Menthol pharmacology and its potential impact on cigarette smoking behavior

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Menthol is the only tobacco additive promoted and advertised by the tobacco industry. Although a considerable body of research has examined the effects of menthol when it is administered alone and unburned, the effects of menthol when burned in cigarette smoke are more complex because it is administered in a matrix of more than 4,000 substances. Therefore, it is difficult to isolate potential pharmacological and toxic effects of menthol when it is administered in a smoke mixture. Menthol properties include cooling and local anesthesia, as well as effects on drug absorption and metabolism, bronchodilation and respiration changes, and electrophysiology. Subjective effects of smoothness and less harshness have been identified as reasons for menthol cigarette smoking, but findings have been inconclusive regarding the effect of menthol on carbon monoxide exposure and smoking topography parameters. Gaps in the research literature and future research areas include the following: (a) What is the role of menthol in tobacco reinforcement and addiction? (b) In the absence of nicotine, is menthol reinforcing? (c) Are the pharmacological and physiological effects of menthol mediated by a menthol-specific receptor or some other central nervous system–mediated action? (d) What are the influences of menthol and menthol metabolism on the metabolic activation and detoxification of carcinogens in tobacco smoke? and (e) Do differences exist in cigarette smoking topography in relation to the interaction of ethnicity, gender, and menthol cigarette preference? Answers to these questions will help to elucidate the function of menthol in cigarettes and its impact on smoking behavior.

Introduction

Tobacco addiction often is what influences the progression of tobacco use and prevents smokers from quitting, thus leading to increased morbidity and mortality from cigarette smoking. Tobacco use causes more than 440,000 deaths each year in the United States (Centers for Disease Control and Prevention [CDC], 2002a). In 2000, smoking prevalence rates were similar by race and ethnicity (Whites = 24.1%, Blacks = 23.2%; CDC, 2002b). However, the majority of Black smokers prefer mentholated cigarettes as evidenced by 76% of Black smokers choosing menthol brands, compared with 23% of White smokers (U.S. Department of Health and Human Services [USDHHS], 1989). More recent data show similar menthol preference rates with 69% of Black smokers and 23% of White smokers reporting menthol cigarette use (Giovino et al., 2004). In addition, the percentage of ever-smokers who had quit was highest for Whites (51%) and lowest for Blacks (37.3%) (CDC, 2002b). Therefore, it is important to examine menthol as one potential factor involved in maintaining smoking behavior.

Menthol may make cigarettes more reinforcing, increase the strength of tobacco addiction, or promote maintenance of cigarette smoking behavior. A number of findings suggest that menthol is involved in tobacco addiction. Black smokers prefer mentholated cigarettes, which are typically higher in tar and nicotine (Federal Trade Commission [FTC], 2000). Also, these cigarettes may deliver more tar and...
nicotine, which could be due to low air dilution of mainstream smoke (Kozlowski et al., 1998). Furthermore, Black smokers have higher levels of cotinine, the primary nicotine metabolite, when compared with Whites and Mexican American smokers (Caraballo et al., 1998; Wagenknecht et al., 1990). Some investigators have found that menthol cigarette use increases cotinine levels regardless of the smoker’s ethnicity (Ahijevych & Parsley, 1999; Clark & Gautam, 1996). In addition, a significant correlation between cotinine and nicotine dependence has been reported (Pomerleau, Pomerleau, Majchrzak, Kloska, & Malakuti, 1990). For example, mentholated cigarette smokers were found to have significantly shorter times to the first cigarette of the day (19 minutes vs. 37 minutes), a widely used measure of nicotine dependence, and higher cotinine levels (239 ng/ml vs. 189 ng/ml) in a study with menthol and nonmenthol smoker groups with a balanced representation of Black and White women (Ahijevych & Parsley, 1999). These findings demonstrate the need for more research on the possible relationship between menthol and tobacco addiction. The fact that menthol cigarettes are higher in nicotine and tar than nonmenthol cigarettes (FTC, 2000) should be considered as a possible confounding factor in future research efforts.

As reported by the tobacco industry, approximately 600 substances are used as cigarette ingredients (Tobacco Reporter, 1994), but menthol is the only one widely advertised by the tobacco industry. Because menthol is classified as generally regarded as safe by the Flavoring Extract Manufacturers Association and is approved for food use by the Food and Drug Administration (FDA) (Opdyke, 1976), the physiological and pharmacological effects of this compound as an additive in tobacco have gained little attention. As purported by the tobacco industry, menthol is added to cigarettes to alleviate harshness and enhance taste and smoothness of cigarette smoke (Perfetti, 1993), but menthol also may aid in the delivery of nicotine and increase the sensory impact of cigarettes (USDHHS, 1998).

Menthol (C_{10}H_{20}O), cyclohexanol-5-methyl-2-(1-methylethyl), the major constituent of peppermint oil, is a monocyclic terpene alcohol that naturally occurs in plants of the Mentha species (i.e., Mentha piperita, peppermint oil; Mentha arvensis, cornmint oil). Menthol has three asymmetric carbon atoms in its cyclohexane ring (Figure 1) and therefore occurs as four pairs of optical isomers: (−) and (+)-menthol, (−)- and (+)-neomenthol, (−)- and (+)-isomenthol, and (−)- and (+)-neoisomenthol (Eccles, 2000). (−)-Menthol is the isomer that occurs most widely in nature and produces the characteristic peppermint odor and cooling sensation when applied to the skin and mucus membranes (Watson, Hems, Rowsell, & Spring, 1978). The other isomers of menthol have a similar odor but do not have the same cooling actions of (−)-menthol (Eccles, 1994, 2000).

Menthol can be applied to cigarettes in a number of ways: It can be applied directly to the tobacco or introduced into the cigarette filter, or it can be applied to the cigarette packaging foil (www.goodhealth.freeservers.com/mentholappliedetc.htm). Because menthol is a volatile compound, the application method influences the extent of its delivery to cigarette smoke. Cigarettes with menthol applied to the filter deliver menthol more efficiently than those with menthol applied to the tobacco (Curran, 1972). The specific method of application used in major U.S. menthol brands (e.g., Kool, Newport, Salem) has not been reported by the tobacco industry. Many synthetic forms of menthol have been developed, which are less volatile and have a longer lasting cooling effect (Perfetti, 1993). These menthol analogs could have an impact on the menthol application and delivery process. A better understanding of menthol application and delivery will help unravel the complexities of how menthol functions in tobacco.

Menthol content varies by product. Best (1993) reported a range of 2.34–2.94 mg menthol per cigarette depending on the age of the cigarette. In our laboratory, we found that Newport menthol cigarettes contained 2.34 mg menthol on average (Ahijevych, Dai, & Chan, 2002) as measured by gas chromatography–mass spectrometry (GC-MS) assay modified from Gelal, Jacob, Yu, and Benowitz (1999). In comparison with mentholated cigarettes, it has been reported that candy lozenges (Altoids, Elmsford, New York) contain approximately 10 mg menthol, and mint tea (Good Earth Peppermint Herb Tea) contains 9.0±1.7 mg menthol/240 ml (Gelal et al., 1999).

Information on menthol delivery is limited. Data from tobacco industry documents show that most major U.S. menthol brands deliver approximately 0.5 mg menthol per cigarette (Cantrell, 1990). Delivery may not necessarily translate into the actual dose consumed by the smoker because dose consumed would depend on the amount of menthol absorbed from the inhaled smoke and how the smoker smokes the cigarette.
This paper reviews what is already known about the pharmacology of menthol as a nontobacco additive and the possible impact of menthol cigarettes on smokers and smoking behavior. Research gaps and future research needs are discussed.

**Physiological impact and pharmacology of menthol**

Menthol is used widely in a number of pharmaceutical and commercial products including tobacco. The compound is used in nontobacco mentholated products because of its minty flavor, aroma, and cooling characteristics, but the reasons for its use in tobacco are not clear. Furthermore, the exact physiological impact of menthol on smoking behavior remains to be understood. In addition to the cooling effects of menthol, it has a number of other documented physiological effects (Table 1), including local anesthetic effects; effects on drug absorption; effects on bronchodilation and respiration; and electrophysiological, central nervous system, and metabolism effects. These effects may explain how menthol functions in tobacco and its physiological impact on smoking behavior (see Table 1).

**Cooling and local anesthesia**

Menthol produces a sensation of coolness in several parts of the body (Green, 1986, 1992; Orani, Anderson, Sant’Ambrogi, & Sant’Ambrogi, 1991; Sant’Ambrogi, Anderson, & Sant’Ambrogi, 1991; Watson et al., 1978) when administered in low to moderate concentrations (Green 1986, 1992). In contrast, menthol in high concentrations has both irritant and local anesthetic effects (Green 1986, 1992). The cooling effects of menthol are attributed to its stimulation of cold receptors in the skin and mucosal surfaces (Hensel & Zotterman, 1951; Watson et al., 1978), whereas menthol is thought to produce its local anesthetic effects by blockade of nociceptors on these same surfaces, thus producing antinociceptive effects (Green 1986, 1992). As described in more detail in the section on electrophysiology, below, menthol has been reported to produce its cooling and local anesthetic effects by inhibiting the efflux of calcium from cold receptors, thus increasing neural discharge by increasing the afferent activity of cold sensors (Schäfer, Braun, & Hensel, 1982; Schäfer, Braun, & Isenberg, 1986; Swandulla, Schäfer, & Lux, 1986).

Until recently, menthol had not been shown to have a receptor site of action by which it produced its sensory effects. New evidence shows that the molecular site of action of menthol is an excitatory ion channel expressed by small-diameter neurons in trigeminal and dorsal root ganglia (McKemy, Neuhausser, & Julius, 2002). This channel has been cloned and is activated by both cold and menthol, indicating that menthol elicits a sensation of cool by serving as a chemical agonist of a thermally responsive receptor. This cold- and menthol-sensitive receptor (CMR1) is a member of the TRP (transient receptor potential) family of excitatory ion channels. Whether these effects of menthol are apparent after inhalation of menthol from smoke is not known. Factors that could affect menthol’s function in tobacco are regulation and distribution of this receptor. For example, several TRP channels are regulated by receptors that couple to phospholipase C (McKemy et al., 2002), a plasma membrane enzyme involved in signal transduction. Whether CMR1 or other menthol-binding sites are expressed in higher brain

### Table 1. Summary of physiological effects from published literature.

<table>
<thead>
<tr>
<th>Physiological effects</th>
<th>References</th>
<th>Possible physiological impact</th>
<th>Possible smoking implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooling and anesthetic</td>
<td>Green, 1986, 1992</td>
<td>Counteract harshness</td>
<td>Permit greater inhalation</td>
</tr>
<tr>
<td>Drug absorption</td>
<td>Kaplan-Frischoff &amp; Touitou, 1997; Kobayashi et al., 1994; Kunta et al., 1997; Shojaei et al., 1999; Benowitz et al., 2002; Madyastha &amp; Srivatsan, 1988; Sellers, 1998; Sellers &amp; Tyndale, unpublished data</td>
<td>Increase absorption and lung permeability of smoke constituents</td>
<td>Increase nicotine intake</td>
</tr>
<tr>
<td>Drug metabolism</td>
<td></td>
<td>Decrease nicotine and cotinine metabolism</td>
<td>Increase addiction potential</td>
</tr>
<tr>
<td>Respiration</td>
<td>Eccles, 1983, 1990</td>
<td>Permit increased lung exposure to nicotine, tar, and other harmful tobacco constituents</td>
<td>Increase addiction and toxicity potential</td>
</tr>
<tr>
<td>Electrophysiological</td>
<td>Eccles, 1994; Schäfer et al., 1986</td>
<td>Increase nerve activity</td>
<td>Enhance tobacco reinforcement</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>Macht, 1939</td>
<td>Stimulant and depressant effects on the central nervous system</td>
<td>Enhance tobacco reinforcement and addiction</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Melikian et al., 2002; Schmeltz and Schlotzhauer, 1968; Zhang et al, 2003</td>
<td>Increase carcinogen production or metabolic activation and exposure</td>
<td>Increase carcinogenic effects</td>
</tr>
</tbody>
</table>
areas also could have important implications on the function of menthol in tobacco. More research on menthol receptor sites will be useful in determining the mechanisms of action of menthol when present in tobacco smoke.

Consistent with the documented cooling effects of menthol, descriptors that smokers use to explain why they smoke mentholated cigarettes are taste, flavor, refreshing, minty, and more taste (Wiseman & McMillan, 1998). Smokers also say that mentholated cigarettes reduce irritating effects, have a soothing effect on the lungs, and are less harsh and smoother. The potential physiological impact that the cooling and local anesthetic effects of menthol could have on menthol cigarette smoking is that it may alleviate the perception of harshness and permit greater inhalation of cigarette smoke.

**Drug absorption**

Menthol, as well as other terpenes, increases the transdermal (Kaplan-Frischoff & Touitou, 1997; Kobayashi, Matsuzawa, Sugiyabayashi, Morimoto, & Kimura, 1994; Kunta, Goskonda, Brotherton, Khan, & Reddy, 1997) and transbuccal (Shojai, Khan, Lim, & Khosravan, 1999) permeability of drugs, demonstrating the absorption-enhancing effects of this compound. Menthol enhances transdermal drug diffusibility by affecting the intracellular lipids or proteins, and increasing the partitioning of drugs into the stratum corneum (Kaplan-Frischoff & Touitou, 1997; Okamoto, Hashida, & Sezaki, 1988; Sasaki, Kojima, Mori, Nakamura, & Shibasaki, 1991).

The mechanism by which menthol influences transbuccal absorption is less clear but appears to enhance the partitioning of drugs across the transcellular pathway (Shojai, et al., 1999). In addition, menthol has been shown to increase salivary flow (Dunér-Engström, Larsson, Lundberg, & Fredholm, 1986). Dunér-Engström et al. (1986) evaluated the effects of nicotine, placebo, and menthol chewing gum on salivation in human subjects. The menthol gum produced a significantly higher amount of stimulated saliva compared with the placebo or nicotine gum. Menthol, by increasing salivary flow, may increase dissolution of drugs in the mouth, thus aiding in the overall absorption of drugs (Clark & Gautam, 1996). If menthol were capable of increasing nicotine absorption across the oral mucosa, then mint-flavored nicotine gum would be expected to deliver nicotine in greater amounts. However, plasma levels of nicotine do not differ between FDA-approved regular- and mint-flavored nicotine gums. Because the FDA requires that both formulations be bio-equivalent with respect to their nicotine dosing (www.fda.gov/ceder/guidance/3616fnl.htm), this information does not prove a lack of an effect of menthol on nicotine absorption.

Because menthol has been shown to increase transdermal and transbuccal absorption of drugs, it is plausible that menthol, when inhaled, increases the diffusibility and permeability of drugs across the lungs as well (Clark & Gautam, 1996; Jarvik, Tashkin, Caskey, McCarthy, & Rosenblatt, 1994; McCarthy et al., 1995; USDHHS, 1998). The effects of menthol on drug absorption may stimulate greater pulmonary absorption and diffusibility of smoke constituents. More research is needed on the dose-response relationship between menthol and nicotine absorption.

**Respiration**

The respiratory effects of menthol have been reviewed extensively elsewhere (Eccles, 1994). Often used as a nasal decongestant, menthol alters perception of breathing patterns, allowing the inhaler to feel that he or she is breathing freely. This increased sensation of airflow has been demonstrated repeatedly in the literature. In a study by Eccles & Jones (1983), total resistance to airflow was measured in 31 subjects before and after a 5-minute exposure to a menthol vapor. The majority of subjects reported an increased sensation of nasal airflow, although menthol inhalation had no measurable effect on nasal resistance to airflow, and no evidence was found to support nasal decongestion by menthol. In a follow-up study by Eccles, Jawad, and Morris (1990), the oral administration of an 11-mg menthol lozenge to subjects suffering from nasal congestion caused a subjective sensation of improved airflow without any change in nasal airway resistance. This effect of menthol is thought to occur via the actions of menthol on cold receptors (Eccles, 1994).

Several reports demonstrate the bronchodilating effects of menthol. The first study identified that the acute inhalation of aromatic vapors (including menthol) improved pulmonary function in volunteers with respiratory tract infections (Cohen & Dressler, 1982). However, because a mixture of aromatic vapors was used, it was difficult to ascertain whether the effects of the mixture were due to menthol. In a more recent study, menthol was shown to attenuate capsaicin- and neurokinin A-induced bronchoconstriction in guinea pigs. In addition, menthol relaxed both acetylcholine and potassium chloride preconstricted bronchi (Wright, Laude, Grattan, & Morice, 1997). A dual mechanism of action for the bronchodilating effects of menthol was proposed, via inhibition of smooth muscle contraction and inhibition of sensory afferents, which might be due to the effects of menthol on calcium conductance.

Other reported respiratory effects of menthol include the surfactant qualities of menthol as demonstrated via in vitro studies on synthetic surfactant film, whereby menthol lowered surface tension (Zanker,
This isolated effect of menthol may, however, be altered in the presence of other cigarette smoke constituents. Menthol also has been shown to significantly increase breath-hold time. A study by Sloan, DeCort, and Eccles (1993) found significantly prolonged breath holding as an involuntary response to menthol inhalation. Breath holding has been shown to increase the onset of central nervous system effects and increase the deposition of tar in the lungs of marijuana smokers (Matthias, Tashkin, Marques-Magallanes, Wilkins, & Simmons, 1997). Similarly, involuntary breath holding may increase uptake of inhaled tobacco smoke constituents from the alveoli of the lungs into the bloodstream. Finally, in the presence of menthol and other essential oils (eucalyptus and pine needle oil), cilia beat frequency is significantly reduced in vitro (Riechelmann, Brommer, Hinni, & Martin, 1997). Cilia, hairlike processes projecting from epithelial cells such as in the bronchi, propel mucus and dust particles from the pulmonary tree. Cigarette smoke (Kitamura, 1987) and menthol may reduce this capability to clear lung airways. The respiratory effects of menthol could potentially permit increased lung exposure to nicotine and toxic smoke constituents including smoke carcinogens.

**Electrophysiological effects on calcium conductance**

One of the main effects of menthol is its ability to produce a sensation of coolness and warmth by stimulating thermoreceptors (i.e., cold and warm receptors) on free nerve endings (Eccles, 1994). This effect of menthol is due to its electrophysiological effects on calcium conductance. It is well known that changes in the calcium concentration around thermoreceptors cause changes in the sensation of temperature (Eccles, 1994). Electrophysiology studies show that the calcium-chelating agent EDTA can increase electrical discharge from cold receptors by decreasing external calcium concentrations and slowing the efflux of calcium from the cold receptor (Eccles, 1994). Menthol exerts its effect on cold receptors by acting in the same manner as EDTA by interfering with the movement of calcium across the cell membrane.

For example, studies show that intravenous infusion of menthol causes an increase in the electrical discharge of cat nasal and lingual cold receptors (Schäfer et al., 1986). This effect of menthol was blocked by intravenous infusion of a calcium solution, indicating that menthol caused receptor depolarization and increased nervous discharge by inhibiting the efflux of calcium from the cold receptor (Schäfer et al., 1986). Other effects of menthol such as bronchodilation, respiration, and central nervous system effects also have been attributed to the effects of menthol on calcium conductance. Calcium is essential for nerve impulse conduction because it aids in the release of neurotransmitters. If menthol affects calcium conductance in nerve cells containing neurotransmitters involved in drug reinforcement, then menthol may affect smoking behavior by enhancing the reinforcing effects of tobacco.

**Central nervous system effects**

Menthol is recognized mainly for its local effects. However, menthol also produces effects in the central nervous system as demonstrated by its ability to produce both stimulant and depressant behavior in mice (Macht, 1939). In this study, when high doses of menthol were administered orally to animals, the animals first became excited and this initial stimulation was followed by a depression of activity, unconsciousness, and then coma.

It could be postulated that the stimulant and depressant effects of menthol on the central nervous system could affect smoking behavior by enhancing the reinforcing and addictive effects of cigarettes. However, the central nervous system effects of menthol cigarettes have not been documented and are highly speculative because it is difficult to isolate the effects of menthol in a cigarette smoke mixture.

**Toxicity**

Little is known about the toxicity profile of menthol, especially when it is burned in conjunction with tobacco. In one study, the pyrolysis of menthol produced a potent carcinogen, benzo[a]pyrene (Schmeltz & Schlotzhauer, 1968). This study identified the pyrolysis products of menthol at two different temperatures, 600°C and 860°C, the latter being the burning temperature of tobacco. After pyrolysis at the lower temperature, most of the menthol (78%) was unchanged; pyrolysis of menthol at the higher temperature resulted in the formation of pyrene, benzo[a]pyrene, and benz[a]anthracene. In contrast to the above findings by Schmeltz and Schlotzhauer, tobacco industry studies found no carcinogen formation after the pyrolysis of menthol. In a study conducted by Philip Morris, little pyrolysis and combustion of menthol occurred during a cigarette puffing procedure (Jenkins, Newman, & Chavis, 1970). In this study, the menthol was unchanged in mainstream smoke, with a mainstream compositional value of 98.9%.

Lorillard Tobacco Company conducted a 13-week inhalation study designed to compare biological responses (i.e., serum nicotine, serum cotinine, carboxyhemoglobin, and nasal discharge) in rats after smoke exposure from mentholated and nonmentholated cigarettes. No significant differences were found in these parameters between the two types of
cigarettes, indicating that menthol does not alter the biological effects attributable to smoke exposure in rats (Gaworski et al., 1997).

The last two tobacco industry studies described suggest that the combustion of menthol does not produce carcinogens or other significant toxic effects. The negative findings from the industry studies on menthol toxicity are not consistent with those from the Schmeltz and Schlotzhauer study. However, they are consistent with data from the National Cancer Institute (1979) that found no evidence of menthol carcinogenicity in rats and mice. This bioassay of D.L-menthol for possible carcinogenicity was conducted by administering menthol in the feed of rats and mice over several weeks. No significant differences were found in tumor development between the treated and control animals.

To our knowledge, the Schmeltz and Schlotzhauer study is the only one that has demonstrated the formation of a carcinogen when menthol is pyrolyzed. Further research is warranted to determine if pyrolysis of menthol increases the carcinogenic effects of cigarette smoking.

**Cardiovascular and subjective effects**

Both menthol and nonmenthol cigarettes produce significant increases in heart rate, as demonstrated in a study that examined the psychophysiological (i.e., electroencephalogram [EEG] and heart rate) and subjective effects of menthol denicotinized cigarettes. This cross-over design study was balanced across 22 participants (Pritchard, Houlihan, Guy, & Robinson, 1999). No significant subjective effects were found for menthol, and little evidence of the central pharmacological effects of menthol was obtained as determined by EEG measurements. Consistent with these findings, a human laboratory repeated-measures design study with 36 participants compared menthol and nonmenthol cigarettes across three levels: commercial average nicotine yield cigarettes, and research high and low nicotine yield cigarettes (Pickworth, Moolchan, Berlin, & Murty, 2002). Results indicated a higher heart rate postcigarette in commercial and high nicotine yield cigarettes. Thus, nicotine was found to have a significant effect on heart rate, but no main effect was found for menthol. Systolic and diastolic blood pressure response was significantly lower in low nicotine yield cigarettes regardless of menthol composition. Carbon monoxide change postcigarette was similar across all cigarette types. Nicotine delivery, not menthol flavoring, determined subjective ratings of strength (Pickworth et al., 2002). These two studies indicate no evidence of cardiovascular effects of menthol.

**Menthol metabolism**

The interaction of menthol and other terpenes with microsomal enzymes in the liver has important pharmacological implications. These enzymes are responsible for the metabolism of terpenes, and paradoxically, these same enzymes are induced by repeated terpene administration. In other words, terpenes, after repeated administration, are capable of accelerating their own metabolism by increasing the metabolic activity of the enzymes responsible for their metabolism. Thus, chronic treatment with a terpene, such as menthol, can accelerate its own metabolism, lower its blood levels, and decrease its effect on enzyme induction. In a study by Madyastha and Srivatsan (1988), repeated oral administration of l-menthol (800 mg/kg/day) to rats for 3 days resulted in an increase of both liver microsomal cytochrome P-450 content and NADPH-cytochrome c reductase activity by almost 80%. Tolerance developed to this effect as further treatment of menthol for up to 7 days reduced enzyme levels considerably (17% and 35% for cytochrome P-450 and NADPH-cytochrome c reductase, respectively) from the initial increase. These findings indicate that the repeated administration of a high dose of menthol initially produced an induction of drug-metabolizing enzymes. This effect increased menthol metabolism and decreased blood levels of menthol. As a result, the effects of menthol on enzyme induction administered under the present conditions were less pronounced over time. In this study, menthol was administered orally and the dose used was much higher than the dose that could potentially be consumed by a pack-a-day smoker (approximately 10 mg; Cantrell, 1990). Because tolerance develops rapidly to this effect of menthol, it may have important implications only in the pharmacological effects of other drugs that are metabolized by these enzyme systems during their initial use.

Menthol is metabolized primarily in the liver, forming menthol glucuronide, which is excreted in the urine (Eccles, 1994). Human studies have measured menthol exposure following oral administration of menthol in capsules and in the form of mint candy and mint tea (Gelal et al., 1999; Kaffenberger & Doyle, 1990). In one study, disposition kinetics of menthol were examined in a cross-over placebo-controlled design that compared oral menthol in a capsule and a placebo capsule, as well as mint tea and a mint lozenge (Gelal et al., 1999). In the first and second phases, 12 nonsmoker subjects received 100 mg l-menthol capsule (641 µmol) or a placebo capsule in random order. Subsequently, half of the group received 10 mg (64 µmol) menthol in a mint lozenge and the remaining six subjects drank mint tea (9.0 ± 1.7 mg menthol/240 ml). Blood samples were obtained at 0, 5, 10, 20, 30, 45, 60, and 90 minutes after dosing to assay for menthol and menthol
glucuronide. Because no menthol was detected, menthol glucuronide was used as an indicator of menthol exposure. Average peak plasma menthol glucuronide concentrations were $16.73 \pm 5.53 \, \text{mmol/l}$ after 100 mg menthol capsule and $2.36 \pm 0.74 \, \text{mmol/l}$ after a lozenge or tea. Peak times ranged from 30 to 120 minutes after a menthol capsule and 20 to 60 minutes after a lozenge or tea.

It is difficult to extrapolate from studies of oral administration of menthol to menthol delivered in a complex matrix of more than 4,000 substances during cigarette smoking. Oral menthol administration may be pharmacologically different from menthol administered through inhalation from a burning cigarette. In addition, constituents in cigarette smoke can induce or inhibit metabolism, a factor not occurring with oral menthol administration. For example, nicotine metabolism was inhibited in the presence of cigarette smoke (Benowitz & Jacob, 2000).

Recently, plasma menthol levels following cigarette smoking were reported in a pilot study (Ahijevych et al., 2002; Table 2). Six Black women smoked two cigarettes of their usual menthol brand consecutively during the protocol. Menthol content of a cigarette was an average 2.34 mg, based on GC-MS analysis. Thus, two mentholated cigarettes contained approximately 4.68 mg (30 µmol) menthol, about one-half of the menthol dose in a lozenge or tea (Gelal et al., 1999). Blood samples for menthol and menthol glucuronide were drawn 1 minute prior to the participant’s first cigarette; at the midpoint of the first cigarette rod; upon completion of the first cigarette; at the midpoint of the second cigarette; upon completion of second cigarette; and 3, 10, 15, 30, 45, and 60 minutes after completion of the second cigarette. Puffing pattern was not controlled, as menthol exposure resulting from natural smoking was being evaluated. Average peak plasma menthol glucuronide concentration of 0.60 µmol/l occurred 3–15 minutes after completion of the second cigarette. These concentrations obtained via cigarette smoke inhalation were lower, and peak time ($T_{\text{max}}$) was significantly shorter than those reported in subjects following oral administration of menthol (Gelal et al., 1999; see Table 2). These preliminary findings suggest a short half-life. The plasma half-life of menthol glucuronide following cigarette smoking was 11.7 minutes, compared with 56 minutes after a menthol capsule and 43 minute after a mint lozenge or tea (Gelal et al., 1999). The 24-hour urinary menthol glucuronide/cigarette (uncorrected for creatinine concentration) ranged from 193 ng/ml to 389 ng/ml per cigarette ($0.58 \pm 1.17 \, \text{µmol/l}$) with a dose-response effect with number of cigarettes per day (Ahijevych et al., 2002). Urinary menthol glucuronide may be a useful marker for assessing menthol exposure in future research. Urinary menthol concentrations could potentially be assessed to examine possible relationships between menthol and concentrations of biomarkers of toxic smoke constituents such as tobacco-specific carcinogens.

### Menthol and nicotine metabolism

Sellers (1998) postulated that menthol and possibly other yet-to-be-identified substances found only in menthol cigarettes may influence nicotine and cotinine metabolism. Sellers posited that menthol might competitively inhibit glucuronidation of cotinine and nicotine and possibly cytochrome P-4502A6 activity for cotinine and nicotine metabolism, which could slow nicotine and cotinine metabolism in menthol smokers. A preliminary examination (Sellers & Tyndale, unpublished data) indicated that menthol can inhibit nicotine metabolism with a moderate affinity ($K_i = 20–40 \, \text{µM}$) but one that is higher than the affinity of nicotine metabolism to cotinine ($K_m = 60 \, \text{µM}$) and much higher than the affinity of cotinine to 3-OH cotinine ($250 \, \text{µM}$) (Ahijevych, Tyndale, Dhatt, Weed, & Browning, 2002). This finding suggests that menthol may be able to inhibit the metabolism of cotinine more extensively than nicotine, which could lead to the accumulation of cotinine in menthol cigarette smokers.

The impact of menthol on nicotine metabolism during cigarette smoking in humans was examined by Benowitz, Herrera, and Jacob (2002) in a cross-over design study with 14 subjects (7 Black and 7 White). During the 3-week study, all participants smoked regular (nonmenthol) cigarettes the first week and then were randomly assigned to menthol or regular

### Table 2. Menthol glucuronide pharmacokinetics comparing oral and cigarette smoke menthol exposure (mean ± SD).

<table>
<thead>
<tr>
<th>Menthol source</th>
<th>Peak plasma concentration (mmol/l)</th>
<th>$T_{\text{max}}$ (minutes)</th>
<th>Plasma half-life (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral dose $^a$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menthol capsule (100 mg)</td>
<td>$16.73 \pm 5.53$</td>
<td>$61 \pm 26$</td>
<td>$56 \pm 8$</td>
</tr>
<tr>
<td>Lozenge or tea (10 mg)</td>
<td>$2.36 \pm 0.74$</td>
<td>$30 \pm 12$</td>
<td>$43 \pm 16$</td>
</tr>
<tr>
<td>Cigarette smoke $^b$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menthol cigarette rod and filter (unsmoked; 2.34 mg/cigarette $\times 2 = 4.68$ mg)</td>
<td>$0.60 \pm 0.14$</td>
<td>$7.3 \pm 5.1$</td>
<td>$11.7 \pm 4.1$</td>
</tr>
</tbody>
</table>

Source. $^a$Gelal et al. (1999); $^b$Ahijevych et al. (2002).
cigarettes in Week 2, with cross-over to the opposite condition in Week 3. On Day 5 of the second and third weeks, subjects received an intravenous infusion of deuterium-labeled nicotine and cotinine. Total clearance of nicotine was significantly slower in the menthol smoking condition compared with regular smoking; cotinine clearance was not affected. In addition, the nicotine glucuronide–to–nicotine ratio was lower with menthol smoking, indicating a potential effect of menthol on glucuronidation. With limited in vitro and in vivo studies of the effect of menthol on nicotine and cotinine metabolism, definitive conclusions are yet to be determined.

**Menthol and carcinogen metabolism**

The possible role of menthol in tobacco carcinogen conjugation may be an important consideration of potential health risks for mentholated cigarette smokers. An important group of conjugation reactions catalyzed by uridine 5'-diphosphate (UDP)-glucuronosyltransferases (UGTs) may affect detoxification pathways of tobacco carcinogens. Menthol is known to be conjugated by UGT2B7 (Blacker et al., 2000) and could serve as a competitive inhibitor of UGT2B7.

In addition, we have found menthol inhibition of 7-hydroxy-benzo[al]pyrene (BP-7) conjugation by human UGT1A9 of 46% by (+)-menthol and 57% by (+)-menthol with a menthol-to-substrate ratio of 100:1 (Ahijevych et al., 2002). Formation of BP-7 glucuronide was significantly *(p < .05)* reduced from 1,915 pmol/min/mg protein with no menthol present to 1,025 pmol/min/mg protein in the presence of 1,000 uM (+)-menthol (Ahijevych & Morse, unpublished data). The two major UGT enzymes identified as responsible for conjugation of NNAL, a tobacco carcinogen, are UGT2B7 and UGT1A9 (Ren, Murphy, Zheng, & Lazarus, 2000). Therefore, menthol may play a role as a competitive inhibitor of enzymes in the detoxification of NNAL. The UGT isoforms involved in nicotine metabolism have not been identified (Ghosheh & Hawes, 2002; Nakajima, Kwon, Tanaka, & Yokoi, 2002).

Benzo[al]pyrene, one of the earliest identified polycyclic aromatic hydrocarbons (PAHs), is considered a complete carcinogen in that it is carcinogenic and also a tumor promoter (Harvey, 1991). Melikian et al. (2002) used urinary 1-hydroxypyrene (1-OH-P) as a marker of benzo[al]pyrene uptake and metabolic activation of PAHs from mainstream cigarette smoke. Yield of benzo[al]pyrene in mainstream smoke of menthol and nonmentholated brands was similar. Significantly higher 1-OH-P concentrations were identified in the urine of smokers of menthol cigarettes than in the urine of nonmenthol smokers (425 ng/g vs. 259 ng/g creatinine, respectively). Subsequently, a gender difference was reported (Zhang et al., 2003). Male menthol smokers experienced a 2.7-fold higher level of urinary 1-OH-P compared with male nonmenthol smokers. No significant difference was noted in women menthol and nonmenthol smokers. However, most male menthol smokers were Black, whereas male nonmenthol smokers were White. This observation suggests that menthol may enhance uptake of PAHs from mainstream smoke and possibly alter metabolism, or that other factors such as lack of air dilution of mainstream smoke in many menthol cigarettes (Kozlowski et al., 1998) may affect exposure to tar and other mainstream smoke constituents. In addition, synergistic mechanisms of tumor promoters and cocarcinogens are likely to be involved in lung carcinogenesis by tobacco smoke (Rubin, 2001).

**Smoking topography and menthol cigarettes**

Cigarette smoking topography is the unique pattern of smoking behavior for each individual cigarette smoker. Components measured include the volume of each puff (milliliters), puff duration (seconds), puff flow (ml/sec), interpuff interval (seconds), the number of puffs per cigarette, and the length of the unsmoked cigarette butt (National Cancer Institute, 1996). Also of interest are the relationships between cigarette smoke constituent exposure and puffing topography parameters. Short-term exposure variables include pre- and postcigarette carbon monoxide (CO) levels and plasma nicotine concentration. CO and nicotine boost refer to this postcigarette change. Plasma cotinine provides information about exposure over time. Instrumentation to measure smoking topography includes a flowmeter cigarette holder through which the individual smokes a cigarette. Information from the differential pressure transducer is integrated by a dedicated software program to generate data on the parameters identified above.

Menthol may affect cigarette smoking topography and ultimately exposure to cigarette smoke constituents via several mechanisms. Stimulation of laryngeal cold receptors may reduce airway irritation (Orani et al., 1991), breath-hold may be increased in the presence of menthol (Sloan et al., 1993), and transbuccal permeation may be enhanced by menthol (Shojai et al., 1999). However, transbuccal permeation and breath holding are not readily measured with current technology. The surgeon general’s report on tobacco use among minority and ethnic populations stated that mentholated cigarettes may promote lung permeability and diffusibility of smoke constituents (USDHHS, 1998).

Interestingly, only six published studies were identified that examined smoking topography and exposure to smoke among smokers of menthol and nonmenthol cigarettes (Ahijevych, Gillespie, Demirici, & Jagadeesh, 1996; Ahijevych & Parsley, 1999; Caskey
et al., 1993; Jarvik et al., 1994; McCarthy et al., 1995; Miller et al., 1994). On the basis of findings of four of these studies, the surgeon general concluded that mentholated cigarettes were not smoked more intensely than regular cigarettes (USDHHS, 1998). Equivocal findings of the six studies are related to sample size and composition and to varied study designs and smoking topography protocols. Three studies were cross-over designs in which each participant smoked both a menthol and a nonmentholated cigarette, whereas one study examined responses to two levels of menthol in a test cigarette (4 mg and 8 mg) compared with a 0-mg menthol cigarette. And finally, two studies were factorial designs with race and menthol preference as factors. Sample sizes ranged from 10 to 95; four studies had fewer than 30 subjects. Research was designed with only men (four studies) or women (two studies) participants. No studies included both men and women. Generally, samples had a balanced representation of Black and White participants. In four studies, participants were from the community, whereas in three studies the sample was drawn from participants of inpatient drug abuse treatment programs. Protocols varied considerably; in four studies, participants smoked their usually preferred brand ad lib. Smoking parameters controlled in the remaining three studies included rapid smoking (40 ml puff every 15 seconds until aversion), 30-second interpuff interval and total volume of 1,200 ml, and a 15-second interpuff interval protocol (Caskey et al., 1993; McCarthy et al., 1995; Miller et al., 1994).

Evidence was inconclusive regarding the relationship of menthol cigarettes and CO boost pre- to postcigarette, which was measured in all studies. For example, the ratio of carboxyhemoglobin to cumulative puff volume was higher after subjects smoked a menthol cigarette in a cross-over design (Jarvik et al., 1994). In a repeated-measures design, CO boost increased with increasing levels of menthol per cigarette (0, 4, and 8 mg) despite controlled volume of smoke exposure (Miller et al., 1994). Similar CO boost occurred despite fewer puffs in the menthol cigarette condition with fixed interpuff interval (McCarthy et al., 1995). Different CO boost findings were identified in 2 two-factor design studies in which one-half of each ethnic group (Black and White) smoked menthol cigarettes (Ahijevych & Parsley, 1999). Significantly higher CO boost was identified in Blacks compared with Whites, and in nonmenthol vs. menthol cigarette preference in a sample of 37 women (Ahijevych et al., 1996). No race-menthol preference interaction effects were found. However, in a larger two-factor study with 95 women, no significant differences in CO boost were reported (Ahijevych & Parsley, 1999).

Smoking topography was controlled as part of the design in three studies (Caskey et al., 1993; McCarthy et al., 1995; Miller et al., 1994). In the remaining three studies, the relationship of topography and menthol cigarettes varied. Smaller puff volumes, fewer puffs, and lower flow rate occurred with menthol cigarettes among 20 men in a cross-over design (Jarvik et al., 1994). In a two-factor study of 37 women, no significant topography differences by race or menthol preference were found (Ahijevych et al., 1996). However, in a larger two-factor study with 95 women, significantly larger puff volumes were identified in menthol smokers (half of whom were Black) compared with nonmenthol smokers (Ahijevych & Parsley, 1999). In addition, menthol smokers had a significantly shorter time to the first cigarette of the day, an indicator of nicotine dependence, and higher cotinine levels (239 ng/ml and 130 ng/ml, respectively).

In summary, in studies with male participants, CO boost increased with increasing levels of menthol per cigarette, was higher in menthol than nonmenthol cigarettes, and did not change despite fewer puffs at fixed intervals. For women, one study found that CO boost was higher among Blacks and nonmenthol smokers, and another found no differences by ethnicity or menthol preference. Puffing topography among men yielded smaller volumes with menthol cigarettes, whereas among women, no difference was noted in one study and larger puff volumes were observed in menthol smokers in another. No topography studies included men and women participants in the same protocol examining menthol effects; therefore, additional research is needed to clarify potential effects of menthol on topography.

Although concerns exist about the representativeness of topography measures of only one cigarette per subject per condition, data on intrasubject variability was obtained during a 6-day inpatient study (Ahijevych, unpublished data). On Days 1 and 4 of the protocol, women smokers (N=24) were in an ad lib smoking condition of their usual cigarettes. Smoking topography and exposure measurements were obtained on the first cigarette of the day and on a cigarette in each of the following intervals: 10 a.m. to noon, 3 p.m. to 5 p.m., and 8 p.m. to 10 p.m. Repeated-measures analysis of average puff volume per cigarette and total puff volume per cigarette revealed no significant within-subject differences or significant differences by time.

Future research
As reviewed in the present paper, much is known about the effects of menthol as a nontobacco additive. However, our understanding of the effects of menthol as a tobacco product additive is limited. In this review, we attempted to extrapolate the actions of menthol actions as a nontobacco additive to its potential pharmacological and physiological effects in cigarettes. Although these effects are speculative, they can
serve as a basis for ascertaining future research directions on the effects of menthol as a tobacco additive. Areas of warranted research are discussed below.

What is the role of menthol in tobacco addiction and reinforcement?

Limited findings suggest an involvement of menthol in tobacco addiction. Menthol smokers have been shown to score higher on a measure of nicotine dependence (Ahijevych & Parsley, 1999), and Black smokers who prefer mentholated cigarette brands have lower quit rates than White smokers (CDC, 2002b). Industry findings also have shown that menthol is capable of increasing nicotine impact in cigarette smokers (Philip Morris, 1995). These findings provide some support for increased tobacco addiction in mentholated cigarette smokers but are still inconclusive. A more thorough investigation of the relationship between menthol and tobacco addiction is warranted.

In the absence of nicotine, is menthol reinforcing?

Not only is it possible for menthol to enhance the addictive and reinforcing effects of tobacco, but also it may have the ability to produce these effects on its own (i.e., in the absence of nicotine). As mentioned above, tobacco industry research has shown that menthol increases nicotine impact in smokers. This same study showed that menthol is capable of producing increases in impact when administered alone (Philip Morris, 1995). More research is needed to determine if menthol is addictive; such research will have important implications in the development of more specific smoking cessation medications for smokers of menthol cigarettes.

Are the pharmacological and physiological effects of menthol mediated by a menthol-specific receptor or some other central nervous system–mediated action?

A menthol receptor site that mediates the sensory effects of menthol was recently identified at the level of the brain stem and spinal cord (McKemy et al., 2002). However, it has not been determined if menthol produces central nervous system effects via specific binding sites in the central nervous system, especially in those areas that mediate tobacco addiction and reinforcement.

What are the influences of menthol and menthol metabolism on the metabolic activation and detoxification of carcinogens in tobacco smoke?

Preliminary reports of menthol competitively inhibiting NNAL detoxification and leading to higher concentrations of a marker of metabolic activation of PAHs from mainstream smoke warrant laboratory and human studies to further explicate potential interactions of menthol and carcinogens.

Do differences exist in cigarette smoking topography in relation to the interaction of race, sex, and menthol cigarette preference?

Gaps identified in the existing literature of smoking topography and menthol cigarettes are studies that include both men and women (Black and White) in the same protocol to provide more comprehensive information on the influences of menthol preference, race, and gender as well as interactions among these variables. In addition, improvements in technology to accurately measure breath-hold and mouth-hold behaviors during smoking would assist in assessing factors affecting exposure. A mechanism to simulate typical blocking of ventilation holes during topography measures is particularly important with non-menthol cigarettes, because many menthol cigarettes have 0% air dilution (Kozlowski et al., 1998). Use of a portable topography device with menthol and non-menthol smokers would provide data in the home and in social and work environments (Plowshare Technology, Baltimore, Maryland). This equipment will improve longitudinal data collection design vs. the more commonly conducted cross-sectional designs.

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References


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