibition closer to physiological conditions, membrane suspensions isolated from rat kidney were incubated in the presence of captopril (positive control) or (-)-epicatechin, dimer, hexamer, high-procyanidin chocolate, or low-procyanidin chocolate [44]. In this specific context, ACE activity in kidney membrane suspensions was inhibited by 100 μM of dimer ($p < 0.001$) or hexamer ($p < 0.001$) but not by (-)-epicatechin. The use of equal volumes of high procyanidin chocolate (634 μM (+)-catechin equivalents) and low-procyanidin chocolate (314 μM (+)-catechin equivalents) inhibited ACE activity by 70% and 45% ($p < 0.001$), respectively.

In a related study, Schewe et al. [45] showed that the exposure of human endothelial cells to (-)-epicatechin resulted in the elevation of cellular NO and cyclic GMP levels and in protection against the oxidative stress elicited by proinflammatory agonists. The authors proposed that endothelial NO metabolism rather than general antioxidant activity is a major target of dietary flavanols and that NADPH oxidase activity is a crucial site of action. Nevertheless, they also noted that besides NADPH oxidase, ACE inhibition [44] appears to play a fundamental role in cardiovascular protection. This supports the hypothesis that flavanols counteract angiotensin II in different ways: by modulating the renin-angiotensin-aldosterone system at the level of ACE, by inhibiting the well known prooxidant actions of this octapeptide and by protecting endothelial cells from its negative effects [15, 44, 45]. Confirming these findings, an experimental study [46] showed a significant and dose-dependent inhibition of ACE activity in cultured human umbilical vein endothelial cells after incubation with (-)-epicatechin and other flavanols. This was paralleled by a significant dose-dependent increment in NO production [46]. Moreover, the same research group [47] reported a significant inhibition of ACE activity (mean 18%) 3 h after the intake of dark chocolate in healthy subjects.

## 9.6 Conclusions

Epidemiological studies report an inverse relationship between flavonoid-rich cocoa, blood pressure and the risk of cardiovascular disease. Experimental data from both in vivo and

in vitro studies suggest that flavonoids and flavanol-rich cocoa products have the biological potential to reduce blood pressure in humans. Clinical intervention studies suggest that the consumption of flavanol-rich cocoa and chocolate reduces the cardiovascular risk by improving endothelial function and decreasing blood pressure. The antihypertensive responses to flavanol-rich cocoa in healthy subjects as well as in pre-hypertensive and hypertensive patients support the inclusion of moderate amounts of flavanol-rich cocoa or chocolate in the daily diet to potentially delay the onset of hypertension or ameliorate its control. Interest in the biological activities of cocoa flavonoids is steadily increasing; nevertheless, the practicability of chocolate or cocoa products as part of a long-term treatment for hypertension has yet to be completely clarified.

Currently, the unanimity of the data cannot be claimed because of the few and very small studies, the paucity of cross-over designed studies and the wide range of flavonoid doses and types tested. In addition, critical attention must be paid to the flavanol content of the finished cocoa products under assessment (manufacturing processes significantly reduce the flavanol concentration) and to the high fat and sugar content of many cocoa snacks and confectionaries. Further investigation on the dose-dependent and long-term effects of cocoa products should clarify these critical points. While physicians and patients await the introduction of cocoa products with "nutraceutical" (nutritional + pharmaceutical) properties, the food industry is encouraged to develop new, low-calorie cocoa-based foods and beverages, labeling the flavonoid content, dose and type.

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# Cocoa, Chocolate and Blood Lipids

# 10

Claudio Galli

## 10.1 Introduction

Chocolate has been around for more than 3000 years. From the use by Aztecs and Mayans, based on the grinding of chocolate beans into hot cacao drinks, the material and recipes on how to convert cacao to chocolate were sent to Spain by the explorer Hernan Cortez, and from Spain it spread to the rest of Europe.

The passion for chocolate conquered the world and the consumption expanded on a global scale. Rich in carbohydrates, chocolate is an excellent source of quick energy; however it also contains minute amounts of bioactive compounds, such as the stimulating alkaloids and other chemically active ingredients, which could lead to the "feel good" feeling and cravings, associated with chocolate. Many food scientists have indeed reported chocolate to be the single most craved food. Also the interest of nutritionists and clinicians for the possibly favorable effects of chocolate on human health, as a form of medicinal treatment has greatly increased over the years [1].

Two issues are very relevant in the evaluation of the impact of cocoa and chocolate consumption on biochemical parameters and risk factors: (a) their composition in terms of nutrients and bioactive compounds; and (b) the levels of consumption in populations on a global scale, in relation to observational studies, and in selected populations.

## 10.2 Components of Cocoa and Chocolate of Nutritional Relevance

The analysis of chocolate reveals notable amounts of the most important nutritional components: rarely are so many natural constituents and so much energy concentrated in such a small space. The detailed composition of chocolates varies depending on the type of

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preparation: e.g., plain, milk and white chocolate. In essence, from a nutritional point of view, the components can be divided in two main categories with diversified, and potentially contrasting effects on health: among the macro nutrients, the features of lipid components and carbohydrates, more than the proteins, and, among micronutrients, vitamins, minerals, methylxanthines e.g., theobromine and especially flavonoids and phytosterols, that are present in appreciable concentrations in enriched brands.

The most relevant components of chocolate differ depending upon the types [39]. In essence the macronutrients are carbohydrates, around 50–60% of the weight, followed by fats, around 32–35% , and proteins (3–7% ). Among the micronutrients, appreciable levels of theobromine in plain and milk chocolate are present, with minor levels of minerals, trace elements and vitamins. Contributions to daily requirements are provided by magnesium, phosphorus and liposoluble vitamins.

Fats in chocolate are largely composed of triglycerides rich in saturated fatty acids. The fatty acid (FA) composition reported in detail by Lipp et al. [2] evaluated in a relatively large number of commercially available brands, is rather uniform in cocoa butter (CB) while there are quite greater variations in cocoa butter equivalents, in relation also to different geographical growing regions, breeding lines and to other vegetable fats used in confectionary manufacturing (cocoa butter equivalents, CBE). CBE levels of minor lipid constituents (tocopherols, trienols, and sterenes, sterol degradation products) provided by the presence of added vegetable fats, are rather variable (not shown). In essence, the main fatty acids are palmitic acid (16:0) in the range of 25–27% in CB and 18–59% in CBE, stearic acid (18:0) 33–38% in CB and 5–44% in CBE, and oleic acid (18:1 n-9) 33–37% in CB and 31–36% in CBE. Contents of the polyunsaturated fatty acid (PUFA) linoleic (LA, 18:2 n-6) are lower (3–4% in both CB and CBE).

The characteristics of the cocoa beans used for the preparation of chocolate add other variables especially concerning the contents of theobromine.

## 10.3 Chocolate Consumption

The impact of chocolate on various parameters, concerning cardiovascular and other systems in populations depends largely on the rates of consumption, that vary appreciably, on a global scale in different countries. Ranges of per capita cocoa consumption in the years 1965–1980 in 18 countries [3] varied from values of about 2.2 kg/year in Germany, followed by Denmark, Switzerland and USA with about 1.8 kg to the lowest values of about 0.1 kg in China and India. Consumption of whole chocolate in the year 1992 varied between almost 10 kg in Switzerland, 7–10 kg/year in Northern European Countries, to about 4.6–4.8 kg/year in the US and around 1.5–1.9 kg/year in Spain, Japan and Italy [4, 5]. In addition, chocolate consumption in the last 40 years underwent a steady increase of 1% per year in developed countries [3].

Also within the same country, the USA, the proportion of chocolate consumers in 1992 [5] was in the range between 5 to around 15% with daily intakes ranging from about 50 g/day to about 110 g/day. Chocolate consumption also varies in relation to gender and age, to seasons, being higher during winter vs summer, to regional areas within the same country,

e.g., Western vs Eastern regions and in different ethnic populations, e.g., higher consumption in whites (around 15 % of the population with average intakes of the order of 50 g/day) vs other racial groups, especially blacks (around 5 % of consumers with however greater average intakes of 110 g/day) in the USA [5]. In another US study, around 20% consumed chocolate regularly with intakes in the order of about 40 g/day [6].

A recent study in an Italian population aimed to investigate the effects of regular chocolate consumption on serum inflammatory biomarkers ([7] and personal communication). From 23,294 recruited subjects, 37% were non consumers, 34% occasional consumers (less than twice/week), 29% regular consumers (more than twice/week). When subdivided in relation to amounts consumed, 35% were in the range of 5–6 g/day, 25% between 6.1 and 10 g/day, 37% between 10 and 20 g/day, and 3% over 20 g/day. It appears therefore that levels of intakes in chocolate consumers in the Italian population is quite a bit lower than in consumers in other countries, e.g., in the USA [5].

In essence it is rather difficult to define high and low chocolate consumers in observational studies on a global scale, and generally a rather small proportion of the population is a regular consumer with high daily intakes.

The relevance of intakes on outcomes is also quite evident in controlled clinical studies. In fact, these are based on the administration of rather different amounts of chocolate for different time periods: from relatively low amounts, e.g., 5–6 g/day for 6 weeks [8], to progressively higher doses, e.g., around 40 g/day for 4 weeks [9] up to about 100 g/day in several randomized controlled trials (RCT), reviewed in a meta-analysis [10]. These values may or not coincide with the situation of chocolate consumers in different countries.

## 10.4 Chocolate Consumption and Risk Factors

A number of studies, especially randomized controlled trials (RCT) based on supplementations of cocoa and/or chocolate have been devoted to investigating the effects on parameters related to the health status, especially functional ones, considering that chocolate consumption has always been related to the feeling of well being (physical and mental). This includes, somewhat indirectly, the effects on cardiovascular parameters and related risk factors.

### 10.4.1 Impact on Cardiovascular Functional Parameters

In general, studies concerning the impact of chocolate and cocoa consumption, on risk factors, e.g., in the cardiovascular system, are mainly devoted to investigating the effects of bioactive components, such as flavonoids, and polyphenols. Chocolate accounts for the major proportion of these compounds in Western diets, as well as of selected added compounds, e.g., plant sterols, rather than those of the lipid composition. In addition, the above components modulate functional parameters in relatively low doses, and the contents can be easily modified in the formulation of chocolates.

A meta-analysis of RCT, in parallel groups or crossover design, aimed to investigate the effects of chocolate consumption on systolic and diastolic blood pressure, in relation to the intake of cocoa products rich in flavonoids for at least two weeks and in the amounts in the range of 100 g/day. Significant reductions of both systolic (around 5 mmHg) and diastolic (around 3 mmHg) blood pressures were seen in four out of five studies [10]. Another review confirmed reduction of BP, in addition to anti-inflammatory activities [11]. Specific favorable effects have been observed in selected studies, e.g., on flow-mediated vasodilation [12], endothelial function [13], the production of vasodilatory nitric oxide [14], platelet function [15] and inflammatory biomarkers [7]. In addition improved insulin sensitivity has been reported [12]. The favorable effects on the above parameters reported in several studies are attributable mainly to the potent effects of the mentioned bioactive ingredients in the chocolate formulations tested and to the relatively high doses of chocolate used.

Some of these studies, although not specifically aiming to assess the effects of cocoa/chocolate on blood lipids, have shown protective effects on blood lipids, not selectively related to the impact of fats in the preparations, but to the bioactive components, mainly flavonoids (including flavonols, flavanols, catechins, epicatechins and procyanidins) endowed of antioxidant activities. In particular, reduced susceptibility of LDL to oxidation [11, 16, 17], reduced concentrations of oxidized LDL [17], reduced lipid peroxidation in vivo [18], and reduced production of isoprostanes, products of non enzymatic lipid oxidation [13], have been reported in studies based on the intakes of chocolates in the range of 15 g/day [17] to 75 g/day [18]. These intakes provided average doses of procyanidins and related compounds of 400 mg/day on average [16], within a range of about 50 to 900 mg /day [19]. Some concern may however be raised on the enrichment of chocolates with these substances since high doses of polyphenols have been shown to exert cytotoxic effects on liver cells, a major metabolic organ in our body [20]. The adverse effect is mainly due to (-)epigallocatechin-3-gallate, a component of polyphenols, which exists in cocoa as well, acts as a pro-oxidant, and is cytotoxic in hepatoma cells [21]. Therefore, higher polyphenol supplementation may counteract their beneficial biological effects on lipid metabolism.

A possible role in reducing the susceptibility of lipoprotein to oxidation can also be attributed to the intakes of saturated and monounsaturated FA with chocolate, i.e., types of FA that are less prone to become oxidized.

## 10.5 Chocolate Consumption and Blood Lipids

### 10.5.1 General Considerations

In the context of coronary artery disease (CAD), namely atherosclerosis, blood lipids and in particular total cholesterol and LDL cholesterol, on one side, and in the opposite direction, HDL cholesterol, have achieved an almost dominant position. However there is considerable debate concerning the type of relationship between these parameters and CAD of atherosclerotic nature, more specifically whether it is a causal relationship or just an association. It is also generally recognized that atherosclerosis is a multi factorial disease and that blood lipids are just one of the various factors involved.

The impact of dietary factors on blood lipids, from available data and a rational point of view, is due to the major components affecting circulating lipids. Dietary fats, namely the fatty acids (FA), are the most relevant components of the diet affecting circulating lipids, in particular those that play major roles as risk factors, i.e. total cholesterol and lipoproteins with opposing impacts (LDL and HDL) and to some extent triacylglycerol (TAG). Dietary FA are the most variable components of the diet both in quantitative and in qualitative terms. In addition, it should be considered that dietary FA, especially polyunsaturated ones (PUFA) that are nutritionally essential, very effectively modulate structural and functional features of biomembranes. These parameters are minimally affected by dietary proteins, provided that essential amino acids are adequately supplied, since the assembly of proteins is almost exclusively dependent upon endogenously controlled, genomic factors.

A large number of animal studies (although not directly equivalent to the human situation) and human studies have indeed clearly shown that the type of dietary FA, i.e., the saturates (SFA), monounsaturates (MUFA) and PUFA, with somewhat opposing effects, modulate serum levels of total, LDL and HDL cholesterol differently, therefore possibly affecting major risk factors e.g., cardiovascular disease (CVD). In essence, there is evidence that higher intakes of SFA enhance serum cholesterol, while PUFA, especially the major dietary component of the omega 6 (or n-6) series, LA (18:2 n-6), reduce serum cholesterol, and MUFA, mainly oleic acid (18:1 n-9) have a somewhat neutral effect on this parameter, although some studies suggest that replacement of SFA with MUFA may exert favorable effects [22]. In addition, the widely studied omega 3 long chain PUFA eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) have been shown to exert protective effects on the cardiovascular system, that are not dependent however on changes in serum cholesterol. Early studies [22] that have associated high dietary intakes of SFA with elevated serum cholesterol, which was proposed to result in enhanced coronary heart disease (CVD) risk, also showed however, that there was a significant difference in absolute risk at the same serum cholesterol level in different cultures. In essence, within Europe similar relative risks for coronary heart disease (CHD) in relation to cholesterol are observed, but with markedly different absolute risks, with high risks in Northern Europe and low risks in Mediterranean, Southern Europe.

These relationships have hence been rather critically reviewed, and while there is substantial agreement that SFA may increase serum cholesterol, there is no convincing evidence concerning the relationship between these two parameters, i.e., that high consumption of SFA is associated with enhanced fatal CHD or CHD events [23].

The complexity of the issue is related to various factors: (a) there is a large variety of FA in the diet, that undergo complex metabolic interactions in the body, in relation also to the structure of the lipid pools in which they are incorporated; and (b) fats are part of a vast mixture of nutrients in any diet, most of them affecting metabolic processes and risk factors, in addition to influencing the bioavailability of fats. Moreover, with regards to observational (epidemiologic) studies, fat consumption on a global scale varies considerably in different geographical areas, ranging from 18.4 E% (49.5 g/day) in Africa to 33.2 E% (122.6 g /day) in developed countries, substantially Europe, North and Central America and Oceania [41]. Also the proportion of vegetable vs animal fat as sources of FA varies appreciably between countries.

Still assuming that SFA enhances total serum cholesterol and LDL cholesterol, there is apparently evidence that palmitic acid (PA, 16:0) enhances these parameters more than stearic acid (18:0) [24]. It should be considered however that other factors concerning SFA, in addition to the strict amounts in foods appear to modulate their effects on blood lipids. For instance intakes by moderately hypercholesterolemic subjects of the same amounts of SFA from dairy fats, resulted in much lower increments of serum total and LDL cholesterol in cheese than in butter, indicating that additional components in cheese, not present in butter, interfere with the hypercholesterolemic effects of SFA [25]. Animal studies have also shown that the sn-position of SFA, independent of the absolute amounts [26], e.g., palmitic acid (PA), mainly in sn-1,3 position in natural palm oil, influences plasma lipid levels, platelet aggregation and fecal excretion very differently than when mainly in the sn-2 position in interesterified oil. FA in position 2 are more efficiently adsorbed, while FA in positions 1 and 3, where SFA are mainly incorporated, instead undergo greater fecal excretion, and these conditions are responsible of the observed differential effects. Since fats in chocolates are rich in PA and various chocolate formulations may contain appreciable proportions of interesterified fats, the above observations may apply to consumption of certain chocolate preparations.

Due to complexity of the relationships between dietary SFA and CVD, the role of reducing their intakes has been recently discussed [27]. The conclusions are that the effect of particular foods on CHD cannot be predicted solely by their content of total SFA because individual SFA may have different CV effects and major SFA sources contain other constituents that could influence CHD risk. Research is needed to clarify the role of SFA in CHD risk and to compare specific foods with appropriate alternatives.

### 10.5.2 Human Data on Chocolate and Blood Lipids

Several studies on the relationships between chocolate consumption and blood levels were carried out in the period 2001–2011 [11]. Studies differed in the number of subjects, design, types of preparation, doses and duration.

#### 10.5.2.1 Effects on HDL Cholesterol

Various studies, including those carried out on a large number of subjects and for a long period of time (five years) [6] observed elevation of HDL-cholesterol, and this effect, also observed in diabetics [28], especially occurred with the administration of preparations enriched in polyphenols over 400 mg /day. Also, in a study comparing the effects of preparations with increasing levels of polyphenols [16] there was a positive relationship between polyphenol intake and HDL-C levels. The daily doses of chocolates were generally higher than 30 g/day. Since cocoa contains a variety of different compounds such as polyphenols (flavan-3-ols, flavonols), sterols, di- and triterpenes, aliphatic alcohols, and methylxanthines [29], it is difficult to determine precisely which compounds present in cocoa would affect the concentration of HDL. It is also likely that compounds other than cocoa polyphenols are responsible for the increase, although the intakes of high amounts of polyphenol rich chocolates, of around 40 g/day [16] up to 75 g/day [18], have greater HDL-cholesterol enhancing effects.

#### 10.5.2.2 Effects on LDL Cholesterol

On the basis of the appreciable contents of SFA in chocolate, it could be predicted that LDL cholesterol levels would be enhanced by its consumption, although the factors modulating the global impact of these FA in the diet on lipid parameters are rather complex as previously mentioned. In this context, the rather high levels of oleic acid in chocolate [2] would also exert opposing effects on cholesterol levels. Various studies have shown LDL lowering effects, which can be attributed to polyphenols, in contrast with somewhat predictable negative impacts on lipoprotein profiles. The LDL cholesterol-lowering effect in plasma has in fact been demonstrated for various polyphenols, including tea catechins, genistein, daizein, naringenin, hesperetin, and polyphenols in red wine [30–32]. These polyphenols in fact have the ability to: (1) inhibit cholesterol absorption in the digestive tract; (2) inhibit LDL biosynthesis by lowering the activity and/or expression of the synthesizing enzymes, and of microsomal transfer protein in the liver; (3) suppress hepatic secretion of apolipoprotein B100; and (4) increase expression of LDL receptors in the liver [17]. These mechanisms may also apply to polyphenols in cocoa powder in humans, although further studies are required to confirm this possibility.

Other studies have reported reduction of LDL and total cholesterol. However, this was associated with the intakes of preparations enriched in phytosterols and plant sterols in general, with daily intakes in the order of at least 1 g/day. The consumption of 1.2 to 2.0 g plant sterols per day has consistently been shown to have favorable effects on total cholesterol and LDL cholesterol levels. The use of sterol-enriched foods to reduce total and LDL cholesterol levels, as well as improve the ratio of LDL to HDL has recently been recommended as an effective dietary strategy in the outpatient management of patients with hypercholesterolemia [33].

It should be noted however that various studies reported that short-term consumption of either free or esterified plant sterols [34] resulted in reduced acute bioavailability of β-carotene and α-tocopherol. Longer-term duration of intakes resulted in reduced plasma carotenoids [35, 36] and lycopene [36]. Yet after adjusting for total cholesterol, other researchers [37] reported that only β-carotene significantly decreased after plant sterol ester intervention (2 g/day plant sterols). In another study [38], consumption of a chocolate product containing sterol esters (1.5 g) twice/day for 6 weeks (3.0 g/day) did not significantly alter plasma and serum lipid adjusted concentrations of vitamins A and E, total lycopene, cryptoxanthin, lutein/zeaxanthin, and β-carotene.

Thus presently, the literature is inconsistent, supporting the controversy that has existed in the plant sterol literature as to whether or not plant sterols influence fat soluble vitamin and antioxidant status.

## 10.6 Conclusions

The impact of chocolate consumption on blood lipids should be considered from a general point of view, rather limited but not negative. As already pointed out, the great differences in the amounts consumed, in different countries and population groups and the role of the composition of the products consumed in relation to the additional incorporation of

bioactive components (polyphenols, carotenoids, phytosterols), make it rather difficult to evaluate the impact of chocolate on blood lipids in observational studies. However, several controlled studies have shown that the intakes of relatively high amounts of chocolate selectively and strategically enriched in the mentioned compounds result in favorable effects on blood lipid parameters. Criteria for recommendation of consumption of selected preparations of chocolate, for the improvement of the lipid profiles have not yet been defined, although chocolate consumption, in the context of strategies based on lipid lowering diets, allow for indulgence of occasional treats without negative effects.

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# Psychological Drivers of Chocolate Consumption

11

Enrico Molinari and Edward Callus

## 11.1 Introduction to the Psychology of Chocolate Consumption

"Chocolate is cheaper than therapy, and the appointment is not necessary" this quote which came from an unknown person, gives an idea of some of the properties associated with chocolate; it gives instant pleasure, it is considered one of the favorite foods consumed during "comfort eating" and it is also one of the most craved foods [1–4].

The pleasure experienced in the act of eating chocolate, cannot be justified solely due to the neurophysiological components produced by it. Even though it has been verified that the presence of simple sugars increase the level of serotonin in the blood and thus lead to an improvement of mood and that the presence of magnesium improves the body's ability to adapt to stress, other qualities must exist to justify its excessive use on such a large scale. In fact, a correlation between the processes of chemical and drug dependency and the consumption of chocolate, have not been demonstrated, even in large quantities.

The secret of the "chocolate craving" must therefore be sought in the psychological aspects to this food, as well as in its chemistry. Chocolate is primarily linked to memories of childhood, the maternal instinct and affection that comes with it in all its forms. It is also the source of a feeling of warmth and protection, reminding us of situations that are pleasant and familiar. In fact, the primary motivation for the purchase and consumption of chocolate is that it is associated with a festive situation, a meeting, the recurrence which occurs in the family and which are emotional. Chocolate is very often linked to moments of celebration, and it assumes a positive value in these types of contexts.

When searching the word "chocolate" in the Ebsco Host Research Database, it appears 142 times. In some articles it is used as a metaphor in the titles indicating the symbolic value attributed to chocolate, it is sometimes included in studies that attempt to determine whether there is a correlation between the consumption of chocolate and some

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particular behaviors, other results suggest research on addiction to chocolate and finally others try to study the advantages and disadvantages associated with it.

In order to highlight the psychological drivers of chocolate consumption, in this chapter the psychological factors that come into play in the decision process regarding the food choice in adults and children will be described. Special attention will be given to chocolate consumption, highlighting the difference between its consumption due to greed (chocolate craving) and during an episode of "emotional eating". Finally the effect of the deprivation of chocolate consumption will be investigated.

## 11.2 Personality and Taste Preferences

When it comes to food choice it is interesting to think about whether as human beings, we are totally free from biological constraints and if our preferences are not only dictated by motivations of a functional nature but are also linked to personal psychology, lifestyle and personality type.

Dieting, being a complex subject, can be seen as the result of an interplay of variables (biological, geographical, cultural, religious, historical, economic and psychological) that assume a specific configuration in the individual. It is clear that biological factors contribute when changing eating habits; the literature in this field suggests that the deprivation of particular substances push the body to seek out foods containing them. Considerable importance is attributed to environmental factors and climate.

Starting with the work of Lewin, who focuses on changing eating habits in relation to the constraints and demands during the war period, several studies have helped to investigate the structure of food preferences [5]. These include the research by Steptoe et al. which indicates that an "internal locus of control" appears to be associated with a high interest in healthy foods and that a high level of neuroticism is often related to choices based on purely emotional and affective aspects [6].

Different tastes evoke different emotional contexts that may correspond more or less with different subjective characteristics. Regarding the preference for fried foods or sweets during infancy, the intake of sweet foods is associated with positive hedonic responses that do not occur when it comes to other flavors.

From an evolutionary perspective, the appreciation for sweetness is related to the fact that this flavor is usually present in foods that are high in nutritional value, and the preference for salty taste is related to the promotion of the intake of sodium and other minerals. The bitter taste seems to be rejected because it is typical of many toxic substances.

Dogana tried to summarize the personality traits that characterize people who prefer the sweet taste and those who prefer the salty taste [7]. The first type more often shows a female type code based on the search of warmth and protection, a tendency to introversion, self-absorbing relationships and the research of consolatory relationships. The latter, on the other hand are characterized by greater energy, assertiveness, dynamism, extroversion, resourcefulness and a relationship style in which the main features consists of being decisive, self-confident, independent and honest.

## 11.3 Factors Determining Food Choice

When one considers the psychological aspects related to chocolate and the link between chocolate and health, it is important to investigate the variables that determine the choice of food and in particular, those variables that should influence the consumption of chocolate. Several factors that influence the preference of food to eat have been identified (Fig. 11.1) [8]:

1) *Sensory hedonic likes:* Some studies have shown that human beings are born with a predilection for specific tastes, the first preference appears to be the one for sweet flavors [9]. During growth however, tastes change, and other flavors are distinguished too. In addition, socio-economic factors and cultural environment have a strong power in influencing the choice of food to consume. Other studies have shown that children learn more easily to choose foods that have high energy values. Chocolate brings together inviting features, including an enticing aroma and unique taste, and for this reason it assumes a strong hedonistic valence which makes it coveted by many people around the world [10].
2) *The situation:* There is a fundamental difference between the desire to eat and the pleasure that derives from it; the desire and pleasure that a person expects from the consumption of a food type are influenced by "perceived appropriateness"; the perception of the appropriateness of a behavior [11]. Social and individual conventions determine the combination of food to the contexts in which it is permissible to eat [8], creating a system of implicit rules for determining the choice of specific foods in specific situations.

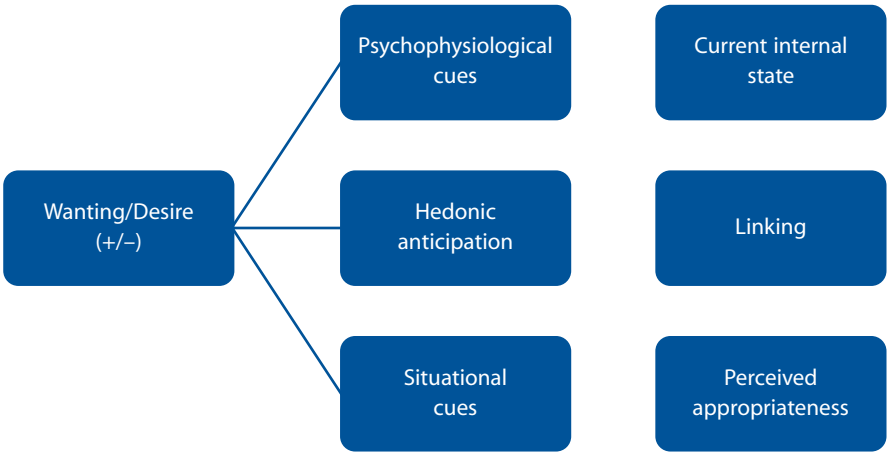


**Fig. 11.1** Determinants of food choice [8]. Schematic diagram of factors influencing the desire for a particular food. The "emotional condition" refers to the immediate psychological state (e.g., mood) or physiological (e.g., thirst) of the moment. "Pleasure" refers to the general pleasure derived from food. "The perceived appropriateness" refers to the usual context (where, when and with whom) in which food is consumed. (Copyright 2000, Society of Chemical Industry, reproduced with modifications)

3) *Emotional state:* Many authors argue that there is a reciprocal relationship between mood and food, if it is true that food changes the mood of the individual it is also true that specific emotional states lead to a preference for a particular food. In the reviews of Ganely and Christensen, multiple mood states such as stress and boredom are handled by the parties through "emotional eating" [12, 13]. The intake of carbohydrates, including chocolate, have a comforting effect by promoting a feeling of general well-being. This also indicates that the preference of food choice is not stable but it is affected by mood and feelings.

## 11.4 Food Choice in Children

There are several elements that influence growth in children; Burden considers the following five factors to be the most important ones [14]:

- parents;
- the school attended;
- friends;
- relatives; and
- TV programs for children.

Since these factors bear a huge influence on children's psychological and behavioral development, it is probable that they also influence attitudes and behaviors related to the consumption of food and chocolate.

The family environment in which a child grows up plays a crucial role in the mechanisms of food choice enacted by children, Young states that children have an active role with regard to eating habits and that the food choice is a process of mutual socialization between parents and children [15].

Although food appears to be a priority topic of conversation within families, nutrition and well-being are underestimated and they are given a low priority. On the other hand, high priority is given to the importance of how to provide food in practical terms with particular reference to what food to choose from [16].

Regarding the attitude towards food, there is a difference between the vision of mothers and their children; it seems that mothers believe that their children perceive food mainly in terms of pleasure, whereas children seem to grasp the nutritious value of food as being necessary for survival [17].

In addition, children between eight and fifteen years of age seem to be aware of "healthy eating" and that foods such as candy and sweets can be eaten in moderation [18].

The social context in which the child grows is very important in determining their dietary preferences. Some studies demonstrate how children learn to dislike foods that represent the conditions for obtaining a reward, and preference for those associated with the awarding of a success, a positive social context or relationship with a friendly adult [19].

Other studies suggest that a ban imposed by parents on a particular food, increases the desirability of that food, increasing attention to these "forbidden" foods. Their increased consumption is not necessarily preceded by a feeling of hunger, which can lead to an increased sense of guilt and negative self-evaluation [19].

In conclusion, children learn to love chocolate when it is used as a reward from parents and restricted access to it paradoxically leads to the development of a strong desire for it.

## 11.5 "Utilitarian" and "Hedonistic" Chocolate Consumption

A study by Kidwell and Jewell that aimed to examine the influence of internal control on health behavior is particularly interesting because it refers specifically to the behavior related to chocolate consumption [20]. Internal control is defined as a determinant of intention that can be measured by a questionnaire consisting of items taken from Armitage et al. (perceived internal control) [21]. Individuals who get a high score in this dimension are characterized by the belief that their behavior is guided by their own decisions and efforts.

Hischaman and Holbrook have identified two different categories of health behaviors [22]:

1) *Utilitarian behavior*: Utilitarian behaviors are seen as practical and useful; the person who engages in such conduct deliberately decides to use active cognitive processes [23];
2) *Hedonistic behaviors*: These behaviors are aimed to satisfy immediate pleasure. The decision to engage in this type of activity can be seen as a "steady stream of fantasies, emotions and fun" [22].

In this study, participants were randomly assigned to four conditions, in two of these chocolate was referred to in utilitarian terms and in the other two chocolate was referred to in hedonistic terms. The consumption of chocolate (at least once a week) was defined as positive (utility) or negative (hedonic) by giving the subjects who were divided into two groups, parcels containing different information.

In the first group, the consumption of chocolate was described as being beneficial to health and the subjects were given information about the usefulness of this behavior. In the second group the intake of chocolate was characterized negatively and as being harmful. The objective was to make people realize the potential damage but also the pleasure derived from eating chocolate.

Kidwell and Jewell argue that internal control has an effect on the two types of behaviors relevant to health with some differences [20]: in the case of utilitarian behavior there is a moderating effect on attitude and self-imposed rules that in turn influence the intention (Fig. 11.2 a). As for hedonistic behaviors, inner control influences the perception of past behavior and affective dimensions, which in turn have a moderating effect on the intention (Fig. 11.2 b).

In particular, the study results confirmed the hypothesis regarding chocolate consumption. In fact, when the consumption of chocolate was a given a positive connotation, the intention of consuming it, in subjects with a high level of internal control was influenced by self-imposed standards. On the other hand, in those subjects with a low level of internal control the variable influencing the intention of consuming chocolate was represented by the attitude towards it.

However, when chocolate was characterized in negative terms, the variable that influenced the intention of the subjects with low internal control to consume chocolate was

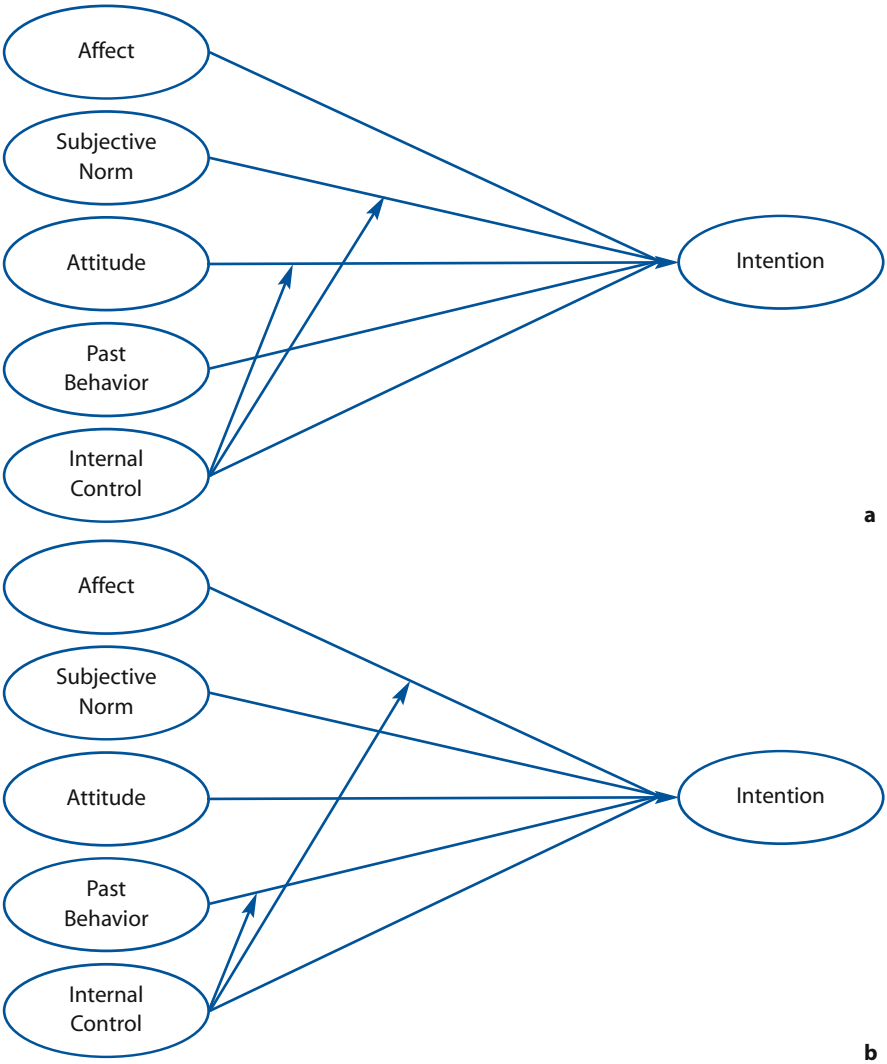


**Fig. 11.2** **a** Conceptual model specification for hypothesized relations of determinants on intention for Utilitarian behaviors; **b** Conceptual model specification for hypothesized relations of determinants on intention for Hedonic behaviors

affection. Past behavior is a useful predictor for the intention of consuming chocolate in subjects with a high degree of inner control.

The following is a summary table on the variables that appear to influence chocolate consumption (Table 11.1).

The experience of "craving" can be defined as an intense desire for a particular thing. In the literature these desires have been investigated in the following ways:

**Table 11.1** Variables that appear to influence chocolate consumption

| "Framing" of chocolate consumption | Internal control | Variables that influence the intention to consume chocolate |
|---|---|---|
| Positive | High | Self-imposed norms |
| | Low | Attitude |
| Negative | High | Past behaviors |
| | Low | Affective dimensions |

- self report [24];
- consumption of food [25];
- rate of consumption [26]; and
- psychophysiological measures such as salivation [27, 28].

None of the methods above is exhaustive in supplying enough information about the craving of certain foods; for this reason it is necessary to use the conjunction of multiple instruments [26].

Chocolate is the most commonly craved food [1–4], and the majority of the people who consume it daily state that the replacement of chocolate with other sweet foods is unsatisfactory [3, 29, 30].

## 11.6 The Effects of Chocolate Deprivation

The craving of chocolate in times of strong appetite could occur due to the internalized repeated experience of the consumption of this food when hungry [31]. Longing for the food cannot be considered an addiction because it does not involve a substance that is needed to get relief from a negative state: the substance is desired for the hedonistic experience that it gives.

"Emotional eating", however, was defined as the search for food when not physiologically hungry or in need of nourishment [32]. Other definitions of this phenomenon include the consumption of large amounts of food (usually junk food) in response to feelings rather than physiological needs. Several authors have developed theories of emotional eating:

1) Timmerman and Acton suggest that to deal with stressful life situations, some individuals resort to the resources of self-care that may be internal or external [33]. When a need is not met for a long time, a deficit that could be mitigated with the consumption of food is created.
2) Another theory proposed by Van Strien suggests that emotional eating is caused by the confusion and anxiety that arises from recognizing and responding accurately to the visceral and emotional states related to hunger and satiety [34].
3) Finally Heatherton et al. suggest that it is an escape from self-awareness, which is caused by discomfort, in which there is a cognitive shift in the subjects that moves attention away from unpleasant thoughts about themselves, to food cues in the environment [35].

All three theories suggest that emotional eating does not produce lasting benefits and indeed, indulging in food can contribute to a worse mood.

It is now necessary to distinguish between chocolate craving and the craving of carbohydrates, which can happen in the context of emotional eating. These two phenomena can coexist in the same individual because chocolate is desired since it provides a unique sensory experience, and because it provides carbohydrates in the moment of emotional eating. It is obvious that each of these phenomena is driven by different motivations, activating different neurotransmitters, producing different results [1].

Research that supports this line of thinking explains how the majority of the chocolate cravers state that no other food can replace chocolate in times of strong desire, while the craving for carbohydrates can be satisfied from any fatty or sweet food, and this includes chocolate [2].

In conclusion, chocolate can be consumed for two entirely different reasons (and of course, for the mere sake of it), on the one hand an episode of "chocolate craving" can only be satisfied by this food. On the other hand, it can be used along with other foods rich in carbohydrates such as cakes and ice cream to get relief from a state of negative mood. Both physical and psychological deprivation are likely precursors of craving for food [26].

In a study by Mann and Ward participants were forbidden to eat particular foods [36]. The prohibition of food led to an increase in its desire, even if participants did not exceed the consumption of these foods when they become available. It is important to specify however, that in this case foods that were easily replaceable and not especially attractive were used (such as rice). As mentioned above, chocolate is often considered as being irreplaceable, and therefore the impact of its deprivation can be very different.

Polivy et al. have examined the effects of deprivation in the consumption of chocolate eaters in "restrained" and "unrestrained" eaters categorized by Herman and Polivy's Restraint Scale [26]. The study also included two control groups, one in which subjects were deprived of food tasting of vanilla, and another group in which there is no restriction was made, in order to test the hypothesis that chocolate is a particular food that is highly coveted and difficult to replace.

Those belonging to "deprivation of chocolate" have eaten more of it when it was finally available, and they were more voracious in the eating. The same thing did not occur for the "vanilla deprivation" group. This confirms that chocolate is a unique food that is more subject to be strongly desired by people.

In addition, the subjects who were deprived of chocolate gave the strongest responses to chocolate cravings after the period of restriction, indicating that the restriction of chocolate consumption in individuals, whose food is monitored, triggers the opposite effect: they end up eating more chocolate when it is available.

## 11.7 Conclusions

Considering all of the above, the following points can be made in summary:

- Family, friends and socioeconomic status have a strong impact on food choices and consumption, including chocolate, in children.

- If parents choose chocolate as a reward or punishment, the child becomes strongly conditioned to it.
- The consumption of chocolate depends on both internal factors (such as mood, attitude) and external factors (such as conditions in which they are found, context, relationships) that interact with the characteristics of the individual (internal control, restriction in feeding).
- As chocolate has a very unusual composition, care must be taken so it is not abused, either when it is coveted as a specific food (chocolate craving) or when it is coveted as a carbohydrate (emotional eating). Its abuse is often correlated with negative moods and people feeling negatively about themselves.
- Finally, it is very important not to deprive someone completely of chocolate if they desire it, even if the subject consumes too much of it (for whatever reason). Research indicates that this will have the opposite effect: when the subject does find the opportunity to consume it, he or she will exaggerate its consumption.

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# Chocolate and Mood

# 12

Gordon B. Parker and Heather L. Brotchie

It is accepted that chocolate has a hedonistic appeal to most people; based on sight, colour, preparation, memories of past chocolate experiences, texture and taste. Yet many people are attracted to chocolate for other reasons, frequently suggesting that it settles stress, anxiety and depression. In essence, that it also has a beneficial impact on mood, and such a claim is the focus of this chapter.

## 12.1 Chocolate Craving

Chocolate's composition and properties have been detailed in earlier chapters of this book. It is likely that differing (but possibly overlapping) neurotransmitter systems are activated by those who merely find chocolate pleasurable, as against those who perceive it, and even crave it, as a mood regulator.

We first focus on the latter group (here termed "cravers"), and overview a study [1] that informs us about some nuances of chocolate craving as a mood regulation strategy. In this study, nearly 3000 individuals who had experienced clinical depression completed a Black Dog Institute web-based online survey of lifetime "treatments" as well as the coping repertoires they employed during depressive episodes. Embedded in the survey were questions about food cravings when depressed. Of the respondents, 70% were female and virtually all respondents had received medication, psychotherapy or counselling for depressive episodes, indicating that their depression was of clinical severity. When depressed, 54% reported craving some type of food. Chocolate was the most commonly craved food, with only 10% of the craver sub-set nominating any other preferred food. Thus, of the whole sample, 45% craved chocolate when depressed, 51% of the females

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and 31% of the males, a distinct gender difference. We provided nine options for participants to rate as reasons for their chocolate craving. The rank order of derived explanations were: its pleasure-enhancing role; its unique taste; its capacity to improve depression, its "feel in the mouth", it decreasing irritation, it decreasing anxiety, its texture, its smell and, finally, its colour. Thus, chocolate cravers noted its hedonistic effects as well as its capacity to settle "emotional dysregulation" (i.e., reducing depression, anxiety and irritability), and this aspect was ranked above aesthetic factors. Some seventy five per cent who rated chocolate's capacity to improve their depressed mood as moderate to very important were also significantly more likely to rate it as making them feel less anxious and less irritated (and thus again underlining its perceived capacity to ameliorate emotional dysregulation).

Study participants also completed the tiered Temperament and Personality questionnaire [2] which assesses molar personality "constructs" (i.e., "neuroticism" and "introversion") and their constituent facets (i.e., "neuroticism" being constituted by "anxious worrying", "irritability", "self-criticism", "rejection sensitivity", "irritability" and "self-focussed"; and "introversion" constituted by "perfectionism", "personal reserve" and "social avoidance") that predispose to depression. An intriguing and important finding from this study was that the chocolate cravers scored significantly higher on five of the personality facets when depressed, with *all five* being facets emerging from the molar neuroticism personality construct. By contrast, cravers and non-cravers did not score differentially on the three constructs underlying introversion (i.e., perfectionism and two expressions of shyness, personal reserve and social avoidance). A logistic regression analysis examined the differential capacity of facet personality scores to predict cravers, with cravers differentiated most distinctly by their irritability and rejection sensitivity scores.

Study results suggest that certain personality and temperament styles predispose to states of emotional dysregulation and which, during actual states of anxiety, irritability and depression, drive the craving for chocolate. Why? The most parsimonious view is that chocolate is judged by such cravers as having homeostatic propensities (i.e., settling emotional dysregulation). We assume that such a mechanism underlies much so-called normative comfort eating and emotional eating but that it also helps explain some psychiatric conditions (especially atypical depression and bipolar II disorder) where hyperphagia or food craving is common, and is also able to be positioned as homeostatic. Thus, we now briefly consider those latter two clinical conditions.

Atypical depression, as currently defined by the DSM-IV–TR system requires mood reactivity (during the depressed state) as a mandatory criterion in combination with at least two of four postulated secondary criteria (i.e., hypersomnia, hyperphagia and weight gain, leaden paralysis, and sensitivity to criticism and rejection). The mandatory status of mood reactivity as the primary distinguishing criterion has been questioned on the basis of two empirical analyses [3, 4] and in an overview [5]. In essence, our revisionist model positions atypical depression as a non-melancholic depressive syndrome and with the primary feature being personality-based interpersonal rejection sensitivity. In situations where such individuals feel rejected or abandoned (and consequently depressed), we have argued [6] that two of the so-called symptoms (i.e., hypersomnia and hyperphagia) more reflect homeostatic self-soothing systems in operation

and with them having some propensity to settle the emotional dysregulation, as occurs in the sub-set of chocolate cravers who have personality styles predisposing to emotional dysregulation.

More specifically, such hypersomnia has been hypothesised [7] to be an adaptive homeostatic response that restores slow wave sleep during times of stress and so has anxiolytic potential. While the hyperphagia [7] is viewed as a compensatory response, being weighted to increased dietary intake and brain uptake of L-tryptophan, and potentially leading to increased brain serotonin (5-HT) levels with consequent increased functional activity of the serotonin receptors in the limbic regions of the brain. A popular hypothesis that links chocolate craving and carbohydrate craving with depressed mood is that such symptomatic behaviours represent a serotonin deficiency. Wurtman and Wurtman [8] suggested that serotonin acts through a biofeedback mechanism to regulate carbohydrate consumption and, when there is a disturbance of that feedback mechanism, the desire for carbohydrate persists in an attempt to compensate through eating more carbohydrates and consequently lifting serotonin levels and thus improving mood.

Consumption of a meal (such as chocolate) that is high in carbohydrate, branch-chain amino acids (such as leucine) and tryptophan, increases insulin production. The insulin facilitates the transport of the branch-chained amino acids into muscle cells, thereby reducing the competition tryptophan faces for the transporter that takes it across the blood brain barrier to be converted to serotonin and increasing the amount of serotonin available to post-synaptic cells of the brain.

There may be some support for this theory in relation to some sub-types of depression such as atypical depression. However, it is important to note that it is not compatible with the general description of melancholic depression, where appetite and weight loss are more likely than food cravings and hyperphagia to be reported, whilst recovery from melancholia is associated with restoration of appetite and renewed pleasure in food. An examination of the relevant literature, reviewed by Hammersley and Reid [9], does however, identify flaws in the serotonin system theory and indicate that additional mechanisms are likely to be involved in explaining differences across the clinical depressive conditions.

Charney et al. [10] hypothesised that eating sugar-rich products which release multiple gut and brain peptides, including cholecystokinin (CCK) and corticotrophin-releasing hormone (which are known to modify cognition), may help to satiate those who have a personality style of sensitivity to rejection and who experience emotional dysregulation and states of atypical depression. It is also recognized that some antidepressants (e.g., the mono-amine oxidase inhibitors) that were long held to be specifically effective in treating atypical depression act on central appetite centres and modify these mechanisms through hypothalamic receptors [10, 11].

In bipolar II disorder (where the individual experiences non-psychotic mood swings) states of emotional dysregulation are evident during the depressed periods, and may similarly explain the hypersomnia and hyperphagia so commonly reported by many individuals during bipolar II depressive states, and argue again for such symptoms having homeostatic propensities.

## 12.2 Non-clinical Mood States

Till now we have focussed on chocolate craving during states of clinical depression, and where a link between personality style, chocolate craving and propensity of chocolate to settle states of emotional dysregulation appears relatively straightforward.

In moving to chocolate's effects and relationships to non-clinical "mood states", the picture is less clear-cut. The literature tends to confound concepts of chocolate craving, carbohydrate craving and emotional eating and to a lesser degree obesity, and self-medication [12]. It is therefore important to try to distinguish the two phenomena of chocolate craving and carbohydrate craving in the context of emotional eating (with the latter involving the eating of large quantities of food, often so-called junk foods, in response to feelings rather than to hunger). It is probable that each phenomenon is driven by different motivations, underpinned by different neurotransmitters, and so producing different outcomes, although apportioning contributions remains difficult.

As we argued in an earlier paper [12], the two craving phenomena can co-exist in the same individual, as chocolate can be desired *specifically* for its unique sensory experience and craved *more generally* as a carbohydrate at times of emotional eating. Chocolate produces a unique effect: when craving chocolate specifically, only chocolate will satisfy that craving, but, when craving carbohydrate any sweet-fat food (including chocolate) will suffice [13]. When experiencing an aversive mood state, any carbohydrate will suffice in an attempt to achieve relief, with chocolate no better than cake or any carbohydrate as a comfort food [14]. Anecdotal reports from "chocoholics" who are also emotional eaters indicate that as chocoholics they only crave chocolate but acknowledge that when feeling stressed, any carbohydrate is craved, and chocolate is not preferred over other carbohydrate. In support of this view, Schuman et al. [15] investigated chocoholics (including former alcoholics who craved sweet food) and non-craving controls, and quantified that 32% of chocoholics were self-medicators compared to 13% of controls and 23% of the former alcoholics. They found that chocolate was not greatly preferred over other sweets for self-medication, even among chocoholics. Self-medication occurred most commonly in response to depression, tension and irritability, less often in response to anxiety, and least often to anger.

Pinel [16] has suggested that humans in modern society are more driven to eat by anticipation of pleasure, than in response to internal energy deficits. Chocolate has a high hedonistic rating, based on a set of extremely appealing sensory characteristics, with an attractive aroma and unique flavour. The hedonistic responses to sweet and fat complexes are interdependent, and the combination of sugar with dairy cream in chocolate increases the hedonistic rating of each [17]. If the appeal of chocolate is the unique sensory combination of chocolate, then chocolate is the only way to satisfy that craving [18].

Although food craving shares some features with drug addiction, including the involvement of common neurotransmitter substrates, and even people describing themselves as chocoholics, there is no consensus that food cravings (including chocolate cravings) qualify as formally addictive. Chocolate contains two analogues of anandamine (which is similar to the cannabinoid responsible for euphoria from cannabis), but any such association from eating chocolate is likely to be indirect as the analogues actually inhibit the breakdown of the endogenous production of anandamine [19]. Other psychoactive

substances in chocolate (the biogenic stimulant amines caffeine, theobromine, tyramine and phenylethylamine) lack such propensities, being in very low concentrations, in fact too low to have a significant psychoactive effect, and are found in higher concentrations in foods that are not craved. Chocolate cravers or chocoholics themselves identify the orosensory properties as the addictive factor [20].

## 12.3 Neurotransmitter Contributions

Chocolate craving is thought to be driven by desire for hedonic reward, rather than to avoid negative consequences of abstinence. Dopamine, being the predominant neurotransmitter involved within the brain's mesolimbic reward system, is the predominant neurotransmitter released after eating chocolate for the purpose of a pleasurable sensory experience. The mesolimbic dopaminergic system is involved in the body's reward system whereby increased levels of dopamine in the nucleus accumbens are central to mediating the rewards of positive reinforcement for drugs of misuse [21]. The positive reinforcing effects of stimulation are remembered for long periods, underpinned by the activation of the brain dopamine systems. Rewards from both food and drugs may depend on similar substrates for motivational processes [14] with the role of dopamine critical in both anticipation of pleasure as well as in withdrawal [22]. The contribution of the dopaminergic system to chocolate craving and eating is likely however, to be general rather than specific to chocolate.

Emotional eating is characterized by carbohydrate cravings, and is motivated by the comforting effect of opioids (endorphins) to alleviate dysphoria and other negative states. The opioid system appears to play a role in the palatability of preferred foods, releasing opioids such as P-endorphins as food is eaten which can enhance the pleasure of eating [23]. Relevant to such theorising is that Fullerton et al. [24] demonstrated that opioid antagonists can suppress stress-induced eating. Opioids released in response to ingestion of sweet and other palatable foods can increase central opioidergic activity, in turn stimulating the immediate release of beta-endorphin in the hypothalamus and producing an analgesic effect that is naltrexone-reversible [25]. Drewnowski et al. [26] suggested that endogenous opioid peptides may be involved in mediating taste responses and preferences especially for sweet and fatty foods, following a study in which they reported that the ion fusion of the opioid antagonist naloxone reduced caloric intake in binge eaters and also reduced taste preference for sweet and high-fat foods such as biscuits and chocolate, in both binge eaters and in controls. Benton [27] reported that poor mood stimulates eating of palatable high carbohydrate foods leading to endorphin release.

The neurotransmitter systems involved may nevertheless be interactive with functional overlaps. It has been suggested that endogenous opioid peptide (EOP) enhances dopaminergic activity in the mesolimbic pathways to alter the reward value of food [28].

Chocolate invokes an anticipatory and consummatory pleasure but for most people, especially those who consume to comfort eat, any mood state benefits of chocolate are likely to be ephemeral. In a retrospective study, Hetherington and Macdiarmid [20] reported that any mood improvement was during consumption only, with negative moods

returning immediately after eating. In a later study, Macdiarmid and Hetherington [29] reported that chocolate addicts were more depressed than controls, and that their negative mood did not improve after chocolate intake. In both studies, chocolate eating resulted in a slight increase in contentment while eating chocolate but ratings of depression or feeling relaxed were not influenced, although following consumption ratings of guilt were increased.

A depressed mood is not necessarily an abnormal state, being a somewhat natural and normal response to a stressor or stressors but clinical depressive conditions represent abnormal states, states that differ by severity, persistence and several relatively distinctive symptoms. Across both realms, chocolate is commonly craved when individuals are depressed (or stressed or irritable) as a compensatory homeostatic mechanism to settle emotional dysregulation and seemingly particularly likely to be so craved in those whose personality or temperament style is vulnerable to such states of dysregulation. Unfortunately, for those who view chocolate as a mood lifter, such emotional eating fails to produce any substantive or lasting benefit to psychological and mood states, while increased or repeated emotional eating may actually contribute to mood dysphoria. In essence, any benefits are at best as ephemeral as attempting to stop a chocolate from melting away by holding it in one's mouth.

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