

Effects of Controlled-Release Alpha Lipoic Acid In Lean, Nondiabetic Patients with Polycystic Ovary Syndrome

Umesh Masharani, M.D.,¹ Christine Gjerde,¹ Joseph L. Evans, Ph.D.,² Jack F. Youngren, Ph.D.,¹ and Ira D. Goldfine, M.D.¹

Abstract

Background:

The purpose of this study was to determine whether a preparation of controlled-release alpha lipoic acid (CRLA) influences features of the polycystic ovary syndrome (PCOS).

Methods:

We administered CRLA 600 mg twice daily for 16 weeks to six lean, nondiabetic patients with PCOS. Insulin sensitivity was measured by the euglycemic, hyperinsulinemic clamp. Plasma lipids were measured by vertical ultracentrifugation. Oxidative stress markers were measured in serum.

Results:

At the end of 16 weeks of CRLA treatment, there was a 13.5% improvement in insulin sensitivity as determined by the euglycemic, hyperinsulinemic clamp ($p < .03$). There was also a lowering of triglyceride levels ($p < .04$) and a shift in the distribution of low-density lipoprotein (LDL) particles toward the larger, more buoyant LDL subclass fraction. Two of the subjects who were not on oral contraception had an increased number of menstrual cycles. Controlled-release alpha lipoic acid treatment, however, was neither associated with an increase in plasma antioxidant capacity nor with a reduction in plasma lipid oxidation products.

Conclusions:

These data suggest that the CRLA has positive effects on the PCOS phenotype. The effects of CRLA, however, may have been exerted through a mechanism not involving changes in oxidative stress.

J Diabetes Sci Technol 2010;4(2):359-364

Author Affiliations: ¹Diabetes Center, Department of Medicine, University of California, San Francisco, Mt. Zion Medical Center, San Francisco, California; and ²Receptor Bio, Inc., Redwood City, California

Abbreviations: (AMPK) 5' adenosine monophosphate-activated protein kinase, (BMI) body mass index, (CRLA) controlled-release alpha lipoic acid, (DHEA) dehydroepiandrosterone, (FSH) follicular stimulating hormone, (HDL) high-density lipoprotein, (hsCRP) highly sensitive C-reactive protein, (iPF2 α -III) 9-iso prostaglandin F $_{2\alpha}$, (LC/MS/MS) liquid chromatography tandem mass spectrometry, (LDL) low-density lipoprotein, (LH) luteinizing hormone, (PCOS) polycystic ovary syndrome, (TBARS) 2-thiobarbituric acid reactive substances, (TRAP) total reactive antioxidant potential

Keywords: cholesterol, insulin resistance, oxidative stress, triglycerides

Corresponding Author: Umesh Masharani, M.D., University of California, San Francisco, 400 Parnassus Ave., ACC 585, Box 1222, San Francisco, CA 94143; email address umeshm@medicine.ucsf.edu

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 4% to 12% of reproductive-age women.¹ Polycystic ovary syndrome is characterized by hyperandrogenemia, amenorrhea, and anovulation.² PCOS patients frequently have insulin resistance, hypertension, and dyslipidemia and are at increased risk for cardiovascular disease and diabetes.² Although most women with PCOS are obese, about 20% of them are of normal weight.^{3–7} Several studies have suggested that the presence of insulin resistance is independent of obesity and is present in lean patients with PCOS.^{8,9} This insulin resistance of PCOS may be pathophysiologically important because lowering insulin resistance with insulin sensitizers can improve reproductive function in some women with PCOS.¹⁰ The molecular mechanisms leading to the insulin resistance in PCOS are not well understood, but oxidative stress has been implicated.^{11–15} Alpha lipoic acid is a potent antioxidant, and controlled-release alpha lipoic acid (CRLA) has been reported to improve glucose control in type 2 diabetes patients,¹⁶ presumably by its effects on reducing oxidative stress and insulin resistance. We hypothesized, therefore, that CRLA, by lowering oxidative stress in women with PCOS, would improve insulin sensitivity and improve reproductive and metabolic abnormalities. Since obesity per se may have additional effects on reproductive function and metabolic parameters, the present study was carried out in nonobese women with PCOS.

Methods

We investigated six nonobese women ages 23 to 34 who met the Rotterdam criteria¹⁷ for diagnosis of PCOS. The women did not have any other medical problems. The patients were relatively lean, with body mass index (BMI) ranging from 18.5 to 26.6 (mean 22 ± 1.4) (Table 1). All the women had a history of oligomenorrhea with less than five spontaneous periods per year since menarche. They gave a history of hirsutism that was improved by using either oral contraceptives and/or spironolactone. Ferriman–Gallwey scores¹⁸ ranged from 1 to 7. The low hirsutism scores reflected the treatments they had received. One patient had a history of a large ovarian cyst.

Measurements of follicular stimulating hormone (FSH), luteinizing hormone (LH), prolactin, dehydroepiandrosterone (DHEA) sulfate, total and free testosterone by liquid chromatography tandem mass spectrometry (LC/MS/MS), and 17 hydroxyprogesterone (LC/MS/MS) were performed on morning blood samples at Quest Diagnostics (Madison, NJ). Patients had normal LH, FSH, DHEA sulfate, prolactin, and 17 hydroxyprogesterone levels (Table 1). The total testosterone levels ranged from 8 to 44 ng/dl (normal range 2–45 ng/dl) and free testosterone levels ranged from 0.3 to 3.6 pg/ml (normal range 0.1–6.4 pg/ml). Four of the six patients were on oral contraceptives, and their free testosterone

Table 1.
Baseline Characteristics and Hormone Levels

Age	BMI	Waist circumference (cm)	17 hydroxy progesterone (ng/dl) (follicular <185; midcycle <225; luteal <285)	Prolactin (ng/ml) (3–30)	FSH (mIU/ml) (follicular 2.5–10.2; midcycle 3.1–17.7; luteal 0.5–16.9)	LH (mIU/ml) (follicular 1.9–12.5; midcycle 8.7–76.3; luteal 0.5–16.9)	DHEA sulfate (mcg/dl) (45–320)	Total testosterone (ng/dl) (2–45)	Free testosterone (pg/ml) (0.1–6.4)	Ferriman–Gallwey score
26 ^a	19.7	67.8	5.5	5.5	5.7	18.1	113	44	3.6	4
26	26	78.8	101	19.8	4.7	18.9	108	53	2.6	1
25 ^a	21.7	71	40	3.8	5.5	7.8	83	22	1.6	1
34	18.5	66	11	10.3	1	1.5	111	21	0.6	4
27	19.6	73.4	21	13.8	0.9	0.4	119	24	1.4	7
23	25.9	80	12	6.3	0.7	0.2	101	8	0.3	2

^a Women not on oral contraception at study entry.

levels ranged from 0.3 to 2.6 pg/ml. The two women who were not on oral contraception at study entry had free testosterone levels of 3.6 and 1.6 pg/ml and total testosterone levels of 44 and 22 ng/dl. The ethnic background was five Caucasians and one Asian.

A standardized oral glucose tolerance test was performed to exclude the diagnosis of either diabetes or impaired glucose tolerance. Individuals with cardiovascular diseases, human immunodeficiency virus, other active infections, thyroid disorders, epilepsy, cancer, hepatitis, cystic fibrosis, sickle cell disease, asthma, or renal disease were also excluded. Subjects were not taking medications known to affect insulin sensitivity, carbohydrate metabolism, or lipid metabolism. These medications included glucocorticoids, adrenergic agonists, psychotropic drugs, diuretics, beta blockers, and 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors.

Subjects were screened with respect to their exercise and physical activity pattern. Only subjects who performed less than 60 min of strenuous exercise per week were recruited—the rationale being that regular strenuous physical activity may attenuate the effect of CRLA on insulin sensitivity. Subjects underwent a dietary history at enrollment and were placed on a weight maintenance diet in order to avoid the confounding effect of weight loss on insulin sensitivity.

The women underwent baseline tests which included (a) a hyperinsulinemic, euglycemic clamp,¹⁹ for which a primed-continuous infusion of regular human insulin was administered at a rate of 40 mU/min/m²/body surface area for 120 min, blood glucose levels were maintained at approximately basal level with a variable infusion of 20% glucose, average glucose infusion rates were determined during the steady state period between 90 and 120 min, and glucose disposal values (M/I) were calculated as milligrams of glucose infused per minute per kilogram of body mass divided by steady state insulin levels (in $\mu\text{U/ml} \times 100$); (b) a cardiovascular risk panel, including low-density lipoprotein (LDL) pattern size and intermittent density lipoproteins measured using a vertical ultracentrifugation technique (VAP[®] panel, Atherotech, Birmingham, AL), and highly sensitive C-reactive protein (hsCRP) and fasting homocysteine levels were also obtained; and (c) measurements of different serum oxidative stress markers, including isoprostane (Kronos laboratories), protein carbonyls, 2-thiobarbituric acid reactive substances (TBARS), and total reactive antioxidant potential (TRAP) (Antioxidants and Mass Spectrophotometry Core Laboratory of

the University of California-Davis, Clinical Nutrition Research Unit).

After completion of the baseline tests, the subjects were administered CRLA 600 mg twice daily for 16 weeks, and the tests repeated. Controlled-release alpha lipoic acid was supplied by the Medical Research Institute, San Francisco, CA.

Ethical Considerations

All subjects gave informed consent. The protocols and consent forms were approved by the University of California, San Francisco, institutional review board and by the Clinical and Translation Science Institute, where the study was conducted.

Statistical Analysis

Comparisons between the baseline and final were made using a Student's *t*-test (two-tailed) and two paired signed rank sum test (Wilcoxon), in which values in each row of the data set were paired. Significance was accepted at $p < .05$. The p values stated are those obtained with Student's *t*-test. Nonparametric analyses of the data did not alter the conclusions. All analyses were performed using GraphPad Prism (MS Windows, version 4.03, GraphPad Software, San Diego, CA). Data are presented as mean \pm standard error of the mean.

Results

Effect of Controlled-Release Alpha Lipoic Acid on Insulin Sensitivity

The subjects were on a weight maintaining diet, and their weights did not change during the study. The subjects did not have any adverse reactions to the alpha lipoic acid. At the end of 16 weeks, there was a significant increase in insulin sensitivity with CRLA as determined by the hyperinsulinemic, euglycemic clamp. The mean insulin mediated glucose disposal increased from 9.7 ± 1.3 mg/min/kg/mU insulin to 11.1 ± 1.7 mg/min/kg/mU insulin ($p = .03$).

Effect of Controlled-Release Alpha Lipoic Acid on Plasma Lipid Values

There was a significant lowering of triglyceride levels with CRLA administration (**Table 2**). Pretreatment levels were 80.3 ± 11.6 mg/dl, and post-treatment levels were 57.8 ± 4.3 mg/dl ($p = .04$). However, there was no change in either total, LDL, or high-density lipoprotein (HDL) cholesterol (**Table 2**). There was no change in either homocysteine or hsCRP levels.

The vertical ultracentrifugation (VAP II) method directly fractionates LDL cholesterol into 4 subfractions (LDL1, LDL2, LDL3, and LDL4). LDL1 and LDL2 contain the large, buoyant LDL particles; LDL4 contains the small, dense LDL particles; and LDL3 is intermediate-sized particles.²⁰ We observed that CRLA therapy was associated with a shift in the distribution of LDL particles toward a larger, more buoyant LDL subclass pattern. Thus LDL4, the most atherogenic of all LDL subfractions, fell from 7.5 ± 0.8 to 4.6 ± 1.3 ($p = .02$).

Effect of Controlled-Release Alpha Lipoic Acid on Oxidative Stress Markers

Since alpha lipoic acid is a potent antioxidant, we measured serum oxidative stress markers before and after CRLA therapy. However, we did not observe an increase in either plasma antioxidant capacity or reduction in plasma lipid oxidation products (Table 3). The TRAP of plasma was 180 ± 13.5 μ M Trolox equivalents before and 170 ± 17.0 μ M Trolox equivalents after CRLA therapy ($p = .63$). The plasma TBARS increased modestly with CRLA therapy (0.7 ± 0.1 before and 0.9 ± 0.1 after), but this was not statistically significant ($p = .12$). There was no statistically significant change in protein carbonyl levels (1.5 ± 0.1 nmol/mg before and 1.7 ± 0.1 nmol/mg protein after CRLA treatment; $p = .26$) or the isoprostane 9-iso prostaglandin F₂ α (iPF₂ α -III; 39.4 ± 9.5 pg/ml before and 45.4 ± 7.6 pg/ml after CRLA treatment; $p = .63$).

Effects of Controlled-Release Alpha Lipoic Acid on Menstrual Cyclicity

Two subjects were not on oral contraception prior to entry into the study. These two subjects had 1.6 and 1.3 periods in the four months prior to study entry. During the 16 weeks of treatment, these subjects had three and four periods, respectively. These two subjects did not have weight changes or increases in physical activity to explain the increased menstrual cyclicity.

There were no changes in the hirsutism scores, but it should be noted that four of the six subjects remained on oral contraception and also that the study duration was only 16 weeks. A study lasting at least six months would be necessary to evaluate the effect of CRLA on hirsutism.

Discussion

Insulin resistance commonly occurs in PCOS patients, and reversal of insulin resistance with either metformin or thiazolidinediones administration improves the PCOS

Table 2.
Effect of Alpha Lipoic Acid on Triglyceride and Cholesterol Levels

	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)
Pre-average	80.3 ± 11.6 (53–131)	179 ± 16.8 (106–205)	67.3 ± 5.2 (51–81)	101 ± 14.3 (47–138)
Post-average	57.8 ± 4.3 (55–77)	175 ± 17.6 (112–217)	61.7 ± 3.2 (52–75)	103 ± 17.3 (46–157)
<i>p</i> value	.04	.60	.17	.82

Table 3.
Effect of Alpha Lipoic Acid on Serum Oxidative Stress Markers

	Protein carbonyls (nmol/mg protein)	TBARS (malondialdehyde μ M)	TRAP (μ M Trolox Equivalents)	iPF ₂ α -III (pg/ml)
Pre-average	1.5 ± 0.1	0.7 ± 0.1	180 ± 13.5	39.4 ± 9.5
Post-average	1.7 ± 0.1	0.9 ± 0.1	170 ± 17.0	45.4 ± 7.6
<i>p</i> value	.26	.12	.63	.63

phenotype in some patients.²¹ Oxidative stress is increased in PCOS patients, and potentially, this factor could contribute to the insulin resistance state.^{12,15} We hypothesized that using a potent antioxidant such as alpha lipoic acid would therefore have beneficial effects on the PCOS phenotype. Thus we administered CRLA to women with PCOS. We recruited lean, nondiabetic women for this study to avoid the confounding effects of obesity or diabetes.

We expected these lean PCOS women to be insulin resistant as measured by the euglycemic, hyperinsulinemic clamp, but in fact, their resistance was not particularly marked. The mean insulin-mediated glucose disposal rate at baseline was 9.7 ± 1.3 mg/min/kg/mU (range 5.2 to 13.6). Twenty-seven nonobese, nondiabetic sedentary subjects studied under identical conditions using the same procedure at our clinical research center had a mean insulin-mediated glucose disposal rate of 9.8 ± 0.6 mg/min/kg/mU (range 4.0 to 16.3). It has been reported previously that, although insulin resistance is a common abnormality in PCOS, it is not a universal feature.^{22,23} There is considerable overlap in insulin

sensitivity with normal controls, and the resistance tends to increase as the BMI increases.²³ It has also been reported that, when obese PCOS women lose weight, their insulin sensitivity improves to the same level as BMI-matched control subjects.²⁴ Despite the absence of severe insulin resistance, we observed that CRLA therapy resulted in an improvement in insulin sensitivity. The patients were on a weight maintenance isocaloric intake, and there was no weight loss to explain the change in insulin sensitivity with CRLA therapy. Metformin and thiazolidinediones have been associated with improvements in insulin sensitivity from approximately 19% to approximately 40% in PCOS patients.^{8,9,25} Some of the studies that showed the greater improvements did not control for changes in either weight or physical activity.^{8,9,25} It is possible, therefore, that longer duration studies with CRLA in more resistant, obese, PCOS subjects will show greater improvements in insulin sensitivity.

Women with PCOS, like patients with type 2 diabetes and the insulin resistance/metabolic syndrome, have increased levels of the atherogenic lipoproteins that predispose them to an increased risk for cardiovascular disease. In animal models of atherosclerosis and dyslipidemia, alpha lipoic acid administration has potent effects on triglyceride and other lipid values.²⁶ In our study, we observed that CRLA both lowered triglycerides levels and increased the LDL particle size. Thus CRLA may have a significant anti-atherogenic effect in PCOS.

Improvements in insulin sensitivity have been associated with improvements in reproduction in women with PCOS. Two of the subjects in this study were not on oral contraceptive medications. Both of these women had improvement in their menstrual cyclicity. Thus CRLA may be useful in restoring normal menstrual cycles in certain PCOS patients.

Our initial hypothesis was that alpha lipoic acid administration would improve insulin resistance by lowering oxidative stress. Thus we were surprised that there was no improvement in serum oxidative stress markers. There are several possible explanations for this finding. First, our assays may have been relatively insensitive to changes in either plasma oxidative capacity or lipid and protein oxidation. This possibility is unlikely, because these same assays have been reported to detect changes due to intake of nutritional antioxidants.²⁷ Second, the circulating oxidative stress markers may not accurately reflect the intracellular oxidative state that could have been affected by CRLA therapy. Third, CRLA

might have had an effect on insulin resistance through a mechanism other than via its antioxidant effect. Thiazolidinediones and metformin activate 5' adenosine monophosphate kinase in muscle and other tissues,²⁸⁻³⁰ and this action is believed to be one mechanism whereby these drugs improve insulin action in patients with PCOS.³¹ Alpha lipoic acid has also been reported to activate 5' adenosine monophosphate-activated protein kinase (AMPK). Alpha lipoic acid increased both fatty acid oxidation and insulin stimulated glucose uptake in obese, diabetic rats.³² Administration of dominant negative AMPK into skeletal muscle prevented these effects of alpha lipoic acid.³¹ In addition to improvements at the level of the muscle, 5' adenosine monophosphate kinase activation with alpha lipoic acid also lowered triglycerides and improved endothelial function.³³ Additional studies are therefore required to determine whether the alpha lipoic acid effects observed in PCOS patients are due to AMPK.

Funding:

This work was supported by the University of California, San Francisco, Division of Endocrinology and Metabolism discretionary funds.

Acknowledgments:

The authors thank the John A. Kerner Foundation and the Medical Research Institute (San Francisco, CA). ClinicalTrials.gov Identifier: NCT00505427

References:

1. Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. *J Clin Endocrinol Metab.* 1998;83(9):3078-82.
2. Ehrmann DA. Polycystic ovary syndrome. *N Engl J Med.* 2005;352(12):1223-36.
3. Chang RJ, Nakamura RM, Judd HL, Kaplan SA. Insulin resistance in nonobese patients with polycystic ovarian disease. *J Clin Endocrinol Metab.* 1983;57(2):356-9.
4. Holte J, Bergh T, Berne C, Lithell H. Serum lipoprotein lipid profile in women with the polycystic ovary syndrome: relation to anthropometric, endocrine and metabolic variables. *Clin Endocrinol (Oxf).* 1994;41(4):463-71.
5. Morales AJ, Laughlin GA, Büttow T, Maheshwari H, Baumann G, Yen SS. Insulin, somatotrophic, and luteinizing hormone axes in lean and obese women with polycystic ovary syndrome: common and distinct features. *J Clin Endocrinol Metab.* 1996;81(8):2854-64.
6. Morin-Papunen LC, Vauhkonen I, Koivunen RM, Ruokonen A, Tapanainen JS. Insulin sensitivity, insulin secretion, and metabolic and hormonal parameters in healthy women and women with polycystic ovarian syndrome. *Hum Reprod.* 2000;15(6):1266-74.

7. Ovesen P, Moller J, Ingerslev HJ, Jørgensen JO, Mengel A, Schmitz O, Alberti KG, Moller N. Normal basal and insulin-stimulated fuel metabolism in lean women with the polycystic ovary syndrome. *J Clin Endocrinol Metab.* 1993;77(6):1636–40.
8. Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, Zanolin E, Muggeo M. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. *J Clin Endocrinol Metab.* 2000;85(1):139–46.
9. Rautio K, Tapanainen JS, Ruokonen A, Morin-Papunen LC. Endocrine and metabolic effects of rosiglitazone in overweight women with PCOS: a randomized placebo-controlled study. *Hum Reprod.* 2006;21(6):1400–7.
10. Goldenberg N, Glueck C. Medical therapy in women with polycystic ovarian syndrome before and during pregnancy and lactation. *Minerva Ginecol.* 2008;60(1):63–75.
11. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes.* 2003;52(1):1–8.
12. Fenkci V, Fenkci S, Yilmazer M, Serteser M. Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. *Fertil Steril.* 2003;80(1):123–7.
13. Gopalakrishna R, Jaken S. Protein kinase C signaling and oxidative stress. *Free Radic Biol Med.* 2000;28(9):1349–61.
14. Polak G, Kozioł-Montewka M, Tarkowski R, Kotarski J. [Peritoneal fluid and plasma 4-hydroxynonenal and malonyldialdehyde concentrations in infertile women]. *Ginekol Pol.* 2001;72(12A):1316–20.
15. Sabuncu T, Vural H, Harma M, Harma M. Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. *Clin Biochem.* 2001;34(5):407–13.
16. Evans JL, Heymann CJ, Goldfine ID, Gavin LA. Pharmacokinetics, tolerability, and fructosamine-lowering effect of a novel, controlled-release formulation of alpha-lipoic acid. *Endocr Pract.* 2002;8(1):29–35.
17. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril.* 2004;81(1):19–25.
18. Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. *J Clin Endocrinol Metab.* 1961;21:1440–7.
19. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* 1979;237(3):E214–23.
20. Kulkarni KR, Garber DW, Jones MK, Segrest JP. Identification and cholesterol quantification of low density lipoprotein subclasses in young adults by VAP-II methodology. *J Lipid Res.* 1995;36(11):2291–302.
21. Sharma ST, Nestler JE. Prevention of diabetes and cardiovascular disease in women with PCOS: treatment with insulin sensitizers. *Best Pract Res Clin Endocrinol Metab.* 2006;20(2):245–60.
22. Cibula D. Is insulin resistance an essential component of PCOS?: The influence of confounding factors. *Hum Reprod.* 2004;19(4):757–9.
23. DeUgarte CM, Bartolucci AA, Azziz R. Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. *Fertil Steril.* 2005;83(5):1454–60.
24. Holte J, Bergh T, Berne C, Wide L, Lithell H. Restored insulin sensitivity but persistently increased early insulin secretion after weight loss in obese women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 1995;80(9):2586–93.
25. Baillargeon JP, Jakubowicz DJ, Luorno MJ, Jakubowicz S, Nestler JE. Effects of metformin and rosiglitazone, alone and in combination, in nonobese women with polycystic ovary syndrome and normal indices of insulin sensitivity. *Fertil Steril.* 2004;82(4):893–902.
26. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes.* 1988;37(12):1595–607.
27. Wang JF, Schramm DD, Holt RR, Ensunsa JL, Fraga CG, Schmitz HH, Keen CL. A dose-response effect from chocolate consumption on plasma epicatechin and oxidative damage. *J Nutr.* 2000;130(8S Suppl):2115S–9S.
28. Hutchinson DS, Summers RJ, Bengtsson T. Regulation of AMP-activated protein kinase activity by G-protein coupled receptors: potential utility in treatment of diabetes and heart disease. *Pharmacol Ther.* 2008;119(3):291–310.
29. Viollet B, Mounier R, Leclerc J, Yazigi A, Foretz M, Andreelli F. Targeting AMP-activated protein kinase as a novel therapeutic approach for the treatment of metabolic disorders. *Diabetes Metab.* 2007;33(6):395–402.
30. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest.* 2001;108(8):1167–74.
31. Lee WJ, Song KH, Koh EH, Won JC, Kim HS, Park HS, Kim MS, Kim SW, Lee KU, Park JY. Alpha-lipoic acid increases insulin sensitivity by activating AMPK in skeletal muscle. *Biochem Biophys Res Commun.* 2005;332(3):885–91.
32. Huong DT, Ide T. Dietary lipoic acid-dependent changes in the activity and mRNA levels of hepatic lipogenic enzymes in rats. *Br J Nutr.* 2008;100(1):79–87.
33. Park KG, Min AK, Koh EH, Kim HS, Kim MO, Park HS, Kim YD, Yoon TS, Jang BK, Hwang JS, Kim JB, Choi HS, Park JY, Lee IK, Lee KU. Alpha-lipoic acid decreases hepatic lipogenesis through adenosine monophosphate-activated protein kinase (AMPK)-dependent and AMPK-independent pathways. *Hepatology.* 2008;48(5):1477–86.