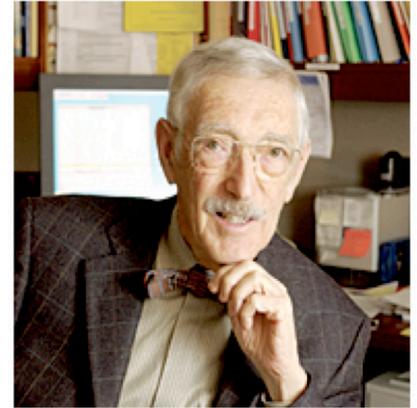


Nutrient-dense food-based supplement bars developed at Children's Hospital Oakland Research Institute (CHORI) to tackle diseases associated with obesity and aging by restoring metabolic balance

U.S. National Medal of Science winner Dr. Bruce N. Ames, after years of studying the interactions of micronutrients (the approximately 40 essential vitamins, minerals, fatty acids, and amino acids) with metabolic processes, became convinced that modest deficiencies in intake of micronutrients could be causing metabolic imbalances that heighten risk for diseases associated with obesity and aging, such as diabetes, cancer and heart disease. The urgency of the problem was brought home to him by the realization that levels of folic acid deficiency known to cause large increases in chromosome breaks (associated with increased cancer risk) were of the same magnitude as common levels of this micronutrient deficiency in disadvantaged populations. Deficiencies in a number of micronutrients are considered by the World Health Organization and other agencies to be a major worldwide public health concern. Widespread, often severe, deficiencies occur in children and adults in developing countries who are unable to consume nutritionally adequate diets. More modest deficiencies are common in developed countries, such as the United States. These deficiencies are compounded by consumption of high calorie low micronutrient-containing diets that promote obesity. The Triage Theory proposed by Ames in 2006 (Ames, 2006, 2010) provided a mechanistic scientific rationale for why modest micronutrient deficiencies might be expected to increase risk of diseases of aging, an idea that has been strongly supported by our recent analyses (McCann & Ames, 2009; 2011).



Dr. Bruce N. Ames

Ames became convinced of the need for an economical, low calorie, food supplement that could restore metabolic balance in people who were not consuming nutritionally optimal diets. In 2003, Ames and his colleague Dr. Mark Shigenaga, an expert in gut health and obesity, embarked on the development of an economical high fiber, low-calorie, micronutrient-fortified nutrition bar to help restore micronutrient adequacy, gut-health, and metabolic balance. There are hundreds of nutrition bars on the market. Some claim to boost energy or protein intake, or to add fiber or omega-3 fatty acids, and many contain some added vitamins and minerals, but no nutrition bar seeks to provide the full range of nutrients needed to restore metabolic balance. It was Ames' vision that if such a food supplement could be developed, it might be made available at low cost to poor, disenfranchised populations, subsidized by market value distribution to more advantaged populations, most of whom also do not consume optimal diets. It was hoped that the bar might serve as a first step in helping people on poor diets to transition to healthier eating habits. There would be almost universal benefit to the restoration of optimal nutrition to avoid the serious developmental and disease consequences of chronic metabolic imbalance.

A team of nutrition scientists and clinicians was assembled, and we also contracted with the local Processed Foods Unit of the United States Department of Agriculture (USDA) to assist in the production of a palatable bar. Over a period of more than 7 years, 3 low-calorie (110-130 kcal) bars were developed and tested in a series of 2-8-week trials in generally healthy adults and adolescents ranging from lean to obese, and eating diets of varying quality. Together these bars improved antioxidant defenses (e.g., increased GSH), and metabolic biomarkers linked to future risk of heart disease (HDL, LDL, Hcy), diabetes (insulin resistance), and cognitive decline (Hcy). A manuscript was recently accepted by FASEB Journal, a major peer-reviewed scientific journal, describing the development and testing of the prototype bar (Bar #1) (Mietus-Snyder et al, 2012). Domestic and international patent applications for the nutrition bars have been filed (Low-calorie Nutritional Compositions for Maintaining Metabolic Balance. U.S. Application No. 61/388,890; International Application No. PCT/US2011/054433).

Current research efforts are described more fully at the end of this document, but in general are directed at combining the most effective attributes of each of the 3 bars into a single bar that will optimally improve the

full range of biomarkers so far detected, at expanding the range of metabolic imbalances improved, at exploring the efficacy of the bar in preventing or improving symptoms of diseases or conditions known to be sensitive to modulation by diet (e.g., asthma, diabetes, hypertension), and at understanding mechanisms through which bar improvements are mediated. We are also developing and refining novel bioassays suitable for use in human trials to expand the range of disease biomarkers that can be monitored.

This article briefly describes the development of the bars and results of pilot trials of Bars #1-3: the challenges and solutions that over the past 7 years led to the development of what we think could become an important tool contributing to the restoration of metabolic health in the obese and in nutritionally disadvantaged people around the world.

CHALLENGES AND SOLUTIONS

In order to ensure that micronutrient intake is adequate by supplementing dietary intake, it is necessary to take a multi-vitamin pill plus additional pills for calcium and magnesium because adding these to a single pill makes it too bulky to consume. On top of that, a fish oil pill is necessary to achieve recommended intakes of the omega-3 long-chain polyunsaturated fatty acids DHA and EPA, and a variety of other supplements are required to supply sufficient fiber, essential amino acids, and other nutrients such as polyphenolics. Thus, Ames and Shigenaga recognized at the outset that a nutrition bar was the only choice to avoid the impractical consumption of multiple pills. Developing such a food nutrition bar, however, presented serious challenges.



Challenge #1: Choosing which nutrients and how much of each to add to the bar

A major challenge was to add micronutrients and other bar components in amounts that reflected the most up-to-date scientific information on optimal nutrition, and did not result in over-supplementation because of intakes already consumed. The goal was to determine amounts of each bar component that would reflect the most recent scientific findings and that would bring individuals on poor diets up to currently recommended intake amounts. Since not all of the 40 micronutrients are deficient, even in people on poor diets, the goal was to focus on micronutrients for which it is most difficult to achieve recommended intakes.

The solution was to utilize advisers that included Dr. Janet King, former chair of the National Academy of Sciences' Food and Nutrition Board, and to assemble a research team of nutrition scientists and clinicians in Ames' research group. By research team consensus it was determined how much of each micronutrient and other constituents to add to the bar. The goal was not to meet the Recommended Daily Allowance (RDA) for every micronutrient, but to complement dietary intakes in order to achieve, overall, intakes that should at least meet the Estimated Average Requirement (EAR) (the RDA is defined as 2 standard deviations above the EAR). This was a conservative goal, but one which respected the importance of not over-supplementing, while responding to the need to correct widespread modest micronutrient deficiencies in most of the U.S. population.

In many cases, amounts of micronutrients chosen were sufficient to meet the EAR, but in some cases higher amounts were added. For example, most scientists now recognize that current recommendations for vitamin D are too low, so higher amounts were added. Higher amounts of vitamin C were also added to protect nutrients sensitive to oxidation. Care was also taken to add micronutrients in their most active biological forms, some of which are not used in multivitamin pills. The mixture of these more bio-available forms is part of the patent application and cannot be disclosed without a confidentiality agreement.

Non-micronutrient components, including certain soluble fibers, polyphenols, and several other ingredients that are part of the patent application, were included to benefit gut health. Since the gut wall represents a

key interface between both the food we consume and bacteria in the intestines that can leak through the gut wall causing inflammation and disease, gut health represents an important determinant of overall health. Unfortunately, the types of diets commonly consumed in western societies (high in trans-fats and refined carbohydrates, low in fiber and many micronutrients and plant material), are detrimental to gut health as well as metabolic balance. As a consequence, the gut barrier becomes weakened and is less resistant to diet-induced stress, a vulnerability that can lead to increased exposure to gut bacteria or their remnant particles (endotoxin). The effects of gram negative bacterial endotoxin on host responses are essentially identical to those often observed in obesity and cardiovascular disease (e.g., chronic inflammation, increased blood pressure, dyslipidemia, insulin resistance).



A soluble fiber was added to the bar that has heart health-promoting qualities and improves gut health. Plant derived polyphenols, including classes of flavonoids present in the bar in fruit extract, walnuts, and chocolate, are associated with many health promoting effects, including improvements in blood lipid profiles. Several other compounds, also part of the patent application, were added to benefit gut energy metabolism. Their support of mitochondrial metabolism and ATP yield helps to promote gut health and resistance to dietary stressors. We think that by placing these substrates in a food matrix containing micronutrients and other complementary dietary agents that help to optimize energy production in response to need, nutrient synergy may be achieved with lower amounts and better efficacy compared to single-nutrient supplements.

Challenge #2: Devising a method to add DHA and other oxidation-sensitive micronutrients to the bar

The long-chain omega-3 polyunsaturated fatty acid DHA is now recognized as being important in brain development and promotion of long-term cardiovascular health. The team felt it was important to include DHA in the bar, but its addition presented a special challenge. DHA is easily oxidized, and is an oily substance with an unpleasant taste unless it is completely pure. It has not been possible to successfully add DHA to nutrition bars. In fact, the only other nutrition bar that attempted to add DHA was withdrawn from the market because of poor taste. The solution to this problem is part of the patent application and cannot be disclosed without a confidentiality agreement.

Challenge #3: Devising a method to deliver sufficient amounts of potassium, since large doses ingested in a bolus amount (such as in a potassium supplement) can result in adverse gastrointestinal side effects

The RDA for potassium is 3.5 grams, a very large quantity when compared to the small amounts needed of most other micronutrients. This amount cannot be ingested as a bolus. If taken orally in pure supplement form, potassium causes gastric irritation. FDA regulations limit the total amount of potassium that can be provided in supplement form to 100 milligrams of pure potassium salt. However, delivered as a component of whole foods, it can be consumed at much higher levels because a whole food matrix has a diluting effect that slows absorption. Potassium is naturally present at fairly high amounts in some foods of plant origin. For example, one banana or a tablespoon of molasses contains about 500 mg of potassium. Nevertheless, potassium deficiency is common in the U.S., as fresh produce is underrepresented in the typical American diet. The team considered it important to find a way to add it to the bar.

The USDA group we contracted with to help us produce a palatable bar has an active program that redirects non-marketable California fruit to other uses, and the solution was to include a fruit matrix in the bar that is high in potassium. Thus, consumption of 2 bars a day (the recommended amount) delivers approximately a banana's worth of potassium, and serves as a supplement to other dietary intake of potassium. Fruit is also an important source of polyphenolics, another key class of bar ingredients.

Challenge #4: Methods had to be devised to accommodate an amount of fiber (both soluble and insoluble) sufficient for biological activity, and to make the bar palatable without adding sugar.

Early prototypes of the bar were inedible. This was due to the strong flavor profiles of B vitamins, DHA, and certain minerals, to the high fiber content, and to the fact that no sweetener was added. Since the goal was to use natural products in the bar, we did not want to add an artificial sweetener. Also, since a hoped for benefit of the bar was to combat obesity, we wanted to keep the caloric content to no more than about 130 calories, and did not want to add natural sweeteners such as sugar or honey.

Palatability was a major challenge. Solving the problem took almost 4 years and required multiple modifications to the bar. The complex of modifications that have resulted in a very tasty bar while preserving its low calorie content are part of the patent application and cannot be disclosed without a confidentiality agreement.

A variety of flavored bars have been produced, including cinnamon/fruit, white-chocolate blueberry, sweet and sour, chocolate mint, and chocolate decaf espresso. We are finding that preferences vary considerably, both among individuals and different ethnic and cultural groups. The design of the recently completed trial in obese parents and their obese adolescent children (described below) permitted participants to choose which bar flavors they would like to eat each week of the trial. This added variety and enhanced enthusiasm and compliance during the trial.

The decision to dose this nutrition bar twice daily made it possible to double the total daily intake of soluble and insoluble fiber deemed palatable in a single bar. The decision to recommend intake of 2 bars a day also drew on research suggesting that the most efficient utilization of nutrients by the body occurs when they are consumed in small amounts over the course of the day. Ingesting 2 bars a day is also beneficial from a weight-control perspective because a consistent effect of the bar is to decrease appetite.

HUMAN TRIALS USING 3 BAR FORMULATIONS RESULTED IN OVERLAPPING BUT ALSO UNIQUE SPECTRA OF METABOLIC IMPROVEMENTS

The aim of a series of 2-8 week pilot trials in lean, overweight, and obese individuals was to test compliance, palatability, and effect of bar consumption on biomarkers linked to future disease risk. Biomarker assays included a standard lipid panel measuring, for example, HDL and LDL (so-called “good” and “bad” cholesterol), as well as state-of-the art lipoprotein phenotyping to determine the size of these lipoprotein particles. Lipid sizing is a sensitive indicator of lipid metabolic status and also relates to insulin sensitivity. Measurements of fasting glucose, insulin, and C-reactive protein (hsCRP, a biomarker associated with inflammation), were also included, as well as a recently developed thiol-redox amino acid metabolomics assay from Dr. J. Suh in our group that is sensitive to shifts in inflammation, oxidative stress, insulin-resistance, and status of several B-vitamins (Suh et al, 2009, 2011).

All 3 bars share the same compositional base. Bar #1 is the basic, “prototype” bar. Bars #2 and #3 are two different modifications of the prototype bar. All 3 bars significantly increased satiety and had no significant effect on weight gain, despite intake of 2 bars a day without guidelines as to whether to use the bar as a meal replacement or supplement. Each bar elicited overlapping, though somewhat unique, improvements in metabolism. Understanding these differences, and developing a single bar that combines the best features of each is a current research priority. Below, results of trials with these 3 bars are briefly described.

Bar #1: The prototype bar: A 2-week trial in 25 generally healthy adults consuming predominantly good diets (Mietus-Snyder et al, 2012)

A manuscript reporting pooled results from 3 identical trials using the prototype bar was recently accepted for publication in FASEB Journal (a peer-review publication of the Federation of American Societies for Experimental Biology) (Mietus-Snyder et al, 2012). Consumption of Bar #1 for 2-weeks significantly raised total HDL (“good cholesterol”) ($p = 0.001$), predominantly its most desirable form, Large HDL (HDL-L, $p < 0.0001$) (**Figure 1A**). The bar also lowered total homocysteine (Hcy, $p = 0.017$), which reflects improved status of several B vitamins (folate, B12, B6) (**Figure 1B**). Higher HDL and lower Hcy are both associated with lower future risk of cardiovascular disease, and lower Hcy is also associated with less future cognitive decline. Bar #1 also raised glutathione (GSH, $p = 0.011$), indicating improved protection against oxidative stress.

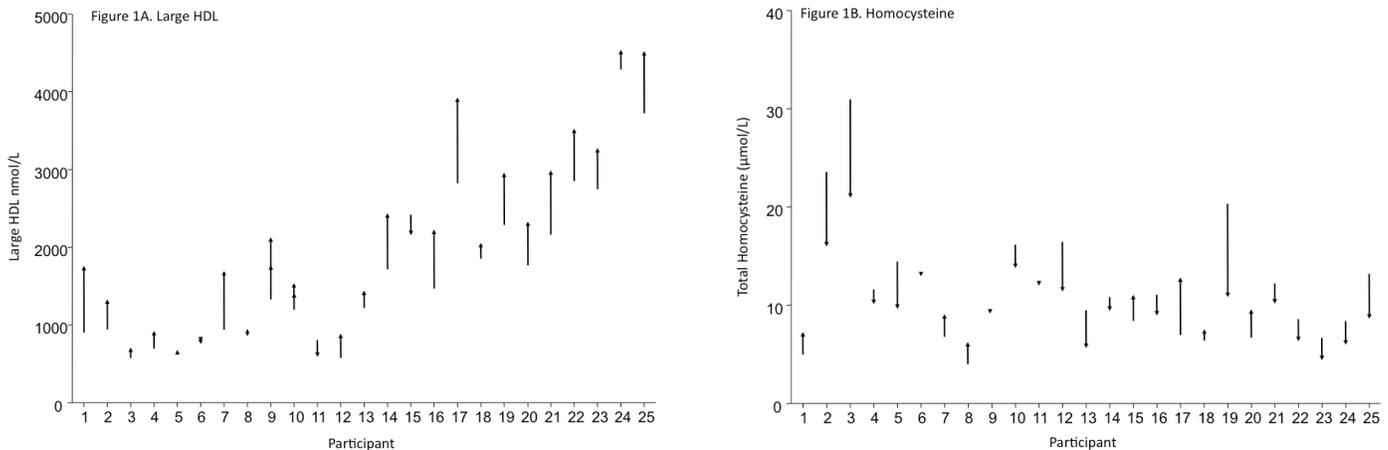


Figure 1A,B (Mietus-Snyder et al, 2012). Large HDL (HDL-L) (Fig. 1A) and total homocysteine (Hcy) (Fig. 1B) plasma concentrations before and after the 2-week trial are shown for each participant, listed in order of increasing total HDL cholesterol (HDL-c) at the beginning of the 2-week trial. Each arrow depicts the direction and magnitude of change.

Bar #2: A 2-month trial of the prototype bar modified by the addition of a different soluble fiber (Shigenaga et al, in preparation)

Participants were similar in weight, age distribution, and dietary habits in both Bar #1 and Bar #2 trials. Bar #2 presented a spectrum of metabolic improvements that was partially overlapping with Bar #1 (Table 1), but also had unique aspects. LDL was significantly decreased at 2-weeks (LDL-c, $\downarrow p = 0.007$), which had not been observed in any trial conducted with Bar #1. But, Hcy was not decreased, which had been a consistent observation with Bar #1. In addition, an increase in HDL-c, observed with Bar #1, was also observed with Bar #2 at 2-weeks, but only in lean participants (HDL-c $\uparrow p = 0.01$), and an increase in GSH, observed with Bar #1, was also observed at 2-weeks with Bar #2, but only in overweight/obese participants (GSH, $\uparrow p = 0.011$). After 8-weeks consumption of Bar #2, both HDL-c and GSH were favorably changed in the full cohort. These results suggest that the new soluble fiber added to Bar #2 was responsible for the new improvement in LDL, but the trade-off was that there was no change in Hcy, and favorable changes in HDL-c and GSH in the full cohort were delayed.

After 2-months consumption of Bar #2, as noted above, improvements in LDL, HDL, and GSH were observed in the entire group. In addition, there was also a borderline significant reduction in insulin resistance in the full cohort (HOMA-IR; $p = 0.043$) that was highly significant in those that were overweight or obese (BMI >25 , $p = 0.0088$) (**Figure 2**). There was little reduction in HOMA-IR in lean participants because their HOMA-IR values were already quite low – i.e., they had nowhere to go. It is possible this

effect on insulin resistance would also have been observed with Bar #1, but a 2-month trial was not conducted using that bar. The 2-week and 8-week results of the Bar #2 trial are summarized in **Table 1**.

Table 1. Bar #2: Metabolic improvements	p-value	
	2 wks	8 wks
Reduced "bad cholesterol" (LDL-c)	**	*
Increased "good cholesterol"	-	
HDL	* ^a	*
Large HDL	-	**
Reduced insulin resistance (HOMA-IR)	-	* ^b
Increased protection against oxidative stress (GSH)	* ^b	*

$p < 0.05 = *$; $p < 0.01 = **$. ^aLean participants only (BMI < 25, $p = 0.01$); ^bOverweight and obese participants (BMI > 25) were most sensitive -- HOMA-IR: $p = 0.008$ at 8 wks ($p < 0.05$ across all participants); GSH: $p = 0.011$ at 2 wks ($p = 0.014$ at 8 wks across all participants).

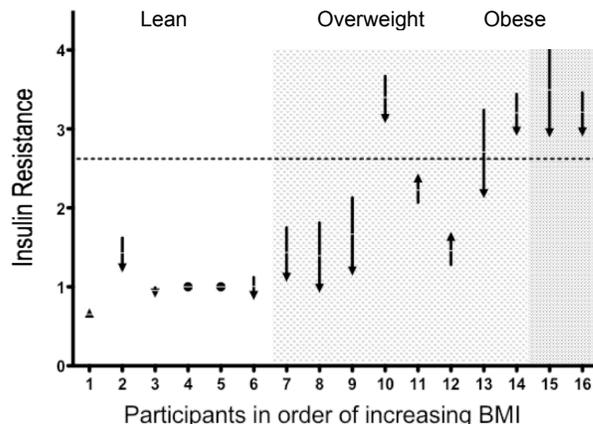


Figure 2. Insulin resistance (measured as HOMA-IR) at baseline and after 2-months consumption of Bar #2, for each participant. The dotted horizontal line indicates the clinical cut-off for insulin resistance.

Bar #3: The prototype bar with several other modifications, including a major change designed to increase natural sweetness in order to encourage compliance by obese individuals conditioned to a high-sugar diet.

Though the metabolic dysregulation of obesity affects all social classes, the greatest prevalence is in those of low socioeconomic status, and also in some ethnic groups, particularly Hispanics and African Americans. Therefore, a major goal is to ensure that CHORI-bars we develop are palatable to and effective in this demographic. To this end, a trial was designed to explore effects of the bar in urban, primarily Hispanic obese adults and adolescents of low socioeconomic status. Eleven obese parent/adolescent dyads were enrolled in a 7-week trial that included weekly nutrition-education and exercise classes. This was the first trial we have conducted in this demographic, the first with almost exclusively obese participants (BMI > 30), and also the first that included adolescents. Thus, it presented new challenges on all 3 fronts. Examples of the extraordinary metabolic dysregulation of obesity are in **Figure 3**, which illustrates the high level of insulin resistance (HOMA-IR), internal inflammation (measured by hsCRP), and DNA damage (measured by the comet assay) at baseline (i.e., prior to eating the CHORI-bar in obese (BMI >30) compared to lean (BMI < 25) individuals.

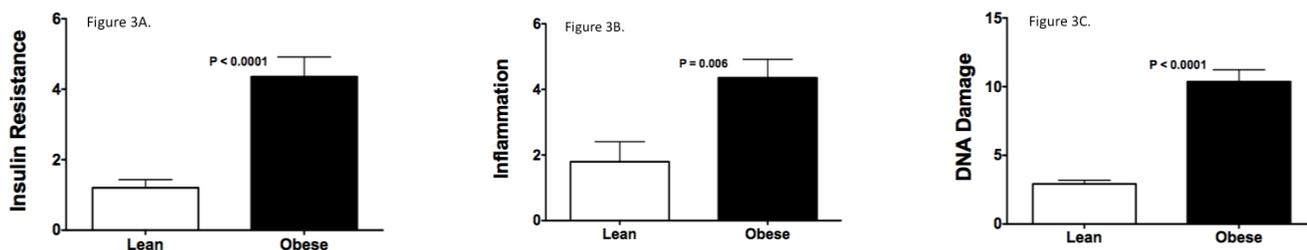


Figure 3A,B,C. Comparison of mean baseline values for (A) insulin resistance (HOMA-IR), (B) inflammation (hsCRP, mg/L), and (C) DNA damage (comet assay, olive tail moment) in lean (BMI < 25) and obese (BMI > 30) participants in CHORI-bar trials. In Figs. 3A and B, lean participants are from all CHORI-bar trials conducted to date (n = 17), and obese (BMI > 30) participants are from the Bar #3 trial (n = 13). In Fig. 3C, participants are the sub-set of those from all CHORI-bar trials for which a DNA damage assay was conducted (Lean, n = 18; Obese, n = 19).

Bar #3 was relatively ineffective in this obese population compared to Bars #1 and #2 in the earlier trials discussed above. We are uncertain whether the relative unresponsiveness of these participants to Bar #3 was due to their obesity, to inaccurate reporting of compliance by this demographic, or to changes made in the bar to accommodate their increased need for sweetness for palatability, or possibly to all three factors. Sorting out these possibilities will be the subject of future studies. The major success of this trial was the family-based study design, which is briefly described below.

A family-based intervention among urban obese minorities designed to motivate a desire to improve dietary habits

The family-based design used for the Bar #3 trial included weekly exercise/nutrition classes. These classes promoted a positive group dynamic and led to a high level of enthusiasm. A number of participants reported anecdotally that eating the bar made them feel better [e.g., more energy, less constipation (common in those eating poor diets)], and some participants began to take steps to improve their diet. Both parents and children appeared to be genuinely and positively affected by the experience. If the bar makes the obese feel better, we believe it may serve as a tool to help transition them to healthier eating habits by showing them that a well-balanced intake of nutrients really does make you feel better. The fact that participants felt better *before* any actual modification of their diets occurred suggests that a nutrient-dense food-based supplement such as the CHORI-bar has great potential to act as a catalyst to motivate real change. This possibility will need to be tested in future trials.



Obese parents and adolescent children who ate Bar #3 for 7-weeks at their last nutrition/exercise class. Our dietician and exercise instructor, Kirsten Graves, is in the front row. Dr. Michele Mietus-Snyder, who led the study, is in the back row.

SUMMARY OF THE MAJOR METABOLIC EFFECTS OF BARS #1, #2, AND #3

Each of the 3 bars improved somewhat overlapping, but also distinct metabolic indicators of health. As shown in **Table 2**, Bar #1 was most effective in raising HDL, Bar #2 was most effective in improving insulin resistance and lowering LDL, and Bar #3 was most palatable and most effective in lowering blood pressure. We are currently, in collaboration with the USDA, developing a bar that combines all of these positive attributes, and that also decreases sub-clinical inflammation, the only major biomarker associated with obesity that the bar has not yet improved.

Table 2. Bars #1, #2, #3: Comparison of metabolic Improvements^a

	Bar #1		Bar #2		Bar #3	
	2-wk	8-wk	2-wk	8-wk	2-wk	7-wk
Lipid metabolism						
HDL-c ↑	+		-	+		-
LDL-c ↓	-		+	+		-
Insulin resistance ↓ (HOMA/IR)	-		-	+		-
Folate/B12/B6 status ↑ (Hcy ↓)	+		-	-		+
Anti-oxidant defense (GSH ↑)	+		-	+		-
Systolic blood pressure (SBP) ↓	-		-	-		+
Palatability	Moderate		Poor		Excellent	

^aEffects observed only in sub-groups (i.e., high or low BMI or hsCRP) are not included. All results indicate statistically significant changes observed over the course of each trial.

CURRENT RESEARCH AND FUTURE PLANS

After 7 years of development, we now have available several precisely formulated nutrient-dense food-based supplement bars that together significantly improve all but one (sub-chronic inflammation) of the classic biomarkers of the metabolic dysregulation (dyslipidemia, homocysteinemia, low anti-oxidant defenses, insulin resistance) of obesity and its related co-morbidities. A patent on the CHORI-bar has been filed (U.S. Application No. 61/388,890; International Application No. PCT/US2011/054433) and efforts have begun to locate partners who can help develop the bar as a marketable product. While making the CHORI-bar available to the public at an affordable cost is an important goal, an equally important goal is to use the bar as a research tool to better understand mechanisms whereby nutrients delivered at physiological doses in a food-based matrix positively impact metabolism linked to future disease risk.

Research in 5 general areas is ongoing or planned to further improve the effectiveness of the CHORI-bars and to elucidate mechanisms:

1. Development of a CHORI-bar that combines the most positive aspects of Bars #1-3 (see Table 2), and that, in addition, improves biomarkers of subclinical inflammation.
2. Conduct of a series of trials to test the efficacy of the CHORI-bar in preventing or ameliorating diseases or conditions associated with obesity that are characterized by metabolic dysregulation known to be favorably impacted by the bar (e.g., asthma, metabolic syndrome, diabetes, hypertension, heart disease). A pilot trial in obese adolescent asthmatics will begin in the Summer of 2012.
3. Deconstruction experiments to determine which bar ingredients or combinations are responsible for improved metabolism. Preliminary deconstruction experiments with Bar #1 suggest that the effect on HDL is due to additive or synergistic interactions of several bar components.
4. Mechanistic studies to determine how bar ingredients interact with metabolic pathways to affect specific disease-relevant biomarkers. For example: a) as predicted by Ames' Triage theory, improved vitamin/mineral status may differentially impact certain enzymes in critical pathways; b) effects of certain soluble fibers in the bar on the gut barrier may mediate improved metabolism; and c) the effect on anti-oxidant defenses could occur endogenously through several alternative pathways.
5. Development of additional biomarker assays targeting metabolic imbalance linked to diseases of aging has been ongoing in the Ames laboratory in parallel with the development of the CHORI-bar. Included are several assays that quantify different types of DNA damage, assess immune status, and measure all of the metals at once in lymphocytes. These biomarker assays are in various stages of development, and when complete, will expand and refine our ability to detect disease-related metabolic changes due to bar consumption. For example, we have found that DNA damage levels in the obese are very high compared to lean individuals (Figure 3), and we are refining several different types of DNA damage assays to better define the type of damage.

CONCLUSION

The potential power of nutrient-dense, properly formulated food-based supplements to restore metabolic balance and thereby prevent or ameliorate disease has not yet been adequately appreciated by the scientific community at large. This is in part because the field of nutraceuticals is tainted by uncritical science, and also because double-blind placebo-controlled randomized controlled clinical trials, considered the "gold standard" for testing drug efficacy, are simply not suitable for food-based supplements. This is because, not only do both controls and intervention groups have to eat, but also designing a true placebo appropriate for a double-blind trial is simply not possible for a food-based supplement.

We believe, however, that these problems should not deter serious investigators because the promise of food-based nutrient-dense supplements to prevent and ameliorate disease is enormous and could be paradigm-shifting. For example, why use an expensive drug with undesirable or unknown side effects to lower cholesterol when the same effect may be possible with a nutrient-dense food-based supplement that has no negative side effects? The full potential of food-based supplements to do the work of some drugs without their side effects is just beginning to be seriously investigated, but it will require critical un-biased scientific investigation and creative experimental designs. These questions motivate the research program at the Nutrition and Metabolism Center at CHORI.

REFERENCES

- Ames, B. N. (2006) Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage. *PNAS* 103:17589-94. doi: 10.1073/pnas.0608757103
- Ames, B. N. (2010) Prevention of mutation, cancer, and other age-associated diseases by optimizing micronutrient intake. *J Nucleic Acids* 1-11. doi: 10.4061/2010/725071
- McCann, J. C., and Ames, B. N. (2009) Vitamin K, an example of triage theory: is micronutrient inadequacy linked to diseases of aging? *Am J Clin Nutr* 90, 889-907. doi: 10.3945/ajcn.2009.27930
- McCann, J. C., and Ames, B. N. (2011) Adaptive dysfunction of selenoproteins from the perspective of the triage theory: Why modest selenium deficiency may increase risk of diseases of aging. *FASEB J* 25:1793-814. doi: 10.1096/fj.11-180885
- Mietus-Snyder, M. L., Shigenaga, M. K., Suh, J. H., Shenvi, S. V., Lal, A., McHugh, T., Olson, D., Lilienstein, J., Krauss, R. M., Gildengoren, G., McCann, J. C., and Ames, B. N. (2012) A nutrient-dense high fiber, fruit-based supplement bar increases HDL, particularly large HDL, lowers homocysteine, and raises glutathione in a 2-week trial. *FASEB J* May 1 (Epub ahead of print). To appear in the August 2012 print issue. doi:10.1096/fj.11-201558
- Suh, J. H., Kim, R., Yavuz, B., Lee, D., Lal, A., Ames, B. N., and Shigenaga, M. K. (2009) Clinical assay of four thiol amino acid redox couples by LC-MS/MS: utility in thalassemia. *J Chromatogr B Analyt Technol Biomed Life Sci* 877:3418-27. doi: 10.1016/j.chromb.2009.06.041
- Suh, J. H., Kim, R.Y., Lee, D.S. (2012) Amino acid/redox metabolomic assay of lipopolysaccharide-activated macrophage inflammation. *J Inflammation*. Accepted pending minor revision, May 2012.