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Thermogenic Drugs as a Strategy for Treatment of Obesity

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There is accumulating evidence to support the hypothesis that a low-energy-output phenotype is at high risk of weight gain and obesity, irrespective of whether this is owing to a low resting metabolic rate and/or physical inactivity. The low-energy-output phenotype is associated with impaired appetite control, which is improved if energy output is increased. This is the background for pharmacologic stimulation of energy expenditure as a tool to improve the results of obesity management. Targets are the leptin receptors, the sympathetic nervous system and its peripheral β-adrenoceptors, selective thyroid hormone derivatives, and stimulation of the mitochondrial uncoupling proteins. Currently available compounds such as recombinant leptin, ephedrine/caffeine, and sibutramine possess thermogenic properties owing to their activation of the sympathoadrenal system. Compounds acting selectively on the human β_3-adrenoceptor are still promising tools to achieve a sustained stimulation of lipolysis and energy expenditure, and several are in the pipeline.

Key Words: β-agonists; thermogenesis; obesity treatment; thyroid hormone; uncoupling protein.

Thermogenic Mechanisms as Targets for Pharmacologic Intervention

Daily energy expenditure represents one side of the energy balance equation. Energy expenditure should be in equilibrium with daily energy intake to ensure weight stability. While much effort is exerted to manipulate diet and appetite, there is also a rationale to support efforts to increase energy expenditure pharmacologically as a tool to enhance the results of obesity management. There is accumulating evidence to support the hypothesis that a low-energy-output phenotype predisposes individuals to weight gain and obesity, whether the low energy output is caused by a low resting metabolic rate (RMR), physical inactivity, or both *(1)*. Increased energy metabolism is therefore an attractive target because it may allow people to maintain food intake at socially more acceptable levels. In addition, there is evidence to support the view that any increase in energy expenditure is not fully counteracted by a similar increase in appetite and energy intake *(2)*, irrespective of whether the increased energy output is achieved through exercise or pharmacologically with selective β_3-agonists *(3)*. Even a slight increase of 2 to 3% in daily energy expenditure may therefore have clinical relevance, particularly in preventing the decline in RMR with weight loss, but also in decreasing the risk of weight regain following weight loss.

Energy expenditure can be stimulated pharmacologically by interfering with any of several steps in the regulatory system—from activation of the central leptin receptor and central nervous system (CNS) regulatory systems to peripheral efferents of the sympathetic nervous system (SNS), thyroid hormones, and cellular mechanisms responsible for thermogenic futile mechanisms such as uncoupling proteins.

Physiology of Energy Expenditure and Contribution of Sympathoadrenal System

Most of the expended daily energy derives from RMR, which comprises 50–80% of the total daily energy expenditure. The remaining energy expenditure is mainly owing to the cost of physical activity and, to a lesser degree, to the thermic effect of food, cold, and thermogenic stimulants in foods and beverages (nicotine, caffeine and derivatives, green tea, and capsaicins). About 70–80% of the variance in RMR can be accounted for by differences in fat-free mass, fat mass, age, and gender *(4,5)*. After accounting for these variables, however, an additional 5–8% of the variance in RMR is accounted for by family membership. This strongly suggests that RMR is influenced by genetic factors. Cross-sectional studies have shown that some of this variance can be explained by individual differences in sympathetic tone and thyroid hormone levels within the normal physiologic range *(5,6)*. Noradrenaline turnover studies have shown that most of the variability in RMR unexplained by body size and composition is related to differences in SNS activity *(5)*. Taken together, these studies show that SNS activity does modulate RMR, the largest component of daily energy expenditure. The other components of 24-h energy expenditure are also influenced by the sympathetic tone. Meal ingestion is accompanied by increased SNS activity, and studies using β-blockade have demonstrated a

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facultative, β-adrenergically mediated component *(7)*. Furthermore, it has been shown, by measurement of plasma noradrenaline turnover, that the increased SNS activity in response to a meal accounts for at least part of the meal-induced thermogenesis. Despite the clear impact of SNS activity on meal-induced thermogenesis, its contribution to total energy expenditure may be too small to have an independent effect on body weight gain *(5)*.

Physical activity also seems to be stimulated by sympathetic activity. The relationship between spontaneous physical activity and noradrenaline appearance rate *(6)* is consistent with the concept that SNS activity is a determinant of individual differences in the level of spontaneous physical activity, i.e., how much people move around, change position, and fidget, all of which contribute to total energy expenditure. So those who are characterized as fidgeters seem to have constitutionally higher SNS activity.

The contribution of the sympathoadrenal system to 24-h energy expenditure has been addressed using measurements in whole-body calorimeters. Administration of the β-antagonist propranolol, which causes some inhibition of the β-adrenergically mediated component, produced a 2–4% decrease in 24-h energy expenditure, suggesting that a total blockade of the system may suppress 24-h energy expenditure by perhaps 4–6%, equivalent to 50–150 kcal/d *(8)*.

SNS Effects on Substrate Metabolism

A stimulation of the SNS appears to influence not only energy expenditure but also the relative amounts of substrate oxidized *(5)*. Accordingly, an inverse relationship has been found between the 24-h respiratory quotient and basal muscle sympathetic nerve activity, independent of body fat. This indicates that low SNS activity is associated with low lipid oxidation. A possible explanation for this observation is the stimulatory effect of noradrenaline on intracellular lipolysis and on nonesterified fatty acids uptake in the muscle, which seems impaired in previously obese subjects *(9,10)*. The stimulatory effect on fat oxidation by β-adrenergic agonists is well established, and β-antagonists accordingly have the opposite effects: that is, they decrease energy expenditure and the relative rate of fat oxidation. The effect of substrate utilization may be partly attributed to the important β-adrenergic stimulatory effect on lipolysis.

The Role of Brown Adipose Tissue, Skeletal Muscle, and Uncoupling Proteins in Thermogenesis in Humans

The regulation of white and brown adipocyte metabolism and gene expression has been characterized in several species. Whereas white adipose tissue (WAT) serves to store energy in the form of triglycerides, brown adipose tissue (BAT) functions to dissipate energy in the form of heat through the action of the mitochondrial uncoupling protein (UCP_1), a proton transporter that is unique to BAT. Heat production by brown adipocytes results from a controlled uncoupling of oxidative phosphorylation by a UCP_1-mediated proton conductance pathway in the inner mitochondrial membrane. A unique β-adrenergic receptor subtype, termed β_3, has been identified on the surface of these adipocytes. This receptor is pharmacologically distinct from the classic β_1 and β_2 receptors. In animal models, the β_3-receptor stimulates lipolysis in WAT and BAT, and in BAT both activates UCP and upregulates the UCP gene, which both result in a further increase in energy expenditure. BAT is abundant in rodents and neonatal humans, in which it is important for thermoregulation. However, the existence and functional role of adult human BAT is controversial because it has been found that morphologically distinguishable BAT is lost as humans mature *(11)*. Nevertheless, recent morphologic and functional studies in humans suggest that small pockets of BAT among WAT depots indicate the possibility of β-adrenergic stimulation and proliferation in vivo *(12)*. This hypothesis is also supported by the observation that patients with the catecholamine-secreting adrenal tumor, pheochromocytoma, experience an increase in metabolic rate and weight loss in conjunction with the appearance of BAT, demonstrating the potential for recruitment and activation of BAT in adult humans under certain circumstances. Moreover, functional β_3-receptors that mediate lipolysis and thermogenesis in humans have also been identified *(13–15)*. It appears that chronic β_3-adrenergic stimulation in WAT increases the expression of newly discovered mitochondrial uncoupling proteins (UCP_2 and UCP_3) and causes a "reawakening" of dormant brown adipocytes.

In humans, however, the major site of catecholamine-induced thermogenesis is skeletal muscle, which can account for 50–60% of the whole-body response *(15)*. Until recently, the cellular thermogenic mechanisms were unknown, but the discovery of UCP_3 mainly expressed in skeletal muscle offers a plausible mechanism for heat dissipation. β_3-Receptors are present in skeletal muscle, where both UCP_2 and UCP_3 are expressed. UCP_3 mRNA expression is increased in response to overfeeding and fasting, and in response to adrenergic stimulation. The *UCP3* and *UCP2* genes are located adjacent to each other in a region implicated in linkage studies as contributing to obesity *(16)*, and a polymorphism in the gene encoding for both UCP_2 and UCP_3 has been associated with reduced RMR and fat oxidation *(17,18)*.

These findings show that tissues other than brown fat play an important role in mediating β_3-adrenergic effects on thermogenesis and substrate oxidation, and this could be a major target for thermogenic drugs.

Role of Low Energy Expenditure in the Development of Obesity

Several studies support the idea that a low RMR is associated with weight gain. A low metabolic rate has been

shown to precede body weight gain in infants and children, as well as in adult Pima Indians and Caucasians *(5)*. Based on the assumption that formerly obese, weight-reduced subjects exhibit the metabolic characteristics that predisposed them to obesity, several studies have compared metabolic rates in formerly obese subjects with those of weight-matched control subjects, who have never been obese. A metaanalysis of 12 such studies corroborates the prospective data by demonstrating a 3–5% lower mean RMR in the formerly obese subjects *(19)*. Moreover, these data indicate that a low RMR is more frequent among formerly obese subjects than among control subjects who were never obese.

Low Sympathoadrenal System Activity Predisposes to Obesity

Studies of the sympathoadrenal activity that have compared lean and obese subjects have yielded conflicting results, probably because comparisons of lean and obese subjects provide only very limited information about the role of the SNS in the etiology of obesity *(8)*. Furthermore, they do not discern between causes and consequences of weight gain. However, longitudinal studies in both Pima Indians and Caucasians have shown a relationship between low urinary noradrenaline excretion and weight gain, as well as a relationship between low urinary adrenaline excretion and the development of central obesity *(5)*. These results strongly suggest that low SNS activity is also a risk factor for weight gain in humans. SNS activity increases in response to weight gain, thereby attenuating the original impairment.

Decreased responsiveness to sympathetic stimuli may also be as important as decreased SNS activity for the development of obesity. Decreased responsiveness could possibly be caused by polymorphisms in the genes encoding the various types of adrenoceptors and uncoupling proteins. A variant in the gene encoding for the $ß_3$-adrenoceptor has been associated with obesity and diabetes in a number of studies, and particularly with lower metabolic rate *(20)*. Genetic polymorphisms in the $ß_2$-adrenoceptor have also been reported, and they seem to be associated with obesity only in individuals with a low energy output *(21)*, which is plausible because these factors represent decreased stimulation of thermogenesis and lipolysis at two different steps in the regulatory pathway. At the intracellular level, cyclic adenosine monophosphate released owing to SNS stimulation may affect the expression and thermogenic function of the UCPs. Individuals homozygous for a variant in the gene that codes for UCP_2 have been shown to exhibit enhanced metabolic efficiency and lower fat oxidation *(18)*, and trained subjects have been found to have lower skeletal muscle expression of UCP_3 and enhanced mechanical efficiency *(22)*. These studies suggest that the UCPs play a functional role in humans and are attractive targets for pharmacologic intervention.

Role of Leptin in Human Obesity

Animals and humans who have a genetic deficiency of the adipocyte-derived hormone leptin or of its receptor exhibit extreme obesity *(23)*. Leptin acts on the hypothalamus to suppress appetite and increase energy expenditure through an activation of SNS, and exogenous administration of leptin in deficient animals and humans reduces energy intake and increases energy expenditure *(24)*. In rodents the negative energy balance produced by leptin is partly mediated by increased SNS outflow to several of organs including BAT. Accordingly, animals with defective leptin biosynthesis or receptor function, such as the *ob*/*ob* mouse, the *db*/*db* mouse, or the *fa*/*fa* rat, exhibit severely reduced SNS activity and gain weight rapidly and become obese. Leptin deficiency is extremely rare in obese humans, and serum concentrations of leptin increase with body fat in all obese persons who do not have a genetic mutation. Leptin levels and body fat are highly correlated, and body fat accounts for approx 50–60% of the variation in serum leptin concentrations among people. The high leptin levels in obese subjects suggests leptin insensitivity and poses questions about the potential for leptin in the treatment of obesity.

Thermogenic Properties of Currently Available Compounds

Leptin as a Weight Loss Agent with Potential Thermogenic Properties

Leptin concentrations in the cerebrospinal fluid increase with body fat but are generally 100 times lower than serum concentrations. The ratio of cerebrospinal fluid to serum leptin concentrations also appears to be lower in obese subjects. Administration of exogenous leptin might therefore affect homeostatic mechanisms of energy regulation and reduce body weight, but the higher serum leptin concentrations in obese subjects may be an indication that exogenous leptin administration may be ineffective in decreasing body fat. Observational studies in humans suggest that leptin stimulates SNS and energy expenditure, as it does in animals *(25)*. Recombinant leptin has indeed proved to be very effective in two children with leptin deficiency, although the investigators did not detect any stimulatory impact of leptin on energy expenditure, possibly owing to the concomitant decrease in body weight *(24)*. However, physical activity level increased from 1.6 to 1.9 after 12 mo of treatment. In normal weight subjects and leptin-intact obese subjects, injections of recombinant leptin produced a dose-response relationship with weight and fat loss, but only a modest weight loss over 24 wk *(26)*. Interestingly, more than 95% of the weight loss seen under the administration of the highest doses was fat. No clinically adverse effects were reported apart from local reactions at the injection site, which may have unblinded the study to some extent *(26)*. However, recent animal studies suggest that

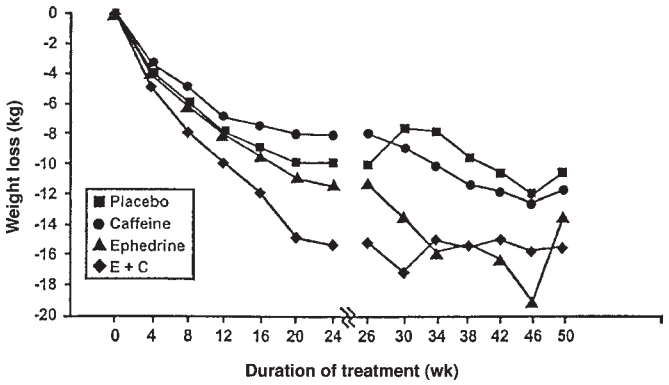

Fig. 1. Effect of a combination of 20 mg of ephedrine and 200 mg of caffeine three times per day as adjuvant to a hypocaloric diet on weight loss and weight maintenance for 1 yr. The first 24 mo involved a randomized, double-blind, placebo-controlled study, whereas all groups received E+C from wk 26 to 50.

leptin also exerts an inhibitory effect on bone formation *(27)*, which serves as a cautionary note about the safety of long-term administration of leptin and analogs as part of the treatment of obesity.

Thermogenic Effects of Ephedrine and Caffeine

Ephedrine (E) decreases body fat in obese subjects by a dual action: suppression of appetite and stimulation of energy expenditure covered by fat oxidation *(28)*. The thermogenic and clinical effects are potentiated by adenosine antagonists such as caffeine (C). Ephedrine is both an indirect sympathomimetic causing release of norepinephrine from the sympathetic nerve endings *(15)* and a direct agonist on β-receptors. Recent experimental studies show that not only β_1 and β_2, but also β_3 are involved in its peripheral thermogenic effect *(14)*. Combinations of E+C have been shown to be effective for the treatment of obesity (Fig. 1). In a study including 180 obese patients, it was found that 20 mg/200 mg t.i.d. of E+C was superior to placebo, caffeine and ephedrine in promoting a dietary-induced weight loss over 24 wk *(29)*. After 24 wk the placebo group had lost 13.2 kg, and E+C improved the results by 3.4 kg to a total weight loss of 16.6 kg. Notably, tachyphylaxis developed to the cardiovascular effects but not to the weight loss-producing effects of the compound. Only a minor influence on blood pressure (BP) and heart rate could be detected when the E+C compound was introduced to the patients, but the effect disappeared after 12 wk where the reductions in BPs were similar to those in the placebo group *(29)*. Stich et al. *(30)* studied the metabolic and hemodynamic responses to submaximal exercise before and after 3 d of E+C treatment in obese patients with a mean body weight of 107 kg. They found no indications of an enhanced exercise-induced increase in BP and heart rate during E+C treatment.

How much of the fat loss produced by E+C can be attributed to the thermogenic action of the compound? A study including measurements of energy expenditure in whole-body calorimeters and of body composition during 8 wk of treatment with a hypocaloric diet and either E+C or placebo suggested that about 25% of the additional fat loss seen in the intervention group could be explained by an enhanced fat oxidation and thermogenic effect *(31)*. Similar results have been found in nonhuman primates, in which E+C caused fat loss and increased sleeping energy expenditure by 20–25% *(32)*.

E+C does not seem to have any long-term effect on glucose and lipid metabolism apart from the beneficial changes that occur secondary to weight loss. Buemann et al. *(33)* reported that E+C prevented the decline in high-density lipoprotein (HDL)-cholesterol associated with weight loss, and increased the ratio of HDL-cholesterol to total-cholesterol, whereas no effect on fasting glucose metabolism was observed.

The clinical studies of E+C clearly show that the compound is effective in the treatment of obesity for up to 1 yr *(34)* (Fig. 1). However, the limited number of patients treated in trials does meet the efficacy and safety requirements of the US Food and Drug Administration or the European Committee on Proprietary Medicinal Products (CPMP) for licensing as a prescription compound, because of the limited interest in the pharmaceutical industry. Various herbal combinations of E+C based on Mia Huang, guarana, and aspirin are sold over the counter in United States, and total sales reached about $950 million in 1999.

Thermogenic Properties of Sibutramine

Sibutramine is a serotonin and noradrenaline reuptake inhibitor, and it causes weight loss in laboratory animals through effects on both food intake and metabolic rate *(35)*. Controlled trials in patients with obesity have consistently shown dose-related weight loss with sibutramine. Typically, weight loss was 3–5 kg greater than placebo at 24 wk, and it was sustained for 2 yr. The proportion of patients losing at least 5% of body weight over 12 mo was 29% with placebo, 56% with 10 mg/d of sibutramine, and 65% with 15 mg/d of sibutramine *(36)*.

Sibutramine causes dose-dependent inhibition of daily food intake in rats owing to the enhancement of satiety, whereas eating patterns and other behaviors remain similar to those exhibited by control animals. Unlike amphetamine, sibutramine does not stimulate locomotor activity. Sibutramine also stimulates thermogenesis in rats, producing sustained (>6 h) elevation of energy expenditure of up to 30% *(35)*. The thermogenic effect of sibutramine results from central stimulation of efferent sympathetic activity because it is inhibited by ganglionic blockade and by high doses of nonselective β-adrenergic antagonists. Sibutramine also decreases food intake in humans by increasing meal-induced satiety *(37,38)*, therefore, sibutramine clearly possesses anorectic properties attributable to both adrenergic and serotonergic properties.

The potential thermogenic properties of sibutramine have been tested in a number of acute tests and long-term trials using indirect calorimetry. Having taken the pharmacokinetic profile of sibutramine and its active metabolites into consideration, Hansen et al. *(39)* observed increases in RMR and in the thermic effect of meals, above that of placebo, and in core temperature in normal weight males. The increased energy expenditure was covered by higher rates of both glucose and fat utilization and was linked to activation of sympathoadrenal activity.

The contribution of the thermogenic effect of sibutramine to weight loss was examined in two trials. Hansen et al. *(38)* studied the chronic effect of sibutramine in 32 obese subjects randomized to 8 wk of treatment with either 15 mg of sibutramine or placebo daily in a double-blind design. Twenty-four-hour energy expenditure was measured before the start and on the last day of treatment. Weight loss was 2.4 kg in the sibutramine group vs 0.3 kg in the placebo group ($p < 0.001$). Despite larger loss of both fat-free mass and fat mass in the sibutramine-treated group ($p < 0.001$), 24-h energy expenditure did not decrease more than in the placebo group (–2.6 *vs* –2.5%, not significant). As expected, the reduction in body weight during the 8 wk was associated with a decrease in 24-h energy expenditure ($r = 0.42$, $p < 0.01$). When the body weight changes were taken into account, 24-h energy expenditure decreased less in the sibutramine than in the placebo group (0.8 vs 3.8%, $p < 0.02$). In a trial by Walsh et al. *(40)*, 19 obese females were instructed to consume a hypocaloric diet for 12 wk and, in a double-blind fashion, received 15 mg of sibutramine or placebo daily. RMR was measured before and after 12 wk of treatment. Adjusted for weight loss, the measurements of RMR suggested that sibutramine may blunt the decline in RMR, although it was not statistically significant. No change in thermogenic response to an infusion of adrenaline was found after chronic treatment with sibutramine.

These studies demonstrate that sibutramine possesses mild thermogenic properties in humans, sufficient to prevent the decline in 24-h energy expenditure that normally occurs in obese subjects during energy restriction and weight loss. Future studies should carefully consider the pharmacokinetics of sibutramine, and study designs should possess sufficient statistical power to detect differences in 24-h energy expenditure on the order of 2–4% *(41)*.

β_3-Adrenoceptor Agonists

In short-term studies in humans, sympathomimetic agents with β-adrenergic effects increase energy expenditure. Lipolysis of WAT, the thermogenic response to catecholamines, and increased fat oxidation have all been shown to be mediated by a combination of β_1-, β_2-, and β_3-receptor subtypes. However, the cardiovascular effects of the β_1-subtype, in particular, and the effects of the β_2-subtype on serum potassium, hepatic glucose production, and myometrium, have directed more focus to the β_3-receptor subtype for the possibility of stimulating fat combustion with a risk of no, or only mild, side effects. After their discovery in the 1980s, β_3-adrenoceptor agonists were shown to have remarkable antiobesity and antidiabetic effects in rodents, but several pharmaceutical problems have since retarded the development of these products into therapeutic agents for human use *(42)*. Those that have been taken forward to clinical studies either have proved ineffective or their activities have been accompanied by significant side effects. Pharmaceutical problems in this area include a poor pharmacokinetic profile with low bioavailability and short half-life, and the failure of prodrugs to be metabolized to selective β_3-agonists *(43)*. More important are the differences between the rodent and human β_3-receptor, because the compounds were selected by screening using the rodent β_3-adrenoceptor and turned out to have a much lower efficacy at the human receptor. Examples are CL 316,243 and ICID7114, compounds that turned out to have no thermogenic effect in human clinical trials *(44,45)*. In humans, treatment with CL 316,243 for 8 wk, in spite of limited bioavailability, induced marked plasma concentration-dependent increases in insulin sensitivity, lipolysis, and fat oxidation in lean volunteers, without causing β_1- or β_2-mediated side effects *(44)*. However, the compound had no effect on 24-h energy expenditure. This finding may be explained by a low intrinsic activity at the human β_3-receptor *(42)*. Some of these problems seem to have been solved with the cloning of the human β_3-receptor, which has made it possible to develop novel compounds directed specifically at the human receptor. Currently, a number of pharmaceutical companies have been successful in developing β_3-agonists with thermogenic efficacy in short-term human studies *(46)*, but none have so far proved sustained efficacy in clinical trials *(44,45)*. A compound from Lilly, LY-377604, has been described as having more than 20% oral bioavailability. A single dose of 120 mg (the highest dose used) of LY-377604 increased RMR by 17.5% in normal weight and obese subjects *(47)*.

The compounds of the new generation in preclinical development are full agonists at the human β_3-receptors. It will be intriguing to discover whether their efficacy in clinical trials will live up to the expectations created by their acute thermogenic effects and, if so, whether their effects will translate into weight loss and improved metabolic control useful in the treatment of obesity and type 2 diabetes.

Some concerns do exist including the major question as to whether the number of biologically active β_3-receptors in adult humans is sufficient to produce a clinically relevant thermogenic response. Are there sufficient β_3-adrenoceptors in non-BAT tissues to achieve a clinical effect in adult humans, or is the clinical efficacy dependent on the existence, also in humans, of a mechanism for recruitment of BAT by chronic, sustained β_3-stimulation? There are some

suggestions that an enhanced thermogenic response to β-adrenergic stimulation can be achieved in humans during chronic β-stimulation *(48)*, but perhaps the pharmaceutical companies should extend trials to assess the effect on 24-h energy expenditure to a duration of at least 8 wk to allow the putative recruitment of dormant BAT. Perhaps, also, expectations of the magnitude of the effect on 24-h energy expenditure should be lowered and compounds such as the Zeneca compound ZD2079, which increases 24-h energy expenditure by 2%, should be considered for clinical development *(45)*.

Other Thermogenic Compounds

Several thermogenic compounds with other modes of action are currently being tested in phase 1 studies. These include derivatives of thyroid hormone, uncouplers related to dinitrophenol, capsaicin from hot chillies *(49)*, and polyphenols from green tea *(50)*. To date, none have gone into clinical trial.

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