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THE POTENTIAL ROLE OF BIOTIN INSUFFICIENCY ON ESSENTIAL FATTY ACID METABOLISM AND CARDIOVASCULAR DISEASE RISK

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ABSTRACT

Among the many contributors to the development of cardiovascular disease are inadequate levels of essential fatty acids. Although the prevalence of essential fatty acid deficiencies (EFAD) have rarely been regarded as problematic in developed nations, studies have shown that insufficient levels of essential fatty acids (EFA) can impact the etiology of cardiovascular disease. Numerous animal studies have shown that clinical symptoms resulting from biotin deficiency are similar to that of EFAD. Many of these studies have also shown low biotin bioavailability in cereal grains. Because the USDA Food Guide Pyramid encourages high carbohydrate intakes and lower consumptions of fat, oil, and protein, not only may EFA level be compromised, but adequate absorption of biotin could be overestimated as well. While static biotin measurements have lead many to believe that nutritional status is adequate, functional biotin status may be accommodated. Due to the coenzymatic activity of biotin in the holocarboxylase complexes, insufficient amounts of exogenous biotin could affect elongation and desaturation of EFA, contributing to endothelial cell dysfunction. The authors suggest that there may be a substantial link between cereal grain intakes and cardiovascular disease stemming from both biotin and EFA insufficiencies.

KEY WORDS: Biotin, Essential Fatty Acids, Bioavailability, Cardiovascular Disease, Cereal Grains, Nutritional Status

INTRODUCTION

Widespread prevalence of CVD in the U.S.:

Cardiovascular disease (CVD) is recognized as the major contributor to morbidity and mortality in the in the United States, with over 7 million people affected. In 1990, one in every three deaths was attributed to CVD, corresponding to a total number exceeding 934,300 (1).

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With smoking, obesity, hypertension and physical inactivity identified as the major risk factors for CVD, recent scientific investigations have examined additional factors which contribute to the development of this disease. Homocysteine and essential fatty acid levels are both related to the evolution of CVD, presumably by disrupting endothelial cell function (2). While homocysteine levels can usually be controlled with adequate intakes of vitamins B_{12}, B_6, and folic acid (3), essential fatty acid nutritional status is commonly overestimated and overlooked. There is a growing body of evidence indicating that essential fatty acids play a substantial role in the development of CVD.

Relationship between EFAD and biotin status:

Although frank essential fatty acid deficiency (EFAD) has been well studied, and recognized to be a minor problem in the United States, more recent studies have found that essential fatty acid (EFA) profiles vary considerably among the national population and may contribute to both hypertriglyceridemia and hyperlipidemia. Fatty acid profiles from more than 500 subjects participating in the Framingham Heart Study showed that more than 20% had evidence of ω3 or ω6 fatty acid deficiency (4). Further, more than 10% of subjects from the Framingham Offspring Study exhibited biochemical markers of essential fatty acid insufficiency (EFAI)*. Consequently, EFAI has become a prevalent nutritional deficiency (5). Additionally, prospective and retrospective studies have shown that EFAI are highly predictive of CVD, with one of numerous mechanisms possibly triggering elevations in low-density lipoproteins and reductions in high-density lipoproteins (5,6).

Multiple studies have shown similar morphological changes related to both EFAD and biotin deficiency (7,8). These similarities suggest a strong association between EFA and biotin metabolism. The more commonly reported resemblance between the two deficiencies included scaly dermatitis, alopecia, and impaired growth (9-12).

BIOTIN BIOAVAILABILITY

Both marginal and frank biotin deficiencies have rarely been reported in the United States. There remains an incomplete knowledge of biotin bioavailability in foods, while biotin nutritional status may have been inappropriately deemed sufficient. In addition to dietary biotin (which appears to be ubiquitous in nature), it has long been recognized that intestinal microflora possess the ability to provide biotin to the host organism. Examination of the literature suggests that biotin nutritional status has previously been overestimated and certain populations may be at high risk for developing biotin insufficiencies.

Methods for assessing biotin bioavailability:

Nutrient bioavailability is commonly assessed by examining its absorption and utilization. Biotin availability is usually determined by methods involving microbiological determination (using *Lactobacillus arabinosus/plantarum*) and/or isotopic dilution of [^{14}C] biotin by extracted

* The term insufficiency has been previously defined as the prevalence of biochemical markers or indicators of a deficiency in the absence of classical signs of that particular deficiency[3]

sample-unlabeled biotin. Biotin digestibility is commonly determined by means of ileal and fecal analysis methods.

Biotin content in cereal grains:

Biotin content in cereal grains vary, but are aggregately very low compared to protein sources (13). Studies have shown that although biotin content appears high in products such as corn (45-94 μg/kg) and barley (110-200 μg/kg), individual variations encompass a wide range (14). Additional evidence shows that although biotin content of some cereal grains in high, absorption and/or digestion is relatively low (14).

Digestibility[†]/absorption of biotin:

Although the content of biotin in cereal grains appear to be high, digestibility and absorption are commonly very low. One study found that corrected ileal digestibilities for soybean meal, canola meal, barley, corn and wheat were 55.4, 3.9, 4.8, 4.0, and 21.6%, respectively (14). Sauer et al. (14) reported that although biotin content of canola meal is quite high, only slight amounts were absorbed. The same study found that only 8-18% of microbially derived biotin was absorbed. Another study found that the apparent digestibilities of biotin in wheat, sorghum, barley, and soybean meal were -3, -123, 18, and 12% in pigs, and -10, -73, 5, and 28% for the same grains in chickens (15).

Kopinski et al. (15) suggest that poor digestion of biotin in cereal grains, particularly sorghum and wheat diets, stems from the presence of unabsorbable biotin complexes. Antinutrients (e.g. tannins in sorghum) are resistant to proteolytic enzymes and decrease absorption of biotin (15).

Bioavailability[‡] of biotin in cereal grains:

Numerous studies have reported low biotin availability in cereal grains (15-18). Table 1 shows the various reports on the bioavailability of biotin in selected cereal grains. Bioavailability of biotin in barley (11-24%), oats (32-48%), sorghum (19.5-39%), triticale (15.9-25.9%), and wheat (0-33.3%) are consistently low, while corn appears to be quite high (95.2-133%, Table 1). It is of interest to note that although bioavailability of biotin from corn was reported at 114% in Blair and Misir's 1989 study, 78.9% of those chicks on the corn diet developed skin lesions along with a 20.8% incidence of mortality (18). Likewise, 71.4% of chicks on a barley diet developed skin lesions, while 100% of those on the wheat diet developed skin lesions concurrent with a 25% incidence of mortality (18).

Biotin bioavailability seems to be consistent in the previously mentioned studies regardless of the species utilized. Studies using rats report similar findings as those reported here, while a recent paper reported that biotin metabolism in rats is similar to that in humans (19). This allows for inferential conclusions to be made from animal studies to human status.

[†] Digestibility is defined as the difference between the amount of the nutrient in the diet and in ileal digesta or feces, divided by the amount in the diet [23]

[‡] Availability is the proportion of the nutrient in the diet that is absorbed in a form suitable for utilization as measured by the slope-ration method [23]

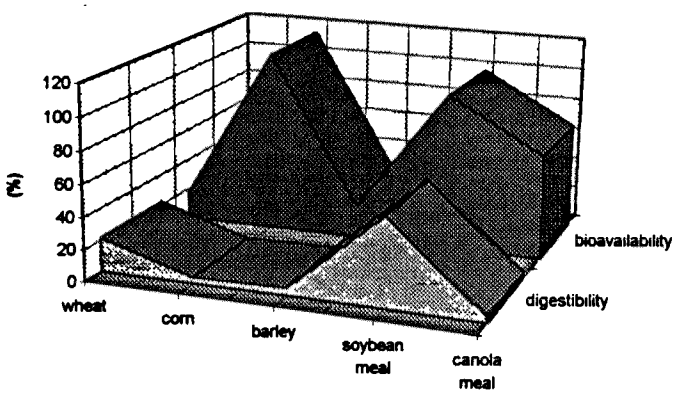
Table 1. Comparison of studies analyzing biotin bioavailability (%) in foodstuffs

	Author(s)					
	Frigg (1976)*	Whitehead et al. (1982)*	Frigg (1984)*	Misir & Blair (1987)#	Misir & Blair (1988)**	Misir & Blair (1989)*
Grains						
Barley	20	11	21.6	19.2	24.0	21
Canola meal	-	62	-	65.4	70.9	66
Corn	107	133	-	95.2	101.2	114
Oats	32	-	40.8	-	-	-
Sorghum	19.5	-	24.5	29.5	25.1	39
Soybean meal	-	108	-	76.8	85.5	98
Triticale	-	-	-	15.9	25.9	20
Wheat	0	5	4	17.0	33.3	17

* chickens, # turkeys, ** swine

Fig. 1

Comparison between biotin bioavailability and digestibility in cereal grains



Data from Sauer et al. (28) and Blair and Misir (14)

Figure 1 shows the discrepancies surrounding studies which have examined bioavailability and digestibility of biotin in some cereal grains. The most obvious incongruity concerns the bioavailability and digestibility of corn and canola meal. It is also clear that digestibiltiy and bioavailability of biotin in both wheat and barley is extremely low. These conflicting data suggest that biotin nutritional status is inadequately defined and warrants further research.

BIOTIN DEFICIENCY

Common reports of biotin deficiency

The most common cases of biotin deficiencies stem from abnormalities in biotinidase activity and inherited disorders associated with biotin-dependent enzymes. Impaired biotin status also frequently occurs during normal human gestation (19). The enzyme biotinidase cleaves endogenous carboxylase-bound biotin, as well as exogenous dietary protein-bound biotin, which is then liberated into the free biotin pool. Cases have been documented showing inadequate biotinidase levels which result in secondary biotin deficiencies (7,20). In these individuals, protein-bound biotin cannot be cleaved from its respective holocarboxylase enzyme, and is therefore subject to lysosomal degradation and excretion.

Inherited disorders of biotin metabolism have also been reported. Individuals expressing multiple carboxylase deficiencies exhibit insufficient activity of the mitochondrial enzymes proprionyl CoA carboxylase (PCC), pyruvate carboxylase (PC), β-methylcrotonyl CoA carboxylase (MCC), and the cytosolic enzyme acetyl CoA carboxylase (ACC). Because all of these enzymes require biotin as a coenzyme, inborn errors in biotin metabolism result in greatly increased requirements of this B vitamin. Previous studies have suggested that these disorders are caused by defective biotin transport and/or holocarboxylase synthetase, the enzyme which binds biotin to the apocarboxylase (21).

Decreases in biotin-dependent enzyme activity

Biotin deficiency commonly results in the decreased activities of the carboxylase enzymes PC, MCC, PCC, and ACC. The B vitamin, biotin, serves as a coenzyme for three enzymes involved in fat synthesis: PC, which catalyzes the conversion of pyruvate to oxaloacetate, and regenerates NADPH, PCC, which metabolizes proprionyl CoA, a precursor for odd-chain fatty acid biosynthesis, and ACC, which is involved in the initial step in fatty acid biosynthesis (22,23). Suchy and colleagues (22) demonstrated that PC, MCC, and ACC activities were all decreased in biotin deficient rats by 12, 12, and 18%, respectively.

POTENTIAL PREVALENCE OF BIOTIN INSUFFICIENCY IN THE AMERICAN DIET

Overestimation of current biotin status:

Studies have shown that oral biotin supplementation in humans result in increased leukocyte carboxylase activities by approximately 200% (34). These data suggest that biotin intake in normal diets may be adequate, however, suboptimal (22). Investigators of another study concluded that the 'safe and adequate' RDA for biotin is higher than is possible to achieve with a 'typical' American diet deplete of organ or glandular meats, or a biotin supplement (25). In fact, no definitive studies have been conducted determining human biotin requirements.

Accurate approaches in the determination of biotin nutritional status remain unsettled. Many human studies continue to analyze urine, plasma, and serum biotin in attempts to determine nutritional status. However, animal studies consistently find significant levels of biotin excreted in the feces. Additionally, studies examining multiple-carboxylase deficiencies agree that PC activity is a solid indicator of functional biotin status (23). Controversy also exists regarding the relative

contribution of total, free, and protein-bound biotin in estimating biotin status (22,23). Although primary and secondary biotin deficiencies are rarely reported in the United States, the potential prevalence of biotin insufficiencies should be of great concern. Although deficiency symptoms may lead many to assume that biotin status is not problematic in the United States, unnoticed long-term biotin insufficiencies will result in the accommodation of biochemical and physiological processes. Impaired carboxylase activity may affect EFA profiles and predispose individuals to future endothelial cell dysfunction.

Increased risks of insufficiencies on high carbohydrate diets:

Not only may a great proportion of the population harbor EFAI, but adherence to the USDA food guidelines may further contribute to inadequate biotin status and EFA profiles (5). Feldman and Wolf (21) suggest that dietary supplementation with fatty acids, along with pharmacological doses of biotin, should be administered to those individuals exhibiting multiple-carboxylase deficiencies. This same advice should be considered for those individual on a high carbohydrate/low fat diet where both biotin bioavailability and EFA intakes may be low. Because eggs, glandular meats, and whole milk are some of the best sources of biotin, diets which commonly exclude these products (high carbohydrate/low fat) place individuals at a higher risk for developing biotin insufficiencies (26). Evidence also indicates that both lactose and sucrose ingestion decrease bacterial synthesis of biotin, suggesting that eggs and glandular meats are the best natural sources of biotin (27).

On a high carbohydrate/low fat diet, consumption of foods which contain trans-fatty acids may further compound EFAI by inhibiting Δ^5 and Δ^6 desaturases (28). Additionally, studies have shown that glucose, and other products of carbohydrate metabolism, decrease Δ^6 desaturase activity, while protein and amino acid ingestion increases Δ^5 and Δ^6 desaturase activity (29). Thus, if biotin intake is low, both elongation and desaturation of EFA may be compromised.

Since hyperglycemia is usually associated with biotin deficiencies (30), it seems apparent that individuals who are insulin resistant, and/or glucose intolerant, should be weary of consuming high carbohydrate/low fat diets (25). Additionally, studies have demonstrated that biotin is involved in glucose metabolism and has been shown to have a beneficial effect in experimental and clinical diabetes mellitus (31-33). These studies suggest that biotin supplementation may help in treating hyperinsulinemia and glucose intolerance, both of which can contribute to the development of CVD. Review of the literature leads the authors to believe that there is a possible connection between biotin insufficiencies and CVD in the United States.

Consequences on fatty acid biosynthesis:

It has been shown that biotin deficiency impairs lipogenesis both in vitro and in vivo (22,23,34). ACC and PCC are involved in the production of malonyl CoA and proprionyl CoA, respectively. As a result of biotin deficiency, decreased activities of these enzymes influence fatty acid biosynthesis. Proud and colleagues (7) showed that biotin deficient rats exhibited a 70% reduction in total fatty acids in skin. Biotin-deficient animals have also demonstrated significant decreases in long-chain saturated and unsaturated fatty acids, suggesting impaired elongation and/or desaturation (7). Suchy et al. (23) reported a significant reduction in serum fatty acids in

their biotin deficient rats. This decrease was largely due to decreased levels of unsaturated fatty acids.

Reduced activity of PCC results in increased proprionyl CoA levels, which is the primary precursor for odd-chain fatty acid biosynthesis. Biotin deficient humans and animals have commonly exhibited increases in odd-chain fatty acids, implicating the elevated production of proprionyl CoA (34-36). Impaired ACC activity results in a decrease in malonyl CoA production, the primary elongation substrate for fat biosynthesis. Puddu et al. (34), and Kramer et al. (36) found that biotin deficiency resulted in increased 15:0, 17:0, 16:1, and decreased 18:0. Marshall (25) demonstrated that the 16:0/18:0 ratio was the highest in their biotin deficient rats. If malonyl CoA production is compromised during biotin deficiency, it would then contribute to the decreases in the longer chain fatty acids. Some studies have found slight increases in long-chain fatty acids during biotin-deficiency, although it has been suggested that this was an adaptive response whereby fat elongation continues due to the reverse activity of β-oxidation, bypassing ACC (23,36).

Contribution to essential fatty acid insufficiencies:

Despite adequate intakes of the essential fatty acids (linoleic and linolenic acid), insufficient biotin intakes adversely influence the synthesis of long-chain EFAs. The decreased capacity of ACC to produce malonyl CoA decreases the elongation of EFAs (7,21,23). Puddu et al. (34) found that although total fat content was unchanged in their biotin deficient rats, the ratio of arachidonic acid (20:4ω6) to linoleic acid (18:2ω6) was significantly decreased. Likewise, a similar study showed that the ratio of dihomo-γ-linolenic acid (DHGLA, 20:3ω6) to linoleic acid was decreased in biotin deficient chicks (12,25,36-38). Watkins (11,28,37) demonstrated that although 20:4ω6 levels were unchanged in his biotin deficient chicks, levels of 20:3ω6 were significantly reduced. Other studies have found increased levels of 18:2ω6 and 18:3ω3 in biotin deficient chicks (11,36). All of these reports suggest that biotin deficiency increases 18:2ω6 and 18:3ω6 levels by decreasing elongation via the ω6 pathway (11,12,28,34,38). Mock and colleagues reported abnormalities in the ω3, ω6 and ω9 pathways resulting from biotin deficiency in human subjects (29). In vitro studies have shown that 18:3ω6 inhibits Δ^6 desaturation of linoleate which further impairs synthesis of long-chain EFAs (39).

Implications for increased risks for cardiovascular disease:

Biotin deficiency in humans is commonly diagnosed and treated with biotin supplementation at an early age. Thus, there is a paucity of data examining the prevalence of CVD in biotin deficiency. Marshall et al. (40) reported a negative correlation between biotin levels and total plasma lipids. Biotin deficient chicks exhibited significantly higher total lipid, free fatty acid, triglyceride and cholesterol (41). Suchy and Wolf (22) examined the effect of biotin deficiency and supplementation upon tissue cholesterol and serum lipoproteins. They reported that biotin deficient rats had increased serum LDL-cholesterol fractions.

A review of the literature suggests that biotin deficiency results in decreased levels of 20:3ω6 and 20:4ω6, both of which serve as precursors for prostaglandin production (28,34). Decreases in DHGLA levels suggest that there may exist a decrease in the ω3 pathway resulting from biotin insufficiency. It would thus appear that biotin plays an important regulatory role in the

production of prostaglandins as well as the integrity of membrane phospholipids (25,39). If functional biotin status is accommodated, structures synthesizing prostaglandins (platelets, endothelial cells) may have a decreased ability to produce thromboxanes and prostacyclin as a result of lowered substrate availability (42). This trail of evidence possibly implicates biotin as an essential component in the development of CVD. Due to the high prevalence of CVD in the United States, we sought to further investigate previous research examining biotin bioavailability and nutritional status.

CONCLUSION

Both biotin and EFA insufficiencies may be considerably more prevalent than currently reported. Not only does there exist a lack of knowledge concerning biotin bioavailability in the American diet, but inconclusive data regarding identification of marginal biotin nutritional status in humans make the consequences of insufficiencies rather enigmatic. Both animal and human biotin studies lead us to believe that there is a strong correlation between biotin and EFA insufficiencies. In this case, individuals consuming diets low in EFA and available biotin would be at elevated risk for developing CVD. Due to the prevalence of high carbohydrate/low fat diets (as recommended by the USDA food guide pyramid), there may be a concomitant increase in the prevalence of both biotin and EFA insufficiencies. Future studies should also consider the role that biotin insufficiencies play in hyperglycemia and diabetes mellitus. Investigators have already identified populations that exhibit an increased prevalence of non-insulin dependent diabetes mellitus in association with the acculturation from traditional hunter-gatherer diets to western diets high in cereal grains (43,44). The relationship between biotin and glucose/insulin metabolism would further implicate this vitamin as an essential nutrient for the prevention of CVD.

The inaccuracies surrounding nutritional status analyses should be cause for great concern. Increased intakes of trans-fatty acids predominantly from vegetable oils, and decreased intakes of polyunsaturated and monounsaturated fatty acids further compound the effects marginal biotin status may play in the development of CVD. The ambiguity associated with the relationships between biotin content, digestibility, absorption, and availability warrants further research in order to accurately establish adequate biotin requirements.

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