

Original Research Paper

# Weight Gain, Conditioning and Metabolic Syndrome: A Sled Dog Model

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**Abstract:** Type II Diabetes (T2D) and insulin resistance are growing national health concerns. Obesity is a risk factor for developing T2D and is associated with chronic and systemic inflammation. Exercise on the other hand has been shown to improve glucose metabolism and insulin sensitivity. We investigated the effects of conditioning and weight gain on adiponectin, tumor necrosis factor and glycosylated hemoglobin, three biomarkers of T2D. We measured the levels of these three biomarkers in racing, sedentary and overweight sled dogs. Exercise did not have a significant impact on the levels of the biomarkers, whereas weight gain had a negative effect. Using a sled dog model, we conclude that weight management is important to reduce the risk of T2D and its consequences.

**Keywords:** Adiponectin, Sled Dogs, TNF, Type II Diabetes, Weight Gain

## Introduction

The prevalence of metabolic syndrome and Type 2 Diabetes (T2D) are increasing around the developed world. Metabolic syndrome consists of a series of five risk factors including a large waistline, high triglyceride levels, low high-density lipoprotein levels, high blood pressure and high fasting blood sugar. These risk factors can lead to health complications like cardiovascular disease, T2D and other metabolic diseases (NHI, 2011).

While genetics are an underlying factor in developing T2D, lifestyle and diet are found to be the main contributing factors for the development of this disease (ADA, 2014). In 2012, the National Diabetes Statistics Report estimated that 29.1 million U.S. citizen had T2D, an increase from the 25.8 million estimated in 2010 (CDCP, 2014). Often T2D requires a lifelong treatment of insulin injections and/or oral medication. The cost associated with T2D is exorbitant and reached \$245 billion U.S. Dollars in 2012 (ADA, 2014). There is an increasing need to develop strategies to lower the prevalence of comorbidities associated with T2D. One lifestyle change shown to be an effective strategy is physical exercise (Pedersen and Saltin, 2015).

Exercise has been shown to decrease body weight and visceral fat accumulation, improve insulin sensitivity, increase high-density lipoproteins,

decrease triglyceride levels and decrease high blood pressure (Lakka and Laaksonen, 2007). Physical activity has both an acute and a chronic effect on insulin sensitivity. Whereas acute exercise improves insulin sensitivity for as long as 48 to 72 hours after the exercise session, an exercise program over the duration of at least three months will help improve insulin sensitivity independently of the acute effects (Kim and Park, 2013; Lakka and Laaksonen, 2007). This suggests the importance of a long-term fitness program to consistently improve insulin sensitivity and, further, the risk of metabolic syndrome was lowered in individuals who remained in a long-term training program (Farinha *et al.*, 2015).

Often the physiological and metabolic benefits are observed whether exercise is accompanied by weight-loss or not. Some studies suggest that exercise accompanied by weight loss provides a stronger effect against insulin resistance and T2D. Both, physical activity and cardiorespiratory fitness without weight-loss have also been shown to enhance insulin sensitivity and thus lower diabetes risk factors (Dobrosielski *et al.*, 2013; Gan *et al.*, 2003; Kim and Park, 2013; Lakka and Laaksonen, 2007). Other studies, however, demonstrated the importance of exercise combined with weight-loss in the prevention and for treatment of T2D (Dobrosielski *et al.*, 2013).

Dogs have been used as a research model for diabetes for over a century, starting as early as 1889 when pancreatic studies were performed on dogs. The genetic diversity of domestic dog makes them an interesting model for human biomedical research. In fact, insulin therapy was used on a diabetic dog before it was used to treat human patients (Catchpole *et al.*, 2005). Today dogs diagnosed with diabetes are treated in the same way as humans (Catchpole *et al.*, 2005). Sled dogs are at low risk for developing T2D; their increased energy expenditure when compared to other elite athletes, make them an ideal model for studying the effects of exercise and metabolism (Hinchcliff *et al.*, 2000). Recent work in our lab has shown that both acute and chronic exercise increases GLUT4, the insulin-sensitive glucose transporter, in mononuclear cells of sled dogs (Schnurr *et al.*, 2014; 2015). Therefore, the aim of this present study was to investigate the effects of exercise or weight gain separately on biomarkers associated with insulin resistance in sled dogs.

Biomarkers typically used to investigate insulin resistance and metabolic changes linked to T2D include adiponectin, Tumor Necrosis Factor (TNF), glycosylated hemoglobin (GHbA1c), leptin, interleukin-1 and interleukin-6. These markers are used to evaluate inflammation related to the metabolic syndrome. In this study, we focused on adiponectin, TNF and GHbA1c as these are reliable markers with relatively affordable testing capabilities.

Adiponectin is an anti-inflammatory adipokine: a cytokine secreted by adipose tissue. Adiponectin promotes insulin sensitivity (Chang *et al.*, 2014b; Ouchi and Walsh, 2007; Schondorf *et al.*, 2005; Tabak *et al.*, 2012a). Plasma levels of adiponectin are notably decreased in individuals with obesity, insulin resistance and/or T2D, but these can be restored upon weight-loss. TNF is a proinflammatory adipokine (Chang *et al.*, 2014a; Popa *et al.*, 2007). Increasing levels of TNF impair glucose tolerance and insulin sensitivity by inhibition of the insulin signaling (Bullo-Bonet *et al.*, 1999; Kaur, 2014).

GHbA1c is a biomarker of impaired glucose regulation found in the blood serum. Long-term hyperglycemia increases GHbA1c concentration. Patients with T2D have higher levels of GHbA1c, which goes along with glucose toxicity in the blood stream (Bianchi *et al.*, 2012; Choi *et al.*, 2011).

Using sled dogs as a model, we investigated the impact of conditioning compared to inactivity and weight gain associated with inactivity. We looked at specific biomarkers of T2D: adiponectin, TNF and GHbA1c. Fluctuation in these biomarkers help us understand the impact from exercise, inactivity and weight gain in attenuating or amplifying the effects of T2D.

## Materials and Methods

### *Animals and Diet*

Privately owned sled dogs were used as test subjects. The protocol of this study was approved by the Institute of Animal Care and Use Committee (IACUC) at the University of Alaska, Fairbanks (#02-14). The dogs were typical mixed breed racing sled dogs. They were fed a high-quality commercial dog food (Performance Chicken and Rice Formula®, Purina) to maintain an ideal body condition score of 4 which is defined as “thin” to “ideal” with easily palpable ribs and abdominal tuck (Laflamme, 1997). The dogs were sampled before and after 2-month of either conditioning or resting. The dogs in the conditioned group (n=8, age range 2.5 to 7 years), were exercised 3-5 times per week for 3-5 miles at approximately 75% of the maximum rate of oxygen consumption during incremental exercise (VO<sub>2</sub>max), which corresponds to a speed of 15mph in front of an All-Terrain Vehicle. The dogs in the sedentary group (n=8, age range 2 to 9 years) were not part of any exercise program for the duration of the study. After this 2-months period, the group of sedentary dogs was fed increased rations to achieve a body condition score of 6-7 on a scale of 9, before they were sampled again. A body condition score of 6-7 is defined as “too heavy” with slight excess fat covering the ribs (Laflamme, 1997). Two dogs in the weight gain group would not eat their full rations and thus did not gain sufficient weight according to the body conditioning score and were not included in the post weight gain analysis, leaving six remaining dogs in the overweight group.

### *Blood Collections*

Blood was drawn after an overnight fast. 6mL of blood was collected into EDTA Vacutainers from the cephalic vein. The tubes were spun at 3600 rpm for 15 minutes and plasma was immediately removed and frozen at -80°C for later analysis.

### *Biochemical Analysis*

The levels of adiponectin, TNF and GHbA1c were measured using ELISA kits following the protocols provided by the manufacturer. The GHbA1c kit for the conditioned Vs. sedentary part of the study was a competitive ELISA (MyBioSource, San Diego, CA) in which concentrations of GHbA1c were inversely proportional to the strength of the color formed. The adiponectin kit for the conditioned vs. sedentary part (Millipore) and TNF (R&D Systems) and adiponectin for the weight gain portion of the study (Cloud-Clone Corp., Houston, TX) were sandwich ELISAs in which the concentration of sample is proportional to the

strength of the color formed. All kits were run in 96 well-plates coated with the specific antibody for the protein measured. The sample added either competes with an added antigen, or directly binds the antibody. For both methods, a secondary antibody linked to horseradish peroxidase was then added. A color forms the absorbance of each sample or standard was read at 450nm to determine quantitative levels of each protein in the samples. All absorbance readings were done using Synergy HT multi-mode microplate reader (BioTek, United States). A standard curve for each biomarker was run with the samples to ensure proper measurements. All samples were run in duplicate at one time on one 96 well-plate per biomarker.

### Statistical Analysis of Data

Samples were analyzed using GraphPad Prism statistical software (version 5.0). Data were analyzed using one-way ANOVA with Tukey post hoc analysis. All results are expressed as mean  $\pm$  SD. Differences were considered significant at  $P \leq 0.05$ .

## Results

We investigated adiponectin and GHbA1c in active and sedentary dogs and found no differences among groups. Because GHbA1c is associated with long-term elevated glucose levels and since these dogs were not at risk for diabetes, we evaluated TNF in the next part of the study and investigated the effect of weight gain. Our previous finding that conditioning increased the insulin sensitive glucose transporter, GLUT4, in mononuclear cells of sled dogs prompted our initial inquiry on the

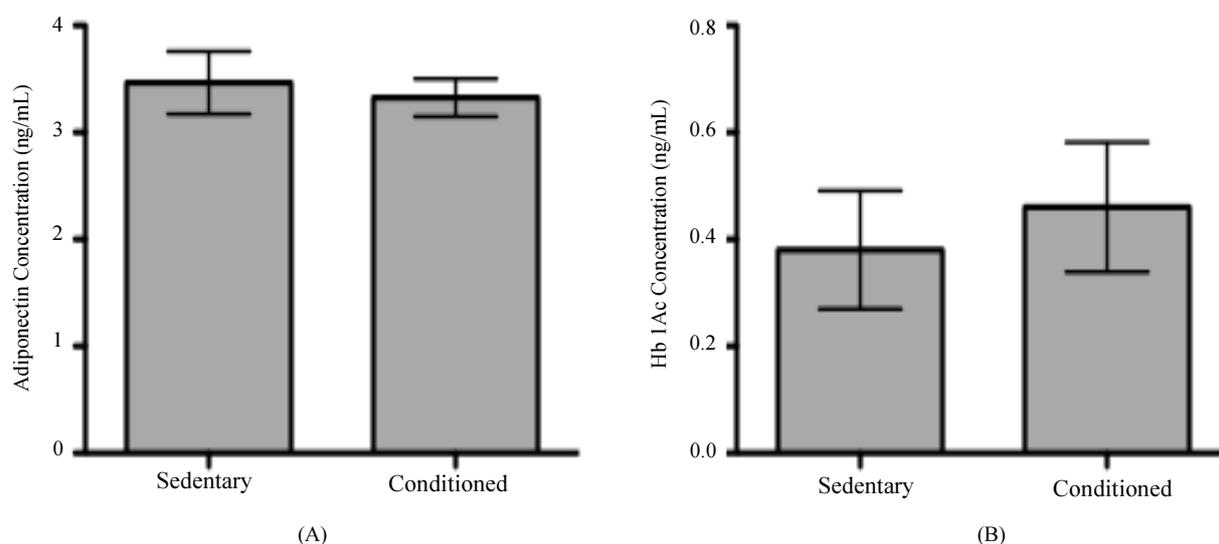
effects of exercise on other biomarkers associated with insulin resistance (Schnurr *et al.*, 2014; 2015). TNF and adiponectin were measured in the sedentary sled dogs after weight gain. Unlike our findings with exercise, weight negatively affected them.

### Conditioned vs. Sedentary Sled Dogs

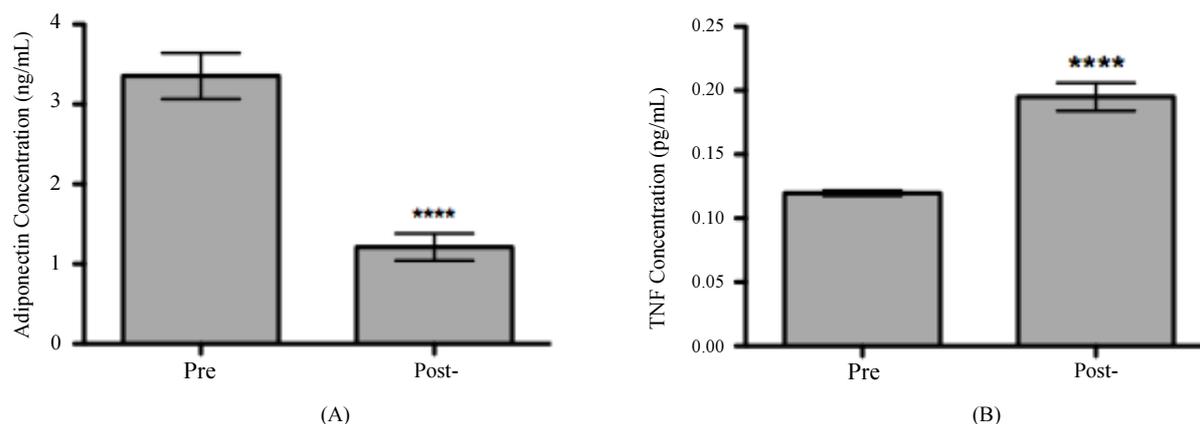
Adiponectin levels were not significantly different in conditioned sled dogs compared to sedentary sled dogs (conditioned average:  $3.325 \pm 0.497$  ng/mL; sedentary average:  $3.466 \pm 0.832$  ng/mL;  $p = 0.63$ ; Fig. 1A). Similarly, GHbA1c were not significantly different in conditioned sled dogs compared to sedentary sled dogs (conditioned average:  $0.460 \pm 0.343$  ng/mL; sedentary average:  $0.358 \pm 0.262$  ng/mL;  $p = 0.69$ ; Fig. 1B). Our results indicate that a long-term exercise program had no effect on the biomarkers we analyzed.

### Weight Gain in Sedentary Sled Dogs

Adiponectin levels were significantly decreased upon weight gain from a body condition score of 3-4 to 6-7 (pre weight gain average:  $3.392 \pm 0.33$  ng/mL; post weight gain average:  $1.208 \pm 0.50$  ng/mL;  $p < 0.001$ ; Fig. 2A). TNF levels were significantly increased upon weight gain from a body condition score of 3-4 to 6-7 (pre weight gain average:  $0.129 \pm 0.014$  pg/mL; post weight gain:  $0.195 \pm 0.027$  pg/mL;  $p < 0.001$ ; Fig. 2B). The sedentary dogs that eventually became overweight showed a negative effect on the measured biomarkers of insulin resistance.



**Fig. 1:** (A) Concentration of adiponectin in conditioned and sedentary dogs (B) Concentration of GHbA1c in conditioned and sedentary dogs



**Fig. 2:** (A) Concentration of adiponectin in sedentary dogs pre and post weight gain (B) Concentration of TNF in sedentary dogs pre and post weight gain

## Discussion

T2D is often associated with obesity, which stems from a lack of physical activity and an increase in visceral fat. Many studies have investigated the importance of physical activity, either including or excluding weight loss and mixed results have come from these studies (Farinha *et al.*, 2015; Kim and Park, 2013; Lakka and Laaksonen, 2007). In the present study, we investigated the independent effect of exercise or weight gain in sled dogs on adiponectin, TNF and GHbA1c, specific biomarkers of T2D. We demonstrated that weight gain significantly affected the levels of these biomarkers while exercise had no influence.

The biomarkers tested for the conditioned group were adiponectin and GHbA1C. Adiponectin is beneficial to insulin signaling, as an anti-inflammatory adipokine it promotes insulin sensitivity and therefore, we expected to find higher level in conditioned dogs (Chang *et al.*, 2014b; Tabak *et al.*, 2012a; 2012b), however this was not the case for sled dogs (adiponectin  $p=0.63$ ). While some studies show increased adiponectin levels with exercise, other studies reported no difference, similar to our findings. (Kriketos *et al.*, 2004; Markofski *et al.*, 2014; Ouchi and Walsh, 2007) GHbA1c is a marker of long-term blood glucose concentration (Byrkjeland *et al.*, 2015; Umpierre *et al.*, 2011). We expected to see lower levels in conditioned dogs, but there were no significant differences when comparing conditioned and sedentary dogs ( $p=0.69$ ). It is likely that the sedentary sled dogs in our study are at higher levels of conditioning than most other domestic dogs from a lifetime of exercise and, therefore, may not be an ideal sedentary model.

The type of conditioning that our dogs underwent during the 2 months conditioning period prior to sampling (exercising 3-5 times per week at 75%  $VO_{2max}$  for 3-5 miles) may not have been strenuous

enough to elicit benefits; Saunders *et al.* (2012) studied vigorous whole-body activity in obese men and saw an increase in adiponectin levels. Other studies which showed an improvement in adiponectin levels used an exercise model that only engaged certain muscles in the body (Sigal *et al.*, 2004). A potential explanation for the diverse findings across studies may be due to the choice of control groups. In some studies, the controls remained sedentary whereas in others the control group was composed of active individuals who remained active (Markofski *et al.*, 2014; Saunders *et al.*, 2012).

Regardless, even in these elite canine athletes, weight gain had negative effects on the biomarkers measured. The biomarkers tested here, in the weight gain group, were adiponectin and TNF. TNF promotes an inflammatory and cytotoxic action. It disturbs the insulin pathway by decreasing tyrosine kinase activity, thus hindering phosphorylation of the insulin. In addition, TNF induces a serine phosphorylation on the Insulin Receptor Substrate (IRS). This serine phosphorylation inhibits proper tyrosine kinase phosphorylation of the insulin receptor, attenuating insulin signaling and thereby inhibiting glucose uptake (Bullo-Bonet *et al.*, 1999; Kaur, 2014; Peraldi *et al.*, 1996; Popa *et al.*, 2007). Elevated levels of TNF are found in individuals with obesity and with T2D (Kaur, 2014; Ouchi and Walsh, 2007). Weight-loss promotes an increase in plasma adiponectin levels, however the benefits of exercise on this parameter have been debated (Ouchi and Walsh, 2007; Saunders *et al.*, 2012). Both adiponectin and TNF levels behaved as we hypothesized; weight gain in sled dogs resulted in decreased adiponectin levels and increased TNF levels (adiponectin:  $p<0.001$ ; TNF:  $p<0.001$ ). These results highlight the importance of weight management in the treatment of T2D and may indicate that adiposity is a higher risk factor for T2D and insulin resistance than physical conditioning.

## Conclusion

Weight gain in sled dogs had a negative effect on certain biomarkers associated with insulin resistance, while physical conditioning had no effect. Even though exercise did not impact the biomarkers investigated in this study, we believe other biomarkers should be tested. TNF and IL-10 are of interest considering their relation to T2D. Physically active individuals have lower plasma concentrations of inflammatory markers such as TNF than inactive or unfit people (Farinha *et al.*, 2015; Lakka and Laaksonen, 2007; Markofski *et al.*, 2014). Also, in the future we intend to investigate the benefits of exercise in conjunction with weight gain or while maintaining excess body fat. This would allow us to see if exercise improves the detriments observed with weight gain.

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## Author's Contributions

**Kriya Dunlap and Arleigh Reynold:** Contribution to the conception and design of the work.

**Aline Collin, Shannon Jimmie and Theresia Schnurr:** Contribution to data collection, analysis and interpretation.

**Aline Collin:** Contribution to drafting this article.

**Kriya Dunlap, Lawrence Duffy and Arleigh Reynolds:** Critical revision of the article.

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## Ethics

The authors have no financial or ethical conflicts of interest regarding this research.

## References

- ADA, 2014. Genetics of Diabetes.
- Bianchi, C., R. Miccoli, R.C. Bonadonna, F. Giorgino and S. Frontoni *et al.*, 2012. Pathogenetic mechanisms and cardiovascular risk: Differences between HbA(1c) and oral glucose tolerance test for the diagnosis of glucose tolerance. *Diabetes care* 35: 2607-2612. DOI: 10.2337/dc11-2504
- Bullo-Bonet, M., P. Garcia-Lorda, F.J. Lopez-Soriano, J.M. Argiles and J. Salas-Salvado, 1999. Tumour necrosis factor, a key role in obesity? *FEBS Letters*, 451: 215-219. DOI: 10.1016/S0014-5793(99)00540-2
- Byrkjeland, R., I.U. Njerve, S. Anderssen, H. Arnesen and I. Seljeflot *et al.*, 2015. Effects of exercise training on HbA1c and VO<sub>2</sub>peak in patients with type 2 diabetes and coronary artery disease: A randomised clinical trial. *Diab. Vasc. Dis. Res.*, 12: 325-333. DOI: 10.1177/1479164115590552
- Catchpole, B., J.M. Ristic, L.M. Fleeman and L.J. Davison, 2005. Canine diabetes mellitus: Can old dogs teach us new tricks? *Diabetologia* 48, 1948-1956. DOI: 10.1007/s00125-005-1921-1
- CDCP, 2014. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States. U.S. Department of Health and Human Services.
- Chang, E., J.M. Choi, W.J. Kim, E.J. Rhee and K.W. Oh *et al.*, 2014a. Restoration of adiponectin expression via the ERK pathway in TNF $\alpha$ -treated 3T3-L1 adipocytes. *Mol. Med. Rep.*, 10: 905-910. DOI: 10.3892/mmr.2014.2278
- Chang, X., Y. Jiao, J. Lu, Y. Wang and K. Abudureyimu *et al.*, 2014b. Adiponectin and visfatin may serve as diagnosis markers for metabolic syndrome in Uyghur population. *Int. J. Clin. Exp. Med.*, 7: 4322-4326.
- Choi, S.H., T.H. Kim, S. Lim, K.S. Park and H.C. Jang *et al.*, 2011. Hemoglobin A1c as a diagnostic tool for diabetes screening and new-onset diabetes prediction: A 6-year community-based prospective study. *Diabetes Care*, 34: 944-949. DOI: 10.2337/dc10-0644
- Dobrosielski, D.A., B. Barone Gibbs, S. Chaudhari, P. Ouyang and H.A. Silber *et al.*, 2013. Effect of exercise on abdominal fat loss in men and women with and without type 2 diabetes. *BMJ Open*. DOI: 10.1136/bmjopen-2013-003897
- Farinha, J.B., F.M. Steckling, S.T. Stefanello, M.S. Cardoso and L.S. Nunes *et al.*, 2015. Response of oxidative stress and inflammatory biomarkers to a 12-week aerobic exercise training in women with metabolic syndrome. *Sports Med. Open*, 1: 3. DOI: 10.1186/s40798-015-0011-2
- Gan, S.K., A.D. Kriketos, B.A. Ellis, C.H. Thompson and E.W. Kraegen *et al.*, 2003. Changes in aerobic capacity and visceral fat but not myocyte lipid levels predict increased insulin action after exercise in overweight and obese men. *Diabetes Care*, 26: 1706-1713. DOI: 10.2337/diacare.26.6.1706
- Hinchcliff, K.W., G.A. Reinhart, R. DiSilvestro, A. Reynolds and A. Blostein-Fujii *et al.*, 2000. Oxidant stress in sled dogs subjected to repetitive endurance exercise. *Am. J. Vet. Res.*, 61: 512-517. DOI: 10.2460/ajvr.2000.61.512

- Kaur, J., 2014. A comprehensive review on metabolic syndrome. *Cardiol Res. Pract.*  
DOI: 10.1155/2014/943162
- Kim, Y. and H. Park, 2013. Does Regular Exercise without Weight Loss Reduce Insulin Resistance in Children and Adolescents? *Int. J. Endocrinol.*  
DOI: 10.1155/2013/402592
- Kriketos, A.D., S.K. Gan, A.M. Poynten, S.M. Furler and D.J. Chisholm *et al.*, 2004. Exercise increases adiponectin levels and insulin sensitivity in humans. *Diabetes Care*, 27: 629-630.  
DOI: 10.2337/diacare.27.2.629
- Laflamme, D., 1997. Nutritional management. *Vet. Clin. North Am Small Anim. Pract.*, 27: 1561-1577.  
DOI: 10.1016/S0195-5616(97)50139-2
- Lakka, T.A. and D.E. Laaksonen, 2007. Physical activity in prevention and treatment of the metabolic syndrome. *Appl. Physiol. Nutr. Metab.*, 32, 76-88.  
DOI: 10.1139/h06-113
- Markofski, M.M., A.E. Carrillo, K.L. Timmerman, K. Jennings and P.M. Coen *et al.*, 2014. Exercise training modifies ghrelin and adiponectin concentrations and is related to inflammation in older adults. *J. Gerontol Biol. Sci. Med. Sci.*, 69: 675-681. DOI: 10.1093/gerona/glt132
- NHI, 2011. What is Metabolic Syndrome? National Heart and Institute.
- Ouchi, N. and K. Walsh, 2007. Adiponectin as an anti-inflammatory factor. *Clin. Chim. Acta.*, 380: 24-30.  
DOI: 10.1016/j.cca.2007.01.026
- Pedersen, B.K. and B. Saltin, 2015. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scand. J. Med. Sci. Sports* 25 Suppl., 3: 1-72. DOI: 10.1111/sms.12581
- Peraldi, P., G.S. Hotamisligil, W.A. Buurman, M.F. White and B.M. Spiegelman, 1996. Tumor necrosis factor (TNF)-alpha inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. *The J. biologi. chem.*, 271: 13018-13022. DOI: 10.1074/jbc.271.22.13018
- Popa, C., M.G. Netea, P.L. van Riel, J.W. van der Meer and A.F. Stalenhoef, 2007. The role of TNF-alpha in chronic inflammatory conditions, intermediary metabolism and cardiovascular risk. *J. Lipid Res.*, 48: 751-762. DOI: 10.1194/jlr.R600021-JLR200
- Saunders, T.J., A. Palombella, K.A. McGuire, P.M. Janiszewski and J.P. Despres *et al.*, 2012. Acute exercise increases adiponectin levels in abdominally obese men. *J. Nutrition Metabolism.*  
DOI: 10.1155/2012/148729
- Schnurr, T.M., A.J. Reynolds, A.M. Komac, L.K. Duffy and K.L. Dunlap, 2015. The effect of acute exercise on GLUT4 levels in peripheral blood mononuclear cells of sled dogs. *Biochem. Biophys. Rep.*, 2: 45-49.  
DOI: 10.1016/j.bbrep.2015.05.002
- Schnurr, T.M., A.J. Reynolds, S.J. Gustafson, L.K. Duffy and K.L. Dunlap, 2014. Conditioning causes an increase in glucose transporter-4 levels in mononuclear cells in sled dogs. *Int. J. Biochem. Cell Boil.*, 55: 227-231.  
DOI: 10.1016/j.biocel.2014.09.009
- Schondorf, T., A. Maiworm, N. Emmison, T. Forst and A. Pftzner, 2005. Biological background and role of adiponectin as marker for insulin resistance and cardiovascular risk. *Clin. Lab.*, 51: 489-494.
- Sigal, R.J., G.P. Kenny, D.H. Wasserman and C. Castaneda-Sceppa, 2004. Physical activity/exercise and type 2 diabetes. *Diabetes care*, 27: 2518-2539.  
DOI: 10.2337/diacare.27.10.2518
- Tabak, A.G., M. Carstensen, D.R. Witte, E.J. Brunner and M.J. Shipley *et al.*, 2012a. Adiponectin trajectories before type 2 diabetes diagnosis: Whitehall II study. *Diabetes Care*, 35: 2540-2547.  
DOI: 10.2337/dc11-2263
- Tabak, O., R. Gelisgen, H. Cicekci, E. Senates and F. Erdenen *et al.*, 2012b. Circulating levels of adiponectin, orexin-A, ghrelin and the antioxidant paraoxonase-1 in metabolic syndrome. *Minerva Medica.*, 103: 323-329.
- Umpierre, D., P.A. Ribeiro, C.K. Kramer, C.B. Leitao and A.T. Zucatti, Azevedo *et al.*, 2011. Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: A systematic review and meta-analysis. *Jama*, 305: 1790-1799.