Anti-diabetic effects of astaxanthin on an STZ-induced diabetic model in rats

Fen Zhuge1),*, Yinhua Ni2),*, Chunyan Wan2), Fen Liu2) and Zhengwei Fu3)

1) Institute of Translational Medicine, The Affiliated Hospital of Hangzhou Normal University, 310015, China
2) College of Biotechnology and Bioengineering, Zhejiang University of Technology, 310014, China

Abstract. Type 2 diabetes mellitus (T2DM), which is characterized by insulin resistance and relative insulin insufficiency, has become the most common chronic metabolic disease threatening global health. The preferred therapies for T2DM include lifestyle interventions and the use of anti-diabetic drugs. However, considering their adverse reactions, it is important to find a low-toxicity and effective functional food or drug for diabetes prevention and treatment. Astaxanthin is a potent antioxidant carotenoid found in marine organisms has been reported to prevent diet-induced insulin resistance and hepatic steatosis. To investigate the anti-diabetic effects of astaxanthin, male Wistar rats were fed a high-energy diet for 4 weeks, followed by a low dose streptozotocin (STZ) injection to induce the diabetes model, and the rats were then fed an astaxanthin-containing diet for another 3 weeks. Astaxanthin significantly decreased blood glucose and total cholesterol (TC) levels, and increased blood levels of high density lipoprotein cholesterol (HDL-C) in STZ-induced diabetic rats in a dose dependent manner. These results were associated with increased expression of insulin sensitivity related genes (adiponectin, adipor1, and adipor2) in vivo, thereby attenuating STZ-induced diabetes. In addition, we also compared the anti-diabetic effects of astaxanthin and monacolin K, which has been reported to downregulate hyperlipidemia and hyperglycemia. The results revealed that astaxanthin and monacolin K showed similar anti-diabetic effects in STZ-induced diabetic rats. Therefore, astaxanthin may be developed as an anti-diabetic agent in the future.

Key words: Diabetes, Astaxanthin, Insulin sensitivity

TYPE 2 DIABETES MELLITUS (T2DM), which is characterized by insulin resistance and relative insulin insufficiency [1], has become the most common chronic metabolic disease threatening global health. The International Diabetes Federation (IDF) estimated that the global diabetes prevalence in 2019 was 9.3% (463 million people), and will increase to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 [2]. Patients with T2DM display increased risk of developing severe complications, including macrovascular complications (cardiovascular disease) and microvascular complications (nephropathy, neuropathy and retinopathy) [3]. In recent years, enormous advances have been made in the field of diabetes prevention and treatment and in glycemic management, but the prevalence of complications remains an important issue in T2DM patients [4].

Currently, there are no drugs to cure diabetes, and the preferred T2DM treatments include the use of anti-diabetic drugs (such as sulfonylureas, glinides, GLP-1R agonists, DPP-4 inhibitors, SGLT2 inhibitors, and insulin) and lifestyle interventions (healthy nutrition and daily physical activity). However, considering their adverse reactions, it is important to find a low-toxicity and effective functional food or drug for the prevention and treatment of diabetes.

A large number of studies have revealed that carotenoids reduce type 2 diabetes risk in men and women [5, 6]. Astaxanthin is a xanthophyll carotenoid found in marine organisms, including salmon, shrimp, crustaceans, and algae such as Haematococcus pluvialis [7], that has been reported to prevent diet-induced obesity and hepatic steatosis in rats [8] and ameliorate insulin resistance by protecting myocytes from oxidative stress [9]. Astaxanthin is safe, with no side effects when it is consumed with food. It is lipid soluble and has been shown to accumulate in tissues after being fed to rats, and no toxic effects were observed [10, 11]. Astaxanthin could reduce the oxidative stress caused by hyperglycemia in pancreatic β-cells and improve glucose and serum...
astaxanthin would inhibit the progression of diabetes. Here, the therapeutic effects of astaxanthin in STZ-induced diabetic rats were investigated. We also compared the anti-diabetic effects of astaxanthin and Monacolin K, an active compound identified from Monascus-fermented products, which has been reported to down-regulate hyperlipidemia and hyperglycemia [18, 19]. Blood parameters and gene expression were measured to explore the anti-diabetic effects. The results revealed that astaxanthin and monacolin K similarly decreased blood glucose levels, increased HDL-C levels and ameliorated insulin resistance, thereby attenuating STZ-induced diabetes.

Materials and Methods

Animals and experimental design

Male Wistar rats (90–100 g) purchased from the China National Laboratory Animal Resource Center (Shanghai, China) were housed under a 12:12-h LD cycle at constant temperature (22 ± 1°C). To evaluate the therapeutic effects of astaxanthin on diabetic rats, the rats were fed with high-energy diet (HD). The HD was prepared by adding 20% sucrose (wt/wt) and 10% lard (wt/wt) to a basic diet (BD). The composition of the BD was described previously [20], and the mineral mix and the vitamin mix were prepared according to AIN-76 [21]. After 4 weeks of HD feeding, the rats were injected intraperitoneally with a low dose of STZ (40 mg/kg) after 14 h of fasting. The rats were then divided into four groups and fed for another 3 weeks as follows: (1) basic diet (BD group); (2) BD diet with 15 mg/kg astaxanthin (BD + AS15); (3) BD diet with 30 mg/kg astaxanthin (BD + AS30); and (4) BD diet with 50 mg/kg astaxanthin (BD + AS50). All rats were maintained on a 12-h light/dark cycle and given free access to food and water.

To compare the anti-diabetic effects of astaxanthin and monacolin K, the diabetic rats were divided into three groups and fed for 3 weeks as follows: (1) basic diet (BD group); (2) BD diet with 30 mg/kg astaxanthin (BD + AS); and (3) BD diet with 11.8 mg/kg monacolin K (BD + MK). All rats were maintained on a 12-h light/dark cycle and given free access to food and water.

All of the rats were sacrificed under anesthesia by an intraperitoneal injection of 45 mg/kg pentobarbital sodium. The tissues were removed quickly, immediately frozen in liquid nitrogen, and stored at −80°C. Blood samples were centrifuged at 6,000 g for 5 min at 4°C and stored at −80°C. All experiments were performed according to international ethical standards, and the study was approved by the Research Committee of Zhejiang University of Technology.

Measurement of serum glucose and lipids

Serum glucose, triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDLC) levels were measured using commercially available kits (Whitman Biotech, Nanjing, China), and an auto-biochemical analysis system (Achtection c8000; Abbott, North Chicago, Illinois, USA) using commercial kits (Whitman Biotech, Nanjing, China).

Quantitative real-time PCR

Total RNA was isolated from the tissues using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions as previously described [14, 41]. Real-time PCR was performed using the SYBR ExScript PCR kit (Takara Biochemicals, Dalian, China) in a total volume of 10 μL. The primers used in the real-time PCR are shown in Table 1. PCR amplification and quantification were performed using an Eppendorf MasterCyclers ep RealPlex4 (Wesseling-Berzdorf, Germany) as described in our previous report [40]. The data were normalized to the amount of GAPDH.

Data analysis

All data are presented as the means ± SEM. The values for the mRNA levels are presented as relative values in all experiments. Statistical significance of mean differences in body weight, serum values, or gene expression was evaluated using one-way or two-way ANOVA followed by the Student-Newman-Keuls test. Values of $p <0.05$ or $0.01$ were considered statistically significant.

Results

Effect of astaxanthin on blood glucose and lipid metabolic parameters in STZ-induced diabetic rats

In this study, we first investigated the therapeutic effect of astaxanthin on the advanced stage of diabetes in rats. The diabetic rats showed increased blood glucose and TC levels and decreased expression of insulin sensitivity-related genes (adiponectin, adiponectin receptor 1 (AdipoR1), adiponectin receptor 2 (AdipoR2), and peroxisome proliferator-activated receptor γ (PPARγ)) compared with those of normal mice (Fig. S1). After diabetes was induced by HD diet feeding and STZ
administration, a BD diet with or without astaxanthin was administered for another 3 weeks. Body weight and TG levels were not changed between the BD and BD + AS groups (Fig. 1A, 1C). Astaxanthin significantly decreased the blood glucose and TC levels of STZ-induced diabetic rats in a dose-dependent manner, but did not affect the TG levels (Fig. 1B, 1D). On the other hand, astaxanthin dose dependently increased blood HDL-C (Fig. 1E) levels.

| Table 1 Primers used in real-time PCR analysis with SYBR Green |
|-----------------|-----------------|
| Gene | Primer sequence 5’ to 3’ |
| GAPDH | Forward, GAC AAC TTT GGC ATC GTG GA  
Reverse, AGG CAG GGA TGA TGT TCT GG |
| Adiponectin | Forward, GGA AAC TGG TGC AGG TTG GAT G  
Reverse, GGG TCA CCC TTA GGA CCA AGA A |
| AdipoR1 | Forward, CAC AGA AAC TGG CAA CAT CTG GA  
Reverse, CTG AAT GAC AGT AGA CGG TGT GGA A |
| AdipoR2 | Forward, GAA GGT CGA TGG CGA GTG A  
Reverse, CAA TGG CAT TTC GGG CAA C |
| PPARγ | Forward, TGT GGT TTC AGA AGT GCC TTG  
Reverse, TTC AGC TGG TCG ATA TCA CTG GAG |
| UCP2 | Forward, CAG AGC ACT GTG GCC TAC AAG  
Reverse, CAA TGG CAT TTC GGG CAA C |
| FAS | Forward, AGC ATA TCC CTG GAA ACA GGT GAC  
Reverse, TCT GTG GAT AGG ACT GAA TGC TGT G |

**Fig. 1** Effects of astaxanthin on streptozotocin (STZ)-induced diabetes on body weight and serum glucose and lipids levels in rats. The body weight (A), serum glucose (B), TG (C), TC (D) and HDL-C (E) levels were compared between the basic diet group and astaxanthin groups. Each value represents the mean ± SE derived from 6 animals. Differences between the different groups are analyzed by one-way ANOVA, *p < 0.05, **p < 0.01 vs. BD group.

**Effect of astaxanthin on tissue gene expression**
We first examined gene expression in adipose tissue, and the results showed that astaxanthin treatment increased the expression of insulin sensitivity-related genes (adiponectin, AdipoR2, and PPARγ) in a dose-dependent manner. Uncoupling protein 2 (UCP2) expression was also increased dose-dependently. AdipoR1 was not affected by astaxanthin administration (Fig. 2). AdipoR1, AdipoR2, PPARγ and UCP2 were all significantly increased after astaxanthin treatment in liver...
Fig. 2  Genes mRNA expression in adipose tissue of diabetic rats. The mRNA amount of all examined genes, (A) adiponectin, (B) AdipoR1, (C) AdipoR2, (D) PPARγ, (E) UCP2, is normalized to GAPDH mRNA. The values for groups are displayed relative to the minimum value of the BD group. Each value represents the mean ± SEM derived from 6 animals. Differences between the different groups are analyzed by one-way ANOVA, * \( p < 0.05 \), ** \( p < 0.01 \) vs. BD group.

Fig. 3  Genes mRNA expression in liver of diabetic rats. The mRNA amount of all examined genes, (A) AdipoR1, (B) AdipoR2, (C) PPARγ, (D) UCP2, (E) FAS, is normalized to GAPDH mRNA. The values for groups are displayed relative to the minimum value of the BD group. Each value represents the mean ± SEM derived from 6 animals. Differences between the different groups are analyzed by one-way ANOVA, * \( p < 0.05 \), ** \( p < 0.01 \) vs. BD group.

(Fig. 3A–D) and muscle (Fig. 4A–D). However, astaxanthin administration did not improve FAS (Fig. 3E) expression in the liver.

**Effect of astaxanthin and monacolin K on blood glucose and lipid metabolic parameters in the STZ-induced diabetes model**

We then compared the anti-diabetic effects of astaxanthin and monacolin K in rats. Consistent with our previous results, body weight and TG levels were not affected
by astaxanthin or monacolin K (Fig. 5A, 5C). Blood glucose was significantly decreased by astaxanthin and monacolin K (Fig. 5B), while TC levels were decreased slightly by astaxanthin and significantly by monacolin K (Fig. 5D) in STZ-induced diabetic rats. However, monacolin K slightly increased blood HDL-C levels, while astaxanthin significantly increased blood HDL-C levels (Fig. 5E). These results indicate that astaxanthin and monacolin K decrease blood glucose and improve dyslipidemia in STZ-induced diabetic rats.

Effect of astaxanthin and monacolin K on gene expression

The effect of astaxanthin on insulin sensitivity-related
Gene expression was similar to that of previous studies. Monacolin K treatment significantly increased UCP2 gene expression and tended to increase adiponectin, AdipoR1, AdipoR2 and PPARγ gene expression in adipose tissue. However, the astaxanthin treated group significantly increased expression of all insulin sensitivity-related genes in adipose tissue, except Adipo R1 (Fig. 6A). Monacolin K significantly increased AdipoR1, AdipoR2 PPARγ and UCP2 gene expression in both the liver (Fig. 6B) and muscle (Fig. 6C), and a similar effect was observed in the astaxanthin treated group. Monacolin K treatment significantly increased FAS expression, a fatty acid synthesis gene, in the liver. However, astaxanthin hardly affected FAS expression.

**Discussion**

Previous studies revealed that dietary factors including antioxidants have important roles in the prevention of T2DM. Most of these studies showed an inverse relation between dietary intake of vegetables and antioxidants and the risk of T2DM [22]. Considering the potent antioxidant effects of natural carotenoids, the role of the potent antioxidant carotenoid, astaxanthin, in T2DM was investigated in this study. We found that astaxanthin exhibited potent therapeutic effects against diabetes in an STZ-induced model. We also compared the anti-diabetic effects of astaxanthin and monacolin K in this study. Monacolin K has been reported to down-regulate hyperlipidemia [18], improve AGE-induced glucose intolerance and protect pancreatic function [19] and has shown anti-inflammatory and anti-obesity properties [18].
Controlling blood glucose is the primary goal of type 2 diabetes treatment. The blood parameter data in the present study showed that astaxanthin attenuated blood glucose, and enhanced the serum levels of high-density lipoprotein cholesterol. Similar effects were also observed by Hussein G et al. in a SHR/NDmc-rp (cp/cp) rat model [14]. However, monacolin K only decreased blood glucose and slightly decreased triglycerides.

Our previous study showed that due to its antioxidant effect, astaxanthin improved dyslipidemia and liver dysfunction and attenuated glucose intolerance and insulin resistance in a NASH model [23]. Monacolin K is an active compound present in Monascus-fermented products. It is an analog of lovastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which has been used as an anti-hyperlipidemic agent [24]. Monacolin K has been reported to downregulate hyperlipidemia in vivo [25]. Studies have shown that monacolin K has the ability to elevate pancreatic PDX-1 and GLUT2 mRNA levels in AGE-treated mice [19], which indicates that the regulatory effect of monacolin K on blood glucose levels is mediated by promoting pancreatic GLUT2 expression. Therefore, monacolin K may work as a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, thereby ameliorating hyperlipidemia; Monacolin K also increases GLUT2 expression thereby improving hypoglycemia.

Adiponectin, which has been recognized as an anti-inflammatory, anti-atherogenic, and anti-diabetic agent and insulin sensitizer, was shown to be decreased in type 2 diabetes, insulin resistance and obesity [26-30]. It is reported that adiponectin plays a key role in promoting ABCA1-dependent cholesterol efflux and in modulating HDL biogenesis via activation of the PPARγ/LXRα signalling pathways in macrophages [31]. Plasma HDL-C levels are positively correlated with plasma adiponectin concentrations in humans, decreased adiponectin levels result in decreased levels of HDL-C [32, 33]. Administration of adiponectin causes glucose-lowering effects and ameliorates insulin resistance in mice [34-36]. In this study, adiponectin expression significantly decreased in diabetes, while astaxanthin significantly increased adiponectin expression, thereby enhanced HDL-C levels. Adiponectin binds to 2 types of receptors, encoded by 2 genes: AdipoR1 and AdipoR2. AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver [37]. Both astaxanthin and monacolin K increased the expression of adiponectin as well as its receptors AdipoR1 and AdipoR2, and improved insulin sensitivity in STZ-induced diabetes.

PPARγ is a master regulator of whole-body lipid metabolism, adipogenesis, and insulin sensitivity [38, 39]. Dysregulation of PPARγ is linked to the development of obesity, type 2 diabetes, atherosclerosis and other disease conditions [40]. Because of its potent insulin-sensitizing effects, PPARγ has been recognized as a major therapeutic target with the identification of thiazolidinediones (TZDs) as high-affinity ligands [41, 42]. Previous evidence has shown that PPARγ is a target for astaxanthin. It has been demonstrated that astaxanthin not only binds to this receptor but also affects its mRNA expression [43]. Astaxanthin administration in this study enhanced PPARγ expression. Therefore, it is a possible that the hypoglycemic effect of astaxanthin, at least in part, is mediated by PPARγ activation.

UCP2, which is widely expressed in human tissues and serves as an uncoupler of oxidative phosphorylation, is involved in the regulation of glucose and lipid metabolism [44, 45]. It has been reported that the combination of resveratrol and quercetin upregulates UCP2 expression in the fat tissue of rats with metabolic syndrome [46]. A lack of UCP2 expression has been reported to confer vulnerability to inflammatory responses [47, 48]. Therefore, the agonism of UCPs might provide benefits for obesity and diabetes that are closely associated with oxidative stress and inflammatory responses [49]. As an inducer of UCP2 in this study, astaxanthin and monacolin K could be considered as a potential treatments for chronic inflammation and/or oxidative stress–mediated diabetes, not only for adiposity control.

A limitation of our study is the lack of protein analyses including western blotting, we only examined the insulin sensitivity related genes expression which are comparatively weak. Therefore further investigations involving the protein analyses are required to confirm the validity of these results. In summary, this study demonstrated that astaxanthin, inhibited and reversed STZ-induced insulin resistance in rats. Therefore, astaxanthin might be a novel and promising treatment for diabetes. In this study, we only supplied astaxanthin for 3 weeks in diabetic rats, and long-term treatment must be evaluated for the prevention of diabetes complications.

Conflicts of Interest

All authors report no conflicts of interest.

Funding

This work was supported by the Program for Changjiang Scholars and Innovative Research Team in University (No. IRT_17R97), the Health Science and Technology Planning Project of Hangzhou Municipal Health Commission (No. 2018A25) and the Research Fund Project from The Affiliated Hospital of Hangzhou Normal University.
Reference

1. Chatterjee S, Khunti K, Davies MJ (2017) Type 2 diabetes. *Lancet* 389: 2239–2251.
2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, *et al.* (2019) Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. *Diabetes Res Clin Pract* 157: 107843.
3. Zheng Y, Ley SH, Hu FB (2018) Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol* 14: 88–98.
4. Pirola L, Balcerczyk A, Okabe J, El-Osta A (2010) Epigentic phenomena linked to diabetic complications. *Nat Rev Endocrinol* 6: 665–675.
5. Ylonen K, Alfthan G, Groop L, Saloranta C, Aro A, *et al.* (2003) Dietary intakes and plasma concentrations of carotenoids and tocopherol in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia dietary study. *Am J Clin Nutr* 77: 1434–1441.
6. Sluijs I, Cadier E, Beulens JW, van der A DL, Spijkerman AM, *et al.* (2015) Dietary intake of carotenoids and risk of type 2 diabetes. *Nutr Metab Cardiovasc Dis* 25: 376–381.
7. Ambati RR, Phang SM, Ravi S, Aswathanarayana RG (2014) Astaxanthin: sources, extraction, stability, biological activities and its commercial applications—a review. *Mar Drugs* 12: 128–152.
8. Ikeuchi M, Koyama T, Takahashi J, Yazawa K (2007) Effects of astaxanthin in obese mice fed a high-fat diet. *Biosci Biotechnol Biochem* 71: 893–899.
9. Ishiki M, Nishida Y, Ishibashi H, Wada T, Fujisaka S, *et al.* (2013) Impact of divergent effects of astaxanthin on insulin signaling in L6 cells. *Endocrinology* 154: 2600–2612.
10. Ranga Rao A, Raghunath Reddy RL, Baskaran V, Sarada R, Ravishankar GA (2010) Characterization of microalgal carotenoids by mass spectrometry and their bioavailability and antioxidant properties elucidated in rat model. *J Agric Food Chem* 58: 8553–8559.
11. Stewart JS, Lignell A, Pettersson A, Elfving E, Soni MG (2008) Safety assessment of astaxanthin-rich microalgal biomass: acute and subchronic toxicity studies in rats. *Food Chem Toxicol* 46: 3030–3036.
12. Uchiyama K, Naito Y, Hasegawa G, Nakamura N, Takahashi J, *et al.* (2002) Astaxanthin protects beta-cells against glucose toxicity in diabetic db/db mice. *Redox Rep* 7: 290–293.
13. Moniruzzaman M, Chin YW, Cho J (2018) HO-1 dependent antioxidant effects of ethyl acetate fraction from Physalis alkekengi fruit ameliorates scopolamine-induced cognitive impairments. *Cell Stress Chaperones* 23: 763–772.
14. Hussein G, Nakagawa T, Goto H, Shimada Y, Matsumoto K, *et al.* (2007) Astaxanthin ameliorates features of metabolic syndrome in SHR/NDmcr-cp. *Life Sci* 80: 522–529.
15. Kim YJ, Kim YA, Yokozawa T (2009) Protection against oxidative stress, inflammation, and apoptosis of high-glucose-exposed proximal tubular epithelial cells by astaxanthin. *J Agric Food Chem* 57: 8793–8797.
16. Manabe E, Handa O, Naito Y, Mizushima K, Akagiri S, *et al.* (2008) Astaxanthin protects mesangial cells from hyperglycemia-induced oxidative signaling. *J Cell Biochem* 103: 1925–1937.
17. Naito Y, Uchiyama K, Aoi W, Hasegawa G, Nakamura N, *et al.* (2004) Prevention of diabetic nephropathy by treatment with astaxanthin in diabetic db/db mice. *Biofactors* 20: 49–59.
18. Fujimoto M, Tsuneyama K, Chen SY, Nishida T, Chen J, *et al.* (2012) Study of the effects of monacolin k and other constituents of red yeast rice on obesity, insulin-resistance, hyperlipidemia, and nonalcoholic steatohepatitis using a mouse model of metabolic syndrome. *Evid Based Complement Alternat Med* 2012: 892697.
19. Hsu WH, Lu SS, Lee BH, Hsu YW, Pan TM (2013) Monacolin K and monascin attenuated pancreas impairment and hyperglycemia induced by advanced glycation end-products in BALB/c mice. *Food Funct* 4: 1742–1750.
20. Endo Y, Fu Z, Abe K, Arai S, Kato H (2002) Dietary protein quantity and quality affect rat hepatic gene expression. *J Nutr* 132: 3632–3637.
21. Bieri JG (1979) AIN-76 diet. *J Nutr* 109: 925–926.
22. Stahl W, Sies H (2005) Bioactivity and protective effects of natural carotenoids. *Biochim Biophys Acta* 1740: 101–107.
23. Ni Y, Nagashimada M, Zhuge F, Zhan L, Nagata N, *et al.* (2015) Astaxanthin prevents and reverses diet-induced insulin resistance and steatohepatitis in mice: a comparison with vitamin E. *Sci Rep* 5: 17192.
24. Choi OS, Park SJ, Seo SW, Park CS, Cho JJ, *et al.* (2005) The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, lovastatin (statin) ameliorates CCK-induced acute pancreatitis in rats. *Biol Pharm Bull* 28: 1394–1397.
25. Musacchio MC, Cappelli V, Di Sabatino A, Morgante G, De Leo V (2013) [Evaluation of the myo-inositol-monacolin K association on hyperandrogenism and on the lipidic metabolism parameters in PCOS women]. *Minerva Ginecol* 65: 89–97 (In Italian).
26. Goropashnaya AV, Herron J, Sexton M, Havel PJ, Stanhope KL, *et al.* (2009) Relationships between plasma adiponectin and body fat distribution, insulin sensitivity, and plasma lipoproteins in Alaskan Yup’ik Eskimos: the Center for Alaska Native Health Research study. *Metabolism* 58: 22–29.
27. O’Rourke RW, Metcalf MD, White AE, Madala A, Winters BR, *et al.* (2009) Depot-specific differences in inflammatory mediators and a role for NK cells and IFN-gamma in inflammation in human adipose tissue. *Int J Obes (Lond)* 33: 978–990.
28. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, *et al.*
al. (2006) Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 116: 1784–1792.

29. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, et al. (2002) Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 8: 731–737.

30. Lautamaki R, Ronnemaa T, Huupponen R, Lehtimaki T, Iozzo P, et al. (2007) Low serum adiponectin is associated with high circulating oxidized low-density lipoprotein in patients with type 2 diabetes mellitus and coronary artery disease. Metabolism 56: 881–886.

31. Hafiane A, Gasbarrino K, Daskalopoulou SS (2019) The role of adiponectin in cholesterol efflux and HDL biogenesis and metabolism. Metabolism 100: 153953.

32. Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, et al. (2004) Adiponectin as a biomarker of the metabolic syndrome. Circ J 68: 975–981.

33. Matsuura F, Oku H, Koseki M, Sandoval JC, Yuasa-Kawase M, et al. (2007) Adiponectin accelerates reverse cholesterol transport by increasing high density lipoprotein assembly in the liver. Biochem Biophys Res Commun 358: 1091–1095.

34. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, et al. (2001) Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci U S A 98: 2005–2010.

35. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, et al. (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipatrophy and obesity. Nat Med 7: 941–946.

36. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE (2001) The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 7: 947–953.

37. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, et al. (2003) Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423: 762–769.

38. Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, et al. (2013) PPARgamma signaling and metabolism: the good, the bad and the future. Nat Med 19: 557–566.

39. Tontonoz P, Spiegelman BM (2008) Fat and beyond: the diverse biology of PPARgamma. Annu Rev Biochem 77: 289–312.

40. Lehrke M, Lazar MA (2005) The many faces of PPARgamma. Cell 123: 993–999.

41. Lehmann JM, Moore LB, Smith- Oliver TA, Wilhelm WO, Willson TM, et al. (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J Biol Chem 270: 12953–12956.

42. Lambe KG, Tugwood JD (1996) A human peroxisome-proliferator-activated receptor-gamma is activated by inducers of adipogenesis, including thiazolidinediones. Eur J Biochem 239: 1–7.

43. Inoue M, Tanabe H, Matsumoto A, Takagi M, Umegaki K, et al. (2012) Astaxanthin functions differently as a selective peroxisome proliferator-activated receptor gamma modulator in adipocytes and macrophages. Biochem Pharmacol 84: 692–700.

44. Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, et al. (1997) Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nat Genet 15: 269–272.

45. Donadelli M, Dando I, Fiorini C, Palmieri M (2014) UCP2, a mitochondrial protein regulated at multiple levels. Cell Mol Life Sci 71: 1171–1190.

46. Castrejon-Tellez V, Rodriguez-Perez JM, Perez-Torres I, Perez-Hernandez N, Cruz-Lagunas A, et al. (2016) The effect of resveratrol and quercetin treatment on PPAR mediated uncoupling protein (UCP-)1, 2, and 3 expression in visceral white adipose tissue from metabolic syndrome rats. Int J Mol Sci 17: 1069.

47. Bai Y, Onuma H, Bai X, Medvedev AV, Misukonis M, et al. (2005) Persistent nuclear factor-kappa B activation in Ucp2–/– mice leads to enhanced nitric oxide and inflammatory cytokine production. J Biol Chem 280: 19062–19069.

48. Sun XL, Liu Y, Dai T, Ding JH, Hu G (2011) Uncoupling protein 2 knockout exacerbates depression-like behaviors in mice via enhancing inflammatory response. Neurosci 192: 507–514.

49. Xu H, Barnes GT, Yang Q, Tan G, Yang D, et al. (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 112: 1821–1830.