Review of Animal Models in Carotenoid Research

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ABSTRACT Foods containing provitamin A carotenoids are the primary source of vitamin A in many countries, despite the poor bioavailability of carotenoids. In addition, epidemiologic studies suggest that dietary intake of carotenoids influences the risk for certain types of cancer, cardiovascular disease and other chronic diseases. Although it would be ideal to use humans directly to answer critical questions regarding carotenoid absorption, metabolism and effects on disease progression, appropriate animal models offer many advantages. This paper will review recent progress in the development of animal models with which to study this class of nutrients. Each potential model has strengths and weaknesses. Like humans, gerbils, ferrets and preruminant calves all absorb β-carotene (βC) intact, but only gerbils and calves convert βC to vitamin A with efficiency similar to that of humans. Mice and rats efficiently convert βC to vitamin A but absorb carotenoids intact only when they are provided in the diet at supraphysiologic levels. Mice, rats and ferrets can be used to study cancer, whereas primates and gerbils are probably more appropriate for studies on biomarkers of heart disease. No one animal model completely mimics human absorption and metabolism of carotenoids; thus the best model must be chosen with consideration of the specific application being studied, characteristics of the model, and the available funding and facilities. J. Nutr. 129: 2271–2277, 1999.

KEY WORDS: • carotenoids • animal models • gerbils • ferrets • preruminant calves

The experimental evaluation of carotenoids is becoming increasingly common. Epidemiologic studies indicate that an increased intake of fruits and vegetables that contain carotenoids is associated with a decreased risk of many types of cancer including lung, breast and those affecting the gastrointestinal tract (Block et al. 1992, Mayne 1996, Steinmetz and Potter 1996), a decreased risk of cardiovascular disease (Kohlmeier and Hastings 1995), a decreased incidence of age-related macular degeneration (AMD)4 (Snodderly 1995) and a decreased incidence of xerophthalmia in areas with low vitamin A (VA) intake (Fawzi et al. 1993). Consumption of certain fruits and vegetables has also been associated with a decreased risk of prostate cancer (Giovannucci et al. 1995), and β-carotene (BC) supplementation has been shown to enhance natural killer cell activity in elderly men (Santos et al. 1996). In contrast, it has been reported that supplementation of BC either with or without VA to high risk populations may increase the risk of lung cancer (Albanes et al. 1996, Omenn et al. 1996). These epidemiologic associations between carotenoid intake and risk of disease should be addressed to determine whether the carotenoids themselves are protective, or if carotenoids are markers for other protective factors in foods.

Figure 1 outlines the various steps required to release carotenoids from the food matrix, for carotenoid uptake by intestinal mucosal cells, and subsequent absorption, transport and metabolism. Mechanisms regulating the following processes are not well understood: 1) the release of carotenoids from the food matrix; 2) the solubilization of carotenoids into micelles; 3) the uptake of carotenoids into intestinal mucosal cells; 4) the absorption of intact carotenoids; 5) the cleavage of pro-VA carotenoids within the enterocyte or within other tissues to yield VA; and 6) the tissue distribution, metabolism and recycling of carotenoids (Boileau, T. et al. 1999, Castenmiller and West 1998).

Of the ~600 naturally occurring carotenoids, ~50 have pro-VA activity and can be cleaved to yield retinol. A few of these carotenoids, BC, α-carotene and β-cryptoxanthin, are found in substantial quantities in foods (Olson 1999). β-Carotene has two unsubstituted β-ionone rings, making it the most VA active of the pro-VA carotenoids, capable of producing two molecules of retinol from one molecule of BC. Other pro-VA carotenoids, such as α-carotene and β-cryptoxanthin, can yield only one molecule of retinol per molecule of carotenoid. β-Carotene is commonly studied in part because it is abundant in a variety of fruits and vegetables and has the greatest potential to prevent VA deficiency, the leading cause of blindness in the world.

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Digestion, absorption and metabolism of carotenoids. As a result of their lipophilic nature, carotenoids are often found complexed in the food matrix with protein, lipids and/or fiber. There are several steps (1-5) necessary for carotenoid absorption to occur: 1) the food matrix must be digested; 2) carotenoids must be released and combined with lipids and bile salts to form micelles; 3) the micelles must move to the intestinal brush border membrane so that carotenoids can be taken up and 4) transported into the enterocyte for subsequent processing; and 5) within the enterocyte, carotenoids can be metabolized, resecreted into the intestinal lumen when the cells turnover or be incorporated into chylomicrons and be secreted into the lymph. Chylomicrons then transport carotenoids to the liver. 6) The action of lipoprotein lipase of extrahepatic tissues may result in extrahepatic tissue uptake of carotenoids by these tissues before hepatic uptake. 7) Chylomicron remnants deliver the majority of absorbed carotenoid to the liver. 8) Carotenoids that are taken up by the liver may be stored there or resecreted in VLDL. 9) Carotenoids in LDL and HDL may also be taken up by extrahepatic tissues, where they may accumulate.

There are several things that must be considered when choosing an animal model for any scientific study. Physiologic similarities of the model to humans with respect to the particular application being studied are very important, yet there are also practical considerations that should be addressed. Table 1 compares animal models used in carotenoid research with respect to some specific applications and their physiologic similarities to humans. Applications compared include the following: carotenoid absorption, carotenoid conversion and metabolism, vitamin A deficiency, cardiovascular disease, immune function, AMD and cancer. The relative desirability of each model for the various applications was estimated, considering both their similarities to humans with respect to the application and their relative use as a model in current research. This table indicates that gerbils, ferrets and preruminant calves would be the best models with which to study carotenoid absorption, metabolism and the potential health aspects of these compounds.

### MODEL SELECTION

Choosing the most appropriate animal model for a study requires careful consideration. We know that humans absorb a variety of dietary carotenoids intact, and that some carotenoids such as βC, β-cryptoxanthin and α-carotene can contribute to the VA status of the individual (de Pee et al. 1998a and 1998b, Olson 1999). Carotenoid involvement in cell-to-cell communication, immune response and reproduction has also been suggested, however the mechanisms involved and the physiologic relevance of these functions are unclear (Olson 1999). The limited knowledge about human carotenoid metabolism makes it especially difficult to choose an appropriate animal model. The ideal model would have the following characteristics: 1) absorb a variety of carotenoids intact at physiologic levels, similarly to humans; 2) have carotenoid distribution in tissues and serum similar to that of humans; 3) represent an appropriate model for the disease state of interest; 4) be readily available; 5) be easily manageable in a laboratory setting; and 6) be affordable. Unfortunately, no one model meets all of these criteria. This paper reviews recent progress in the development of animal models with which to study carotenoid absorption, metabolism and the potential health aspects of these compounds.

### Table 1

<table>
<thead>
<tr>
<th>Animal</th>
<th>Carotenoid absorption</th>
<th>Carotenoid metabolism</th>
<th>Vitamin A deficiency</th>
<th>Cardiovascular disease</th>
<th>Immune function</th>
<th>AMD</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>*1</td>
<td>*</td>
<td>*2</td>
<td>&quot;</td>
<td>****</td>
<td>NA</td>
<td>****</td>
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<tr>
<td>Rat</td>
<td>*</td>
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<td>***</td>
<td>NA</td>
<td>****</td>
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<tr>
<td>Gerbil</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>*</td>
<td>**</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Preruminant calf</td>
<td>****</td>
<td>**</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Nonhuman primate</td>
<td>* to ****3</td>
<td>* to ****3</td>
<td>NA</td>
<td>****</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ferret</td>
<td>****</td>
<td>***</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>NA</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

1 Relative use/desirability: * (not frequently used/less desirable)–***** (frequently used/more desirable); NA, not applicable.
2 This species is not generally used or is not appropriate for this application.
3 These characteristics vary greatly depending on the species of nonhuman primate used.
When conducting any scientific study, it is necessary to have large enough sample groups to achieve statistical power. The Mongolian gerbil (Meriones unguiculatus) is a good model to choose in these situations because they are small, easily maintained in large numbers and readily available. Gerbils also possess several characteristics that make them an appropriate model for carotenoid research.

This laboratory was the first to demonstrate that gerbils absorb βC intact when fed at physiologic levels (Pollack et al. 1994). We found that serum βC peaked 4 h after a βC dose and that βC was found in the liver, spleen, kidney + adrenal, adipose and lung tissue of gerbils (Pollack et al. 1994). Recent work in our laboratory has also shown that Mongolian gerbils absorb dietary lycopene, resulting in accumulation in the liver, adrenal and spleen, and trace amounts in the kidney and heart (Heintz, unpublished data).

Intestinal absorption and tissue accumulation of βC in gerbils has been studied extensively in our laboratory (Lee et al. 1998, Thatcher et al. 1998), and by others (House et al. 1997). It has also been shown that βC contributes to both hepatic and extrahepatic VA stores in Mongolian gerbils (House et al. 1997, Lee et al. 1998, Thatcher et al. 1998) and that gerbils convert dietary βC to VA with efficiency similar to that currently estimated for humans (Lee et al. 1998). It has also been shown that substantial amounts of hepatic βC that had accumulated when gerbils were fed a diet that contained βC were rapidly depleted once βC was removed from the diet, regardless of the VA status of the gerbils or the VA content of the diet (Thatcher et al. 1998). These data suggest that Mongolian gerbils are an appropriate animal model for studying carotenoid absorption, metabolism and the availability of VAs from carotenoid precursors. However, knowledge about the VA requirement of this species is limited, thus comparing the VA value of a compound in this species to humans must be done with caution.

To our knowledge no one has used gerbils to study the consequences of VA deficiency. Studies from our laboratory have shown that it is difficult to produce clinical VA deficiency in this species. Weanling gerbils maintain liver stores of VA (120–138 nmol) even after 84 d of consuming a VA-free diet (Lee et al. 1998, Thatcher et al. 1998). In contrast, hepatic VA stores in weanling rats are negligible after 28–33 d (Corey and Hayes 1972, Gardner and Ross 1993), and rats exhibit clinical signs of VA deficiency after only 35 d of consuming a VA-free diet (Corey and Hayes 1972). Gerbils have the potential to be valuable models for evaluating VA deficiency, but it requires months to deplete hepatic VA stores. This time might be shortened by the development of a second generation, VA-deficiency model, as has been developed for the rat (Gardner and Ross 1993, Roodenburg et al. 1995).

Mongolian gerbils are an established model for cholesterol and lipid metabolism because their serum lipid profile responds to dietary changes similarly to humans (DiFrancesco et al. 1990, Nicolaoli et al. 1981, Pronczuk et al. 1994, Sullivan et al. 1993, Tasker and Potter 1993). Because gerbils also appear to metabolize βC similarly to humans, they may be a promising model with which to evaluate any possible relationship between βC intake and hyperlipidemia, a risk factor for developing coronary heart disease.

Overall, gerbils appear to be an appropriate model with which to study carotenoid absorption and metabolism, and they are a practical species for laboratory research.

**Domestic ferrets**

Gerbils may be a convenient model for many applications; however, they are small. If a study requires surgical manipulation, a larger model, such as the ferret, may be more appropriate. Ferrets may not be as practical as gerbils, due to their cost and housing requirements, but they are still easily managed in a research setting.

Ferrets are larger than gerbils (adult male ferrets weigh ∼1200 g, and adult male gerbils 80 g), and carotenoid absorption can be measured directly by surgical cannulation of the
lymphatics and the portal vein without extreme difficulty (Boileau, A. et al. 1999, Wang et al. 1992). Ferrets are also resilient under halothane anesthesia and can be maintained easily in a surgical plane of anesthesia for several hours in terminal procedures (Boileau, A. et al. 1999).

Domestic ferrets are used in many areas of carotenoid research, including absorption and bioavailability, isomer metabolism and dietary interactions. Our laboratory has developed a pelleted, purified ferret diet containing adequate VA and negligible βC (White et al. 1993b). This diet has been used in our laboratory to standardize dietary treatment and deplete tissues of carotenoids.

Ferrets absorb dietary βC intact and accumulate βC in tissues and serum in a dose-dependent fashion (Ribaya-Mercado et al. 1989). Ferrets also absorb a variety of other carotenoids including α-carotene, lycopene and canthaxanthin (Tang et al. 1993, White et al. 1993a). In addition, ferrets convert βC to VA in the intestine (Wang et al. 1991) and in homogenates of liver, lung and adipose tissue (Wang et al. 1992); tissue distribution of carotenoids in ferrets is also similar to that of humans (Gugger et al. 1992, Ribaya-Mercado et al. 1992 and 1989). Absorption and tissue distribution of βC isomers, specifically 9-cis βC, in ferrets also appear to be similar to what is reported in humans (Erdman et al. 1998).

Conversion of dietary βC to VA in ferrets requires a weight ratio of βC/VA > 15:1 (Lederman et al. 1998), much higher than the estimated 6:1 ratio required for humans (NRC 1989). Therefore, ferrets are not an ideal model with which to evaluate the conversion of dietary βC to VA. Ferrets also have high concentrations of retinyl esters in their serum (Lederman et al. 1998, Ribaya-Mercado et al. 1992). Under normal dietary conditions, humans have negligible concentrations of serum retinyl esters. This suggests differences between ferret and human VA metabolism (Bankson et al. 1986). Despite these differences, ferrets do absorb βC intact and accumulate it in their tissues; they should be an appropriate animal model for studying the biological effects of tissue βC.

The association between βC and lung cancer is currently an active area of carotenoid research. Recently, a smoke-exposed ferret model was used to evaluate the effects of smoke exposure and/or βC supplementation on lung tissue morphology, and plasma βC and retinoid concentrations (Wang et al. 1999). These researchers found increased proliferation of alveolar cells and macrophages, elevated activator protein-1 expression (AP-1 is a transcription factor usually associated with increased lung carcinogenesis), and down-regulation of retinoic acid receptor-β in the lungs of βC-supplemented, smoke-exposed ferrets. These data indicate that ferrets are a good model with which to study the relationship between βC, smoking, and lung cancer, and the specific mechanisms.

Ferrets may also be a suitable model with which to evaluate the possible relationship between carotenoids and stomach cancer. High intakes of fruits and vegetables, which contain carotenoids, have been associated with a decreased risk of stomach cancer (Block et al. 1992, Mayne 1996, Steinmetz and Potter 1996), and a number of studies have suggested that ferrets are a good model with which to study certain types of stomach cancer (Erdman et al. 1997, Fox et al. 1997).

**Preruminant calves**

It is often necessary to obtain larger quantities of tissue and/or multiple samples from the same animal. For these situations, preruminant calves may be a more realistic model. Preruminant calves are monogastric and, like humans, absorb βC intact. It is possible to obtain multiple blood samples, and a liver biopsy procedure that allows multiple sampling of hepatic tissue from this species has been developed recently in our laboratory (Swanson, unpublished data).

It has been demonstrated the preruminant calf is an appropriate animal model for carotenoid research (Bierer et al. 1995, Hoppe and Schoner 1987, Poor et al. 1992). Following a βC-containing meal, preruminant calves demonstrate carotenoid absorption kinetics similar to what has been reported for human infants (Poor et al. 1992). The following characteristics have been demonstrated by preruminant calves: 1) they absorb other carotenoids such as canthaxanthin, lutein, lycopene and α-carotene (Bierer et al. 1995), although βC is preferentially absorbed; 2) dietary βC can be used as a source of VA (Hoppe et al. 1996); and 3) the serum appearance and tissue distribution of 9-cis βC are similar to what has been reported in humans (Bierer et al., unpublished data). Preruminant calves have also been used to evaluate the role of β-carotene in reproductive function (Chew et al. 1984, Wang et al. 1988). Our laboratory is currently in the process of defining the VA requirement of calves (Swanson et al., unpublished data). This information should prove valuable in formulating diets for carotenoid studies.

Preruminant calves are ideal for absorption studies because of the availability of multiple blood and liver samples. However, they are not readily available, they are more expensive than many other models, they are not easily maintained in a laboratory setting because they require special facilities for housing, and they are difficult to obtain in large numbers (sample size), thus creating a challenge in achieving statistical power.

**Rats and mice**

Rats and mice are used in many areas of scientific research, including the study of carotenoids. Specific applications for these species include cancer studies (Sato et al. 1997, Thompson et al. 1993) and studies evaluating immune function (Bendich and Shapiro 1986, Jyonouchi et al. 1994). These rodents are well characterized and there are many strains, including knock-outs and transgenics available that have been established as models for many of the diseases epidemiologically associated with carotenoid intake. However, rats and mice absorb carotenoids differently than humans; thus extrapolation of data to humans must be done with caution.

Rats have been used extensively (Biesalski and Weiser 1993, Grolier et al. 1995, Mittal 1983) to evaluate the efficiency of βC conversion to VA by monitoring changes in liver VA stores. Rats are high efficiency converters of βC to VA at the level of the enterocyte (Fig. 1, step 4). Therefore, they do not readily absorb carotenoids intact (Fig. 1, step 5).

If fed diets containing supraphysiologic levels of carotenoids (≥0.02% of diet), rats can absorb a variety of carotenoids including βC (Krinsky et al. 1990, Ribaya-Mercado et al. 1989, Warmer et al. 1985), canthaxanthin and lycopene (Mathews-Roth et al. 1990), and accumulate βC in tissues in a dose-dependent manner (Shapiro et al. 1984). (When normalized for body weight, feeding βC at 0.02% of diet is equivalent to a 70-kg person eating 163 carrots per day.) This is in contrast to humans, who absorb small, physiologic doses of a variety of carotenoids, including βC, intact.

Rats and mice may not be the most appropriate models for studying carotenoid absorption and bioavailability, but rats are a desirable model with which to study the effects of VA deficiency. Dietary restriction of VA and βC in rats causes a rapid depletion of hepatic VA stores (28–33 d) and the onset of clinical VA deficiency (35 d) (Corey and Hayes 1972,
Gardner and Ross 1993). A second-generation model has also been developed in this species, resulting in more rapid development of VA deficiency, (Gardner and Ross 1993, Roedenburg et al. 1995).

Despite shortcomings, rodents are used extensively to evaluate the effect of dietary carotenoids on the development of cancer (Moon 1989, Nagasawa et al. 1995, Nairn et al. 1996 and 1998, Schwartz and Shklar 1987, Youping et al. 1997) and immune function (Bendich 1989, Bendich and Shapiro 1986, Jyonouchi et al. 1994, Lingen et al. 1959, Rosales and Ross 1998). Generally, high levels of dietary carotenoids are fed in these studies to achieve adequate tissue levels. Therefore, relevance to humans must be considered with caution.

Nonhuman primates

Nonhuman primates have been used to evaluate carotenoid accumulation in the macula of the eye (especially lutein and zeaxanthin) to study age-related macular degeneration because they have a macula lutea similar to that of humans (Handelman et al. 1992, Snodderly et al. 1991). These species have also been used to study many aspects of cardiovascular disease (Bond et al. 1980, Williams et al. 1991), making nonhuman primates a possible model with which to study the association between carotenoids and cardiovascular disease. However, there are differences in carotenoid absorption between different species of nonhuman primates compared with humans. Primate species include both high and low absorbers of carotenoids (Krinsky et al. 1990, Slikha et al. 1999, Snodderly et al. 1990). Thus, care must be taken when choosing the species of nonhuman primate to be used.

Advantages to using these species include the following: 1) they are more closely related to humans than other models from an evolutionary standpoint; and 2) it is possible to obtain multiple blood samples from the same animal.

Despite their close relationship with humans, nonhuman primates are not commonly used because they are extremely expensive, they are not easily managed in a laboratory setting, they require special facilities and their availability is limited.

Other species

Many other animal species have been, or are currently being used in carotenoid research. Pigs have been used to evaluate BC absorption and uptake (Chew et al. 1991) and the role of BC on reproductive function (Chew 1993). However, it has also been reported that this species does not absorb appreciable quantities of intact BC and is not a good model with which to study postabsorptive metabolism of BC (Poor et al. 1987). Hamsters have been used for carotenoid studies investigating cancer (Schwartz and Shklar 1987) and toxicology (Beems et al. 1987). Chicks have been evaluated as a carotenoid bioavailability model (Erdman et al. 1986, Poor et al. 1987), and have been used for absorption studies (Tyczkowski and Hamilton 1986). Rabbits have been used to evaluate carotenoid absorption and distribution (Yap et al. 1997), and the effect of BC on the development of atherosclerotic lesions (Sun et al. 1997). Dogs are currently being used to study carotenoids and immune function (Chew et al. 1998, Kim et al. 1998). Quail, a relatively new model, has been used to study carotenoids and AMD because the quail retina is similar to the human macula. Because this species ages rapidly, disease progression is accelerated (Dorey et al. 1998, Kunert et al. 1997, Toyoda et al. 1996).

SUMMARY

Choosing the most appropriate animal model for a specific application can be challenging. No one animal model completely mimics human absorption and metabolism of carotenoids. The best model must be chosen considering the specific application, characteristics of the individual models, and the available funding and facilities. It may also be desirable to verify findings in more than one species to increase confidence in extrapolating the results of animal studies to humans.

LITERATURE CITED


Erdman, J. W., Jr., Thatcher, A. J., Hofmann, N. E., Lederman, J. D., Block, S. S.,


