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Relation between the changes in IL-6 and glucose in response to exercise test and investigation of physicochemical properties of IL-6 and its ligand binding site

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Abstract

Obesity is known to be associated with systemic inflammation. In this study, we investigated serum Interleukin 6 and glucose concentration (IL-6) in response to a stepwise cycling test in obese men. For this purpose, serum IL-6 and glucose concentration were measured in eighteen none-trained adult obese men. Serum IL-6 increased significantly and glucose concentration decreased by exercise test when compared to pre test. Changes in IL-6 were negatively correlated with change in glucose concentration. In present study, also, the structure of protein IL-6 was selected from the protein data bank (PDB code 1ALU). We choose Monte Carlo and AMBER force fields for molecular mechanics calculations. Temperatures of calculation were kept 288-315 K. Moreover, additional parameters were calculated using the QSAR Properties Module of HyperChem 8.6 software. We measured partial derivative of each subunit in constant and variable volume and temperature. This survey results will help us find the best thermodynamics properties for IL6 and finding the best active site for activity in different state of temperature.

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Introduction

A growing body of evidence supports the notion that chronic low-grade inflammation may contribute to the pathogenesis of diseases such as atherosclerosis, type I- and type-II diabetes and the other metabolic diseases (Hotamisligil, 2006; Libby, 2006). Recently, researchers have reported that IL-6 did not increase whole-body glucose disposal in either healthy subjects or patients with type 2 diabetes, whereas it reduced insulin concentrations in the patients to values comparable with those of the healthy subjects, indicating that IL-6 might have favorable effects on insulin action (Petersen *et al.*, 2005). It has been demonstrated that IL-6 increases insulin-stimulated glucose transport (Stouthard *et al.*, 1996) or glycogen synthesis (Weigert *et al.*, 2004) in myotubes and/or adipocytes, but others (Rotter *et al.*, 2003) have found opposite effects; thus, further studies are warranted. In general, though, IL-6 is known as an inflammatory cytokine and most studies point out that the levels of inflammatory cytokines in increases obese patients and obesity related diseases, but some recent findings have indicated that this cytokine, increases insulin-induced glucose transport (Carey *et al.*, 2006). According to the population studies, it has been indicated that the protective effects of regular exercise against diseases such as cardiovascular disease, type 2 diabetes, colon cancer and breast cancer (Lamonte *et al.*, 2005; Thune *et al.*, 2001). Since, the simultaneous responses of serum IL-6 and glucose concentration to a single bout exercise were not studied by researchers, and this is the main aim of the present study. We hypothesized that increased Inteleukine-6 is associated with decreased glucose concentration in response to a single bout cycling exercise and finding relationship between structure of IL-6 and its activities.

Comparative protein structure modeling and experimental efforts complement each other with the goal of providing structural models for diverse applications in biomedical research. Stable, accurate, reliable and fully automated modeling pipelines are required to provide structural information for the rapidly growing amount of sequence data (Nicolas *et al.*, 2009).

Hence, we carry out computer simulations in the hope of understanding the properties of assemblies of molecules in terms of their structure and the microscopic interactions between them. This serves as a complement to conventional experiments, enabling us to learnsomething new, something that cannot be found out in other ways. Monte Carlo (MC), Molecular Dynamics (MD) and Langevin Dynamics (LD) are three methods to simulate some molecules and macromolecules for understanding their structures and binding sites which can interact with other molecules.

Material and methods

In this study, eighteen healthy non-trained adult (38 ± 6 years) obese men participated in the study. All subjects were non-trained and non-smoker. Written consent was obtained from each subject after the experimental procedures and possible risks and benefits were clearly explained.

Subjects included individuals with no cardiovascular diseases, gastrointestinal diseases, kidney and liver disorders or diabetes. In addition, if any of the people had been participating in regular exercise or diet program during the past 6 months, they were excluded study. In addition, exclusion criteria included inability to exercise and supplementations that alter carbohydrate-fat metabolism. The subjects were advised to avoid any physical activity or exercise 48 hours before the exercise test.

Weight was measured by an electronic balance and height by a stadiometer. Height of the barefoot subjects was measured to the nearest 0.1 cm. Body mass index was calculated as body mass (in kilograms) divided by height squared (in square meters). Venous blood samples were obtained before and after a single bout incremental cycling test in order to measuring serum IL-6 and glucose concentration. Cycling exercise test was a YMCA standard test on leg ergometery cycle (Mullis *et al.*, 1999). Glucose was determined by the oxidase

method (Pars Azmoun, Tehran, Iran). Serum Il-6 was determined by ELISA method ((Enzyme-linked Immunosorbent Assay for quantitative detection of human IL-6.

Statistical analysis

Statistic analysis was done with SPSS 15.0 for Windows. Normal distribution of data was analyzed by the Kolmogorov-Smirnov normality test. Data were compared using the student "t" test and paired "t" test. Pearson correlations and partial correlations were used to establish the relationship between the changes in glucose and IL-6 by exercise test. All statistical tests were performed and considered significant at a $P \leq 0.05$.

In addition, we choose Molecular Mechanics to use a classical Newtonian calculation method instead of a quantum mechanical method. The crystal structures of proteins were from the Brookhaven protein data bank. The structure of protein human IL-6 was selected from the protein data bank (PDB code 1ALU).All modeling procedures, including energy minimization and molecular dynamics, were performed using the hyperchem 8.6 software.

Energy calculations were carried out using the MM+ force fields for molecular mechanics calculations. We could use any of these methods for single point, Geometry optimization and molecular Dynamics calculation. We choose Monte Carlo on the compute menu. Moreover, additional parameters were calculated using the QSAR Properties Module of HyperChem 8.6 software. Computation also was done in vacuo with the GROMOS 96 $43B_1$ parameter set without reaction field by using Swiss PDB viewer programs.

Results and dissection

Anthropometrical measurements showed that all participants are overweight or obese. In this study, at first we determined serum IL-6 in relation to glucose concentration at baseline. A borderline significant negative association was observed between serum IL-6 and glucose concentration in pretest condition ($p = 0.56$, $r = 0.25$). Single bout cycling exercise resulted in significant increase in serum IL-6 in studied subjects (3.73 ± 0.69 vs 6.23 ± 1.44 pg/ml, $p = 0.021$). Compared to pre-test, glucose concentration decreased significantly after exercise test (105 ± 12 vs 97 ± 11 mg/dl, $p = 0.033$). The statistical analysis by Pearson showed that changes in serum IL-6 after training was significant negatively related to changes in glucose concentration ($p = 000$, $r = -0.74$, Fig 1).

We have reported findings for eight different temperatures of various sizes and topologies. Results are presented in Table- 1 that was indicated energy and QSAR parameters of IL-6 in various temperatures. Low reduced temperatures promote complex structure stability, whereas high reduced temperatures oppose it. Single point energy IL-6 in eight different temperatures from 290k to 315k showed that the 290k had the lowest energy (table 1). In conclusion, these results agree with this theory: if the environmental temperature increases, the kinetic energy also increases. The effect of temperature in Monte Carlo simulations is primarily to modulate the strength of intermolecular interactions, since temperature enters the simulation only through the Boltzmann factor $\exp(-\Delta E/kT)$ .in 315k IL-6 was showed lowest Electrostatic potential, The results of electrostatic potential calculations can be used to predict initial attack positions of protons (or other ions) during a reaction. Volume in 315K was showed the most increase .molecule surface in 306K was showed the most elevation, but in 296k showed the most decreasing. We also were done in vacuo with PDB viewer programs and one of the aims of this study is to investigate the computing of the energy (force field) IL6 wild and mutate type molecule. We mutate 182 Arg→ASP in active binding site of molecule and compute energy of molecule then compare these with together (Table 2). The main purpose of the changes is to show the most stability or the most activity of molecule. Ramachandran plot this molecule (fig8) is depicted .this plot only values for currently selected amino acids of current layer. By using the Ramachandran plot we can altered the backbone. This plot can be used to directly modify a Phi/Psiangle of a residue with a direct feedback of

modification.

The protective of exercise training was reported against all cause mortality and there is evidence from randomized intervention studies that physical training is effective as a treatment in patients with chronic heart diseases, type 2 diabetes and symptoms related to the metabolic syndrome (Petersen *et al.*, 2006). IL-6 is the first cytokine present in the circulation during exercise and the appearance of IL-6 in the circulation is by far the most marked and its appearance precedes that of the other cytokines (Petersen *et al.*, 2006). Obesity is associated with a chronic inflammatory response characterized by abnormal cytokine production, increased synthesis of acute-phase reactants and activation of inflammatory signaling pathways (Moschen *et al.*, 2010).

Table 1. Energy and QSAR parameters of IL-6.

Temperature(k)	Energy	gradient	Surface area	volume	Bond	Angle	Dihedral	Vdw	electrostatic
290	-938.84	20.21	11538	29559	890.2	1283.6	1619.21	-766.81	-3965.15
292	-915.12	20.35	11541	29534	888.9	1275.1	1642.1	-722.19	-3999.26
296	-880.84	20.51	11447	29547	885.6	1299.5	1639.1	-697.1	-4008.06
298	-823.21	20.73	11498	29546	928.6	1285.7	1656.6	-767.7	-3926.43
302	-837.57	20.68	11551.8	29573	926.6	1305	1661.8	-737.6	-3993.95
306	-768.47	21.28	11587	29571	957.2	1340.6	1652.5	-725.8	-3993.06
310	-768.76	20.88	11496	29563.5	920.48	1356.8	1651.2	-726.6	-3970.67
315	-733.79	21.46	11477.8	29855	977.8	1367.9	1679.6	-726.80	-4032.32

Serum IL-6 levels as inflammation cytokine are elevated in obese, diabetic patients (Ontana *et al.*, 2004; Kopp *et al.*, 2003). Recent findings demonstrate that Regular physical activity, independently of BMI, is associated with lower risk of all cause mortality (Hu *et al.*, 2005). But, the response of inflammation cytokines to acute exercise is not fully understood. A marked increase in circulating levels of IL-6 was reported after exercise without muscle damage (Petersen *et al.*, 2006). Plasma-IL-6 increases in an exponential fashion with exercise and is related to exercise intensity, duration, the mass of muscle recruited, and one's endurance capacity (Pedersen *et al.*, 2003; Nishimoto *et al.*, 2004). In present study, serum IL-6 levels were also significantly increased in response to acute exercise when compared with baseline levels. This data were observed in some previous studies (Ostrowski *et al.*, 1998; Philippou *et al.*, 2009). On the other hand, Single cycling test resulted in significant decrease in glucose concentration compared to pre-exercise.

Table 2. Energy computation of IL6 with the GROMOS 96(kj/mol).

	Bonds	angles	torsion	improper	nonbond	electrostatic	total
Wild type	742.52	1175.911	912.724	388.47	-3663.76	-5911.42	-6354.56
Mutate type	723.02	1150.84	901.36	387.04	-3651.40	-5656.78	-6145

There is also the question whether decreased glucose levels in response to exercise is caused by increased serum IL-6, and or changes in levels of these two parameters in this study in response to cycling activity, are independent of each other. It is possible that the increase in serum levels of IL-6 has somehow brought about a decrease in blood glucose concentration.

In present study, serum IL-6 was not significant related to fasting glucose in studied obese subjects in baseline. In this area, a number of studies have

demonstrated that at resting conditions, acute IL-6 administration at physiological concentrations did not impair whole-body glucose disposal, net leg-glucose uptake, or increased endogenous glucose production in resting healthy young humans (Petersen *et al.*, 2005). But, we observed a significant negatively correlation between them after cycling test. In other words, Changes in serum IL-6 was negatively correlated with change in glucose concentration in response to cycling exercise. Decreased plasma insulin was observed in response to IL-6 infusion, suggesting an insulin sensitizing effect of IL-6 (Petersen *et al.*, 2005). Additionally, several studies have reported that IL-6 increased glucose infusion rate (Carey *et al.*, 2006) and glucose oxidation without affecting the suppression of endogenous glucose production during a hyperinsulinemic euglycemic clamp in healthy humans (Petersen *et al.*, 2006).

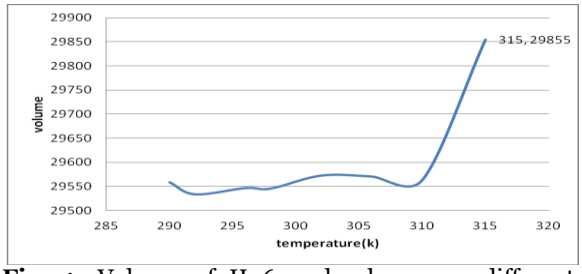

Fig. 1. Volume of IL-6 molecule versus different temperature.

The contracting skeletal muscle is a major source of circulating IL-6 in response to acute exercise (Julia *et al.*, 2010). Physiological concentrations of IL-6 stimulate the appearance in the circulation of the anti-inflammatory cytokines.

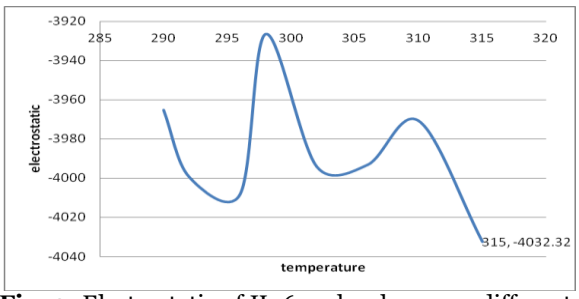

Fig. 2. Electrostatic of IL-6 molecule versus different temperature.

IL-1ra and IL-10, and inhibit the production of the pro-inflammatory cytokine TNF-α (Julia *et al.*, 2010).

Therefore, the anti-inflammatory effects of exercise may offer protection against TNF-induced insulin resistance (Julia *et al.*, 2010). On the other hand, Several studies have suggested that Blocking IL-6 in clinical trials with patients with rheumatoid arthritis leads to enhanced cholesterol and plasma glucose levels, indicating that functional lack of IL-6 may lead to insulin resistance and an atherogenic lipid profile rather than the opposite (Nishimoto *et al.*, 2004; Choy *et al.*, 2002).

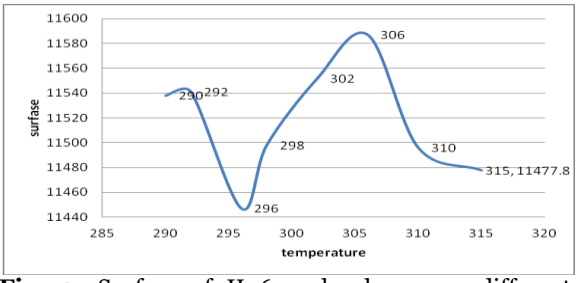

Fig. 3. Surface of IL-6 molecule versus different temperature.

It is reported that IL-6 alone markedly increases both lipolysis and fat oxidation identify IL-6 as a novel lipolytic factor (Petersen *et al.*, 2006). The anti-inflammatory effects of IL-6 are also established that IL-6 stimulates IL-1ra and IL-10 production (Steensberg *et al.*, 2003). However, it is not known whether the effects of IL-6 on glucose and lipid metabolism are mediated by activation of AMPK. A recent study has established that IL-6 increases AMP-activated protein kinase (AMPK) in both skeletal muscle and adipose tissue (Kelly *et al.*, 2004). AMPK may increase glucose uptake (Fisher *et al.*, 2002) via mechanisms thought to involve enhanced insulin signaling transduction (Jakobsen *et al.*, 2001).

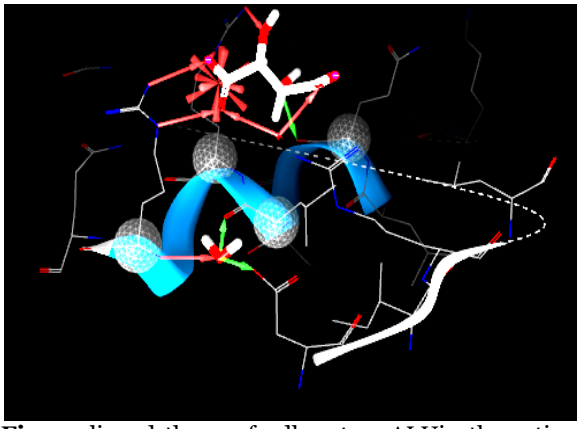

Fig. 4. ligand tla300of pdb entry 1ALUin the active site.

In a general summary, this study showed that a single bout cycling exercise would lead to increased serum levels of IL-6. Increased serum IL-6 was observed while the reduction in glucose concentration was yet another favorable benefit of this exercise protocol. Although according to these findings it can not be stated with certainty that the reduction in blood glucose during exercise occurs in response to increased IL-6. But observation of a negative association between increased IL-6 serum and decreased blood glucose levels in response to exercise somehow supports direct or indirect role of IL-6 in blood glucose concentration. If so, we can conclude that although in most cases IL-6 has been recognized as an inflammatory cytokine, but increase in its serum levels after one session exercise has anti-inflammatory characteristics. Because some other studies too have reported that increased its levels after exercise would lead to increased levels of IL-10 which has anti-inflammatory properties. The potential role of IL-6 on the activity of AMPK, that is one effective factor in membrane transport of glucose, should not be ignored. In addition, some other studies have supported the role of IL-6 as an inhibitor of TNF-α secretion following a one-session exercise. According to the findings of this study and previous studies it may be concluded that changes in IL-6 in response to exercise, directly or indirectly leads to a decrease in blood glucose concentration. Despite all these findings, understanding the molecular mechanisms responsible for this process still requires extensive studies in this field.

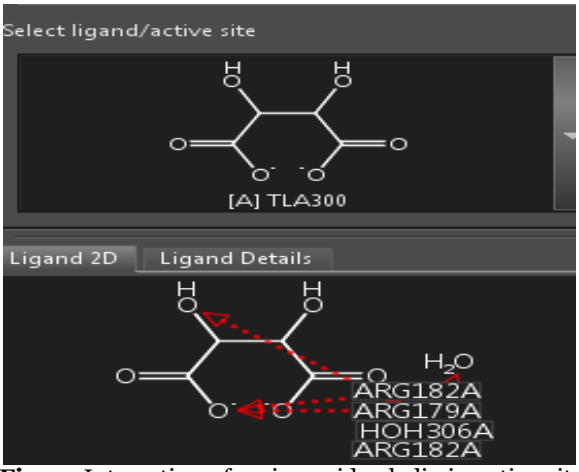

Fig. 5. Interaction of amino acid α-helix in active sit with ligand.

Quantitative Structure –Activity (QSAR) are attempts to correlate molecular structure, or properties derived from molecular structure, with a particular kind of chemical or biochemical activity. The kind of activity is a function of the interest of the user: QSAR is widely used in pharmaceutical, environmental, and agricultural chemistry in the search for particular properties. The calculations are empirical, and so, generally, are fast.

The strategies of drug research did not change too much from the late 19th century till the seventies of the 20 th century. New compounds were synthesized and tested in animals or organ preparations, following some chemical or biological hypotheses. Although synthetic output was relatively low, the real bottleneck was the biological test models.

Pharmacological experiments, using dozens of animals for every new compound, most often needed more time for biological characterization than for chemical synthesis. This situation started to change about thirty years ago. Slowly rational approaches developed, like QSAR and molecular modeling. The consequence was a lower output in such projects, when certain chemical structures had to be synthesized that were proposed by these methods. On the other hand, in vitro test systems like enzyme inhibition or the displacement of radio-labeled ligands in membrane preparations enabled a much faster investigation of new analogs (Hugo K, 2010). Active site of IL-6 has a α-helix that interact whit ligand (fig 7&8). ARG 179,182 and H2O 306 in active site (fig8) interact with ligand. If mutate these amino acids in active site, it will be altered the biological properties.

The present study provided a set of thermodynamically information allowing for a better understanding of specific behavior of interleukin 6 in computational study.

References

Allen MP, Tildesley DJ. 1987. Computer Simulation of Liquids. Clarendon Press; Oxford; Ch

9, 261.

Carey AL, Steinberg GR, Macaulay SL, Thomas WG, Holmes AG, Ramm G. 2006. Interleukin-6 Increases Insulin-Stimulated Glucose Disposal in Humans and Glucose Uptake and Fatty Acid Oxidation In Vitro via AMP-Activated Protein Kinase. Diabetes **55(10),** 2688-97. http://dx.doi.org/10.2337/db05-1404

Choy EH, Isenberg DA, Garrood T. 2002. Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis: a randomized, double-blind, placebo-controlled, dose-escalation trial. Arthritis & Rheumatology **46,** 3143-3150. http://dx.doi.org/10.1002/art.10623

Fisher JS, Gao J, Han DH, Holloszy JO, Nolte LA. 2002. Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. American Journal of Physiology - Endocrinology and Metabolism **282,** 18–23.

Hotamisligil GS. 2006. Inflammation and metabolic disorders. Nature **444,** 860–867. http://dx.doi.org/10.1038/nature05485

Hu G, Tuomilehto J, Silventoinen K, Barengo NC, Peltonen M, Jousilahti P. 2005. The effects of physical activity and body mass index on cardiovascular, cancer and all-cause mortality among 47 212 middle-aged Finnish men and women. International journal of obesity and related metabolic disorders **8,** 894-902. http://dx.doi.org/10.1038/sj.ijo.0802870

Jakobsen SN, Hardie DG, Morrice N, Tornqvist HE. 2001. 5 -AMP-activated protein kinase phosphorylates IRS-1 on Ser-789 in mouse C2C12 myotubes in response to 5-aminoimidazole-4-carboxamide riboside. Journal of Biological Chemistry **276,** 46912–46916. http://dx.doi.org/10.1074/jbc.C100483200

Julia W, Karen C, Javier R, Ascension M. 2010. Role of physical activity on immune function Physical activity, exercise and low-grade systemic inflammation. Proceedings of the Nutrition Society **69,** 400–406. http://dx.doi.org/10.1017/S0029665110001928

Kelly M, Keller C, Avilucea PR, Keller P, Luo Z, Xiang X. 2004. AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. Biochemical and Biophysical Research Communications **320,** 449–454. http://dx.doi.org/10.1016/j.bbrc.2004.05.188

Kopp HP, Kopp CW, Festa A. 2003. Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. Arteriosclerosis, Thrombosis, and Vascular Biology **23,** 1042-7. http://dx.doi.org/10.1161/01.ATV.0000073313.16135.21

Lamonte MJ, Blair SN, Church TS. 2005. Physical activity and diabetes prevention. Journal of Applied Physiology **99,** 1205-1213. http://dx.doi.org/10.1152/japplphysiol.00193.2005

Libby P. 2006. Inflammation and cardiovascular disease mechanisms. American Journal of Clinical Nutrition **83,** 456S–460S.

Moschen AR, Molnar C, Geiger S, Graziadei I, Ebenbichler CF, Weiss H *et al*. 2010. Anti-inflammatory effects of excessive weight loss: potent suppression of adipose interleukin 6 and tumour necrosis factor {alpha} expression. Gut **59(9),** 1259-64. http://dx.doi.org/10.1136/gut.2010.214577

Mullis R, Campbell IT, Wearden AJ, Morriss RK, Pearson DJ. 1999. Prediction of peak oxygen uptake in chronic fatigue syndrome. British Journal of Sports Medicine **33(5),** 352-6. http://dx.doi.org/10.1136/bjsm.33.5.352

Nicolas G, Manuel CP, Torsten S. 2009. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: A historical perspective. Electrophoresis **30,** 162–173. http://dx.doi.org/10.1002/elps.200900140

Nishimoto N, Yoshizaki K, Miyasaka N. 2004. Treatment of rheumatoid arthritis with humanized anti-interleukin-6 receptor antibody: a multicenter, double-blind, placebo-controlled trial. Arthritis & Rheumatology **50,** 1761-1769. http://dx.doi.org/10.1002/art.20303

Ontana L, Eagon JC, Trujillo ME. 2007. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes **56,** 1010-13. http://dx.doi.org/10.2337/db06-1656

Ostrowski K, Rohde T, Zacho M. 1998. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. Journal of Physiology **508(3),** 949–953. http://dx.doi.org/10.1111/j.1469-7793.1998.949bp.x

Pedersen BK, Steensberg A, Fischer C. 2003. Searching for the exercise factor - is IL-6 a candidate. Journal of Muscle Research and Cell Motility **24,** 113-119. http://dx.doi.org/10.1023/A:1026070911202

Petersen AM, Pedersen BK. 2006. The role of IL-6 in mediating the anti-inflammatory effects of exercise. Journal of Physiology & Pharmacology **57(10),** 43-51.

Petersen EW, Carey AL, Sacchetti M, Steinberg GR, Macaulay SL, Febbraio MA, Pedersen BK. 2005. Acute IL-6 treatment increases fatty acid turnover in elderly humans in vivo and in tissue culture in vitro. American Journal of Physiology - Endocrinology and Metabolism **288,** 155–162. http://dx.doi.org/10.1152/ajpendo.00257.2004

Philippou A, Bogdanis G, Maridaki M. 2009. Systemic cytokine response following exercise-induced muscle damage in humans. Clinical Chemistry and Laboratory Medicine **47,** 777–782. http://dx.doi.org/10.1515/CCLM.2009.163

Rotter V, Nagaev I, Smith U. 2003. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. Journal of Biological Chemistry **278,** 45777-84. http://dx.doi.org/10.1074/jbc.M301977200

Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK. 2003. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. American Journal of Physiology - Endocrinology and Metabolism **285,** 433-437.

Stouthard JM, Oude Elferink RP, Sauerwein HP. 1996. Interleukin-6 enhances glucose transport in 3T3–L1 adipocytes. Biochemical and Biophysical Research Communications **220,** 241–245. http://dx.doi.org/10.1006/bbrc.1996.0389

Thune I, Furberg AS. 2001. Physical activity and cancer risk: dose-response and cancer, all sites and site-specific. Medicine Science and Sports Exercise **33,** 530-550. http://dx.doi.org/10.1097/00005768-200106001-00025

Weigert C, Brodbeck K, Staiger H, Kausch C, Machicao F, Haring HU, Schleicher ED. 2004. Palmitate, but not unsaturated fatty acids, induces the expression of interleukin-6 in human myotubes through proteasome dependent activation of nuclear factor-kappaB. Journal of Biological Chemistry **279,** 23942–23952. http://dx.doi.org/10.1074/jbc.M312692200