How to Activate your Lazy Fat

Exercise induced mechanisms to improve insulin sensitivity in obese humans

Rebecca Johanna Henricus Maria Verheggen

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Chapter 1

General Introduction

Obesity and (metabolic) disease

"Obesity is not only a disease itself, but also the harbinger of others." Hippocrates (460 – 370 BC) was one of the first to recognize the threat to health that obesity embodies.[1] He observed that the life expectancy of obese individuals is shorter when compared to those who are lean.[2,3] Obesity, derived from the Latin '*obesus*': *one who has become plump through overeating*, has not always been associated with health risks.[4] For a significant part of the history of humankind, humans were living in conditions in which food was scarce. Therefore, obesity was looked upon as a sign of wealth, and in women, of fertility.[5] The first sculptures of humans ever created (around 40.000 BC) depicted obese women, probably to emphasize the importance of their fertility (Figure 1A).[6] In the Middle Ages and during the Renaissance, obesity was a sign of prosperity and was relatively common in the upper societal classes. Again, this served as an inspiration for artists of whom Rubens (1577-1640) is most famous for his paintings of full-bodied ('*Rubenesque*') women (Figure 1B).[7] Since the 18th century, being obese has become less attractive as the health risks associated with an excess of adipose tissue have become increasingly clear.[7]

Figure 1.A. One of the first human sculptures ever created: Venus von Willendorf (25.000 B.C)[6]; 1.B. Mars, Venus and Cupid by Peter Paul Rubens (1630-1635)[8]

A **B**

Nowadays, obesity is recognized as a major threat to public health. Its prevalence worldwide is still increasing and tripled since 1975. Currently, more than 1.9 billion adults are overweight (Body Mass Index (BMI)>25 kg/m2) and more than 650 million adults suffer from obesity (BMI >30 kg/m2).[9] In the Netherlands, almost half of the adult population (49.2%) is overweight and one in six adults suffers from obesity.[10] The causes of obesity are widely recognized as being multifactorial: besides genetic predisposition,[11,12] a lack of physical activity and caloric overconsumption play a significant role in its etiology.[13,14]

The impact of obesity on health is based on the relation between obesity and the development of metabolic and cardiovascular disease. Overwhelming evidence shows that obesity is an independent risk factor for diabetes mellitus, hypertension, coronary artery disease and stroke, but also for some types of cancer, obstructive sleep apnea syndrome, osteoarthritis, fatty liver disease and kidney disease.[15-17] Specifically for metabolic disorders, previous work found that overweight and obesity play a central role in the pathogenesis of insulin resistance, a crucial element in the etiology of type 2 diabetes mellitus.[18] Eighty percent of patients suffering from diabetes mellitus is overweight or obese.[16] Recently, data from two large health surveys (National Health Interview Survey (NHIS; n = 282,322) and National Health And Nutrition Examination Survey (NHANES; n =21,814) identified diabetes mellitus as the 3rd cause of death in the United States.[19] In 2019, diabetes mellitus was the 9th cause of death globally, causing over 2 million deaths worldwide anually.[20]

Taken together, obesity contributes to the development of various non-communicable diseases, but strong links are especially demonstrated between obesity and metabolic disorders in general, and diabetes mellitus type 2 in particular. This has been demonstrated with use of data from the Behavorial Risk Factor Surveillance System (BRFSS; n=195,005) that showed that the odds ratio (OR) for diabetes in subjects with obesity is 7.37, which is higher than for other conditions (Hypertension: OR 6.38; Hypercholesterolemia OR 1.88).[21] In the published vision for Dutch healthcare from 2025 onwards (Medisch Specialist 2025), The Dutch Federation of Medical Specialists (FMS) advocates to place more emphasis on prevention of disease by lifestyle interventions in daily medical practice.[22] Since the burden of type 2 diabetes mellitus for the general population has risen to pandemic proportions,[19,20] it has become pivotal not only to aim at treatment of diabetes mellitus type 2 but also to prevent it. This thesis specifically focuses on this topic.

The pathogenesis of insulin resistance in obesity

Crucial in the pathogenesis of diabetes mellitus type 2 is the occurrence of insulin resistance: the body's inability to respond to the presence of insulin at cellular level.[23] Different mechanisms have been proposed to play a role in the pathogenesis of insulin resistance in humans with obesity. As obesity is characterized by an excess of adipose tissue, molecular processes in the adipose tissue are altered to a large degree.[24] Below, some of the most frequently discussed mechanisms are listed to explain why obesity is related to insulin resistance.

1. An excess of visceral adipose tissue

The localization of excess adipose tissue plays a pivotal role in the development of insulin resistance in obesity. Especially an excess of visceral adipose tissue (VAT) is strongly related to metabolic health risks and all-cause mortality.[25] This relation might be explained by the fact that the drainage of VAT occurs in the portal vein.[24,26] Adipose tissue is able to secrete metabolically active proteins (cytokines or more specifically: adipokines) that have endocrine effects in various other tissues in the body. Adipokines secreted by VAT reach the liver in a direct way, causing both liver and systemic insulin resistance.[27] In addition to the abundance in VAT, fat storage as a result of an excess in energy can also occur in other tissues, such as the liver and the heart. This ectopic lipid deposition is also strongly correlated with insulin resistance.[26]

2. Adipose tissue as an endocrine organ

Adipose tissue is able to exert endocrine effects in other organs that play a central role in glucose homeostasis, such as the liver and skeletal muscle. This was first discovered with the ability of adipose tissue to secrete free fatty acids (FFAs) into the bloodstream. In obesity, the secretion of FFAs is increased. When FFAs reach skeletal muscle, the organ responsible for 80% of insulin-stimulated glucose uptake, local insulin actions are inhibited. In the liver, circulating FFAs inhibit insulin suppression of hepatic glucose production, resulting in higher glucose concentrations. FFAs therefore cause insulin resistance at both the hepatic and systemic level.[28]

More recently, the ability of adipose tissue to secrete cytokines was described.[29] In obesity, hypertrophy of adipocytes occurs when fat mass grows. This process itself is associated with an alteration in homeostasis in adipose tissue, but also contributes to diminishing oxygen supply as adipose tissue further expands, leading to hypoxia. This elicits necrosis which causes a release of signaling factors that contributes to the presence of inflammation in adipose tissue.[30] In response to an altered homeostasis and local inflammation, adipose tissue secretes pro-

inflammatory cytokines, which contribute to a state of low-grade systemic inflammation, which is characteristic for obesity. This inflammation has strongly been linked to insulin resistance.[30,31]

3. Altered gut microbiome

In recent years, the important role of gut microbiota in (metabolic) health and disease has become increasingly clear.[32] The human gut is occupied by at least 100 trillion different microorganisms that account for expression of 150 times as many genes as the human genome: the gut microbiome. [33-35] The gut microbiome has a vital function in energy homeostasis by influencing the way the body harvests energy from (indigestible) food and how this energy is stored.[36] Furthermore, it plays a significant role in maturation of the human immune system and in defense mechanisms against infections.[35] In recent years, increasing evidence has emerged showing that obesity is associated with an altered gut microbiome.[36] Its composition shows less alpha-diversity when compared to the gut microbiome of lean humans. These alterations are believed to cause a change in how the body digests food and stores energy obtained from food.[36,37] The altered gut microbiome in obesity is believed to contribute to the pathogenesis of diabetes mellitus. Targeting the gut microbiome, therefore, seems a potent strategy to decrease obesity related health risks.[35]

Taken together, the different functions of different adipose tissue depots and having a 'healthy' gut microbiome might explain why some obese humans are metabolically healthy whilst others suffer from a number of diseases. Therefore, targeting loss of body weight or fat mass alone may not lead to significant health improvements in obesity. To accomplish a decrease in insulin resistance, alterations in the location of fat storage, ability to secrete cytokines and release of FFAs need, at least in part, be altered.

Exercise training in obesity and its effects on insulin sensitivity

In humans, exercise training has been proven to be an excellent non-pharmacological tool to decrease all-cause mortality.[38] Especially in unfit, obese subjects, exercise training has enormous effects on cardiorespiratory fitness levels.[39] The beneficial effects of exercise in the management of obesity were first observed by Indian surgeon Susruta (600BC), who linked the presence of diabetes mellitus to being obese and sedentary. He was one of the first to prescribe physical activity as treatment for diabetes mellitus.[40] To support this strategy, increasing cardiorespiratory fitness levels with an exercise intervention is still an often prescribed and highly effective therapy for people with obesity and type 2 diabetes

mellitus.[41] Aerobic exercise training has multiple beneficial effects on general health and causes a decrease in insulin resistance, independent of weight loss. The effects of exercise training on insulin sensitivity are multifactorial:

1.The effects of exercise on insulin sensitivity– effects on visceral adipose tissue

In obese humans, exercise contributes to a restoration of the energy balance, which can, but not always does, result in loss of body weight. In general, by inducing a negative energy balance, in which energy expenditure exceeds energy intake, exercise training will cause a decline in adipose tissue mass.[42] Aerobic exercise, rather than resistance exercise, causes a decline in VAT mass.[43] This latter effect is accompanied by an improvement in insulin sensitivity.

2. The effects of exercise on insulin sensitivity – cytokines and inflammation

The acute and chronic effects of exercise on inflammation seem to show a paradoxical effect. During exercise training, when a negative energy balance is induced, hypertrophy of adipocytes will decrease, as fat mass declines. This will cause an improvement in adipose tissue health. In general, this will result in an anti-inflammatory effect, as the secretion of pro-inflammatory cytokines will diminish during chronic exercise training.[44] Intriguingly, an acute exercise bout causes a pro-inflammatory response, which is for example demonstrated by the higher risk of occurrence of fever after performing a marathon. Evidence suggests that this is caused by an increase in the release of pro-inflammatory cytokines.[45,46] The difference between the pro-inflammatory effect of an acute exercise bout *versus* the anti-inflammatory effect of training (i.e. repeated exercise bouts) suggests the presence of adaptive mechanisms in the secretion of cytokines that mediate inflammation. However, relatively little work explored these potential adaptive responses. To examine adaptations in secretory organs such as adipose tissue gene expression analysis can be used. This technique provides the opportunity to study transcriptional responses to an exercise (training) stimulus and has been used extensively to study exercise induced effects on different tissue types.[47,48] With this technique, novel adaptive signaling pathways can be discovered, that will contribute to our understanding of how adaptation during exercise training occurs and elicits its effects on improving insulin sensitivity.

3. The effect of exercise on insulin sensitivity – gut microbiome

To date, data in humans on the effects of exercise training on the gut microbiome is scarce. A cross-sectional observational study in humans reported higher alpha-diversity of the microbiome in elite rugby players compared to sedentary controls.[49] A few prospective studies explored the potential benefit of exercise training on

gut microbiome but lacked outcomes about metabolic health.[50,51] Insight into the effects of exercise on insulin sensitivity in relation to effects on gut microbiome may contribute to our understanding of how exercise elicits improvements in insulin sensitivity.

Outline of this thesis

The general aim of this thesis is to obtain better insight in exercise-induced mechanisms that contribute to a decrease in insulin sensitivity in obese individuals, thereby reducing the risk of developing type 2 diabetes mellitus.

An excess of adipose tissue and more particular abundance of visceral adipose tissue (VAT), is strongly associated with the pathogenesis of diabetes mellitus and cardiovascular disease. Lifestyle interventions such as dietary caloric restriction and exercise training provide powerful tools to reduce the amount of adipose tissue. Previous meta-analyses revealed that caloric restriction is more effective in reducing body weight when compared to exercise training. However, it is unknown whether exercise training or caloric restriction causes a larger decrease in visceral adipose tissue. Therefore, the aim of **chapter 2** is to investigate the effect of caloric restriction *versus* exercise training on visceral adipose tissue in overweight and obese individuals with use of a systematic review and meta-analysis. Since previous work showed that body weight is a poor marker for the quantity of visceral adipose tissue, the second aim of this study is to examine the relation between changes in body weight and changes in visceral adipose tissue.

Overweight and obesity is characterized by a chronic inflammatory state. An acute exercise bout causes a transient rise in circulating pro-inflammatory cytokines whilst exercise training has anti-inflammatory effects. This suggests the presence of adaptive mechanisms that occur in repeated exercise sessions that cause a decline in inflammation in obese individuals. In a study design of repeated exercise bouts, adaptive mechanisms can be examined. Therefore, in **chapter 3** we examine the effects of repeated prolonged exercise on circulating cytokines and compare these effects between lean and overweight individuals. We hypothesize that, in contrast to lean healthy humans, overweight subjects show a larger pro-inflammatory response to acute exercise with a blunted reaction to repeated exercise.

Exercise training causes improvement in insulin sensitivity via a number of metabolic adaptations. Part of these adaptations may be related to changes in circulating inflammatory cytokines, produced in contracting skeletal muscle. In **Chapter 4** we examine circulating levels and changes in RNA-expression in skeletal

muscle of novel and traditional cytokines after a 6-month aerobic exercise program in obese, insulin resistant women and lean controls and whether these changes correlate with improvements in insulin sensitivity.

In addition to skeletal muscle, adipose tissue plays a crucial role in glucose homeostasis. Exercise training elicits numerous effects in both tissues that eventually cause an improvement in insulin sensitivity. In **chapter 5** we investigate changes in gene expression levels in adipose tissue after an 8-week aerobic exercise intervention in obese, insulin resistant humans. This analysis allows us to illuminate molecular signaling pathways in and between both tissues that contribute to an improvement in insulin sensitivity during an aerobic exercise intervention.

Obesity is characterized by an altered gut microbiome composition and content. Lack of gut microbiome diversity is believed to contribute to the pathogenesis of type 2 diabetes mellitus in obese humans. Based on cross-sectional data in humans and prospective data in rodents, we hypothesize that exercise causes an improvement in insulin sensitivity by means of changing the gut microbiome. Therefore, in **chapter 6** we examine the effects of an 8-week aerobic exercise intervention in obese humans on gut microbiome diversity and composition and relate these effects to changes in insulin sensitivity.

Finally, in **chapter 7** a general discussion of the findings of this thesis and perspectives for future research will be provided.

References

1. Haslam DW, James WPT. Obesity. *The Lancet*. 2005;366:1197-1209. doi: https://doi.org/10.1016/S0140-6736(05)67483-1
2. Cheng TO. Hippocrates and cardiology. *American heart journal*. 2001;141:173-183. doi: 10.1067/mhj.2001.112490
3. Katz AM, Katz PB. Diseases of the heart in the works of Hippocrates. *British heart journal*. 1962;24:257-264.
4. Barnett R. Obesity. *Lancet (London, England)*. 2005;365:1843. doi: 10.1016/s0140-6736(05)66604-4
5. Haslam D. Obesity: a medical history. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2007;8 Suppl 1:31-36. doi: 10.1111/j.1467-789X.2007.00314.x
6. Baaij d. Wat is het oudste beeld ter wereld? *https://kunstvensterscom/2017/01/30/wat-is-het-oudste-beeld-ter-wereld/*. 2017.
7. Ferrucci L, Studenski SA, Alley DE, Barbagallo M, Harris TB. Obesity in aging and art. *The journals of gerontology Series A, Biological sciences and medical sciences*. 2010;65:53-56. doi: 10.1093/gerona/glp166
8. Hillyard H. "Peter Paul Rubens, Venus, Mars and Cupid," in Smarthistory, . https://smarthistory.org/rubens-venus-mars-and-cupid/. Accessed July 30, 2023,.
9. Organization WH. World Health Organization Fact Sheet 311 Obesity and Overweight. 2017.
10. (RIVM) RvVeM. Leefstijlmonitor CBS. 2017.
11. Elks CE, den Hoed M, Zhao JH, Sharp SJ, Wareham NJ, Loos RJ, Ong KK. Variability in the heritability of body mass index: a systematic review and meta-regression. *Frontiers in endocrinology*. 2012;3:29. doi: 10.3389/fendo.2012.00029
12. Qasim A, Turcotte M, de Souza RJ, Samaan MC, Champredon D, Dushoff J, Speakman JR, Meyre D. On the origin of obesity: identifying the biological, environmental and cultural drivers of genetic risk among human populations. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2017. doi: 10.1111/obr.12625
13. Hambrecht R, Gielen S. Essay: Hunter-gatherer to sedentary lifestyle. *Lancet (London, England)*. 2005;366 Suppl 1:S60-61. doi: 10.1016/s0140-6736(05)67856-7
14. Pereira MA, Kartashov AI, Ebbeling CB, Van Horn L, Slattery ML, Jacobs DR, Jr., Ludwig DS. Fast-food habits, weight gain, and insulin resistance (the CARDIA study): 15-year prospective analysis. *Lancet (London, England)*. 2005;365:36-42. doi: 10.1016/s0140-6736(04)17663-0
15. Kopelman P. Health risks associated with overweight and obesity. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2007;8 Suppl 1:13-17. doi: 10.1111/j.1467-789X.2007.00311.x
16. NIH. Health risks of being overweight. 2017.
17. Booth FW, Roberts CK, Laye MJ. Lack of exercise is a major cause of chronic diseases. *Compr Physiol*. 2012;2:1143-1211. doi: 10.1002/cphy.c110025
18. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 2006;444:840-846.
19. Stokes A, Preston SH. Deaths Attributable to Diabetes in the United States: Comparison of Data Sources and Estimation Approaches. *PloS one*. 2017;12:e0170219. doi: 10.1371/journal.pone.0170219

20. organization Wh. The top 10 causes of death. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. december 2020.
21. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, Marks JS. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *Jama*. 2003;289:76-79.
22. Specialisten FM. Medisch Specialist 2025. 2017.
23. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *The Lancet*. 2005;365:1415-1428. doi: https://doi.org/10.1016/S0140-6736(05)66378-7
24. Kajimura S. Advances in the understanding of adipose tissue biology. *Nature reviews endocrinology*. 2017;13:69-70.
25. Ross R, Bradshaw AJ. The future of obesity reduction: beyond weight loss. *Nat Rev Endocrinol*. 2009;5:319-325. doi: 10.1038/nrendo.2009.78
26. Chait A, den Hartigh LJ. Adipose Tissue Distribution, Inflammation and Its Metabolic Consequences, Including Diabetes and Cardiovascular Disease. *Frontiers in Cardiovascular Medicine*. 2020;7. doi: 10.3389/fcvm.2020.00022
27. Kolb H. Obese visceral fat tissue inflammation: from protective to detrimental? *BMC Medicine*. 2022;20:494. doi: 10.1186/s12916-022-02672-y
28. Boden G. Obesity and Free Fatty Acids (FFA). *Endocrinology and metabolism clinics of North America*. 2008;37:635-ix. doi: 10.1016/j.ecl.2008.06.007
29. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab*. 2004;89:2548-2556. doi: 10.1210/jc.2004-0395
30. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, Furukawa S, Tochino Y, Komuro R, Matsuda M, et al. Adipose Tissue Hypoxia in Obesity and Its Impact on Adipocytokine Dysregulation. *Diabetes*. 2007;56:901-911. doi: 10.2337/db06-0911
31. Ye J. Emerging role of adipose tissue hypoxia in obesity and insulin resistance. *International Journal of Obesity*. 2009;33:54-66. doi: 10.1038/ijo.2008.229
32. Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. *Genome Med*. 2016;8:51. doi: 10.1186/s13073-016-0307-y
33. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science (New York, NY)*. 2005;307:1915-1920. doi: 10.1126/science.1104816
34. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. Metagenomic analysis of the human distal gut microbiome. *Science (New York, NY)*. 2006;312:1355-1359. doi: 10.1126/science.1124234
35. Bermon S, Petriz B, Kajeniene A, Prestes J, Castell L, Franco OL. The microbiota: an exercise immunology perspective. *Exerc Immunol Rev*. 2015;21:70-79.
36. Kallus SJ, Brandt LJ. The intestinal microbiota and obesity. *J Clin Gastroenterol*. 2012;46:16-24. doi: 10.1097/MCG.0b013e31823711fd
37. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006;444:1027-1031. doi: 10.1038/nature05414
38. Blair SN, Kohl HW, Barlow CE, Paffenbarger RS, Gibbons LW, Macera CA. Changes in physical fitness and all-cause mortality. *Jama*. 1995;273:1093-1098.
39. Shaw KA, Gennat HC, O'Rourke P, Del Mar C. Exercise for overweight or obesity. *Cochrane database of systematic reviews*. 2006.
40. Tipton CM. Susruta of India, an unrecognized contributor to the history of exercise physiology. *Journal of Applied Physiology*. 2008;104:1553-1556. doi: 10.1152/japplphysiol.00925.2007

41. Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, Horton ES, Castorino K, Tate DF. Physical Activity/Exercise and Diabetes: A Position Statement of the American Diabetes Association. *Diabetes Care*. 2016;39:2065-2079. doi: 10.2337/dc16-1728

42. Franz MJ, VanWormer JJ, Crain AL, Boucher JL, Histon T, Caplan W, Bowman JD, Pronk NP. Weight-loss outcomes: a systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up. *J Am Diet Assoc*. 2007;107:1755-1767. doi: 10.1016/j.jada.2007.07.017

43. Ismail I, Keating SE, Baker MK, Johnson NA. A systematic review and meta-analysis of the effect of aerobic vs. resistance exercise training on visceral fat. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2012;13:68-91. doi: 10.1111/j.1467-789X.2011.00931.x

44. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. *J Appl Physiol (1985)*. 2005;98:1154-1162. doi: 10.1152/japplphysiol.00164.2004

45. Niemelä M, Kangastupa P, Niemelä O, Bloigu R, Juvonen T. Acute Changes in Inflammatory Biomarker Levels in Recreational Runners Participating in a Marathon or Half-Marathon. *Sports Medicine - Open*. 2016;2:21. doi: 10.1186/s40798-016-0045-0

46. Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, Sugawara K. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exercise immunology review*. 2002;8:6-48.

47. McGee SL, Hargreaves M. Exercise adaptations: molecular mechanisms and potential targets for therapeutic benefit. *Nature Reviews Endocrinology*. 2020;16:495-505.

48. Rönn T, Volkov P, Tornberg Å, Elgzyri T, Hansson O, Eriksson KF, Groop L, Ling C. Extensive changes in the transcriptional profile of human adipose tissue including genes involved in oxidative phosphorylation after a 6-month exercise intervention. *Acta physiologica*. 2014;211:188-200.

49. Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, Hogan A, Hayes P, O'Reilly M, Jeffery IB, Wood-Martin R, et al. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut*. 2014;63:1913-1920. doi: 10.1136/gutjnl-2013-306541

50. Liu Y, Wang Y, Ni Y, Cheung CKY, Lam KSL, Wang Y, Xia Z, Ye D, Guo J, Tse MA, et al. Gut Microbiome Fermentation Determines the Efficacy of Exercise for Diabetes Prevention. *Cell metabolism*. 2020;31:77-91.e75. doi: 10.1016/j.cmet.2019.11.001

51. Munukka E, Ahtiainen JP, Puigbó P, Jalkanen S, Pahkala K, Keskitalo A, Kujala UM, Pietilä S, Hollmén M, Elo L, et al. Six-Week Endurance Exercise Alters Gut Metagenome That Is not Reflected in Systemic Metabolism in Over-weight Women. *Frontiers in Microbiology*. 2018;9. doi: 10.3389/fmicb.2018.02323

Chapter 2

A systematic review and meta-analysis on the effects of exercise training versus hypocaloric diet: distinct effects on body weight and visceral adipose tissue

Rebecca J.H.M. Verheggen, Martijn F.H. Maessen, Daniel J. Green, Ad R.M.M. Hermus, MD,PhD, Maria T.E. Hopman, Dick H.T. Thijssen

Obes Rev. 2016 Aug;17(8):664-90.

ABSTRACT

Exercise training ("exercise") and hypocaloric diet ("diet") are frequently prescribed for weight loss in obesity. Whilst body weight changes are commonly used to evaluate lifestyle interventions, visceral adiposity (VAT) is a more relevant and stronger predictor for morbidity and mortality. A meta-analysis was performed to assess the effects of exercise or diet on VAT (quantified by radiographic imaging). Relevant databases were searched through May 2014. 117 Studies (n=4,815) were included. We found that both exercise and diet cause VAT loss ($P<0.0001$). When comparing diet *versus* training, diet caused a larger weight loss ($P=0.04$). In contrast, a trend was observed towards a larger VAT decrease in exercise ($P=0.08$). Changes in weight and VAT showed a strong correlation after diet ($R^2=0.737$, $P<0.001$), and a modest correlation after exercise ($R^2=0.451$, $P<0.001$). In the absence of weight loss, exercise is related to 6.1% decrease in VAT, whilst diet showed virtually no change (1.1%). In conclusion, both exercise and diet reduce VAT. Despite a larger effect of diet on total body weight loss, exercise tends to have superior effects in reducing VAT. Finally, total body weight loss does not necessarily reflect changes in VAT and may represent a poor marker when evaluating benefits of lifestyle-interventions.

INTRODUCTION

The worldwide prevalence of obesity, characterized by an excess in adipose tissue, has grown to pandemic proportions.[1, 2] Multiple reviews have demonstrated that accumulation of adipose tissue in general, and in the visceral area in particular, is strongly and positively correlated with all-cause morbidity and mortality.[3] Since obesity is an important, but also modifiable, risk factor for cardiovascular and metabolic diseases [4, 5] the WHO has recommended lifestyle interventions to aim at 5-10% reduction in body weight as treatment for obesity.[6]

Caloric restriction and exercise training cause a reduction in body weight by inducing a negative energy balance in which energy expenditure exceeds caloric intake. When comparing hypocaloric diet and exercise training, previous meta-analyses revealed that dietary restriction has superior effects on weight reduction.[7, 8] However, a growing body of evidence shows that excess visceral adipose tissue (VAT) may result in more detrimental obesity-related health effects than excess body weight.[9] Indeed, increased VAT is strongly associated with insulin resistance, atherogenic dyslipidemia, and cardiovascular disease.[3, 10, 11] Moreover, a reduction in VAT improves cardiovascular and metabolic risk.[3, 12] Hence, changing VAT is considered to be more important than weight reduction in the management of obesity.

In patients with obesity, physical exercise training leads to a healthier metabolic and cardiovascular phenotype.[13-15] Whilst exercise training does not always aim to reduce body weight, exercise training in general and aerobic exercise training in particular, have potent effects on reducing VAT.[16-18] Previous meta-analyses have evaluated only the effects of caloric restriction and aerobic exercise on weight loss. The effects of these interventions on VAT have not yet been compared. Therefore, we aimed to conduct a systematic review and meta-analysis to investigate the effect of caloric restriction versus aerobic exercise training on visceral adiposity loss in overweight and obese adults. For this purpose, we included studies that examined VAT after: 1. Caloric restriction only, 2. Exercise training only, and 3. Aerobic exercise training versus caloric restriction. We hypothesize that, in marked contrast to body weight loss, caloric restriction and exercise training have comparable effects on reducing VAT. With the use of a meta-regression analysis we aim to further explore the impact of intervention (e.g. duration, intensity, frequency) and subject (e.g. age, sex, baseline body weight) characteristics on the magnitude of changes in VAT.

Several international guidelines recommend lifestyle interventions aimed at a reduction in body weight of at least 5% as treatment for obesity.[6, 19, 20] Previous work, however, demonstrated that a reduction in body weight is a poor marker for VAT change.[9] Accordingly, changes in VAT may occur irrespective of changes in body weight. A hypocaloric diet causes a reduction in skeletal muscle mass, which along with a reduction in fat mass, contributes to weight loss.[21, 22] Aerobic exercise training, however, may be associated with an increase in lean body mass and/or plasma volume.[23-25] Assuming that fat mass decreases with exercise training, training may still not lead to weight loss.[24, 26] Therefore, we hypothesize that the relation between changes in body weight and changes in VAT differs between caloric restriction and exercise interventions.

METHODS

Data sources and searches

The systematic literature search and documentation of literature was performed with the use of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement .[27] Databases systematically searched were Pubmed, Cochrane, Web of Science and Embase. The following search strategy was used, with adaption for each database: (("Energy Intake"OR"Diet Therapy"OR"(calori*AND restrict*)"OR(low AND calori*)OR"dietary intervention*"OR"diet intervention*)"AND ("Overweight"OR"obes*")AND("Abdominal Fat"OR("Adipose Tissue"AND("intra-abdom"*ORintraabdom*ORabdom*ORvisceral*))OR"Body Composition"OR"abdominal adipos*ORvisceral adipos*ORintra-abdominal fat"OR"abdominal fat"OR"total body fat"OR"adipose tissue distribution))OR(("Overweight"OR"obes*")AND("Motor Activity"OR"Exercise"OR"Running"OR"Swimming"OR"Walking"OR"Warm-Up Exercise"OR"Exercise Therapy"OR"Motion Therapy, Continuous Passive"OR"Sports"OR "Athletic Performance"OR"Bicycling"OR"Physical Exertion"OR"running" OR"bicycling"OR"cycling"OR"walking"OR"swimming"OR"training"OR"physical activity"OR" exercis*"OR"cardio-training")AND("Abdominal Fat"OR("Adipose Tissue" AND (intra-abdom*OR"intraabdom*"OR"abdom*"OR"visceral*"))OR"Body Composition" OR" abdominal adipos"*OR"visceral adipos"*OR"intra-abdominal fat"OR"abdominal fat"OR"total body fat"OR"adipose tissue distribution")). Randomized Controlled Trials (RCTs), Non-randomized Controlled Trials (non-RCTs) OR Clinical Trials (CT) published in English, German and Dutch were included from January 1th, 1987 to May 5th, 2014. Reference lists of included articles were manually checked by RV for possible eligible studies that were missed during the literature search (Figure 1). This

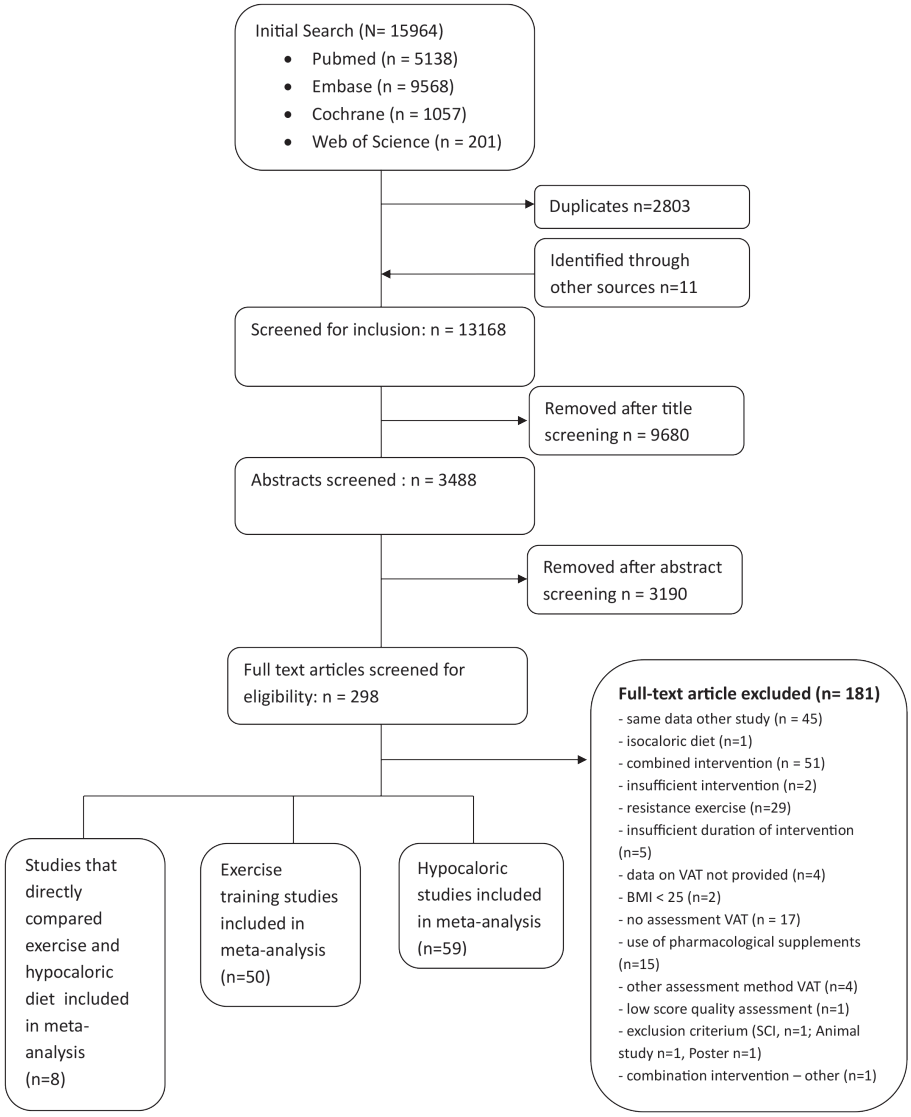
represents a valid and frequently used method to further increase the number of potentially eligible studies.

Study selection

To standardize the selection procedure by two independent reviewers (RV and MM), investigators received a standardized protocol previous to the selection of studies,. After the elimination of duplicates, one investigator (RV) screened study titles for eligibility with use of the in- and exclusion criteria in the review protocol, which are listed below. Two reviewers (RV, MM) independently screened the abstracts of the remaining studies. 389 studies were assessed in full text (Figure 1). Inter-reviewer disagreements were resolved through consensus or by consulting a third reviewer (MH). When study characteristics or viable information was missing, an attempt was made to request missing information from the authors by email (n=6 studies; authors of n=2 studies provided requested information). Studies were included when the mean age at entry was ≥18 years and mean BMI was ≥25 kg/m^2. Studies of HIV-infected individuals were excluded because of the interference of anti-retroviral drugs with abdominal adipose tissue.[16] Because spinal cord injuries are associated with changes in body composition, studies conducted in spinal cord injured individuals were also excluded.[28] Studies with one or more arms assigned to an aerobic exercise intervention or a hypocaloric diet were eligible for inclusion. For the first aim, Clinical Trials and Randomized Controlled Trials (RCTs) with one arm assigned to exercise or caloric restriction were selected. Furthermore, in order to directly compare duration- and energy deficit- matched exercise training with caloric restriction, RCT's with an exercise- and a diet-arm were included. To identify exercise and subject characteristics that predict the magnitude of change in VAT using the meta-regression analysis, clinical trials and RCT's with one arm assigned to exercise or caloric restriction were selected. Finally, diet and exercise studies that provided baseline and post-intervention results for VAT and weight were included for the correlation analysis. Exercise training was defined as a program including voluntary aerobic exercise at a low to vigorous intensity for at least two times per week during a minimum period of four weeks and with a minimum duration of 20 minutes per session. Caloric restriction was defined as a daily reduction in energy (caloric) intake of at least 10% of the habitual intake (2000 kcal for women, 2500 kcal for men) during a minimum period of four weeks. Interventions combining exercise and diet therapy or adding resistance exercise or bariatric surgery to an intervention arm were excluded. Studies in which a pharmacological dietary supplement was used were excluded from our analysis. Studies were eligible when VAT was measured with the use of Computerized Tomography (CT) or Magnetic Resonance Imaging (MRI). which are both considered to be the gold standard for

the quantitative measurement of VAT[29]. Studies that used another measurement technique were excluded.

Figure 1. PRISM Flowchart of outcomes of search strategy



Data extraction and Quality assessment

Baseline and post-intervention mean VAT area or volume and standard deviation or standard error was independently recorded by two authors (RV, MM). When VAT was measured at multiple sites, the measurement at the 4th and/or 5th lumbar vertebrae was recorded for further analysis, since this region is most strongly correlated with body adiposity.[28] Based on changes in visceral abdominal fat area or volume, percentage change in VAT for each study was calculated by one of the authors (RV) for the correlation analysis. Percentage weight loss was also calculated based on pre- and post-intervention values. Furthermore, publication year, journal, study design, sample size, age, sex, weight, BMI, and intervention details (duration, intensity and frequency (exercise studies), caloric deficit (diet studies) were extracted from all included studies. When results were depicted in figures only (n=14 studies), data were extracted with the use of GetData Graph Digitizer. A request by email was send to the authors, when key information was not included in the published manuscript (n=6 studies). Two out of six authors responded to our repeated email requests, thus the remaining 4 studies were excluded from further analysis.

The quality of each eligible study was independently assessed by two authors (RV and MM), with the use of a modified version of The Critical Review Form for Quantitative Studies, from Law et al.[30]. One item ("contamination was avoided") was not applicable for the studies included in this meta-analysis and was therefore removed for analysis. Only studies with a minimum score of 10 out of 14 items were eligible for inclusion (Figure 1).

Data synthesis and analysis

To account for potential heterogeneity between studies, a random-effects model (specified a priori) was used to determine the overall effect size of the intervention (exercise training or hypocaloric diet) on VAT. Effect sizes for RCTs and clinical trials were calculated as the standardized mean difference (SMD) with corresponding 95%-CI. A correlation of 0.5 between the outcomes measured in each study arm (i.e. exercise, diet, or control) was assumed. When a study contained multiple study arms, all were included in the statistical analysis, whereby the different intervention groups were individually compared against the control group. Analyses to assess the following comparisons: (1) diet versus control; (2) exercise versus control; (3) diet versus exercise were performed. The Cochrane's Q statistic and I^2 were calculated to assess the degree of heterogeneity across studies. Publication bias was assessed using visual analysis of the funnel plot asymmetry using the 'trim and fill' and the 'Classic fail 'n safe' algorithms. All calculations and plots were performed in CMA-2 (Comprehensive Meta-Analysis 2nd version, Biostat, Englewood, NJ, USA).

Meta-regression analysis

To assess the effects of subject and intervention characteristics on VAT loss, random-effects meta-regression analysis with SMD as dependent variable was calculated. The weighted inverse variance (with correction for total n) was used as weight factor. For the purpose of meta-regression analysis, the aerobic exercise arms (n=86) were separated from the hypocaloric diet arms (n=87). In both study types, duration of the exercise training or diet intervention (weeks), measurement technique (CT or MRI), body weight at baseline, age and sex were defined as a covariate. Duration was assessed as a categorical variable (duration <16 weeks versus duration of ≥16 weeks). In the exercise studies, intensity of the intervention was examined as a covariate. Intensity was categorized in 'vigourous intensity' (i.e. largely performed at 70% of maximal heart rate (maxHR) or >55% of maximal oxygen uptake (VO2max) or 60-80% of the heart rate reserve (HRR)), 'moderate intensity' (60-70% of maxHR, 45-55% of VO2max or at the lactate threshold), and 'low intensity' (<60% of HRmax or <45% of VO2max) based on previous work.[18] This categorization is somewhat different from the often used and more practical categories based on METs as proposed in the ACSM and AHA guidelines (i.e. light <3.0 METs, moderate (3.0-6.0 METs), vigorous (>6.0 METs).[31, 32] Only two studies included in our meta-analysis provided data on METs. Therefore, we adopted the aforementioned strategy to divide studies based on intensity. In hypocaloric diet studies, "intensity" was divided in 'very low calorie diets' (VLCDs; reduction to maximal 800 kcal/day) and 'low calorie diets (LCDs; caloric restriction to 800-2000 kcal/day). Lastly, frequency (times spent in training per week) was added as covariate in exercise studies.

Correlation analysis

To examine correlations between weight loss and VAT improvement a Pearson correlation coefficient was calculated. The formula of the corresponding trend line was retrieved with the use of linear regression. Meta-regression analyses and correlation analysis were conducted with use of SPSS version 20.0.

RESULTS

Selection of studies for the meta-analysis

The original search resulted in 15,964 studies. Eleven more studies were found from the reference lists of the included full text papers. After removal of duplicates and elimination of papers based on the eligibility criteria and quality assessment, 50 aerobic exercise studies and 59 hypocaloric diet studies were included (Figure 1).

For the analysis of a direct comparison between caloric restriction and exercise training, 8 studies were included. (Table 1; see below) One study, which directly compared exercise training with caloric restriction was excluded as duration and energy deficit did not match between the two intervention arms. This study was included for the separate analysis of diet or exercise training only.

Cohort characteristics

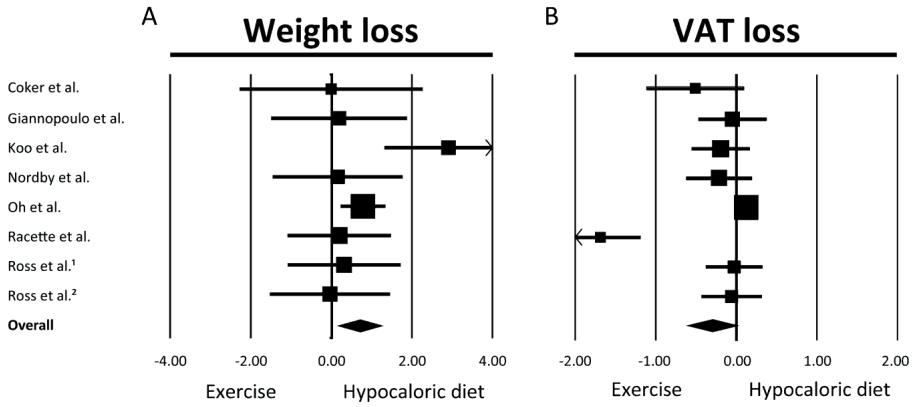
A total number of 4,815 individuals (2,404 in the exercise studies, 2,411 in the hypocaloric diet studies) participated in the interventions. (Table 1) In the 8 RCT's that directly compared exercise training and caloric restriction, a total of 400 individuals were included (200 in the exercise arm, 200 in the diet arm). (Table 2) 28 studies exclusively included male subjects, whereas in 39 studies females were exclusively included. 55 studies included both sexes. Some studies recruited specific populations, which included older (aged 50-80 years) individuals (n=4); patients with type 2 diabetes (n=11), impaired glucose tolerance (n=3) and metabolic syndrome (n=3). (Table 1)

Meta-analysis

The SMD of change in VAT after exercise training was -0.47 (95%CI -0.56 to -0.39, P<0.0001). (Figure S1, see below) Heterogeneity analysis showed significant heterogeneity (Cochran's Q=265.4; I^2=68.0). Through a funnel plot of standard error by Hedge's g and the Trim 'n Fill algorithm, publication bias was assessed. With the use of the Classic Fail 'n Safe approach, it became clear that there was no significant publication bias since 7427 missing studies would be required to achieve a p-value above 0.05. The SMD of change in VAT after caloric restriction was –0.63 (95%CI -0.71 to -0.55, P<0.0001) (Figure S2, see below), whilst significant heterogeneity was present (Cochran's Q= 236.0; I^2= 63.6). In these studies, no publication bias was present since there would be 4096 studies required to achieve a p-value above 0.05.

Based on the studies that directly compared exercise training and caloric restriction, exercise training caused a non-significantly larger decrease in VAT (-0.59, 95%CI -1.248 to 0.071; P=0.08), whilst caloric restriction caused a significantly larger weight loss than exercise training (SMD 0.308, 95%CI 0.02 to 0.60; P=0.04) (Figure 2). Heterogeneity analysis showed significant heterogeneity (Cochran's Q=51.9; I^2=86.5). Publication bias was assessed with the Trim 'n Fill method and showed no change in SMD when adding trimmed studies, for both the weight loss as VAT loss data.

Figure 2. Forest plot of the effect size (SMD) of (A) exercise training versus caloric restriction on weight loss and (B) exercise training versus caloric restriction on VAT loss. The effect size (SMD) and 95%CI for individual studies and the pooled estimate (assessed with the use of Random Effects Model) are depicted.



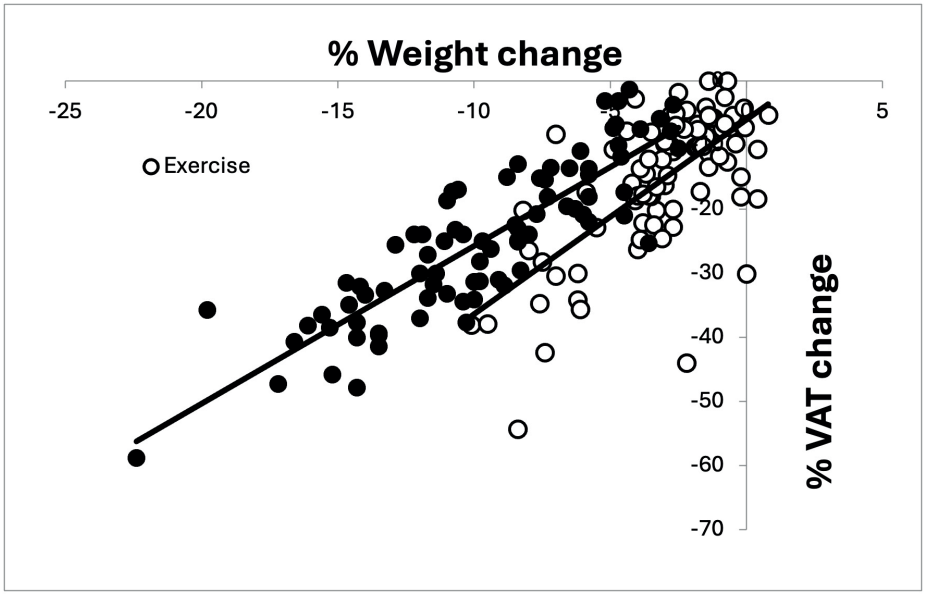
Meta-regression analysis

No effect of measurement technique on the SMD was observed for studies that performed exercise training or diet (data not shown). In the exercise studies, univariate analysis revealed that the SMD for VAT improvement was significantly influenced by sex (R^2=0.11; 95%CI 0.06 – 0.472; P=0.012); duration (R^2=0.073; 95%CI -0.449 to -0.055; P=0.013) and frequency (R^2=0.084; 95%CI -0.157 to -0.030; P=0.004). The multivariate regression analysis, which included the factors that revealed a significant impact in the univariate analysis, identified an impact of male sex on SMD (R^2=0.20; 95%CI 0.066 to 0.467; P=0.01). In hypocaloric diet studies, univariate analysis showed a significant effect of male sex only (R^2=0.09; 95%CI 0.116 to 0.632; P=0.005).

Correlation analysis

For exercise studies, a moderate correlation was found between changes in weight versus changes in VAT after exercise training (R^2=0.453, P<0.001), whilst diet-interventions showed a strong correlation between the change in weight versus change in VAT (R^2=0.737, P<0.001) after caloric restriction. Exercise training showed a somewhat steeper slope compared to diet (-3.04x versus -2.41x, respectively), and a larger Y-axis intercept (-6.1 versus -1.1, respectively, Figure 3).

Figure 3. Correlation between %VAT improvement and %weight loss for exercise studies (R^2=0.4531, P<0.001; trendline: y = -3.03x – 6.1), and caloric restriction studies (R^2=0.737, P<0.001; trendline: y = -2.46x - 1.1).



DISCUSSION

The present work is the first meta-analysis to compare the effect of caloric restriction and aerobic exercise training on visceral adipose tissue (VAT) loss in overweight and obese individuals. We present the following findings. First, our results confirm that both caloric restriction and exercise training successfully reduce VAT. Second, in studies that provided a direct comparison of caloric restriction and exercise training, a hypocaloric diet resulted in significantly larger weight loss. Interestingly, these studies reveal a different story for VAT. Exercise training tends to show a larger decrease in VAT compared to caloric restriction. The distinct effects of both interventions on total body weight and VAT are supported by the correlation analysis. Only a moderate correlation was found for the exercise training cohort between changes in weight and VAT. Furthermore, in the absence of weight loss, exercise training results in a 6.1% decrease in VAT, whilst a hypocaloric diet leads to virtually no change (1.1%). This suggests that evaluating only total body weight changes could lead to spurious conclusions when evaluating the efficacy of a lifestyle intervention in overweight and obese individuals since health benefits occur independent of body weight changes. Indeed, even in the absence of weight loss after exercise training, health benefits such as a reduction in VAT are present.

In line with previous meta-analyses, we found caloric restriction to have a larger effect on weight loss than exercise training.[7, 8] We extended this finding by a direct comparison of studies with matched duration and energy deficit in order to more accurately compare the impact of both interventions. In marked contrast to the superior effect of caloric restriction on weight loss, no difference in VAT reduction was observed between caloric restriction and exercise training. In fact, exercise training tended to have a superior effect on VAT reduction compared to caloric restriction. A possible mechanism underlying these different effects on weight and VAT could relate to distinct changes in body composition during these lifestyle interventions. During caloric restriction, both muscle mass and fat mass are lost, resulting in a marked decline in weight.[21, 22] During exercise training, however, lean body mass and circulating plasma volume increase, whilst fat mass decreases.[21, 23, 25, 26, 33] Previous work that directly measured these factors indeed showed that an increase in lean body mass counteracts loss of fat mass after 8 weeks of exercise training.[34] These opposing effects resulted in the absence of total body weight loss.[34] Appreciating and understanding these effects are important to acknowledge that exercise training effectively reduces VAT, despite the absence of a reduction in body weight.

In this meta-analysis, a large number of studies were included. Multivariate meta-regression analysis on these data showed that male sex is associated with a larger decrease in VAT, in both exercise and diet interventions. Other subject and intervention characteristics did not influence the magnitude of VAT loss in the multivariate model. A possible explanation that underlies the larger effect of exercise training and caloric restriction on VAT in men is that men typically have larger VAT stores than women. As a result, this makes male participants more likely to lose VAT than female participants.[35] However, our meta-regression analysis showed no effect of baseline VAT area on the magnitude of VAT decrease. The exact underlying mechanisms should be subject for future research.

The distinct effects of diet and exercise training on weight and VAT suggest the presence of a different correlation between changes in body weight and VAT after caloric restriction in comparison to exercise training. Indeed, whilst a strong correlation between changes in body weight and VAT was found after caloric restriction, this correlation was only moderate for exercise training studies. This means that a change in weight after hypocaloric diet predicts a substantial effect on VAT, whereas changes in weight after exercise training only modestly predict the change in VAT. Furthermore, the trend lines for these correlations show important differences. The Y-intercept for the correlation of exercise studies is 6.1%, meaning that the absence of weight loss after exercise training is still correlated with a

significant and meaningful reduction in VAT of 6.1%. In marked contrast, studies examining the impact of hypocaloric diet revealed a Y-intercept of only 1.1%, which means that in the absence of weight loss only 1.1% of VAT is lost. Furthermore, the steepness of the correlation for exercise training is slightly higher than that observed after hypocaloric diet. Taken together, these data strongly indicate that a change in weight, which is currently recommended by international guidelines for the management of obesity, does not necessarily reflect changes in VAT.

Limitations

The presence of heterogeneity of the included studies may represent a potential limitation when interpreting the results of this review. However, to correct for this heterogeneity a random effect approach was selected to perform the meta-analyses, which was specified *a priori*. Furthermore, analysis of publication bias with use of the Classic Fail 'n Safe method showed that an unrealistically large number of studies is needed to influence the significant results obtained in our meta-analyses. Therefore, we are confident that the heterogeneity observed in the studies included in this analysis does not impact the major conclusions of our study. Another limitation might be that our study provides no insight into the potential impact of ethnicity on our observation, since this subject information was often lacking in the included studies. However, our analysis is not biased by the inclusion of a single ethnic group only, since we included studies that were conducted on all continents.

Clinical relevance

As treatment for obesity, international guidelines including WHO and ACSM guidelines, recommend a minimum of 5% loss of body weight loss.[4, 6, 20] Although in common clinical practice a combination of training and hypocaloric diet is often prescribed, it is highly relevant to understand the separate effects of these interventions. Indeed, our study reveals that effects on weight loss and VAT loss are different in training and diet interventions. For example, a 5% reduction in body weight after hypocaloric diet has a different effect on VAT than a similar reduction in body weight after exercise training. Indeed, 5% loss in body weight is associated with 21.3% reduction in VAT after exercise training, but only with 13.4% reduction in VAT after a hypocaloric diet. To reduce VAT by 13.4% after exercise training, weight loss of only 2.4% is needed. Moreover, the absence of a reduction in body weight after exercise training may lead physicians to *incorrectly* conclude that the intervention has failed. This is in accordance with the ACSM position statement on appropriate physical intervention strategies for weight loss, which also emphasized that exercise training entails health benefits beyond the effects on body weight.[20]

In fact, it is likely that a clinically relevant VAT reduction (of 6.1%) is present in the absence of weight loss after exercise training, which may lead to reductions in cardiovascular risk and improvement in metabolic health. Therefore, it seems incorrect to recommend a 5% weight loss for all lifestyle interventions.

In conclusion, our systematic review and meta-analysis provide evidence that exercise training, despite smaller effects on reducing body weight, tends to have superior effects on reducing visceral adipose tissue compared to diet interventions in overweight and obese subjects. This suggests that changes in body weight represent a poor marker for adaptation in visceral adipose tissue, especially when performing exercise training. Our data therefore strongly indicate that, in clinical practice, caution should be taken when interpreting (lack in) changes of body weight after exercise training interventions. Incorrect conclusions can potentially lead to recommendations or suggestions that the exercise intervention was unsuccessful, despite the presence of a marked effect on body composition. Setting the correct targets for evaluating the health benefits of lifestyle interventions is therefore recommended.

Table 1 Overview of the characteristics of the included Exercise training (n=50) and Hypocaloric diet (n=59) studies.

Data depicted as: Mean ± standard deviation or Mean (standard error). Post value –(x) represents absolute decrease in VAT or weight (unless stated otherwise).

Abbreviations: M=male; F=female; BMI=body mass index; NR= not reported; maxHR = maxium heart rate; HRR = heart rate reserve; min = minutes; CT = Computed Tomography; MRI = magnetic resonance imaging; LCD=low calorie diet; VLCD = very low calorie diet; IGT = impaired glucose tolerance

Table 1. Overview of the characteristics of the included Exercise training (n=50) and Hypocaloric diet (n=59) studies.

Exercise Training Studies					
Reference	**Groups**	**N (M/F)**	**Age (years)**	**BMI (kg/m^2)**	**Intensity**
Baria et al.[36]	Centre-based exercise	10 (10/0)	52.1 ± 11.4	30.8 ± 5.1	Personal ventilatory treshold
	Home-based exercise	8 (8/0)	50.8 ± 7.7	30.9 ± 3.9	
	Control	10 (10/0)	53.4 ± 9.6	29. ± 1.9	
Boudou et al.[37]	Exercise	8 (8/0)	42.9 ± 5.2	28.3 ± 3.9	75% of VO2peak & 5 x 2 min 85% of VO2peak
	Control	8 (8/0)	47.9±8.35	30.85±5.2	alternated by 3x 50% of VO2peak
Cho et al.[38]	Low-intensity exercise	15 (0/15)	42.4 ± 7.6	25. 6 ± 1.7	40-50% of VO2max
	High-intensity exercise	15 (0/15)	45.6 ± 4.6	25.1 ± 2.0	70-75% of VO2max
	Control	15 (0/15)	49.2 ± 8.7	26.1 ± 2.7	
Cuff et al.[39]	Aerobic exercise	9 (0/9)	59.4 ± 1.9	32.5 ± 1.4	60-75% maxHR
	Control	9 (0/9)	60.0 ± 2.9	36.7 ± 2.0	
Davidson et al.[40]	Aerobic exercise	37 (17/20)	68.8±6.0(m) 69.1±6.5 (f)	29.9 ± 3.0 29.2 ± 3.7	60-75% of VO2peak
	Control	28 (11/17)	67.4±3.8(m) 66.7 ± 3.7(f)	30.5 ± 2.0 30.4 ± 3.2	
Dekker et al.[41]	Obese with T2D	8 (8/0)	51.0 (3.0)	29.9 (1.2)	60% VO2max
	Obese	8 (8/0)	47.1 (3.1)	32.4 (0.6)	
Despres et al.[42]	Aerobic exercise	13 (0/13)	38.8 ± 5.3	34.5 ± 4.3	55% of VO2max
Dipietro et al.[43]	Aerobic exercise	9 (2/7)	72 (1)	27.5 (2.7)	55% of maxHR during 1 month 75% during 3 months
	Control	7 (1/6)	73 (1)	26.8 (1.5)	
Donges et al.[44]	Aerobic exercise	13 (13/0)	45.4 (1.7)	32.0 (1.3)	75% of age-predicted maxHR during first 4 weeks, thereafter 80% of maxHR
	Control	8 (8/0)	49.5 (2.6)	29.6 (2.1)	
Donges et al.[45]	Aerobic	41 (16/25)	NR	30.0 ± 5.5	70-75% of max HR
	Control	26 (13/13)		28.3 ± 4.1	

	Frequency/ duration per session	Duration (weeks)	Assessment VAT	Results VAT (pre / post)	Weight (kg) (pre/post)
	3x/week / 30-60min	12	CT L4-L5 (mm)	113.1 ± 24.1 / 106.6 ± 22.8 115.2 ± 20.5 / 107.4 ± 17.0 92.1 ± 25.9 / 97.0 ± 23.9	86.2 ± 19.4 / 86,1 ± 20.7 90.9 ± 12.4 / 89.3 ± 11.9 84.8 ± 7.8 / +1.5kg
	2x/week / 45 min & 1x/week / 19 min	8	MRI L4-L5 (cm^2)	153.25± 38.55/ 84.20±21.30 156.85±23.40 / 150.35±23.3	86.90 ± 13.4/ 85.00 ±13.8 85 ± 13.75 / 88.75 ± 1.30
	3/week (duration depending on energy expenditure)	12	CT L4-L5 (cm^2)	99 ± 41 / 79 ± 40 90 ± 26 / 83 ± 30 106 ± 33 / 103 ±28	64.4 ± 6.0 / 62.3 ± 5.5 63.2 ± 6.4 / 60.4 ± 6.4 63.0 ± 7.8 / 64.5 ± 7.0
	3x /week / 75 min	16	CT L4-L5 (cm^2)	215.7 (25.8) / -8.8 (5.4) 225.8 (8.9) / - 0.4 (12.0	81.2 ± 3.8 / -1.2 (0.7) 95.6 ± 6.5 / +2.0 (1.2)
	7x/week / 30 min	26	MRI (kg)	-11.0 (1.9)% -0.7 (2.5)%	NR / -2.7 (3.1)% NR / -0.1 (0.7)%
	5x/week / 60 min	12	MRI L4-L5 (kg)	3.8 (0.3) / 3.1(0.4) 4.0 (0.4) /3.4 (0.4)	93.5 (2.9) / 93.9 (3.2) 97.6 (3.4) / 97.2 (0.6)
	4-5x/week / 90 min	12	CT L4-L5 (cm^2)	124.7 ± 48.6 / 121.3 ± 45.5	90.0 ± 11.8 / 86.3 ± 9.6
	4x/week / 40-60min	17	CT L4-L5 (cm^2)	116 (31) /106 (24) 136(28) / 118 (27)	65 (5) / 64(4) 69 (4) / 69(4)
	3x/week / 40-50min	12	CT L4 (cm^2)	1371 (113) / 1222 (100) 1383 (164) / 1349 (145)	103.1 (4.6) / -1.9 (0.7)% 92.2 (6.9) / + 0.1(0.6)%
	3x/week / 30-50min	10	DEXA (kg)	1.49 ± 0.55 / 1.38 ± 0.58 1.44 ± 0.43 / 1.44 ± 0.45	84.8 ± 18.6 / -0.8 ± 1.9 83.2 ± 13.4 / +0.6 ± 1.3kg

Table 1. Continued

Exercise Training Studies					
Reference	**Groups**	**N (M/F)**	**Age (years)**	**BMI (kg/m^2)**	**Intensity**
Donnelly et al.[46]	Exercise Group men Control Group men Exercise group women Control group women	16 (16/0) 15 (15/0) 25 (25/0) 18 (18/0)	22 ± 4 24 ± 4 24 ± 5 21 ± 4	29.7 ± 2.9 29.0 ± 3.0 28.7 ± 3.2 29.3 ± 2.3	60% of HRR with a gradual increase to 75% at 6 months
Friedenreich et al.[47]	Aerobic exercise Control	160 (0/160) 160 (0/160)	61.2±5.4 60.6±5.7	29.14.5 29.3 ± 4.3	70-80% of maxHR
Gan et al.[48]	Aerobic exercise	18 (0/18)	37.4 (1.3)	30.9 (0.7)	55-70% VO2max
Giannopoulou et al.[49]	Aerobic exercise	11 (0/11)	57 (entire study)	35.9 (2.2)	70% HRR
Halverstadt et al.[50]	Aerobic exercise	83 (34/49)	57.9 ± 0.6	36.0 ± 1.1 (% body fat)	50% of VO2max with a gradual increase to 70% of VO2max (for at least 14 w)
Haus et al.[51]	Aerobic exercise	16 (5/11)	65 ± 1	33 ± 1	60-65% max HR with a gradual increase to 80-85% week 4
Heydari et al.[52]	Aerobic exercise Control	25 (25/0) 21 (21/0)	24.7 ± 4.8 25.1 ± 3.9	28.4 ± 0.5 29 ± 0.9	80-90% of max HR during 8 sec sprint, whereafter 12 sec recovery
Hutchison et al.[53]	Obese PCOS	8 (0/8) 14 (0/14)	NR	36.9 (2.1)	75-85% of maxHR OR HIIT: 6-8 x 5 minutes at 95-100% of maxHR – 1-2 min recovery
Irving et al.[54]	Low-intensity exercise High-intensity exercise Controls	11 (0/11) 9 (0/9) 7 (0/7)	51 ± 9 (entire group)	34.7 ± 7.5 34.7 ± 6.8 32.7 ± 3.8	At lactate threshold Midway between lactate threshold and VO2max (3 days) ; lactate threshold (2 days)

Frequency/ duration per session	Duration (weeks)	Assessment VAT	Results VAT (pre / post)	Weight (kg) (pre/post)
5x/week / 20-45min	65	CT L4-L5 (cm^2)	97.9 ± 22.5 / 75.5 ± 18.3 91.7 ± 29.7 / 85.4 ± 39.7 60.6 ± 25.5 / 57.4 ± 28.4 62.9 ± 21.8 / 66.0 ± 13.9	94.0 ± 12.6 / 88.8 ± 9.5 94.1 ± 11.4 / 93.16 ± 11.6 77.0 ± 11.4 / 77.6 ± 12.8 79.9 ± 8.1 / 82.8 ±9.2
3.6x/week / 45min	52	CT umbilicus (cm^2)	101.4 ± 55.4 / -16.5 103.2 ± 56 / -1.6	75.6 ± 13.0 / -2.3 76.3 ± 12.7 / -0.5
4-5x/week / 40min	9.7	MRI L4-L5 (l)	2.23 (0.12) / 2.11 (0.12)	94.1 (2.0) / 92.8 (2.0)
3x/week / 60-75min	12	MRI (cm^3)	5204 (598) / 4675 (550)	92.9 (6) / 91.2 (5.6)
3x/week / 20-40min and addition of one extra low intensity exercise session	24	CT (cm^2)	127.8 (4.5) / -14.4 (2.4)	80.6 ± 1.6 / -1.1 (0.3)
5x/week / 50-60min	12	CT (cm^2)	182.4 ± 21.5 / 134.5 ± 15.9	95.7 ± 4.1 / 91.9 ± 3.8
3x/week / 20min	12	CT L4/L5 (g)	62.6 (6.2) / 51.8 (5.1) 69.7 (9.7) / 67.3 (8.4)	87.8 ± 2.7 / 86.3 ± 2.7 89 ± 2.9 / 89.4 (3.1)
3x/week / 60 min (alternating between HIIT and continuous)	12	CT L4-L5 (cm^2)	135.1 (15.7) / 132.7 (18.1) 119.5 (16.1) / 107.6 (15.1)	99.4 (5.4) / 96.9 (4.5) 96.9 (4.8) / 95.3 (4.8)
5x/week / duration depended on energy expenditure	16	CT L4/L5 (cm^2)	153 ± 51 / 146 ± 49 173 ± 73 / 148 ± 59 157 ± 71 / 155 ± 71	97.2 ± 22 / 95.1 ± 19.3 93.5 ± 18.3 / 90.0 ± 15.6 89.6 ± 11.2 / 88.7 ± 10.6

Table 1. Continued

Exercise Training Studies					
Reference	**Groups**	**N (M/F)**	**Age (years)**	**BMI (kg/m²)**	**Intensity**
Irwin et al.[55]	Exercise	87 (0/87)	60.7 ± 6.7	30.4 ± 4.1	Start 40% of maxHR with a gradual increase to 60-75% by week8
	Control	86 (0/86)	60.6 ± 6.8	30.5 ±3.7	
Janssen et al.[56]	Aerobic exercise in black men	84 (84/0)	Not depicted for entire groups	27.0 ± 4.8	75% of VO2max
	Aerobic exercise in white men	255 (255/0)		26.7 ± 4.9	
	Aerobic exercise in black women	160 (0/160)		28.2 ± 6.1	
	Aerobic exercise in white women	243 (0/243)		24.9 ± 4.8	
Johnson et al.[57]	Aerobic exercise	12 (N.R.)	49.1 (2.3)	32.2 (0.8)	Week 1: 50% of VO2p Week 2: 60% VO2p Week 3 and 4: 70% VO2p
	Stretching control	7 (N.R.)	47.3 (3.6)	31.1 (1.1)	
Jung et al.[58]	Moderate intensity	8 (0/8)	56.8 ± 8.2	25.5 ± 1.5	Goal: intensity at 3.5- 5.2 METs
	Vigorous intensity				
	Control	8 (0/8)	48.4 ± 6.1	25.9 ± 1.6	Goal: intensity at > 5.3 METs
		12 (0/12)	55.5 ± 7.6	27.7 ± 3.4	
Karstoft et al.[59]	Continuous walking group	12 (8/4)	60.8 ± 2.2	29.9 ± 1.6	>55% of peak energy expenditure
	Interval walking group	12 (7/5)	57.5 ± 2.4	29.0 ± 1.3	Walking at 70% of peak energy expenditure for 3 minutes, alternated with 3 minutes of slow walking
	Control group	8 (5/3)	57.1 ± 3.0	29.7 ± 1.9	
Kim et al.[60]	Aerobic exercise	24 (24/0)	49.4 ± 9.6	30.7 ± 3.3	Gradual increase of 50-60% of maxHR to 60-70%
Ku et al.[61]	Aerobic exercise	15 (0/15)	55.7 ± 7.0	27.1 ± 2.4	40-50% of maximal exercise capacity
	Control	16 (0/16)	57.8 ± 8.1	27.4 ± 2.8	
Kwon et al.[62]	Aerobic exercise	13 (0/13)	55.5 ± 7.5	27.0 ± 2.5	Anaerobic threshold
	Control	14 (0/14)	57.5 ± 8.6	27.5 ± 3.0	
Lee et al.[63]	Obese	8 (8/0)	47.1 ± 8.1	32.4 ± 1.6	~60% of VO2peak
	T2D	9 (9/0)	51.0 ± 8.0	29.9 ± 3.2	

Frequency/ duration per session	Duration (weeks)	Assessment VAT	Results VAT (pre / post)	Weight (kg) (pre/post)
5x/week / 45min	52	CT L4-L5 (g/cm^2)	147.6 (134.3-161) / -8.5 147.6 (135.4-159.8) / +0.1	81.4 ± 14.1 / -1.3% 81.7 ± 12.1 / 0.1%
3x/week / 50 min	20	CT L4-L5 (cm^2)	77.5 ± 5.1 / 71.9 ± 52.2 109.5 ± 63.6 / 102.4 ± 61.2 69.1 ± 40.8 / 65.4 ± 37.9 75.4 ± 52.7 / 72.2 ± 49.1	83.9 ± 16.3 / - 0.5 ± 2.4 84.3 ± 16.3 / -0.3 ± 2.1 73.8 ± 16.3 / -0.4 ± 3.0 67.0 ± 13.6 / -0.1 ± 2.1
3x/week / 30-45 min (interval:15 min training, 5 min rest)	4	MRI L4-L5 (cm^2)	154.3 (18.3) / 143.6 (18.7) 154.3 (21.2) / 158.6 (23.9)	94.4 (3.8) / 94.1 (4.0) 98.8 (6.0) / 98.6 (6.3)
5x/week / 60min (moderate intensity) vs. 30min (vigorous intensity)	12	CT L4-L5 (cm^2)	15784.6±4662.7 / 13262.5 ± 3217.8 13726.6±3011.8 / 12447.4 ± 2252.6 17790.2±5621.7 / 17372.7 ± 5235.7	63.7 ± 5.0 / -2.9% ± 2.5% 62.9 ± 4.4 / -2.5% ± 2.3% 67.3 ± 9.8 / -1.5% ± 1.6%
5x/week / 60min	17	MRI, below diaphragm (l)	4.5 ± 0.3 / 4.2 ± 0.4 4.7 ± 0.8 / 4.2 ± 0.7 4.7 ± 0.4 / 4.6 ± 0.4	88.2 ± 4.7 / 87.5 ± 4.8 84.9 ± 4.9 / 80.7 ± 4.1 88.5 ± 4.7 / 89.2 ± 5.2
3x/week / 60min	12	CT L4-L5 (cm^2)	197.1 ± 61.9 / 165.7 ± 57.0	87.7 ± 11.2 / -4.2%
5x/week / 60min	12	CT (g)	15890 ± 4593 / 15038 ± 3369 17530 ± 4747 / 17362 ± 4728	66.3 ± 6.0 / -1.9 ± 1.2 67.6 ± 7.5 / -0.6 ± 0.7
5x/week / 60min	12	CT L4-L5	16291.5±4808/ 14682.7±3494 17204.5±4674/ 17216.3±4560	66.3 ± 6.5 / NR 68.0 ± 7.9 / NR
5x/week / 60min	13	MRI 5cm below to 15 cm above L4-L5 (kg)	9.2 ± 1.2 / 8.3 ± 1.1 7.5 ± 1.3 / 6.7 ± 1.5	97.6 ± 8.9 / 97.2 ± 8.9 93.5 ± 7.6 / 93.9 ± 8.5

Table 1. Continued

Exercise Training Studies					
Reference	**Groups**	**N (M/F)**	**Age (years)**	**BMI (kg/m^2)**	**Intensity**
Liao et al.[64]	Aerobic exercise	32 (9/23)	55.8 (1.8)	25.6 (0.8)	50% of HRR, with a gradual
	Stretching control group	32 (17/15)	52.2 (1.8)	26.6 (0.8	increase to 70%
Malin et al.[65]	Aerobic exercise	35 (16/19)	66.8 ± 0.8	35.1 ± 0.7	60-65% of maxHR first four weeks, thereafter 80-85%
Malin et al.[66]	Impaired fasting glucose	12 (8/4)	65.1 ± 0.6 (entire group)	33.8 ± 1.0	60-65% of maxHR first four
	IGT	9 (4/5)		32.7 ± 1.1	weeks, thereafter increase to
	Combined glucose intolerance	22 (7/15)		35.6 ± 1.0	80-85%
	Normal glucose tolerant	15 (4/11)		32.3 ± 1.2	
	Type 2 diabetes	18 (7/11)		34.1 ± 1.3	
McKenzie et al.[67]	Males, GG genotype	29 (29/0)	58 ± 1	28.7 ± 0.7	50-70% of HRR
	Males, GT + TT genotype	21 (21/0)	61 ± 1	27.3 ± 0.8	
	Females, GG genotype	38 (0/38)	57 ± 1	27.7 ± 0.7	
	Females, GT + TT genotype	20 (0/20)	58 ± 1	27.9 ± 1.0	
McTiernan et al.[68]	Women, aerobic exercise	49 (0/49)	54.4 ± 7.1	28.9 ± 5.5	60-85% of maxHR
	Women, controls	51 (0/51)	53.7 ± 5.6	28.5 ± 4.5	
	Men, exercisers	51 (51/0)	56.2 ± 6.7	29.7 ± 3.7	
	Men, controls	51 (51/0)	56.6 ± 7.6	30.1 ± 4.8	
Miyatake et al.[69]	Aerobic exercise	25 (25/0)	NR	28.5 ± 2.3	60% of maximum HR and walking of an extra 1000 steps/day
Moghadesi et al.[70]	Aerobic exercise	8 (8/0)	NR	30.3 ± 2.1	Walking 2 miles on 40-59%
	Control	8 (8/0)		32.0 ± 5.3	VO2max
O'Leary et al.[71]	Aerobic exercise	16 (5/11)	63 (1)	33.2 (1.4)	Start at 60-65% of maxHR with a gradual increase to 80-85%

Frequency/ duration per session	Duration (weeks)	Assessment VAT	Results VAT (pre / post)	Weight (kg) (pre/post)
3x/week / 60min	26	CT (cm^2)	86.3 (8.1) / -16.1 112.3 (9.9) / -14.5	66.1 (2.9) / -2.7 (0.4) 69.7 (2.6) / -0.9 (0.3)
5x/week / 50-60min	12	CT (cm^3)	151.4 (14) / -30.6	99.0 ± 2.4 / - 8.1 ± 0.7
5x/week / 50-60min	12	CT (cm^2)	139.9 ± 16.8 / 86.6 ± 14.4 215.9 ± 76.6 / 140.9 ± 45.2 187.7 ± 19.1 / 172.2 ± 19.9 137.4 ± 23.9 / 90.5 ± 21.2 139.9 ± 23.7 / 109.0 ± 18.1	100 / 89.9 94.5 / 87.3 96.9 / 90.1 90.1 / 84.5 94 / 90.4
3-4x/week / 20-40min	24	CT (cm^2)	150 (129-175) / -20.1 (5.6) 131 (110-156) / -29.9 (9.2) 110 (100-121) / -7.9 (3.1) 111 (97-127) / -0.2 (5.6)	91.1 ± 2.7 / -1.3 86.7 ± 3.1 / -2.3 73.8 ± 2.0 / -0.4 76.0 ± 2.8 / -1.1
6x/week / 60min	52	CT L4-L5 (cm^2)	105.9 ± 60.8 / 100.1 ± 58.8 102.6 ± 55.8 / 104.2 ± 59.6 161.8 ± 66.3 / 149.6 ± 76.6 176.7 ± 79.1 / 170.5 ± 73.3	78 ± 17.8 / -1.4 ± -1.8% 77.9 ± 12.8 / +0.7 ± 0.9% 94.8 ± 14.9 / -1.8 ± -1.9% 97.4 ± 18.2 / +0.7 ± 0.9%
1x/week supervised and daily walking / duration NR	52	CT (cm^2)	109.8 ± 57.2 / 82.7 ± 42.6	81.3 ± 7.9 / 78.1 ± 7.4
4x/week / 30min	12	MRI L4-L5 (cm^3)	651.1 ± 31.8 / 602.2 ± 13.7 688.4±106.2/ 692.2 ± 108.8	86.1 ± 4.6 / 84.1 ± 4.3 90.4 ± 13.9 / 90.6 ± 14.1
5x/week / 50-60min	12	CT L4-L5(cm^2)	175.6 (20.2) / 136.2 (16.9)	94.1 (4.3) / 90.9 (4.0)

Table 1. Continued

Exercise Training Studies					
Reference	**Groups**	**N (M/F)**	**Age (years)**	**BMI (kg/m^2)**	**Intensity**
Park et al.[72]	Aerobic exercise	10 (0/10)	42.2 ± 1.91	25.3 ± 1.74	60-70% of maxHR
	Control	10 (0/10)	43.1 ± 1.67	25.5 ± 0.86	
Prior et al.[73]	Aerobic exercise	34 (34/0)	62 ± 1	28.9 ± 0.7	50-70% of VO2max
Pritchard et al.[74]	Aerobic exercise	14 (14/0)	21.0 ± 0.8	26.2 ± 5.5	50-55% of VO2max
Redman et al.[75]	Aerobic exercise	8 (0/8)	25 ± 1	32.0 ± 1.6	55% of VO2max
Reichkendler et al.[76]	Moderate dose aerobic exercise	18 (18/0)	30 ± 2	28.6 ± 0.4	VO2max > 70%
	High dose aerobic exercise	18 (18/0)	28 ± 1	27.6 ± 0.3	VO2max 50-70%
	Control	17 (17/0)	31 ± 1	28.0 ± 0.6	
Sasai et al.[77]	Moderate intra-abdominal fat	33 (33/0)	52.9 ± 10.6	29.2 ± 3.1	Anaerobic treshold
	High intra-abdominal fat	24 (24/0)	53.5 ± 9.5	30.3 ± 3.1	
Sasai et al.[78]	Low volume exercise	19 (19/0)	49.7 ± 8.2	31.0 ± 4.1	65-80% of maxHR
	High volume exercise	18 (18/0)	45.4 ± 8.6	29.3 ± 2.0	
Schwartz et al.[79]	Young men	13 (13/0)	28.2 ± 2.4	26.0 ± 3.5	50-60% of HRR with a gradual
	Older men	15 (15/0)	67.5 ± 5.8	26.2 ± 2.7	increase to 85%
Shojaee-Moradie et al.[80]	Aerobic exercise	10 (10/0)	47 ± 3	27.6 ± 0.6	60-85% of VO2max
	Control	7 (7/0)	55 ± 4	27.6 ± 0.9	
Sigal et al.[81]	Aerobic training group	60 (39/21)	53.9 ± 6.6	35.6 ± 10.1	60-75% of max HR
	Control	63 (41/22)	54.8 ± 7.2	35.0 ± 9.5	

Frequency/ duration per session	Duration (weeks)	Assessment VAT	Results VAT (pre / post)	Weight (kg) (pre/post)
6x/week / 60min	24	CT umbilicus	195.0 ± 12.55 / 112.4±10.50 182.9 ± 16.81 / 190.4±15.74	63.7 ± 2.58 / - 4.7 kg 65.2 ± 1.87 / +0.6kg
3x/week / 20-45min	26	CT L4-L5 (cm^2)	154 (13) / 138.3	91.4 ± 2.4 / -1.6%
7x/week / 57min	13	CT L4-L5 (cm^2)	80.8 ± 19.0 / 52.1 ± 22.4	82.1 ± 19.9 / 77.1 ± 19.0
5x/week / 23min week 1-4; gradual increase to 58min week 12-16	16	MRI (kg)	1.3(0.9-1.9) / 1.2 (0.7-1.7)	84.6 ± 5.8 -1 ± 2%
3x/week 4x/week / (duration depended on EE)	11	MRI L4-L5 (kg)	2.2 ± 0.8 kg / 1.9 ± 0.6 kg 2.0 ± 0.7 kg / 1.6 ± 0.4 kg 2.0 ± 0.6kg / 2.1 ± 0.6 kg	93.2 ± 1.9 / 89.6 ± 2.0 91.3 ± 1.7 / 88.8 ± 1.6 92.8 ± 2.1 / 92.9 ± 2.1
3x/week / 90min	12	CT (cm^2)	149.7 ± 35.4 / 134.6 ± 43.1 242.4 ± 34.4 / 199.1 ± 39.7	80.9 ± 10.1 / -2.3 ± 2.2 88.8 ± 11.3 / -3.2 ± 3.0
3x/week / 30-60min	12	CT (cm^2)	188.1 ± 53.9 / 170.3 ± 46.6 167.9 ± 44.3 / 137.9 ± 40.6	89.8 ± 13.4 / -2.7 ± 3.1 85.7 ± 9.6 / -3.4 ± 2.6
5x/week / 45min	27	CT (cm^2)	66.3 ± 37.1 / 54.8 ± 33.6 144.5 ± 49.4 / 109.0 ± 44.9	85.1 ± 15.0 / 84.6 ± 13.4 79.6 ± 7.9 / 77.1 ± 7.8
3x/week / >20min	6	CT L4-L5 (cm^2)	169.8 ± 13.1 / 139.2 ± 10.0 197.0 ± 25.6 / 181.4 ± 26.7	87.4 ± 2.8 / 87.6 ±2.6 84.1 ± 2.5 / 83.3 ± 2.4
3x/week /15-45min	22	CT L4-L5 (cm^2)	257 ± 161 / 244 ± 161 252 ± 147 / 250 ± 147	103.5 ± 31.0 /100.9 ± 30.2 101.3 ± 28.6 /101.0 ± 27.8

Table 1. Continued

Exercise Training Studies					
Reference	**Groups**	**N (M/F)**	**Age (years)**	**BMI (kg/m^2)**	**Intensity**
Slentz et al.[82]	Low Amount, moderate intensity	40 (22/18)	54.0 ± 5.5	29.8 ± 3.2	40-55% of VO2max in order to reach walking 19.2km/week
	Low Amount, vigorous intensity	46 (23/23)	53.0 ± 7.0	29.7 ± 3.1	65-80% of VO2max in order to reach jogging 19.2km/week
	High amount, vigorous intensity	42 (23/19)	51.5 ± 5.3	29.1 ± 2.4	65-80% of VO2max in order to reach levels of jogging 32.0km per week
	Control	47 (23/24)	52.3 ± 7.7	29.8 ± 3.0	
Solomon et al.[83]	Aerobic exercise and low glycemic index isocaloric diet	10 (2/7)	67 (2)	34.9 (1.1)	~85% of maximum heart rate
	Aerobic exercise and high glycemic index isocaloric diet	12 (5/7)	64 (1)	34.1 (1.1)	
Yassine et al.[84]	Aerobic exercise	12 (NR)	64 ± 2	35.3 ± 5.8	Initially 60-65 of maxHR with a gradual increase to 80-85%
Yoshimura et al.[85]	High liver fat group	13 (5/8)	NR	30.2 ± 5.7	Lactate treshold
	Low liver fat group	14 (6/8)		25.5 ± 3.2	

Hypocaloric diet studies				
Reference	**Groups**	**N (M/F)**	**Age (years)**	**BMI (kg/m^2)**
Alvarez et al.[86]	Obese, old	6 (6/0)	60 (2.7)	28.9 (1.1)
	Obese, young	6 (10/0)	32.9 (2.3)	30.4 (1.0)
Banasik et al.[87]	VLCD	15 (2/13)	39.6 ± 13.4	36.2 ± 6.3
Bosy-Westphal et al.[88]	LCD	30 (0/30)	31.4 ± 6.0	35.5 ± 4.9
Brochu et al.[89]	LCD	71 (0/71)	58.0 ± 4.7	32.2 ± 4.6

Frequency/ duration per session	Duration (weeks)	Assessment VAT	Results VAT (pre / post)	Weight (kg) (pre/post)
Duration and frequency depended on set goal for distance/intensity	34-39	CT at L4 pedicle	173 ± 72 / +1.7 ± 19.7%	88.0 ± 16.3 / -0.7%
			154 ± 55 / +2.5 ± 21.3%	85.0 ± 13.4 / -0.8%
			168 ± 64 / -6.9 ± 20.8%	85.7 ± 12.2 / -2.6%
			165 ± 68 / +8.6 ± 17.2%	86.9 ± 14.2 / -1.0%
5x/week / 60min	12	CT (cm^2)	106.9 (12.7) / 78.7 (12.1)	97.4 (3.8) / 89.6 (3.4)
			117.5 (26.3) / 73.0 (18.5)	94.7 (4.4) / 85.7 (4.1)
5x/week / 50-60min	12	CT (cm^2)	192.3 ± 104.3 / 158.4 ± 87.0	99.7 ± 15.7 / 95.9 ± 14.6
3/week / 60min	12	CT L4-L5 (cm^2)	213 ± 63 / 187 ± 66 139 ± 59 / 116 ± 61	78.3 ± 17.1 / -3.6% 66.0 ± 11.8 / -3.3%

Caloric restriction	Duration (weeks)	Assessment VAT	Results VAT (pre / post)	Weight (kg) (pre/post)
Reduction of 500-800 kcal/day	13	CT (cm^2)	184 (27) / 140 (31) 135 (17) / 107 (14)	91.2 (4.1) / 83.9 (4.0) 97.9 (4.3) / 90.2 (3.8)
Restriction to 800 kcal/day	4	CT L4-L5 (cm^2)	139.8 ± 82 / 120.8±85.9	104.1 ± 26.8 / 97.3 ± 26.4
Restriction to 800-1000 kcal/day	14.2	MRI (cm^3)	1757 **± 826 /** 1530 **± 755**	101.0 **± 18.3/** 91.2 ± 17.4
Reduction of 500-800 kcal of baseline resting metabolic rate (determined by indirect calorimetry)	26	CT L4-L5 (cm^2)	186 **± 56 /** -23 **± 30**	83.6 ± 14.4 / - 5,1 **± 4,7**

Table 1. Continued

Hypocaloric diet studies				
Reference	**Groups**	**N (M/F)**	**Age (years)**	**BMI (kg/m²)**
Chan et al.[90]	Hypocaloric diet	20 (20/0)	46 ± 8 (entire group)	35 ± 1.0
	Isocaloric diet	15 (15/0)		31 ± 0.7
Colles et al.[91]	VLCD	32 (19/13)	47.5 ± 8.3	47.3 ± 5.5
Collins et al.[92]	LCD	30 (3/27)	53	56.0 (1.0)
Conway et al.[93]	VLCD and LCD in black women	8 (0/8)	34.8 ± 7.2	40.0 ± 5.0
	VLCD and LCD in white women	10 (0/10)	38.6 ± 6.3	38.2 ± 8.1
Cooper et al.[94]	LCD	(2/43)	47.5 ± 6.2	44.0 ± 6.6
Dengo et al.[95]	LCD	36 (15/11) (combined groups)	61.2 (0.8)	30.0 (0.6)
	Control		66.1 (1.9)	31.8 (1.4)
Trussardi Fayh et al.[96]	LCD only	18 (6/12)	30.1 ± 5.5	34.7 ± 2.4
Fisher et al.[97]	LCD	29 (0/29)	NR	28 ± 1
Fujioka et al.[98]	LCD in visceral fat obesity	14 (0/14)	39.6 ± 9.4	34.3 ± 3.2
	LCD in subcutaneous fat obesity	26 (0/26)	37.1 ± 9.9	36.0 ± 5.7
Gasteyger et al.[99]	LCD in women	85 (0/85)	Median 43 (21-67)	Median 37.3 (31.4 -48.8)
	LCD in men	26 (26/0)	41 (20-61)	36.6 (33.5 – 41.9)
Giannopoulou et al.[49]	LCD	11 (0/11)	57 (no SE)	35.9 (2.2)

Caloric restriction	Duration (weeks)	Assessment VAT	Results VAT (pre / post)	Weight (kg) (pre/post)
reduction in energy intake by ~33%	16	MRI (kg)	7.1 (0.5) / 5.4 (0.4) 6.9 (0.4) / 6.7 (0.4)	109 (2) / 96 (3) 105 (3) / 109 (2)
Restriction to 456-680 kcal/day	12	CT and MRI L2-L3 (cm^2)	346.3 ± 103.3 / 285.1 ± 89.3	139.8 ± 11.0/ 125.0 ± 11.7
Restriction to 800kcal/day	9	CT (cm^2)	388.0 (31.2) / 342.1 (23)	NR
During first twelve weeks: restriction to 800 kcal/day During week 12-24: restriction to 1200-1500 kcal/day	24	CT L4-L5(cm^2)	105 (25) / 74 (23) 160 (70) / 105 (63)	NR
Restriction to 1200-2100 kcal/day	52	CT (cm^2)	186.9 ± 62.9 / -28.7 ± 46.	118.6 ± 16.6/ -8.8 ± 5.9
Restriction to 1200-1500 kcal/day	12	CT (cm^2)	177 (15) / 133 (12) 188 (18) / 186 (17)	84.6 (2.6) / 77.5 (2.2) 91.0 (4.8) / 90.4 (4.9)
Reduction of 500-1000 kcal/day	11.4	CT L4-L5 (cm^2)	136.1 ± 64.0 / 112.5 ± 54.0	95.8 ± 13.7 / 91.5 ± 14.2
Restriction to 800 kcal/day	8	CT L4-L5 (cm^2)	93 ± 35 / 58 ± 26	78 ± 8 / 66 ± 7
Gradual decrease over 8 weeks to 800 kcal/day restriction, and rise to ~1100 kcal/day before discharge	8	CT (l)	6.9 ± 3.1 / 4.3 ± 2.9 3.9 ± 1.7 / 2.6 ± 1.1	83.9 ± 12.8 / 71.9 ± 10.4 87.6 ± 17.3 / 75.3 ± 15.1
Restriction to 800-1000 kcal/day	8	MRI L4-L5 (cm^2)	123 (44-288) / -23.7% 162 (73-265) / -38.4%	Only %loss: -6 ± 5% 0 ± 2%
Reduction of 600 kcal/day	14	MRI (cm^3)	4785 (480) / 4425 (435)	92.4 (5.9) / 88.8 (5.7)

Table 1. Continued

Hypocaloric diet studies				
Reference	**Groups**	**N (M/F)**	**Age (years)**	**BMI (kg/m²)**
Goss et al.[100]	High glycemic load LCD	29 (14/15)	34.6 ± 8.1	30.9 ± 4.5
	Low glycemic load LCD	40 (17/23)	35.6 ± 4.3	32.4 ± 4.1
Gray et al.[101]	VLCD	10 (0/10)	37 ± 4	35.1 ± 2.1
Gu et al.[102]	VLCD	46 (27/19)	NR	32.6 ± 0.6
Haufe et al.[103]	Reduced carbohydrate LCD	52 (8/44)	Subgroups: 42 ± 9 and 45 ± 8	Subgroups: 32.0 ± 3.3 and 35.6±4.7
	Reduced fat LCD	50 (10/40)	44 ± 9 and 46 ± 9	31.9 ± 3.9 and 33.9 ± 3
Ibanez et al.[104]	LCD	12 (0/12)	51.4 ± 5.5	34.6 ± 3.4
	Control	9 (0/9)	50.2 ± 6.8	35.0 ± 3.6
Jang et al.[105]	LCD	177 (NR)	40.0 (1.04)	27.1 (0.22)
Janssen et al.[106]	Men, LCD	10 (10/0)	45.6 (2.1)	31.6 (0.9)
	Women, LCD	10 (0/10)	39.6 (2.4)	34.5 (1.4)
Kanai et al.[107]	LCD	26 (0/26)	50 ± 13	33.7 ± 3.1
Kim et al.[108]	LCD in wild type	224 (144/110) (entire study)	52.7 (1.31)	25.9 (0.29)
Kim et al.[108]	LCD in only UCP3 variant		52.4 (1.05)	25.8 (0.29)
	LCD in only β3-AR variant		55.4 (1.52)	25.9 (0.64)
	LCD in both variants		54.3 (1.65)	25.4 (0.51)
Kim et al.[109]	LCD	27 (27/0)	45.8 (1.7)	30.5 (0.7)
Kockx et al.[110]	LCD	50 (25/25)	38.4 ± 5.5	31.3 ± 4.5
Laaksonen et al.[111]	VLCD	20 (9/11)	46.7 ± 8.7	35.8 ± 9.5

Caloric restriction	Duration (weeks)	Assessment VAT	Results VAT (pre / post)	Weight (kg) (pre/post)
8 weeks eucaloric diet 8 weeks hypocaloric diet with a 1000 kcal-deficit	8	CT L4-L5 (cm^2)	80.6 ± 48.3 / 82.4 ± 57.9 89.5 ± 46.3 / 81.5 ± 49.4	94.3 ± 20.4 / 89.4 ± 20.9 98.4 ± 17.9 / 92.9 ± 18.1
Restriction to 650 kcal/day	10	MRI (cm^2)	96 ± 36 / 70 ± 26	90.6 ± 8.1 / -10.6 ± 3.8
Restriction to < 800 kcal/diet	8	MRI L4-L5 (cm^2)	113.9 (5.8) / 79.8 (3.7)	96.1 (2.7) / 87.4 (2.5
Reduction of ~30% baseline food (to a minimum of 1200 kcal)	26	MRI (kg)	1.8 ± 1.1 / 1.4 ± 0.9 1.9 ± 1 / 1.5 ± 0.9	95.0 ± 15.9 / 89.5 ± 14.9 93.6 ± 17.3 / 89.4 ± 17.0
Reduction of 500 kcal/day	16	MRI (cc)	3340 ± 977 / 2724 ± 1052 3175 ± 1122 / 3157 ± 1073	88.0 ± 15.2 / 82.3 ± 14.0 88.9 ± 11.4 / 88.8 ± 10.5
Reduction of 300 kcal/day	12	CT L4 (cm^2)	88.3 (2.81) / 77.8 (2.58)	71.1 (0.69) / 67.8 (0.58)
Reduction of 1000 kcal/day from baseline isocaloric diet	16	MRI 5 cm below L4-L5 to 15cm above L4-L5 (cm^2)	188 (22) / -58 (10) 142 (17) / -51 (7)	98.1 (3.5) / -12% 92.9 (5.0) / -12%
Restriction to 1200 kcal/day	12	CT umbilicus (cm^2)	168 ± 12 / 124 ± 65	81.3 ± 12.1 / 71.9 ± 10.0
Reduction of 300 kcal/day	12	CT L1 (cm^2)	274.1 (10.4) / 254.0 (10.6) 296.8 (10.5) / 276.7 (9.6) 281.9 (10.5) / 273.2 (9.6) 281.0 (10.5) / 272.3 (10.5)	69.3 (1.17) / 65.9 (1.17) 69.4 (1.06) / 66.1 (1.04) 67.4 (1.69) / 63.9 (1.68) 70.0 (2.13) / 66.7 (2.09)
Average restriction to 1547 kcal/day	12	CT umbilicus (cm^2)	195.1 (14.2) / 129.4 (10.9)	89.4 (2.4) / 79.9 (2.7)
Reduction of 1000 kcal/day	13	MRI (cm^2)	98 ± 31 / 66 ± 26	85.9 ± 8.8 / 74.9 ± 8.9
Restriction to 800 kcal/day	9	CT L4 (cm^2)	216 ± 49 / 148 ± 31	101.3 ± 12.0 /86.4 ± 9.6

Table 1. Continued

Hypocaloric diet studies				
Reference	**Groups**	**N (M/F)**	**Age (years)**	**BMI (kg/m^2)**
Langendonk et al.[112]	VLCD in lower body obese	8 (0/8)	35.0 (1.7)	33.2 (1.6)
	VLCD in upper body obese	8 (0/8)	38.3 (2.9)	33.9 (1.1)
Larson-Meyer et al.[113]	Control	11 (5/6)	37 (7)	27. 8 (2.0)
	LCD	12 (6/6)	39 (5)	27.8 (1.4)
Lee et al.[114]	LCD	33 (0/33)	32.4 ± 8.5	27.1 ± 2.3
Leenen et al.[115]	LCD in women	33 (0/33)	39 ± 5	31.3 ± 2.2
	LCD in men	27 (27/0)	40 ± 6	30.7 ± 2.2
Maki et al.[116]	LCD with diacylglycerol supplements	65 (25/40)	49.9 ± 11.4	34.5 ± 3.7
	LCD with triacylglycerol supplements	62 (25/38)	48.1 ± 11.2	33.9 ± 3.7
Murakami et al.[117]	LCD	18 (10/8)	48.2 (1.9)	27.8 (0.5)
Ng et al.[118]	LCD	20 (20/0)	NR	35.2 (1.0)
Nicklas et al.[119]	LCD	34 (0/100)	58.4 ± 6.0	33.9 ± 4.0
Okhawara et al.[120]	LCD	9 (9/0)	50.1 ± 12.9	27.9 ± 2.3
Okura et al.[121]	LCD in intra-abdominal fat obesity	31 (0/31)	NR	29.4 ± 3.2
	LCD in subcutaneous fat obesity	34 (0/34)		27.8 ± 2.0
Pierce et al.[122]	LCD	26 (15/11)	49.5 (2.5)	29 (1)
	Control	14 (9/5)	40.8 (3.3)	31 (1)

Caloric restriction	Duration (weeks)	Assessment VAT	Results VAT (pre / post)	Weight (kg) (pre/post)
Restriction to 478 kcal/day	17	MRI L4-L5 (cm^2)	303 (37) / 155 (25) 583 (77) / 359 (47)	93.4 (5.0) / 79.2 (4.7) 94.1 (3.0) / 79.7 (2.3)
Reduction of -25% from baseline energy requirements	24	CT L4-L5 (kg)	2.9 (0.4) / 2.8 (0.4) 3.2 (0.5) / 2.3 (0.4)	81.8 (2.8) / 81.9 (2.8) 81.0 (3.3) / 72.6 (3.1)
Restriction to 1200 kcal/day	12	CT L4-L5 (cm^2)	79.6 ± 28.3 / 76.9 ± 29.1	70.2 ± 8.2 / 68.2 ± 6.4
Reduction of 1000 kcal/day	13	MRI (cm^2)	103 ± 35 / -33 ± 21 155 ± 38/ -61 ± 26	86.9 ± 7.6 / -12.4 ± 4.3 97.4 ± 8.0 / -13.5 ± 3.5
Individual diet with reduction of 500-800 kcal/day	24	CT L4-L5 (cm^2)	150.4 ± 10.7 / -38 ± 3 160.6 ± 9.9 / -17 ± 8	NR
Restriction to 1000-1500 kcal/day (women); 1500-1700 kcal/day (men)	12	CT (cm^2	130.6 (16.1) / 97.9 (11.4)	72.5 (2.2) / 66.4 71.5 (2.0) / 62.9 (1.8)
Restriction to 1467 kcal/day	14	MRI (kg)	7.1 (0.5) / 5.4 (0.4)	109.3 (2.3) / 96.0 (2.7)
Reduction of 400 kcal/day	20	CT L4-L5 (cm^3)	2369 ± 870 / -612 ± 338	91.8 ± 10.4 / -11.8 ± 4.1
Restriction to 1680 kcal/day	13	CT umbilicus (cm^2)	186 ± 41.9 / 97 ± 17.7	81.1 ± 5.6 / 69.4 ± 4.4
Restriction to 1130 kcal/day	14	CT L4-L5 (cm^2)	148 ± 41 / -37 ± 19 68 ± 24 / -23 ± 17	71.5 ± 8.8 / -7.0 ± 2.4 67.5 ± 5.9 / -7.9 ± 3.6
Individualized diet with pre-set weight loss goal (minimum calories 1200 kcal/day)	12	CT L4-L5 (cm^2)	128 (10) / 84 (7) 150 (19) / 154 (19)	85 (3) / 76(2) 94 (3) / 95(3)

Table 1. Continued

Hypocaloric diet studies				
Reference	**Groups**	**N (M/F)**	**Age (years)**	**BMI (kg/m²)**
Purnell et al.[123]	LCD	21 (21/0)	65 (60-75)	31 (27-37)
Purnell et al.[124]	LCD	13 (5/8)	NR	35 ± 4.8
Riches et al.[125]	LCD	12 (12/0)	NR	34.1 (1.0)
	Isocaloric diet	14 (14/0)		34.6 (0.7)
Ross et al.[26]	LCD	11 (11/0)	46.8 ± 7.6	31.6 ± 2.7
Rossi et al.[126]	LCD	24 (13/11)	46.7 ± 14.3	35.4 ± 4.5
Ryan et al.[127]	LCD in NGT	29 (0/29)	60 (1)	32.8 (0.9)
	LCD in IGT	17 (0/17)	65 (2)	32.7 (1.2)
Ryan et al.[128]	LCD	23 (0/23)	56 (1)	Range: 25-48
Saiki et al.[129]	LCD	22 (16/6)	53.6 ± 8.4	30.4 ± 5.3
Shin et al.[130]	LCD in MAO	106 (0/106)	39.8 ± 12.2	28.0 ± 2.6
	LCD in MHO	23 (0/23)	36.4 ± 11.2	27.2 ± 1.94
Snel et al.[131]	VLCD	14 (8/6)	53 (2)	35.2 (1.1)
Stallone et al.[132]	LCD	11 (0/11)	52 (no SD)	37.0 ± 4.5
Svendsen et al.[133]	VLCD	10 (0/10)	Median (range) 34 (28-27)	NR(minimum for each subject: 28)
Tchernof et al.[134]	LCD	25 (0/25)	57.2 ± 5.5	35.3 ± 4.0

Caloric restriction	Duration (weeks)	Assessment VAT	Results VAT (pre / post)	Weight (kg) (pre/post)
Restriction to 1200 kcal/day	13	CT umbilicus (cm^2)	201 ± 51 / 153 ± 49	96 ± 11 / 86 ± 11
Restriction to 1000 kcal/day for 3 months, thereafter gradual transition during 2 weeks to a solid diet	13	CT umbilicus (cm^2)	146 ± 57 / 77 ± 47	99 (no SD) / 82
Restriction to 1200 kcal/day	14	MRI L3 (cm^2)	322.8 (23.4) / 222.1 (22.1) 309.6 (20.3) / 296.7 (15.1)	106.3 (4.1) / 95.9 (4.0) 108.2 (2.4) / 109.1 (2.6)
Reduction of 1000 kcal/day	16	MRI (l)	4.7 ± 1.6 / -1.5 ± 0.8	Only %loss -11.5%
Reduction of 500 kcal below daily energy expenditure	13-26	MRI L4-L5 (cm^2)	174.8 ± 94.7 / 118.9 ± 76.3	98.4 ± 15.9 / 89.7 ± 14.8
Reduction of 500 kcal/day	26	CT L4-L5 (cm^2)	146.9 (12.6) / 127.1 (11.1) 148.7 (11.6) / 126.5 (9.7)	88.3 (2.8) / 81.9 (2.9) 84.4 (3.7) / 77.0 (3.3)
Reduction of 250-350 kcal/day	26	CT L4-L5 (cm^2)	140.4 (12.1) / 115.1 (11.5)	88.8 (3.8) / 83.6 (3.7)
Restriction to 740 or 970 kcal/day	4	CT L4-L5 (cm^2)	233.1 ± 66.5 / 191.0 ± 67.0	85.2 ± 17.0 79.0 ± 17.2
Reduction of 300 kcal/day	12	CT L4 (cm^2)	95.1 ± 34.0 / 89.5 ± 33.4 69.0 ± 18.5 / 63.6 ± 15.5	71.2 ± 8.3 / -3.16±4.08% 70.5 ±5.1 / -2.83±2.74%
Restriction to 450 kcal/day	16	MRI L5 (ml)	553 (47) / 228 (46)	107 (4) / 83 (4)
3 months restriction 400-800 kcal/day, 2 months refeeding, 1 month 1200-1500 kcal/day	26	CT L4 (cm^2)	148 ± 75.4 / -52.9 ± 38.0	94.8 ± 10.8 / -18.8 ± 6.9
Restriction to 500-600 kcal/day	8	CT umbilicus (cm^2)	125.9 ± 115.2 / 109.8 ± 90.3	Only %loss: -11%
Restriction to 1200 kcal/day	13.9	CT L4-L5 (cm^2)	202 ± 73 / 128 ± 57	93.0 ± 10.7 / 79.5 ± 11.0

Table 1. Continued

Hypocaloric diet studies				
Reference	**Groups**	**N (M/F)**	**Age (years)**	**BMI (kg/m^2)**
Tiikainen et al.[135]	Women with high liver fat	11 (0/11)	37 (1)	33 (1)
	Women with low liver fat	12 (0/12)	37 (2)	32 (10)
Toledo et al.[136]	LCD	7 (3/7)	46.1 (2.0)	33.4 (1.2)
Van Dam et al.[137]	VLCD in ovalutory responders	9 (0/9)	30 (2.5)	37.5 (1.6)
	VLCD in ovalutory non-responders	6 (0/6)	30 (1.8)	41.9 (3.6)
Van der Kooy et al.[138]	Obese women	40 (0/40)	39 ± 6	31.3 ± 2.3
	Obese men	38 (38/0)	40 ± 6	30.7 ± 2.3
Viljanen et al.[139]	VLCD	16 (4/12)	45 (2.5)	33.3 (1.1)
Vissers et al.[140]	LCD	20 (5/15)	45.5 ± 13.1	32.9 ± 3.1
	Control	21 (5/16)	44.8 ± 11.4	30.8 ± 3.4
Wahlroos et al.[141]	VLCD (n=13)	13 (0/13)		45 ± 7
Weinsier et al.[142]	LCD in white women	23 (0/23)	37.0 ± 5.9	29.0 ± 1.5
	LCD in black women	23 (0/23)	35.5 ± 5.9	28.7 ± 1.8
Zamboni et al.[143]	VLCD and LCD	16 (0/16)	38.8 ± 14.1	38.2 ± 6.9

Caloric restriction	Duration (weeks)	Assessment VAT	Results VAT (pre / post)	Weight (kg) (pre/post)
Reduction of 600-800 kcal/day	18-19	MRI (cm^3)	1665 ± 141 / -383 ± 67 1497 ± 167 / -441 ± 122	Only %loss: -8.4 (0.2)% -8.3 (0.2)%
Reduction of 25% of calorie intake (both groups)	19.2	CT L4-L5 (cm^2)	207.9 (24.7) / 172.1 (32)	95.0 (4.3) / 84.4 (2.7)
Restriction to 470 kcal/day	8	MRI L4-L5 (cm^2)	138 (17) / 91 (18) 166 (29) / 114 (217)	NR
Reduction of 1000 kcal/day	13	MRI (cm^2)	106 ± 50 / -37 ± 29 154 ± 40 / -61 ± 25	86.5 ± 8.7 / -12.6 ± 3.9 98.3 ± 7.2 / -13.3 ± 3.0
Restriction to 550 kcal/day	6	MRI L2/L3 (kg)	1.6 (0.2) / 1.2 (0.1)	95.7 (3.3) / 84.6 (2.9)
Reduction of -600 kcal/day (for all diet groups)	26	CT L4-L5 (cm^2)	134.8 ± 57.3 / -26/3 ± 29.2 111.5 ± 47.6 / -3.6 ± 20.5	92.1 ± 11.1 / - 6.1 ± 4.6 88.6 ± 15.9 / + 0.9 ± 3.4
Restriction to 450-800 kcal/day	6	MRI L4-L5 (mm^2)	22400±11300/ 18300±8700	118.8 ± 16.6/ 110.0 ± 17.5
Restriction to 800 kcal/day	22	CT L4-L5 (cm^2)	113.0 ± 39.2 / 67.0 ± 23.8 67.6 ± 18.0 / 41.8 ± 16.9	79.1 ± 5.0 / 66.0 ± 4.8 78.2 ± 8.9 / 65.6 ± 7.7
First two weeks restriction to 307 kcal/day (VLCD) LCD for a mean duration of 14 weeks with restriction to 1003 kcal/day	16	CT L4 (cm^2)	167 ± 80.3 / 93.3 ± 61.6	104.3 ± 18.1 88.1 ± 11.6

Table 2. Characteristics of included studies (n=8) that directly compared exercise training with hypocaloric diet

Reference	Groups	N (M/F)	Age (years)	BMI (kg/m^2)	Intensity (exercise studies)Caloric restriction (hypocaloric diet studies)
Christiansen et al.[144]	Aerobic exercise LCD	19 (9/10) 19 (10/9)	37.2 ± 7 35.6 ± 7	33.3 ± 4 35.3 ± 4	70% of HRR Restriction to 600 kcal during 8 weeks
Coker et al.[145]	Aerobic exercise LCD Control	6 (2/4) 6 (3/3) 5 (3/2)	55 (2) 58 (2) 59 (2)	32 (1) 30 (0) 31 (1)	50% of VO2peak Reduction of 1000 kcal/week in week 1, and a further addition of 500 kcal each week until a reduction of 2500 kcal/week was reached
Koo et al.[146]	LCD Aerobic exercise Control	19 (0/19) 13 (0/13) 18 (0/18)	57 ± 8 59 ± 4 57 ± 8	27.1 (no SD) 25.5 28.5	Restriction 1200 kcal/day Depending on energy expenditure
Nordby et al.[147]	Aerobic training LCD Control	12 (12/0) 12 (12/0) 12 (12/0)	28 (1) 32 (2) 31 (2)	28.3 (0.3) 28.0 (0.4) 28.0 (0.4)	65% HRR, alternated with HIIT (bouts at 85% HRR) Reduction of 600 kcal/day
Oh et al.[148]	Aerobic exercise LCD	108 (108/0) 104 (104/0)	NR (adults)	29.2 (0.3) 29.4 (0.4)	60-85% of maxHR Restriction to 1680 kcal/day
Racette et al.[149]	LCD Aerobic exercise Control	19 (7/12) 19 (7/12) 10 (4/6)	55.6 (0.8) 58.8 (0.6) 56.0 (0.9)	27.2 (0.6) 27.2 (0.4) 27.9 (0.4)	Reduction of 16% of caloric intake 3 months, reduction of 20% 9 months Depending on energy deficit (same reduction as diet groups)
Ross et al.[150]	LCD Aerobic exercise Control	15 (0/15) 17 (0/17) 10 (0/10)	43.9 ± 4.9 43.2 ± 5.1 43.7 ± 6.4	31.9 ± 2.8 32.8 ± 3.9 32.4 ± 2.8	Reduction of 500 kcal/day ~80% maxHR
Ross et al.[151]	LCD Aerobic exercise Control	14 (14/0) 16 (16/0) 8 (8/0)	42.6 ± 9.7 45.0 ± 7.5 46.0±10.9	30.7 ± 1.9 32.3 ± 1.9 30.7 ± 1.6	Reduction of 700 kcal/day ~75% max HR

Data depicted as: Mean ± standard deviation or Mean (standard error). Post value –(x) represents absolute decrease in VAT or weight (unless stated otherwise).
Abbreviations: M=male; F=female; BMI=body mass index; NR= not reported; maxHR = maxium heart rate; HRR = heart rate reserve; min = minutes; CT = Computed Tomography; MRI = magnetic resonance imaging; LCD=low calorie diet

	Frequency/duration per session (for exercise only)	Duration (weeks)	Assessment VAT	Results VAT(pre / post)	Results weight (kg) (pre / post)
	60-75 min per session, 3x/week	12 12 (4 weeks mainte-nance)	MRI	3038.3±1086.1 / -18.4% ± 2.8 3437.5±1516.2 / -30.2% ± 3.2	100.9 / -3.5kg 107.8 / -12.3
	Depending on energy expenditure (week 1: 1000 kcal with a gradual increase to 2500 kcal/week)	12	CT (cm^2)	245 (31) / 228 (24) 199 (12) / 170 (11) 198 (17)/ 170 (11)	91 (3) / 91 (3) 86 (2) / 81 (2) 89 (4) / 91 (4)
	7 days/week, 120 minutes	12	CT L4-L5 (cm^2)	157.8 / 151.7 162.4 / 146.9 172.4 /163.4	67.4 (no SD) / 62.4 64.0 / 62.4 66.0 / 65.8
	7 days/week, duration depended on energy expenditure (600 kcal per session)	12	MRI T11-L5 (L)	1.60 (0.12) / -0.53 1.83 (0.18) / -0.25 2.12 (0.21) / -0.02	94.5 (2.3) / 88.6 (2.3) 91.2 (1.8) / 85.9 (2.2)* 92.2 (2.7) / 92.1 (2.5)
	3 days/week, 40-60 minutes	12	CT (cm^2)	178.1 (5.5) / 156.4 (5.0 159.0 (6.2) / 123.3 (5.2)	85.2 (1.0) / 82.6 (1.0) 84.9 (1.3) / 77.7 (1.2)
	7 days /week, duration depending on energy deficit	52	MRI (cm^3)	824.7 ± 143,4 / 633.5 ± 95.6 1123.5 ± 131.5 / 513.9 ± 107.6 1159.4 ± 203.4 / 1004 ± 155	78.5 (2.3) / 70.5 (2.3) 77.5 (2.4) / 71.0 (2.4) 81.9 (3.7) / 80.0 (3.7)
	7 days/week, 63 minutes	14	MRI (kg)	2.4 ± 1.2 / 1.9 ± 1.0 2.3 ± 0.8 / 1.6 ± 0.7 2.3 ± 0.9 / 2.2 ± 0.9	86.6 ± 10.9/ 80.1 ± 11.2 86.8 ± 10.9/ 80.9 ± 10.8 88.1 ± 8.2 / 88.6 ± 7.4
	7 days per week, 60.4 minutes	14	MRI L4-L5 (kg)	3.2 ± 1.0 / -25.2 (2.0)% 3.9 ± 1.0 / -27.5 (1.9)% 4.1 ± 1.7 / -1.9 (2.7)%	96.1 ± 8.7 / -7.7 (0.2)% 101.5±7.7 / -7.5 (0.3)% 96.7 ± 9.0 / -0.2 (0.4)%

Supporting Information

Figure S1. Forest plot of the effect size (SMD) of exercise training on VAT loss. The effect size (SMD) and 95% CI for individual studies and the pooled estimate (assessed with the use of Random Effects Model) are depicted.

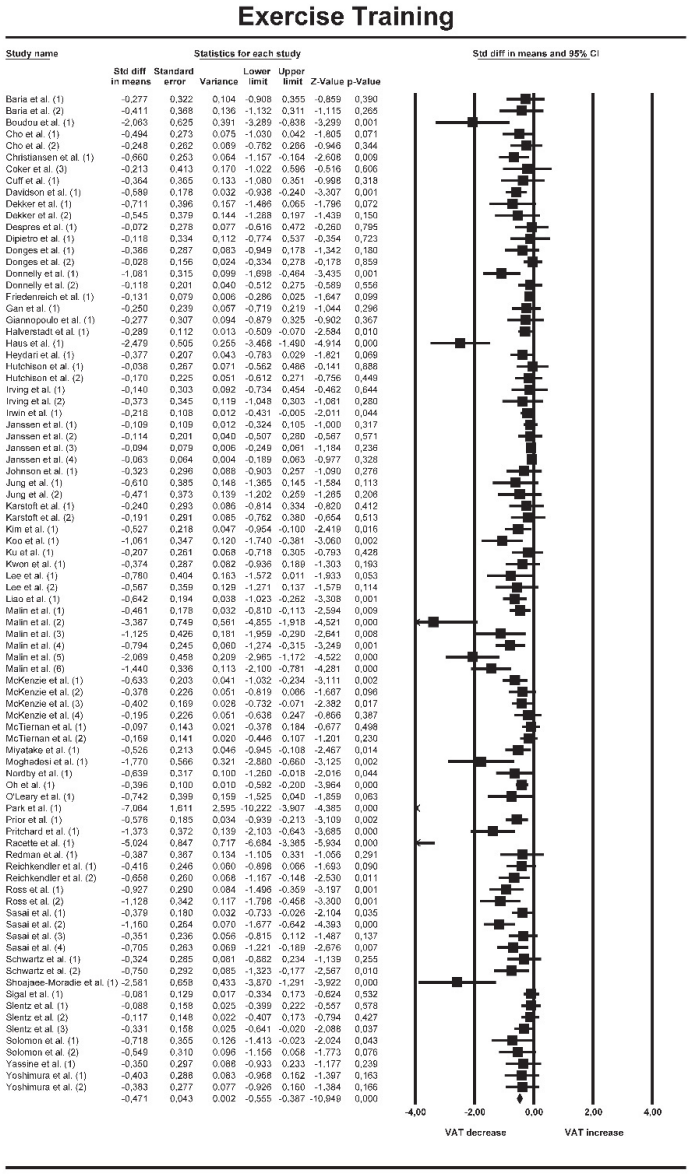
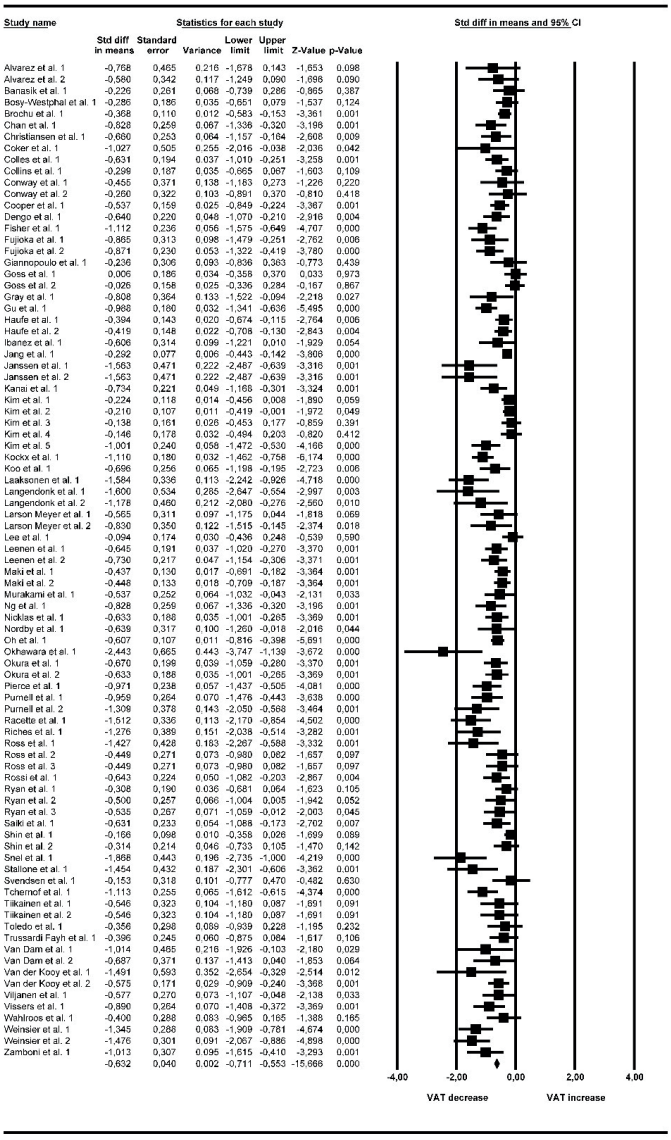


Figure S2. Forest plot of the effect size (SMD) of hypocaloric diet on VAT loss. The effect size (SMD) and 95% CI for individual studies and the pooled estimate (assessed with the use of Random Effects Model) are depicted.



REFERENCES

1 Haslam DW, James WP. Obesity. *Lancet*. 2005; 366: 1197-209.

2 World Health Organisation. Global database on body mass index. *http://appswhoint/bmi/indexjsp*. 2014.

3 Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. *Physiol Rev*. 2013; 93: 359-404.

4 Eckel RH, Krauss RM. American Heart Association call to action: obesity as a major risk factor for coronary heart disease. AHA Nutrition Committee. *Circulation*. 1998; 97: 2099-100.

5 Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 2006; 444: 840-6.

6 World Health Organisation Western Pacific Region International Association for the Study Obesity International Obesity Task Force. Redefining Obesity and its treatment. *http://wwwwhoint/nutrition/publications/obesity/09577082_1_1/en/*. 2000.

7 Franz MJ, VanWormer JJ, Crain AL, *et al.* Weight-loss outcomes: a systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up. *J Am Diet Assoc*. 2007; 107: 1755-67.

8 Miller WC, Koceja DM, Hamilton EJ. A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. *Int J Obes Relat Metab Disord*. 1997; 21: 941-7.

9 Ross R, Bradshaw AJ. The future of obesity reduction: beyond weight loss. *Nature reviews Endocrinology*. 2009; 5: 319-25.

10 Jensen MD. Role of body fat distribution and the metabolic complications of obesity. *J Clin Endocrinol Metab*. 2008; 93: S57-63.

11 Mathieu P, Poirier P, Pibarot P, Lemieux I, Despres JP. Visceral obesity: the link among inflammation, hypertension, and cardiovascular disease. *Hypertension*. 2009; 53: 577-84.

12 Despres JP, Lemieux I, Bergeron J, *et al.* Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. *Arterioscler Thromb Vasc Biol*. 2008; 28: 1039-49.

13 Farrell SW, Cheng YJ, Blair SN. Prevalence of the metabolic syndrome across cardiorespiratory fitness levels in women. *Obes Res*. 2004; 12: 824-30.

14 Katzmarzyk PT, Church TS, Janssen I, Ross R, Blair SN. Metabolic syndrome, obesity, and mortality: impact of cardiorespiratory fitness. *Diabetes Care*. 2005; 28: 391-7.

15 Wildman RP, Muntner P, Reynolds K, *et al.* The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). *Arch Intern Med*. 2008; 168: 1617-24.

16 Ismail I, Keating SE, Baker MK, Johnson NA. A systematic review and meta-analysis of the effect of aerobic vs. resistance exercise training on visceral fat. *Obesity Reviews*. 2012; 13: 68-91.

17 Kay SJ, Fiatarone Singh MA. The influence of physical activity on abdominal fat: A systematic review of the literature. *Obesity Reviews*. 2006; 7: 183-200.

18 Vissers D, Hens W, Taeymans J, Baeyens JP, Poortmans J, Van Gaal L. The Effect of Exercise on Visceral Adipose Tissue in Overweight Adults: A Systematic Review and Meta-Analysis. *PLoS ONE*. 2013; 8.

19 Jensen MD, Ryan DH, Apovian CM, *et al.* 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Circulation*. 2014; 129: S102-38.

20 Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Med Sci Sports Exerc*. 2009; 41: 459-71.

21 Janssen I, Fortier A, Hudson R, Ross R. Effects of an energy-restrictive diet with or without exercise on abdominal fat, intermuscular fat, and metabolic risk factors in obese women. *Diabetes Care*. 2002; 25: 431-38.

22 Weiss EP, Racette SB, Villareal DT, *et al.* Lower extremity muscle size and strength and aerobic capacity decrease with caloric restriction but not with exercise-induced weight loss. *Journal of applied physiology (Bethesda, Md : 1985)*. 2007; 102: 634-40.

23 Brooks GA. Exercise physiology: Human bioenergetics and its applications 1996.

24 Boule NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *Jama*. 2001; 286: 1218-27.

25 Scholten RR, Hopman MT, Lotgering FK, Spaanderman ME. Aerobic Exercise Training in Formerly Preeclamptic Women: Effects on Venous Reserve. *Hypertension*. 2015; 66: 1058-65.

26 Ross R, Rissanen J, Pedwell H, Clifford J, Shragge P. Influence of diet and exercise on skeletal muscle and visceral adipose tissue in men. *Journal of applied physiology (Bethesda, Md : 1985)*. 1996; 81: 2445-55.

27 Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *Annals of Internal Medicine*. 2009; 151: 264-69.

28 Spungen AM, Adkins RH, Stewart CA, *et al.* Factors influencing body composition in persons with spinal cord injury: a cross-sectional study. *Journal of applied physiology (Bethesda, Md : 1985)*. 2003; 95: 2398-407.

29 Shuster A, Patlas M, Pinthus JH, Mourtzakis M. The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. *The British journal of radiology*. 2012; 85: 1-10.

30 Law M SD, Letts L, Pollock N, Bosch J, et al. Critical Review Form, Quantitative Studies. *McMaster University*: 1998.

31 Ainsworth BE, Haskell WL, Whitt MC, *et al.* Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc*. 2000; 32: S498-504.

32 Haskell WL, Lee IM, Pate RR, *et al.* Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Med Sci Sports Exerc*. 2007; 39: 1423-34.

33 Convertino VA. Blood volume: its adaptation to endurance training. *Med Sci Sports Exerc*. 1991; 23: 1338-48.

34 Watts K, Beye P, Siafarikas A, *et al.* Exercise training normalizes vascular dysfunction and improves central adiposity in obese adolescents. *J Am Coll Cardiol*. 2004; 43: 1823-7.

35 Micklesfield LK, Goedecke JH, Punyanitya M, Wilson KE, Kelly TL. Dual-energy X-ray performs as well as clinical computed tomography for the measurement of visceral fat. *Obesity (Silver Spring)*. 2012; 20: 1109-14.

36 Baria F, Kamimura MA, Aoike DT, *et al.* Randomized controlled trial to evaluate the impact of aerobic exercise on visceral fat in overweight chronic kidney disease patients. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*. 2014.

37 Boudou P, Sobngwi E, Mauvais-Jarvis F, Vexiau P, Gautier JF. Absence of exercise-induced variations in adiponectin levels despite decreased abdominal adiposity and improved insulin sensitivity in type 2 diabetic men. *European Journal of Endocrinology*. 2003; 149: 421-24.

38 Cho JK, Lee SH, Lee JY, Kang HS. Randomized controlled trial of training intensity in adiposity. *International journal of sports medicine*. 2011; 32: 468-75.

39 Cuff DJ, Meneilly GS, Martin A, Ignaszewski A, Tildesley HD, Frohlich JJ. Effective Exercise Modality to Reduce Insulin Resistance in Women With Type 2 Diabetes. *Diabetes Care*. 2003; 26: 2977-82.

40 Davidson LE, Hudson R, Kilpatrick K*, et al.* Effects of exercise modality on insulin resistance and functional limitation in older adults: a randomized controlled trial. *Arch Intern Med*. 2009; 169: 122-31.

41 Dekker MJ, Lee S, Hudson R*, et al.* An exercise intervention without weight loss decreases circulating interleukin-6 in lean and obese men with and without type 2 diabetes mellitus. *Metabolism: Clinical and Experimental*. 2007; 56: 332-38.

42 Despres JP, Pouliot MC, Moorjani S*, et al.* Loss of abdominal fat and metabolic response to exercise training in obese women. *American Journal of Physiology - Endocrinology and Metabolism*. 1991; 261: E159-E67.

43 DiPietro L, Seeman TE, Stachenfeld NS, Katz LD, Nadel ER. Moderate-intensity aerobic training improves glucose tolerance in aging independent of abdominal adiposity. *Journal of the American Geriatrics Society*. 1998; 46: 875-79.

44 Donges CE, Duffield R, Guelfi KJ, Smith GC, Adams DR, Edge JA. Comparative effects of single-mode vs. duration-matched concurrent exercise training on body composition, low-grade inflammation, and glucose regulation in sedentary, overweight, middle-aged men. *Appl Physiol Nutr Metab*. 2013; 38: 779-88.

45 Donges CE, Duffield R, Drinkwater EJ. Effects of resistance or aerobic exercise training on interleukin-6, C-reactive protein, and body composition. *Medicine and Science in Sports and Exercise*. 2010; 42: 304-13.

46 Donnelly JE, Hill JO, Jacobsen DJ*, et al.* Effects of a 16-month randomized controlled exercise trial on body weight and composition in young, overweight men and women: the Midwest Exercise Trial. *Arch Intern Med*. 2003; 163: 1343-50.

47 Friedenreich CM, Woolcott CG, McTiernan A*, et al.* Adiposity changes after a 1-year aerobic exercise intervention among postmenopausal women: a randomized controlled trial. *International journal of obesity (2005)*: 2011; 427-35.

48 Gan SK, Kriketos AD, Ellis BA, Thompson CH, Kraegen EW, Chisholm DJ. 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*. 2003; 26: 1706-13.

49 Giannopoulou I, Ploutz-Snyder LL, Carhart R*, et al.* Exercise is required for visceral fat loss in postmenopausal women with type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism*. 2005; 90: 1511-18.

50 Halverstadt A, Phares DA, Ferrell RE, Wilund KR, Goldberg AP, Hagberg JM. High-density lipoprotein-cholesterol, its subfractions, and responses to exercise training are dependent on endothelial lipase genotype. *Metabolism*. 2003; 52: 1505-11.

51 Haus JM, Solomon TP, Marchetti CM*, et al.* Decreased visfatin after exercise training correlates with improved glucose tolerance. *Med Sci Sports Exerc*. 2009; 41: 1255-60.

52 Heydari M, Freund J, Boutcher SH. The effect of high-intensity intermittent exercise on body composition of overweight young males. *Journal of Obesity*. 2012; 2012.

53 Hutchison SK, Stepto NK, Harrison CL, Moran LJ, Strauss BJ, Teede HJ. Effects of exercise on insulin resistance and body composition in overweight and obese women with and without polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*. 2011; 96: E48-E56.

54 Irving B, Weltman J, Davis C, *et al.* Effects of exercise training intensity on abdominal fat in abdominally obese individuals with the metabolic syndrome. *Diabetes*. 2006; 55: A238-A38.

55 Irwin ML, Yasui Y, Ulrich CM, *et al.* Effect of exercise on total and intra-abdominal body fat in postmenopausal women: A randomized controlled trial. *Journal of the American Medical Association*. 2003; 289: 323-30.

56 Janssen I, Katzmarzyk PT, Ross R, *et al.* Fitness alters the associations of BMI and waist circumference with total and abdominal fat. *Obesity Research*. 2004; 12: 525-37.

57 Johnson NA, Sachinwalla T, Walton DW, *et al.* Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology (Baltimore, Md)*: 2009; 1105-12.

58 Jung JY, Han KA, Ahn HJ, *et al.* Effects of aerobic exercise intensity on abdominal and thigh adipose tissue and skeletal muscle attenuation in overweight women with type 2 diabetes mellitus. *Diabetes & metabolism journal*. 2012; 36: 211-21.

59 Karstoft K, Winding K, Knudsen SH, *et al.* The effects of free-living interval-walking training on glycemic control, body composition, and physical fitness in type 2 diabetic patients: A randomized, controlled trial. *Diabetes Care*. 2013; 36: 228-36.

60 Kim MK, Tomita T, Kim MJ, Sasai H, Maeda S, Tanaka K. Aerobic exercise training reduces epicardial fat in obese men. *Journal of Applied Physiology*. 2009; 106: 5-11.

61 Ku Y, Kwon HR, Seok HG, *et al.* Effect of resistance exercise on muscular subfascial adipose tissue and retinol-binding protein-4 concentration in individuals with diabetes. *Diabetologia*. 2009; 52 (S1): S270-S71.

62 Kwon HR, Min KW, Ahn HJ, *et al.* Effects of aerobic exercise on abdominal fat, thigh muscle mass and muscle strength in type 2 diabetic subject. *Korean diabetes journal*. 2010; 34: 23-31.

63 Lee S, Kuk JL, Davidson LE, *et al.* Exercise without weight loss is an effective strategy for obesity reduction in obese individuals with and without Type 2 diabetes. *Journal of Applied Physiology*. 2005; 99: 1220-25.

64 Liao D, Asberry PJ, Shofer JB, *et al.* Improvement of BMI, body composition, and body fat distribution with lifestyle modification in Japanese Americans with impaired glucose tolerance. *Diabetes Care*. 2002; 25: 1504-10.

65 Malin SK, Solomon TPJ, Blaszczak A, Finnegan S, Filion J, Kirwan JP. Pancreatic beta-cell function increases in a linear dose-response manner following exercise training in adults with prediabetes. *American Journal of Physiology - Endocrinology and Metabolism*. 2013; 305: E1248-E54.

66 Malin SK, Kirwan JP. Fasting hyperglycaemia blunts the reversal of impaired glucose tolerance after exercise training in obese older adults. *Diabetes, Obesity and Metabolism*. 2012; 14: 835-41.

67 McKenzie JA, Witkowski S, Ludlow AT, Roth SM, Hagberg JM. AKT1 G205T genotype influences obesity-related metabolic phenotypes and their responses to aerobic exercise training in older Caucasians. *Experimental Physiology*. 2011; 96: 338-47.

68 McTiernan A, Sorensen B, Irwin ML, *et al.* Exercise effect on weight and body fat in men and women. *Obesity (Silver Spring)*. 2007; 15: 1496-512.

69 Miyatake N, Nishikawa H, Morishita A, *et al.* Evaluation of exercise prescription for hypertensive obese men by ventilatory threshold. *Journal of the Chinese Medical Association*. 2003; 66: 572-78.

70 Moghadasi M, Mohebbi H, Rahmani-Nia F, Hassan-Nia S, Noroozi H. Effects of short-term lifestyle activity modification on adiponectin mRNA expression and plasma concentrations. *European journal of sport science*. 2013; 13: 378-85.

71 O'Leary VB, Marchetti CM, Krishnan RK, Stetzer BP, Gonzalez F, Kirwan JP. Exercise-induced reversal of insulin resistance in obese elderly is associated with reduced visceral fat. *Journal of Applied Physiology*. 2006; 100: 1584-89.

72 Park SK, Park JH, Kwon YC, Kim HS, Yoon MS, Park HT. The effect of combined aerobic and resistance exercise training on abdominal fat in obese middle-aged women. *Journal of Physiological Anthropology and Applied Human Science*. 2003; 22: 129-35.

73 Prior SJ, Joseph LJ, Brandauer J, Katzel LI, Hagberg JM, Ryan AS. Reduction in midthigh low-density muscle with aerobic exercise training and weight loss impacts glucose tolerance in older men. *Journal of Clinical Endocrinology and Metabolism*. 2007; 92: 880-86.

74 Pritchard J, Despres JP, Gagnon J*, et al.* Plasma adrenal, gonadal, and conjugated steroids following long-term exercise-induced negative energy balance in identical twins. *Metabolism: Clinical and Experimental*. 1999; 48: 1120-27.

75 Redman LM, Elkind-Hirsch K, Ravussin E. Aerobic exercise in women with polycystic ovary syndrome improves ovarian morphology independent of changes in body composition. *Fertility and Sterility*. 2011; 95: 2696-99.

76 Reichkendler MH, Auerbach P, Rosenkilde M*, et al.* Exercise training favors increased insulin-stimulated glucose uptake in skeletal muscle in contrast to adipose tissue: A randomized study using FDG PET imaging. *American Journal of Physiology - Endocrinology and Metabolism*. 2013; 305: E496-E506.

77 Sasai H, Katayama Y, Nakata Y, Ohkubo H, Tanaka K. Obesity phenotype and intra-abdominal fat responses to regular aerobic exercise. *Diabetes Research and Clinical Practice*. 2009; 84: 230-38.

78 Sasai H, Katayama Y, Nakata Y*, et al.* The effects of vigorous physical activity on intra-abdominal fat levels: A preliminary study of middle-aged Japanese men. *Diabetes Research and Clinical Practice*. 2010; 88: 34-41.

79 Schwartz RS, Shuman WP, Larson V*, et al.* The effect of intensive endurance exercise training on body fat distribution in young and older men. *Metabolism: Clinical and Experimental*. 1991; 40: 545-51.

80 Shojaee-Moradie F, Baynes KC, Pentecost C*, et al.* Exercise training reduces fatty acid availability and improves the insulin sensitivity of glucose metabolism. *Diabetologia*. 2007; 50: 404-13.

81 Sigal RJ, Kenny GP, Boule NG*, et al.* Effects of aerobic training, resistance training, or both on glycemic control in type 2 diabetes: a randomized trial. *Ann Intern Med*. 2007; 147: 357-69.

82 Slentz CA, Aiken LB, Houmard JA*, et al.* Inactivity, exercise, and visceral fat. STRRIDE: A randomized, controlled study of exercise intensity and amount. *Journal of Applied Physiology*. 2005; 99: 1613-18.

83 Solomon TP, Haus JM, Kelly KR*, et al.* A low-glycemic index diet combined with exercise reduces insulin resistance, postprandial hyperinsulinemia, and glucose-dependent insulinotropic polypeptide responses in obese, prediabetic humans. *Am J Clin Nutr*. 2010; 92: 1359-68.

84 Yassine HN, Marchetti CM, Krishnan RK, Vrobel TR, Gonzalez F, Kirwan JP. Effects of exercise and caloric restriction on insulin resistance and cardiometabolic risk factors in older obese adults--a randomized clinical trial. *The journals of gerontology*. 2009; Series A, Biological sciences and medical sciences. 64: 90-95.

85 Yoshimura E, Kumahara H, Tobina T*, et al.* A 12-week aerobic exercise program without energy restriction improves intrahepatic fat, liver function and atherosclerosis-related factors. *Obesity Research and Clinical Practice*. 2011; 5: e249-e57.

86 Alvarez GE, Davy BM, Ballard TP, Beske SD, Davy KP. Weight loss increases cardiovagal baroreflex function in obese young and older men. *American Journal of Physiology - Endocrinology and Metabolism*. 2005; 289: E665-E69.

87 Banasik JL, Walker MK, Randall JM, Netjes RB, Foutz MS. Low-calorie diet induced weight loss may alter regulatory hormones and contribute to rebound visceral adiposity in obese persons with a family history of type-2 diabetes. *Journal of the American Association of Nurse Practitioners*. 2013; 25: 440-8.

88 Bosy-Westphal A, Kossel E, Goele K*, et al.* Association of pericardial fat with liver fat and insulin sensitivity after diet-induced weight loss in overweight women. *Obesity*. 2010; 18: 2111-17.

89 Brochu M, Malita MF, Messier V*, et al.* Resistance training does not contribute to improving the metabolic profile after a 6-month weight loss program in overweight and obese postmenopausal women. *J Clin Endocrinol Metab*. 2009; 94: 3226-33.

90 Chan DC, Watts GF, Ng TWK, Yamashita S, Barrett PHR. Effect of weight loss on markers of triglyceride-rich lipoprotein metabolism in the metabolic syndrome. *European Journal of Clinical Investigation*. 2008; 38: 743-51.

91 Colles SL, Dixon JB, Marks P, Strauss BJ, O'Brien PE. Preoperative weight loss with a very-low-energy diet: Quantitation of changes in liver and abdominal fat by serial imaging. *American Journal of Clinical Nutrition*. 2006; 84: 304-11.

92 Collins J, McCloskey C, Titchner R*, et al.* Preoperative weight loss in high-risk superobese bariatric patients: A computed tomography-based analysis. *Surgery for Obesity and Related Diseases*. 2011; 7: 480-85.

93 Conway JM, Yanovski SZ, Avila NA, Hubbard VS. Visceral adipose tissue differences in black and white women. *Am J Clin Nutr*. 1995; 61: 765-71.

94 Cooper JN, Columbus ML, Shields KJ*, et al.* Effects of an intensive behavioral weight loss intervention consisting of caloric restriction with or without physical activity on common carotid artery remodeling in severely obese adults. *Metabolism: Clinical and Experimental*. 2012; 61: 1589-97.

95 Dengo AL, Dennis EA, Orr JS*, et al.* Arterial destiffening with weight loss in overweight and obese middle-aged and older adults. *Hypertension*: 2010; 855-61.

96 Trussardi Fayh AP, Lopes AL, Fernandes PR, Reischak-Oliveira A, Friedman R. Impact of weight loss with or without exercise on abdominal fat and insulin resistance in obese individuals: a randomised clinical trial. *Br J Nutr*. 2013; 110: 486-92.

97 Fisher G, Hyatt TC, Hunter GR, Oster RA, Desmond RA, Gower BA. Effect of diet with and without exercise training on markers of inflammation and fat distribution in overweight women. *Obesity*. 2011; 19: 1131-36.

98 Fujioka S, Matsuzawa Y, Tokunaga K*, et al.* Improvement of glucose and lipid metabolism associated with selective reduction of intra-abdominal visceral fat in premenopausal women with visceral fat obesity. *International Journal of Obesity*. 1991; 15: 853-59.

99 Gasteyger C, Larsen TM, Vercruysse F, Pedersen D, Toubro S, Astrup A. Visceral fat loss induced by a low-calorie diet: A direct comparison between women and men. *Diabetes, Obesity and Metabolism*. 2009; 11: 596-602.

100 Goss AM, Goree LL, Ellis AC*, et al.* Effects of diet macronutrient composition on body composition and fat distribution during weight maintenance and weight loss. *Obesity*. 2013; 21: 1133-38.

101 Gray DS, Fujioka K, Colletti PM*, et al.* Magnetic-resonance imaging used for determining fat distribution in obesity and diabetes. *Am J Clin Nutr*. 1991; 54: 623-7.

102 Gu Y, Yu H, Li Y*, et al.* Beneficial effects of an 8-week, very low carbohydrate diet intervention on obese subjects. *Evidence-based Complementary and Alternative Medicine*. 2013; 2013.

103 Haufe S, Kast P, Engeli S*, et al.* The influence of a low fat versus Low carbohydrate hypocaloric diet on intrahepatic fat content in overweight and obese human subjects. *Obesity Reviews*. 2011; 12: 216.

104 Ibáñez J, Izquierdo M, Martínez-Labari C, *et al.* Resistance training improves cardiovascular risk factors in obese women despite a significative decrease in serum adiponectin levels. *Obesity (Silver Spring, Md)*: 2010; 535-41.

105 Jang Y, Kim OY, Lee JH, *et al.* Genetic variation at the perilipin locus is associated with changes in serum free fatty acids and abdominal fat following mild weight loss. *International Journal of Obesity*. 2006; 30: 1601-08.

106 Janssen I, Ross R. Effects of sex on the change in visceral, subcutaneous adipose tissue and skeletal muscle in response to weight loss. *International Journal of Obesity*. 1999; 23: 1035-46.

107 Kanai H, Tokunaga K, Fujioka S, Yamashita S, Kameda-Takemura K, Matsuzawa Y. Decrease in intra-abdominal visceral fat may reduce blood pressure in obese hypertensive women. *Hypertension*. 1996; 27: 125-29.

108 Kim OY, Cho EY, Park HY, Jang Y, Lee JH. Additive effect of the mutations in the beta3-adrenoceptor gene and UCP3 gene promoter on body fat distribution and glycemic control after weight reduction in overweight subjects with CAD or metabolic syndrome. *Int J Obes Relat Metab Disord*. 2004; 28: 434-41.

109 Kim MK, Tanaka K, Kim MJ, *et al.* Comparison of epicardial, abdominal and regional fat compartments in response to weight loss. *Nutrition, Metabolism and Cardiovascular Diseases*. 2009; 19: 760-66.

110 Kockx M, Leenen R, Seidell J, Princen HMG, Kooistra T. Relationship between visceral fat and PAI-1 in overweight men and women before and after weight loss. *Thrombosis and Haemostasis*. 1999; 82: 1490-96.

111 Laaksonen DE, Kainulainen S, Rissanen A, Niskanen L. Relationships between changes in abdominal fat distribution and insulin sensitivity during a very low calorie diet in abdominally obese men and women. *Nutrition, Metabolism and Cardiovascular Diseases*. 2003; 13: 349-56.

112 Langendonk JG, Kok P, Frolich M, Pijl H, Meinders AE. Decrease in visceral fat following diet-induced weight loss in upper body compared to lower body obese premenopausal women. *European Journal of Internal Medicine*. 2006; 17: 465-69.

113 Larson-Meyer DE, Heilbronn LK, Redman LM, *et al.* Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. *Diabetes Care*. 2006; 29: 1337-44.

114 Lee HO, Yim JE, Lee JS, Kim YS, Choue R. The association between measurement sites of visceral adipose tissue and cardiovascular risk factors after caloric restriction in obese Korean women. *Nutrition research and practice*. 2013; 7: 43-8.

115 Leenen R, Van Der Kooy K, Seidell JC, Deurenberg P, Koppeschaar HPF. Visceral fat accumulation in relation to sex hormones in obese men and women undergoing weight loss therapy. *Journal of Clinical Endocrinology and Metabolism*. 1994; 78: 1515-20.

116 Maki KC, Davidson MH, Tsushima R, *et al.* Consumption of diacylglycerol oil as part of a reduced-energy diet enhances loss of body weight and fat in comparison with consumption of a triacylglycerol control oil. *American Journal of Clinical Nutrition*. 2002; 76: 1230-36.

117 Murakami T, Horigome H, Tanaka K, Nakata Y, Katayama Y, Matsui A. Effects of diet with or without exercise on leptin and anticoagulation proteins levels in obesity. *Blood Coagulation and Fibrinolysis*. 2007; 18: 389-94.

118 Ng TW, Watts GF, Barrett PH, Rye KA, Chan DC. Effect of weight loss on LDL and HDL kinetics in the metabolic syndrome: associations with changes in plasma retinol-binding protein-4 and adiponectin levels. *Diabetes Care*. 2007; 30: 2945-50.

119 Nicklas BJ, Wang X, You T, *et al.* Effect of exercise intensity on abdominal fat loss during calorie restriction in overweight and obese postmenopausal women: a randomized, controlled trial. *The American journal of clinical nutrition*. 2009; 89: 1043-52.

120 Ohkawara K, Nakata Y, Numao S, *et al.* Response of coronary heart disease risk factors to changes in body fat during diet-induced weight reduction in Japanese obese men: A pilot study. *Annals of Nutrition and Metabolism*. 2010; 56: 1-8.

121 Okura T, Nakata Y, Lee DJ, Ohkawara K, Tanaka K. Effects of aerobic exercise and obesity phenotype on abdominal fat reduction in response to weight loss. *International Journal of Obesity*. 2005; 29: 1259-66.

122 Pierce GL, Beske SD, Lawson BR, *et al.* Weight loss alone improves conduit and resistance artery endothelial function in young and older overweight/obese adults. *Hypertension*. 2008; 52: 72-79.

123 Purnell JQ, Kahn SE, Albers JJ, Nevin DN, Brunzell JD, Schwartz RS. Effect of weight loss with reduction of intra-abdominal fat on lipid metabolism in older men. *J Clin Endocrinol Metab*. 2000; 85: 977-82.

124 Purnell JQ, Cummings D, Weigle DS. Changes in 24-h area-under-the-curve ghrelin values following diet-induced weight loss are associated with loss of fat-free mass, but not with changes in fat mass, insulin levels or insulin sensitivity. *International Journal of Obesity*. 2007; 31: 385-89.

125 Riches FM, Watts GF, Hua J, Stewart GR, Naoumova RP, Barrett PH. Reduction in visceral adipose tissue is associated with improvement in apolipoprotein B-100 metabolism in obese men. *J Clin Endocrinol Metab*. 1999; 84: 2854-61.

126 Rossi AP, Fantin F, Zamboni GA, *et al.* Effect of moderate weight loss on hepatic, pancreatic and visceral lipids in obese subjects. *Nutrition and Diabetes*. 2012; 2.

127 Ryan AS, Ortmeyer HK, Sorkin JD. Exercise with calorie restriction improves insulin sensitivity and glycogen synthase activity in obese postmenopausal women with impaired glucose tolerance. *American Journal of Physiology - Endocrinology and Metabolism*. 2012; 302: E145-E52.

128 Ryan AS, Nicklas BJ, Berman DM. Aerobic exercise is necessary to improve glucose utilization with moderate weight loss in women. *Obesity*. 2006; 14: 1064-72.

129 Saiki A, Nagayama D, Ohhira M, *et al.* Effect of weight loss using formula diet on renal function in obese patients with diabetic nephropathy. *Int J Obes (Lond)*. 2005; 29: 1115-20.

130 Shin MJ, Hyun YJ, Kim OY, Kim JY, Jang Y, Lee JH. Weight loss effect on inflammation and LDL oxidation in metabolically healthy but obese (MHO) individuals: low inflammation and LDL oxidation in MHO women. *Int J Obes (Lond)*. 2006; 30: 1529-34.

131 Snel M, Jonker JT, Hammer S, *et al.* Long-term beneficial effect of a 16-week very low calorie diet on pericardial fat in obese type 2 diabetes mellitus patients. *Obesity*. 2012; 20: 1572-76.

132 Stallone DD, Stunkard AJ, Wadden TA, Foster GD, Boorstein J, Arger P. Weight loss and body fat distribution: a feasibility study using computed tomography. *Int J Obes*. 1991; 15: 775-80.

133 Svendsen PF, Jensen FK, Holst JJ, Haugaard SB, Nilas L, Madsbad S. The effect of a very low calorie diet on insulin sensitivity, beta cell function, insulin clearance, incretin hormone secretion, androgen levels and body composition in obese young women. *Scandinavian Journal of Clinical and Laboratory Investigation*. 2012; 72: 410-19.

134 Tchernof A, Nolan A, Sites CK, Ades PA, Poehlman ET. Weight loss reduces C-reactive protein levels in obese postmenopausal women. *Circulation*. 2002; 105: 564-69.

135 Tiikkainen M, Bergholm R, Vehkavaara S, *et al.* Effects of identical weight loss on body composition and features of insulin resistance in obese women with high and low liver fat content. *Diabetes*. 2003; 52: 701-07.

136 Toledo FG, Menshikova EV, Azuma K*, et al.* Mitochondrial capacity in skeletal muscle is not stimulated by weight loss despite increases in insulin action and decreases in intramyocellular lipid content. *Diabetes*. 2008; 57: 987-94.

137 van Dam EW, Roelfsema F, Veldhuis JD*, et al.* Retention of estradiol negative feedback relationship to LH predicts ovulation in response to caloric restriction and weight loss in obese patients with polycystic ovary syndrome. *American journal of physiology Endocrinology and metabolism*. 2004; 286: E615-20.

138 van der Kooy K, Leenen R, Seidell JC, Deurenberg P, Droop A, Bakker CJ. Waist-hip ratio is a poor predictor of changes in visceral fat. *Am J Clin Nutr*. 1993; 57: 327-33.

139 Viljanen AP, Lautamaki R, Jarvisalo M*, et al.* Effects of weight loss on visceral and abdominal subcutaneous adipose tissue blood-flow and insulin-mediated glucose uptake in healthy obese subjects. *Ann Med*. 2009; 41: 152-60.

140 Vissers D, Verrijken A, Mertens I*, et al.* Effect of long-term whole body vibration training on visceral adipose tissue: A preliminary report. *Obesity Facts*. 2010; 3: 93-100.

141 Wahlroos S, Phillips ML, Lewis MC*, et al.* Rapid significant weight loss and regional lipid deposition: Implications for insulin sensitivity. *Obesity Research and Clinical Practice*. 2007; 1: 7-16.

142 Weinsier RL, Hunter GR, Gower BA, Schutz Y, Darnell BE, Zuckerman PA. Body fat distribution in white and black women: different patterns of intraabdominal and subcutaneous abdominal adipose tissue utilization with weight loss. *Am J Clin Nutr*. 2001; 74: 631-6.

143 Zamboni M, Armellini F, Turcato E*, et al.* Effect of weight loss on regional body fat distribution in premenopausal women. *Am J Clin Nutr*. 1993; 58: 29-34.

144 Christiansen T, Paulsen SK, Bruun JM*, et al.* Comparable reduction of the visceral adipose tissue depot after a diet-induced weight loss with or without aerobic exercise in obese subjects: a 12-week randomized intervention study. *European journal of endocrinology / European Federation of Endocrine Societies*. 2009; 160: 759-67.

145 Coker RH, Williams RH, Yeo SE*, et al.* The impact of exercise training compared to caloric restriction on hepatic and peripheral insulin resistance in obesity. *Journal of Clinical Endocrinology and Metabolism*. 2009; 94: 4258-66.

146 Koo BK, Min KW, Kim H-J*, et al.* Changes of Abdominal, Thigh Adipose Tissue and Skeletal Muscle Attenuation in Response to Energy Restriction or Exercise in Overweight Type 2 Diabetes. *Obesity*. 2008; 16: S228-S28.

147 Nordby P, Auerbach PL, Rosenkilde M*, et al.* Endurance training per se increases metabolic health in young, moderately overweight men. *Obesity*. 2012; 20: 2202-12.

148 Oh S, Tanaka K, Warabi E, Shoda J. Exercise reduces inflammation and oxidative stress in obesity-related liver diseases. *Med Sci Sports Exerc*. 2013; 45: 2214-22.

149 Racette SB, Weiss EP, Villareal DT*, et al.* One year of caloric restriction in humans: feasibility and effects on body composition and abdominal adipose tissue. *The journals of gerontology Series A, Biological sciences and medical sciences*. 2006; 61: 943-50.

150 Ross R, Janssen I, Dawson J*, et al.* Exercise-induced reduction in obesity and insulin resistance in women: a randomized controlled trial. *Obesity Research*. 2004; 12: 789-98.

151 Ross R, Dagnone D, Jones PJ*, et al.* Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. *Ann Intern Med*. 2000; 133: 92-103.

Chapter 3

Cytokine responses to repeated, prolonged walking in lean versus overweight/obese individuals

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ABSTRACT

Objectives. Obesity is characterized by a pro-inflammatory state, which plays a role in pathogenesis of metabolic and cardiovascular disease. An exercise bout causes a transient increase in pro-inflammatory cytokines, whilst training has anti-inflammatory effects. No previous study examined whether the exercise-induced increase in pro-inflammatory cytokines is altered with repeated prolonged exercise bouts and whether this response differs between lean and overweight/obese individuals.

Design. Lean (n=25, BMI 22.9±1.5kg/m^2) and age-/sex-matched overweight/obese (n=25; BMI 27.9±2.4kg/m^2) individuals performed walking exercise for 30, 40 or 50 km per day on four consecutive days (distances similar between groups).

Methods. Circulating cytokines (IL-6, IL-10, TNF-α, IL-1β and IL-8) were examined at baseline and <30 minutes after the finish of each exercise day.

Results. At baseline, no differences in circulating cytokines were present between groups. In response to prolonged exercise, all cytokines increased on Day 1 (IL-1β: P=0.02; other cytokines: P<0.001). IL-6 remained significantly elevated during the 4 exercise days, when compared to baseline. IL-10, TNF-α, IL-1β and IL-8 returned to baseline values from exercise day 2 (IL-10, IL-1β, IL-8) or exercise day 3 (TNF-α) onward. No significant differences were found between groups for all cytokines, except IL-8 (Time*Group Interaction P=0.02).

Conclusion. These data suggest the presence of early adaptive mechanisms in response to repeated prolonged walking, demonstrated by attenuated exercise-induced elevations in cytokines on consecutive days that occurs similar in lean and overweight/obese individuals.

INTRODUCTION

In individuals with obesity, a chronic state of low grade-inflammation is present which is characterized by elevated circulating levels of cytokines.[1] This chronic inflammation is associated with the pathogenesis of cardiovascular and metabolic diseases, which are strongly associated with obesity.[2, 3] Exercise training represents a potent non-pharmacological intervention with strong anti-inflammatory effects, leading to lower levels of circulating pro-inflammatory cytokines and increased expression of anti-inflammatory cytokines.[4] Paradoxically, an acute exercise bout elicits a pro-inflammatory response, characterized by a transient rise of pro-inflammatory cytokines.[5, 6] The response of cytokines to acute exercise seems dose-dependent, as higher cytokine levels are observed after exercise of higher intensity and/or longer duration.[6] To support these observations, flu-like symptoms have been reported in relation to an exhaustive acute exercise bout, such as a marathon, which are accompanied by a (transient) rise in circulating cytokines.[6] Even exercise bouts of lower intensity have shown to cause a rise in pro-inflammatory cytokines.[5]

The pro-inflammatory effects of acute exercise versus the anti-inflammatory effect of regular exercise training imply the presence of an adaptive mechanism. Repeated exposure to the pro-inflammatory effects of acute exercise may induce an adaptive response, leading to an attenuated exercise-induced release of cytokines, as was previously demonstrated for Interleukin-6 (IL-6) in trained cyclists performing repeated exercise bouts of prolonged duration and moderate intensity (~72% of maximal heart rate).[7] In recent years an increasing number of voluntary exercise events, characterized by repeated prolonged exercise on consecutive days (e.g. walking, swimming, hiking, cycling), is organized. Since the release of cytokines in response to acute exercise seems to increase with longer duration and higher intensity,[6] it is highly relevant to examine physiological responses of cytokines to repeated prolonged exercise during such events.

Obesity is characterized by the presence of low grade inflammation.[3] Accordingly, the acute changes in cytokines in response to prolonged exercise may be affected in overweight individuals because of the presence of higher circulating cytokine levels in resting conditions.

Therefore, the aim of this study is to examine differences in the effect of repeated moderate-intensity prolonged exercise (i.e. prolonged walking 30, 40 or 50km on four consecutive days during the Nijmegen Four Day Marches, a voluntary walking event) on circulating cytokine levels (IL-6, IL-10, Tumor necrosis factor (TNF)-α, IL-1β,

and IL-8) and between lean and overweight/obese individuals. We hypothesize that the presence of low-grade inflammation at baseline in overweight/obese subjects leads to exaggerated increases in pro-inflammatory cytokines in response to prolonged exercise when compared to lean individuals.

METHODS

A total of 50 adult participants of the Nijmegen Four Days marches were included. Subjects were recruited form a cohort of participants in the Nijmegen 4 Day Marches that filled out a questionnaire as part of the Nijmegen Exercise Study. Subjects with a chronic inflammatory disease (e.g. inflammatory bowel disease, rheumatoid arthritis) and participants that used anti-inflammatory drugs (non-steroidal anti-inflammatory drugs, corticosteroids) were excluded from participation since these conditions can cause a change in circulating cytokines independent from overweight/obesity. All participants completed a distance of 30, 40 or 50 km per day on four consecutive days at a self-selected pace. Every participant was assigned to an individual distance (30, 40 or 50 km) and completed the same distance on the four consecutive exercise days. To answer our research question, subjects were allocated either to a lean (BMI <25 kg/m^2) or overweight/obese (BMI ≥ 25 kg/m^2) cohort. Furthermore, subjects were individually matched based on age, sex and walking distance and were selected for recruitment accordingly. Since exercise intensity is known to influence cytokine levels, participants were also matched based on exercise intensity, calculated based on individually recorded heart rate during the walking event. Written informed consent was obtained from all participants prior to the start of the study. This study was approved by the Medical Ethical Committee of the Radboud University Nijmegen Medical Center, and was conducted in accordance with the declaration of Helsinki.

Baseline data (subject characteristics and blood sample; day 0) were collected 1 or 2 days prior to the start of the event after a minimum resting period of 24 hours. During Day 1, exercise intensity was assessed with the use of a 2-channel chest band system (Polar Electro Oy, Kempele, Finland). At baseline, height and body weight (Seca 888 scale, Hamburg, Germany) were measured to calculate body mass index (BMI). Waist and abdominal circumference were measured with a measuring tape (Seca 201, Chino, USA) to calculate waist-to-hip ratio. A four-point skinfold thickness measurement (biceps, triceps, sub-scapular, supra-iliac) was obtained by a well-trained, experienced researcher to calculate the body fat percentage as previously described.[8] Resting heart rate and blood pressure were measured in supine position, after a 5 minute rest period.

Habitual daily energy intake, macronutrient and anti-oxidant intake were assessed with use of an online validated 180-item semi-quantitative Food Frequency Questionnaire (FFQ).[9] The FFQ reference period was one month, and portion sizes were estimated using standard portions. Intake of total energy and nutrients was calculated using the Dutch Food Composition Database.[10]

At baseline, physical activity levels were assessed with the use of the Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH), a validated tool to assess physical activity levels in the Dutch population.[11]

Heart rate was measured with a 2-channel ECG chest band system (Polar Electro Oy, Kempele, Finland) at every 5 km point during Day 1. Exercise intensity was calculated for each measurement by dividing the mean heart rate during exercise by the maximal predicted heart rate (208-0.7*age).[12] By calculating the mean of these percentages of maximal heart rate, the mean intensity for the exercise bout was recorded for each participant.

Venous blood was sampled at baseline (between 9.00 AM to 4.00 PM after a minimum resting period of 24 hours) and at each walking day within 30 minutes after completion of the exercise bout by venepuncture. Blood was centrifuged at 3000 RPM for 15 minutes and plasma was stored at -80ºC until analysis. Cytokines (IL-6, IL-10, TNF-α, Il-1β and IL-8) were simultaneously analyzed using the ultrasensitive MesoScale Discovery (MSD) QuickPlex SQ 120 Instrument with Multi-spot assay (Human Proinflammatory Panel 1, K15049D, MSD) according to the manufacturer's recommendations. The lower detection limit varied per plate and was 0.029–0.159 (IL-6), 0.025–0.051 (IL-8), 0.021–0.042 (IL-10), 0.008–0.061 (IL-1β), and 0.034–0.079 (TNF-α) pg/ml. 34 of the 250 (<15%) samples for IL-1β were below the lower detection limit. These samples were excluded from further analysis. The other cytokines were all above the detection limit.

Statistical Analysis. Data were checked for normality with use of the Shapiro-Wilk test and visual inspection of Q-Q plots. Baseline characteristics were normally distributed and therefore assessed with use of a one-way ANOVA. Cytokine data that was not normally distributed was transformed with use of square root transformation (IL-6 and TNF-α) or inverse transformation (IL-10). Cytokine data were analyzed using a time (exercise day) X group (lean vs. overweight) linear mixed model analysis. Post hoc analysis (Bonferroni) per group was performed when a significant effect was found. The level of statistical significance was defined at α=0.05. Data are presented as mean±SD, unless stated otherwise. The statistical

analyses were conducted in SPSS 25 (Statistical Package for Social Sciences 25.0, SPSS Inc., Chicago, Illinois, USA)

RESULTS

Subject characteristics are presented in Table 1. We found significant differences between the lean and overweight/obese subgroups for weight, BMI, body fat percentage and waist-hip-ratio, whilst no differences in age and sex distribution were present due to selective matching. Furthermore, the groups reported comparable habitual physical activity levels, daily energy intake and intake of macronutrients and anti-oxidants (Table 1).

Table 1. Physiological characteristics

	Lean subjects (n=25)	**Overweight/Obese subjects (n=25)**	**P-value***
Baseline characteristics			
Age (years)	56.4 ± 14.4	58.4 ± 11.9	0.60
Male sex (%)	56%	56%	1.00
Weight (kg)	69.3 ± 7.7	84 ± 12.6	<0.0001
Body mass index (kg/m^2)	22.9 ± 1.5	27.9 ± 2.4	<0.0001
Body fat percentage (%)	27.3 ± 6.6	33.5 ± 6.7	0.002
Waist-to-hip ratio	0.89 ± 0.1	0.95 ± 0.1	0.02
Systolic blood pressure (mmHg)	139 ± 21	142 ± 16	0.59
Diastolic blood pressure (mmHg)	86 ± 12	89 ± 9	0.92
Resting heart rate (bpm)	62 ± 8	63 ± 7	0.54
Daily physical activity levels			
Total SQUASH score	6342 ± 3974	7397 ± 4687	0.41
METmin/day	968 ± 522	1130 ± 629	0.32
Habitual dietary intake			
Caloric intake (kJ)	9592 ± 2516	9570 ± 3441	0.98
Total protein (g)	82 ± 21	87 ± 30	0.48
Total fat (g)	93 ± 34	89 ± 35	0.72
Saturated fat (g)	31 ± 13	31 ± 15	0.87
Total carbohydrates (g)	249 ± 64	244 ± 101	0.86
Fibre (g)	27 ± 7	25 ± 10	0.34
Dietary anti-oxidant intake			
Retinol (µg)	616 ± 369	655 ± 446	0.74

Table 1. Continued

	Lean subjects (n=25)	Overweight/Obese subjects (n=25)	P-value*
Vitamine E (mg)	16 ± 5	16 ± 7	0.71
Vitamine C (mg)	121 ± 54	115 ± 59	0.71
Exercise characteristics			
Exercise intensity (%HR_{max})	66 ± 5	69 ± 5	0.11
Exercise distance 30 km **(n)40 km (n)50 km (n)**	5 15 5	5 15 5	- - -
Exercise duration day 1 (minutes)	510 ± 129	444 ± 167	0.12
Exercise duration day 2 (minutes)	534 ± 83	522 ± 98	0.64
Exercise duration day 3 (minutes)	508 ± 140	509 ± 114	0.97
Exercise duration day 4 (minutes)	565 ± 112	540 ± 124	0.46

**One-way ANOVA between lean and overweight subgroups*

All subjects successfully completed the four exercise days. No group differences were present for exercise intensity and exercise duration (Table 1). At baseline, circulating levels of IL-6, IL-10, IL-8, IL-1β and TNF-α were not significantly different between the lean and overweight groups (Figure 1).

Repeated prolonged exercise resulted in a significant change of all cytokines (Figure 1). For all cytokines, except for IL-8 (interaction effect P=0.02), we found no differences in the post-exercise levels between lean and overweight/obese subjects (all P>0.05, Figure 1). Specifically, IL-6 showed a significant increase that remained elevated on all exercise days (P<0.001), with no differences between groups. In contrast, IL-10 increased significantly on exercise day 1(lean group: P = 0.005; overweight/obese group: P=0.003), but post-exercise levels were similarly declined to baseline on subsequent exercise days in both groups (interaction-effect P>0.05). For TNF-α, a significant effect of exercise was only present at exercise day 1 and 2 in the overweight/obese group (P<0.001 day 1, P = 0.02 day 2), whilst the lean group exhibited no change after exercise on any of the exercise days. IL-1β was significantly higher on day 1 (P=0.04) in the overweight/obese group, whilst no post-exercise increases were found in the lean group. For IL-8 a significant Time*Group Interaction effect (P=0.02) was found. Both groups showed an increase in IL-8 on day 1 that returned to baseline on subsequent days. The lean group demonstrated a significantly larger decline resulting in below-baseline levels on day 4 (P=0.001). (Figure 1)

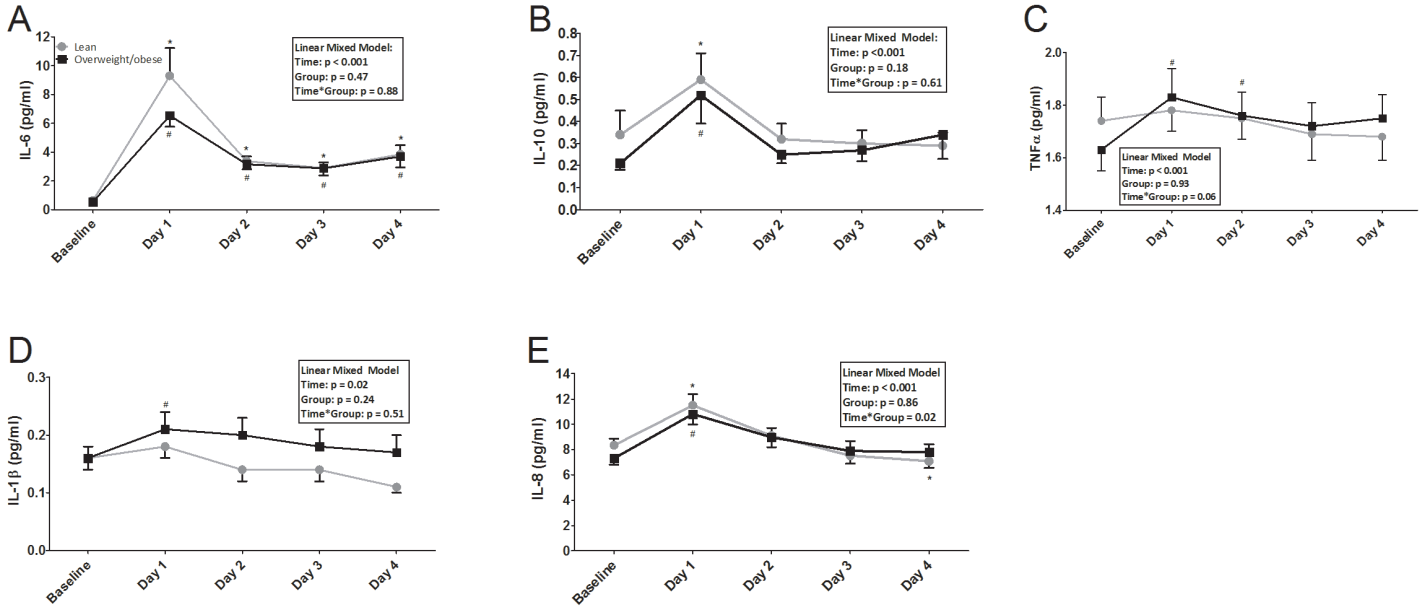

Figure 1. Mean circulating cytokine levels of IL-6 (A); IL-10 (B); TNFα (C); IL-1β (D) and IL-8 (E) at baseline and after each exercise day, with data being presented for lean subjects (●) and overweight/obese subjects (■). Error bars represent the standard error of the mean. * Significantly different from baseline in lean group (P <0.05); # Significantly different from baseline in overweight/obese group (P <0.05)

DISCUSSION

This study presents the following findings. First, prolonged exercise induced an immediate increase in pro- and anti-inflammatory cytokines, and the magnitude of this response was not different between lean and overweight/obese individuals. The exercise-induced elevation in cytokine levels was attenuated following exercise on consecutive days. Except for IL-8, no differences in cytokine responses between lean and overweight/obese individuals were found. Our data suggest the presence of early adaptive mechanisms in inflammatory cytokines in response to repeated prolonged exercise bouts performed on consecutive days, which did not markedly differ between lean and overweight/obese individuals.

In contrast to our hypothesis, no differences in plasma cytokines between the lean and overweight/obese group were present at baseline. In this study, relatively fit subjects were included since all subjects participated in a 4-day walking event. Previous work has shown that overweight and obese subjects with higher cardiorespiratory fitness levels, as a result of higher levels of physical activity, demonstrate lower levels of circulating pro-inflammatory cytokines, compared to unfit individuals.[13] Furthermore, we included subjects with only modest obesity (range BMI: 25-32.9 kg/m^2). Higher levels of BMI are significantly related to higher levels of inflammation.[14] Last, the individuals in the overweight/obese cohort report similar caloric and macronutrient intake when compared to the individuals in the lean cohort, despite being overweight/obese. It can be hypothesized that the reported dietary intake of the overweight/obese cohort is relatively healthy because these are fit individuals who perform exercise on a regular basis. Therefore, the relatively high level of fitness, modest level of obesity and similar dietary intake when compared to lean controls in our study may explain the absence of differences in baseline levels of cytokines between the overweight/obese and lean group.

To our knowledge, this is the first human study that examined responses of different cytokines to repeated exercise bouts on subsequent days and whether these responses differ between lean and overweight/obese individuals. We found no differences between lean and overweight/obese individuals in responses of IL-6, IL-10, TNF-α and IL-1β to repeated exercise. Exercise caused a subsequent rise in circulating IL-6 across the four consecutive exercise days in both groups. Of all known cytokines, IL-6 shows the largest response to exercise.[15] This might explain why IL-6 plasma levels remain elevated throughout the four-days of walking. Furthermore, previous work has shown that expression and circulating levels of IL-6 remain elevated at least 24 hours after cessation of an exercise bout, which might

also have contributed to the persistent rise of circulating IL-6 in our study and why no group differences were found.[16] Anti-inflammatory IL-10 showed a significant rise after the first exercise day. The release of IL-10 into the circulation is induced by the presence of IL-6, which was previously observed in both in vitro and in vivo work.[17, 18] This might explain the rise in IL-10 we observed after the first exercise day in both groups. However, IL-10 returns to baseline levels after the subsequent exercise days in both groups, despite the elevated levels of IL-6 on all 4 exercise days. It has been hypothesized previously that IL-6 levels have to reach a certain threshold to cause IL-10 production by leukocytes.[17] Possibly this threshold was not reached on exercise day 2-4, since IL-6 levels are lower on exercise day 2-4 when compared to exercise day 1, which may explain the return to baseline of IL-10 levels from exercise day 2 onwards.

We observed a significant change in cytokines on day 1 (IL-8, TNF-α and IL-1β) and day 2 (TNF-α in the overweight/obese cohort) when compared to baseline, that was no longer present on the consecutive exercise days. This suggests an attenuated acute response to exercise of pro-inflammatory cytokines (TNF-α, IL-8 and IL-1β) after repeated bouts of prolonged exercise. In discordance with our hypothesis, we found no differences in this attenuation between lean and overweight/obese individuals, except for IL-8. Our time-effects results show a transient rise in IL-1β on day 1 in the overweight/obese group, whilst IL-1β in the lean group shows no change. The modest response of IL-1β to exercise might relate to the presence of a persistent rise in IL-6. Previous work postulated that under influence of IL-6, the presence of IL-1receptor antagonist (IL1-ra) in the circulation is induced,[4, 19] which subsequently causes a decrease in IL-1β by competitively binding to the IL-1re ceptor.[19] The presence of elevated levels of IL-6, therefore, may contribute to the attenuated exercise-induced increase in IL-1β in the overweight/obese group.

IL-8 is a cytokine involved in chemotaxis and phagocytosis. IL-8 is elevated in individuals with obesity and related to constitutes of the metabolic syndrome, such as waist circumference and insulin resistance (i.e. HOMA-IR). [20] The difference between the lean and overweight/obese group in IL-8 response to repeated prolonged exercise seems to be caused by the decrease in IL-8 in the lean cohort on exercise day 4 when IL-8 decreases below baseline. This attenuated response of IL-8 suggests the presence of early adaptations to repeated bouts of prolonged exercise. This is in line with previous work that found a decrease in IL-8 after exercise training, although the exercise stimulus in our study is different due to the prolonged duration.[21]

Based on our data, one may speculate that the shift from the pro-inflammatory effects of a single bout of prolonged exercise to the known anti-inflammatory effects of exercise training is mediated by a change in cytokine secretion in response to repeated prolonged exercise bouts. During acute prolonged exercise, cytokines are secreted from adipose tissue[22] and skeletal muscle.[23] Exercise training is known to change gene expression in these tissues, which eventually results in altered secretion patterns of cytokines.[24-27] Gene expression in skeletal muscle is altered during each prolonged exercise bout because of altered contractile activity,[23] but is also believed to be influenced by the increased respiratory capacity in skeletal muscle that occurs by aerobic exercise training.[28] These adaptive responses, where responses to acute bouts of exercise relate to subsequent adaptation, have been referred to as hormesis: a biological process in which exposure to a low amount of a damaging factor leads to an adaptive beneficial effect in the organism.[29] Pro-inflammatory cytokines, i.e. the pro-inflammatory state which occurs during and after a single bout of exercise could be classified as a "hormesis stimulus", where the acute responses to exercise mediate an adaptive response contributing to health benefits when performed repeatedly.[30] The attenuated response of cytokines we observed in our study fits well in this hypothesis. This is further supported by a study that examined responses of IL-6 mRNA expression in skeletal muscle after a 3-h exercise protocol, before and after 10 weeks of exercise training in untrained men. A decrease in IL-6 mRNA expression levels in response to prolonged exercise from 76-fold (before training) to 8-fold (after the training period) was observed.[2727] Although it is important to emphasize that our design does not resemble the typical exercise training response, our data support the presence of an attenuated magnitude in exercise-induced changes in circulating cytokines when subjects repeat the same exercise stimulus on subsequent days.

Some limitations must be considered. Due to practical reasons, it was impossible to measure cytokines directly before the start of the walking exercise on the four consecutive days. Baseline levels were measured one or two days prior to the start of the walking event. Therefore, we were unable to assess potential adaptations in resting levels of cytokines (prior to each exercise bout). However, the primary goal of this study was to investigate differences between overweight and lean individuals in cytokine responses to repeated prolonged exercise bouts, which were therefore assessed immediately after cessation of such a bout. In our study, a prolonged exercise stimulus was used to examine cytokine responses to repeated exercise. Because of the duration of the exercise bouts (8.6 ± 2.1 hours) this design is not intended as a training study but rather as a model to examine physiological changes in response to repeated exercise stimuli.

CONCLUSION

This study demonstrated that prolonged exercise induces an immediate increase in pro- and anti-inflammatory cytokines in lean and overweight/obese individuals while repeated bouts of prolonged exercise lead to an attenuated exercise-induced cytokine response. Our data suggest that overweight/obese subjects, when matched for sex, age and fitness, largely show comparable exercise-induced changes in levels of cytokines across consecutive days of prolonged walking exercise. Therefore, our data suggest the presence of early adaptive mechanisms in circulating cytokines in response to repeated exercise bouts..

Practical Implications

- Cytokines are circulating factors that play a role in inflammation in the human body. Inflammation contributes to the development of metabolic and cardiovascular disease. Our study reveals that a prolonged walking exercise results in a rise in these cytokines that attenuates when this exercise bout is repeated.
- Our study demonstrates that both lean and overweight individuals largely show comparable exercise-induced changes of cytokines across four days of repeated prolonged walking.
- The attenuation of cytokine IL-8 occurs delayed in overweight individuals when compared to lean controls.

REFERENCES

1. Hotamisligil GS. Inflammation and metabolic disorders. *Nature.* 2006; 444(7121):860-867.
2. Eckel RH, Krauss RM. American Heart Association call to action: obesity as a major risk factor for coronary heart disease. AHA Nutrition Committee. *Circulation.* 1998; 97(21):2099-2100.
3. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature.* 2006; 444(7121):840-846.
4. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. *Journal of applied physiology (Bethesda, Md. : 1985).* 2005; 98(4):1154-1162.
5. Ostrowski K, Hermann C, Bangash A, Schjerling P, Nielsen JN, Pedersen BK. A trauma-like elevation of plasma cytokines in humans in response to treadmill running. *The Journal of physiology.* 1998; 513 (Pt 3):889-894.
6. Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, Sugawara K. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exercise immunology review.* 2002; 8:6-48.
7. Robson-Ansley P, Barwood M, Canavan J, et al. The effect of repeated endurance exercise on IL-6 and sIL-6R and their relationship with sensations of fatigue at rest. *Cytokine.* 2009; 45(2):111-116.
8. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *The British journal of nutrition.* 1974; 32(1):77-97.
9. Siebelink E, Geelen A, de Vries JH. Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults. *The British journal of nutrition.* 2011; 106(2):274-281.
10. RIVM/Voedingscentrum. NEVO-tabel 2016. http://nevo-online.rivm.nl/.
11. Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. *Journal of clinical epidemiology.* 2003; 56(12):1163-1169.
12. Tanaka H, Monahan KD, Seals DR. Age-predicted maximal heart rate revisited. *Journal of the American College of Cardiology.* 2001; 37(1):153-156.
13. Hong S, Dimitrov S, Pruitt C, Shaikh F, Beg N. Benefit of physical fitness against inflammation in obesity: role of beta adrenergic receptors. *Brain, behavior, and immunity.* 2014; 39:113-120.
14. Festa A, D'Agostino R, Jr., Williams K, et al. The relation of body fat mass and distribution to markers of chronic inflammation. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity.* 2001; 25(10):1407-1415.
15. Fischer CP. Interleukin-6 in acute exercise and training: what is the biological relevance? *Exercise immunology review.* 2006; 12:6-33.
16. Louis E, Raue U, Yang Y, Jemiolo B, Trappe S. Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. *Journal of applied physiology (Bethesda, Md. : 1985).* 2007; 103(5):1744-1751.
17. Cullen T, Thomas AW, Webb R, Hughes MG. Interleukin-6 and associated cytokine responses to an acute bout of high-intensity interval exercise: the effect of exercise intensity and volume. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme.* 2016; 41(8):803-808.
18. Nieman DC, Henson DA, Davis JM, et al. Blood leukocyte mRNA expression for IL-10, IL-1Ra, and IL-8, but not IL-6, increases after exercise. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research.* 2006; 26(9):668-674.

19. Lancaster GI, Febbraio MA. The immunomodulating role of exercise in metabolic disease. *Trends in immunology.* 2014; 35(6):262-269.

20. Kim CS, Park HS, Kawada T, et al. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. *International journal of obesity (2005).* 2006; 30(9):1347-1355.

21. Troseid M, Lappegard KT, Claudi T, et al. Exercise reduces plasma levels of the chemokines MCP-1 and IL-8 in subjects with the metabolic syndrome. *European heart journal.* 2004; 25(4):349-355.

22. Keller C, Keller P, Marshal S, Pedersen BK. IL-6 gene expression in human adipose tissue in response to exercise--effect of carbohydrate ingestion. *The Journal of physiology.* 2003; 550(Pt 3):927-931.

23. Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund Pedersen B. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *The Journal of physiology.* 2000; 529 Pt 1:237-242.

24. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nature reviews. Endocrinology.* 2012; 8(8):457-465.

25. Stanford KI, Middelbeek RJ, Goodyear LJ. Exercise Effects on White Adipose Tissue: Beiging and Metabolic Adaptations. *Diabetes.* 2015; 64(7):2361-2368.

26. Bluher M, Williams CJ, Kloting N, et al. Gene expression of adiponectin receptors in human visceral and subcutaneous adipose tissue is related to insulin resistance and metabolic parameters and is altered in response to physical training. *Diabetes care.* 2007; 30(12):3110-3115.

27. Fischer CP, Plomgaard P, Hansen AK, Pilegaard H, Saltin B, Pedersen BK. Endurance training reduces the contraction-induced interleukin-6 mRNA expression in human skeletal muscle. *American journal of physiology. Endocrinology and metabolism.* 2004; 287(6):E1189-1194.

28. Hawley JA, Hargreaves M, Joyner MJ, Zierath JR. Integrative biology of exercise. *Cell.* 2014; 159(4):738-749.

29. Mattson MP. Hormesis defined. *Ageing research reviews.* 2008; 7(1):1-7.

30. Ost M, Coleman V, Kasch J, Klaus S. Regulation of myokine expression: Role of exercise and cellular stress. *Free radical biology & medicine.* 2016; 98:78-89.

Chapter 4

Exercise improves insulin sensitivity in the absence of changes in cytokines

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ABSTRACT

Purpose. The benefits of aerobic exercise training on insulin sensitivity in subjects with the metabolic syndrome (MetS) are, at least in part, associated with changes in cytokines. Recent studies identified novel cytokines (e.g. fractalkine, omentin and osteopontin) that are strongly involved in glucose homeostasis and therefore potentially contribute in the exercise-induced changes in insulin sensitivity. Therefore, we aim to examine changes in skeletal muscle RNA expression and plasma levels of novel cytokines after exercise training, and correlate these changes to the exercise-induced changes in insulin sensitivity.

Methods. Women with the metabolic syndrome (MetS, n=11) and healthy women (n=10) participated in a 6-month aerobic exercise training intervention (3/week, 45min per session at 65%-85% of individual heart rate reserve). Before and after training, we examined insulin sensitivity (M-value during hyperinsulinemic euglycaemic clamp), circulating blood levels of cytokines (venous blood sample; leptin, adiponectin, omentin, fraktalkin, osteopontin). Skeletal muscle RNA-expression of these cytokines (muscle biopsy) was examined in two subgroups (MetS n=6; healthy women n=6).

Results. At baseline, plasma levels of omentin (85.8±26.2ng/ml) and adiponectin (5.0±1.7µg/ml) levels were significantly higher in controls compared to MetS (51.1±27.1; 3.6±1.1 respectively), and leptin levels were lower in controls (18.7±11.5ng/ml vs 53.0±23.5). M-value was significantly higher in controls (8.1±1.9mg/kg/min) than in MetS (4.0±1.7). Exercise training significantly improved M-values in both groups ($P<0.01$). Exercise training did not alter plasma and skeletal muscle RNA-expression levels of cytokines, whilst no correlation was observed between changes in cytokine level/RNA-expression and M-values ($P>0.05$).

Conclusion. Whilst exercise training successfully improves insulin sensitivity in MetS and healthy women, we found no change in plasma and mRNA expression levels of novel cytokines.

INTRODUCTION

The metabolic syndrome (MetS) comprises a set of interrelated risk factors resulting in an increased risk for development of type 2 diabetes mellitus and cardiovascular diseases.[1,2] The pathophysiology of MetS is characterized by the presence of peripheral insulin resistance, which is caused by a low physical activity level and excessive central adiposity.[3] Several previous intervention studies have demonstrated that exercise training is a powerful, non-pharmacological tool to improve insulin sensitivity via a number of metabolic adaptations.[4,5] Part of these adaptations may be related to exercise-induced alterations in cytokines. These pro- and anti-inflammatory factors play a pivotal role in the presence of low-grade inflammation that underlies a decline in insulin sensitivity.[6,7] Therefore, the measurement of cytokines prior to and after exercise training may provide potential insight in the mechanisms underlying the benefits of exercise training on insulin sensitivity.

Recent studies have identified novel cytokines, which may play an important role in glucose homeostasis and the presence of inflammation. Fractalkine is secreted in both adipose tissue and skeletal muscle,[8,9] and plays a role in the regulation of pancreatic islet β cell function. In humans, fractalkine has been proven to modulate monocyte adhesion in adipose tissue, thereby influencing chronic inflammation processes,[9] and is independently associated with markers of insulin resistance (HOMA-IR).[10] Omentin is a cytokine mainly secreted by visceral adipose tissue. Circulating omentin levels are negatively correlated with insulin resistance and are decreased in obesity and type 2 diabetes.[11] Osteopontin is biosynthesized in numerous cell types including macrophages and myoblasts in skeletal muscle.[12] In rodents, osteopontin influences macrophage recruitment in adipose tissue and thereby contributes to the inflammatory state. In obesity models in mice, circulating osteopontin levels are increased, whilst mice with a lack in osteopontin display improved insulin sensitivity.[13] Finally, vaspin is a cytokine that is mainly secreted in visceral adipose tissue and is reported to have insulin-sensitizing effects in rodents. [14,15] Currently, it is not known whether the benefits of exercise training on insulin sensitivity are related to changes in these novel cytokines.

The first aim of this study was to examine whether 6 months of aerobic exercise training alters circulating levels as well as RNA-expression levels in skeletal muscle of a set of selected novel (i.e. fractalkine, omentin, osteopontin, vaspin) and traditional (i.e. adiponectin, leptin and interleukin-6) cytokines, and whether these changes are associated with exercise training-induced adaptations in insulin

sensitivity in subjects with MetS and healthy controls. We hypothesize that the benefits of aerobic exercise training on insulin sensitivity in MetS may be associated with alterations in levels of these cytokines.

METHODS

Subjects

Eleven women with MetS and ten lean, age-matched, sedentary control women were included in this study. Metabolic syndrome was defined as having at least three out of five criteria as defined in the Joint Scientific Statement for Harmonizing the Metabolic Syndrome, including waist circumference >88 cm, triglycerides >1.7 mmol/l, High Density Lipoprotein (HDL)-cholesterol <1.3 mmol/l, blood pressure >130/85 mmHg and/or the use of antihypertensive medication, and fasting glucose levels >6.1 mmol/l.[16] Lean women were defined as having a body mass index (BMI) <25 kg/m^2 and the absence of all metabolic syndrome criteria. Pre-, peri- and postmenopausal women were included. Women were considered peri-menopausal when they experienced a persistent change in menstrual cycles of at least seven days, or a period of amenorrhea of 60 days or more. Post-menopause was defined as a period of amenorrhea of 12 months or more.[17] We excluded women with a medical history of known diabetes mellitus and/or cardiovascular diseases, liver or renal diseases, smoking, who consume more than two units of alcohol (10 g) a day, or perform regular physical activity >2 hours a week. Before participation, written informed consent was obtained. This study was approved by the Medical Ethical Committee of the Radboud university medical center, and was conducted in accordance with the Declaration of Helsinki (2000).

Study design

All subjects who participated in this study were engaged in a six month aerobic exercise training intervention. Before and after the intervention, a venapunction, hyperinsulinemic, euglycemic clamp, vastus lateralis muscle biopsy and a maximal cycling test was conducted in each participant.

Exercise training

During this training study, all women trained three times a week under the supervision of an experienced researcher. Training consisted of cycling exercise on an ergometer (Lode, Groningen, the Netherlands) starting with a 10 minute warming-up, followed by 30 minutes of exercise at 65% of the individual heart rate reserve (HRR) and ending with a cooling-down of 5 minutes. Workload was

increased based on improvements in physical fitness level across the six month intervention. Exercise intensity was monitored and documented with the use of heart rate monitors (Polar). Women had to attend at least 90% of the training session during this six month period to be eligible for inclusion of the statistical analysis.

Measurements

Insulin sensitivity. Peripheral tissue sensitivity to exogenous insulin was measured using a hyperinsulinemic euglycemic clamp as previously described.[18] The clamp was performed at least 48 hours after cessation of the last exercise bout. After an overnight fast (10 hours), the subject was placed in the supine position in a quiet, temperature controlled (22 – 24 ºC) room. Insulin (Actrapid, Novo-Nordisk, Copenhagen, Denmark) was infused intravenously in a dose of 430 pmol·m^{-2}·min^{-1} (60 mU · m^{-2} · min^{-1}) for 120 minutes. Insulin 50 U · ml^{-1} was diluted in 48 ml NaCl 0.9% with the addition of 2 ml blood from the subject to a concentration of 1 U·ml^{-1}. Venous plasma glucose concentrations were clamped at 5.0 mmol·L^{-1} by a variable glucose 20% infusion rate, adjusted depending on venous plasma glucose level measured at 5-minute intervals. Venous plasma glucose was measured in duplicate, in samples that were immediately centrifuged during 10 seconds, with use of the glucose oxidation method (Beckman Glucose Analyzer 2, Beckman Instruments Inc, Fullerton, CA 92634, USA). Insulin was measured in duplicate conform international standard 83/500 by an in-house radio-immunoassay (RIA) with the use of an anti-human insulin antiserum raised in guinea pig and radio-iodinated insulin as a tracer. Bound/free separation was carried out by addition of sheep anti-guinea-pig antiserum and precipitation by means of polyethylene glycol (PEG). Between and within-run coefficients of variation were 4.6% and 5.8% respectively, at a level of 33mU·L^{-1}. Whole body glucose disposal during the last 30 minutes of the euglycemic clamp was calculated as the M-value.

Plasma cytokine levels. Fasting venous blood samples collected prior to the start of the hyperinsulinemic euglycemic clamp were used to determine glucose, cholesterol and triglycerides via standard laboratory methods. Fasting venous blood was sampled at least 48 hours after cessation of the last exercise bout. Adiponectin and leptin were measured in duplicate by using DuoSet ELISA development system kits (R&D systems, Minneapolis, USA), free fatty acids (FFA) using Cobas Mira Plus (Roche Diagnostics Ltd., Basal, Switzerland), inflammatory marker C-reactive protein by Dako high-sensitivity ELISA (Glostrup, Denmark) and fractalkine, omentin. osteopontin, vaspin and interleukin 6 (IL-6) by Luminex assay (Austin, Texas, USA).

Skeletal muscle cytokine RNA gene expression. Before and after the six month training period, biopsies from the vastus lateralis muscle were taken after a standardized 250 kcal breakfast (79% carbohydrates, 11.2% protein, 9.8% fat). The muscle biopsies after the exercise training period, were taken at least 48 hours after the last exercise session. From muscle biopsies of six women of each group total RNA was isolated and purified. The physiological characteristics of these subgroups were representative for the whole group (Table, Supplemental Digital Content 1). RNA concentration and purity were measured with a Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA,USA). RNA integrity was analyzed on an Agilent Bioanalyser (Santa Clara, CA, USA). RNA gene expression profiling was performed using Affymetrix GeneChip Human Gene 1.0 ST arrays (Affymetrix Inc., Santa Clara, CA, USA), according to the manufacturer's instruction. The average fluorescence intensity of all genes was calculated using the Robust Multiarray Analysis (RMA) Algorithm, including a quantile normalization and using a background correction for GC-content.[19] Microarray analysis was performed using MADMAX pipeline for statistical analysis of microarray data.[20] Quality control was performed and all arrays met our criteria, except arrays from three participants. Data of these participants was excluded from further analysis. Microarray data were filtered, and probe sets with expression values higher than 20 on more than 5 arrays were considered to be expressed and were selected for further statistical analysis. Significant differences in expression levels were assessed using Intensity-Based Moderated T-statistics (IBMT).[21] Gene expression was defined as significantly changed when the p-value was <0.01, an alpha that is commonly used in gene expression studies.[8,21] The protocol used was compliant with the MIAMI guidelines, and data have been submitted to the Gene Expression Omnibus (GEO) repository under no. GSE43760.

Anthropometry. Before and after six months of endurance training we examined height and body weight (Seca 888 Scale, Seca, Hamburg) to calculate body mass index (BMI). Waist and abdominal circumference were measured with a measuring tape (Seca 201, Chino, USA) to calculate waist-to-hip ratio. Plasma cholesterol, triglyceride and glucose were determined in fasting venous blood samples using standard laboratory methods. Before and after the training period a total body Dual-X-ray Absorptiometry (DXA) scan was performed to determine lean body mass, total fat mass and trunk fat mass (QDR 4500 densitometer, Hologic Inc. Waltham, MA).

Daily activity levels. At baseline, daily activity levels were assessed with an accelerometer (SenseWear Pro3 Armband, Body Media Inc., Pitssburgh, PA, USA). From each 24 hour interval, data were analyzed from 0700 to 2300 h with a

minimum on-body time of 85%. At least three days had to fulfill these criteria to be used for analysis. Time per day spent in vigorous intensity activities (>6.0 METs) and time per day in very vigorous intensity activity (>9.0 METs) were calculated.[22]

Dietary intake. During the training intervention, women were instructed not to change their caloric intake. To assess potential changes in daily food intake, women were asked to record 3-day dietary intake records before and in the last week of the training intervention. Dietary records were analyzed with Eetmeter Software (Voedingscentrum, the Hague, Netherlands). Only data of women who completed the dietary records on at least three days before and at the end of the training period was included for analysis.

Cardio-respiratory fitness level. Women performed a maximal exercise test on an electrically braked leg-cycling ergometer (Lode, Angio 300, Groningen, the Netherlands) using an incremental protocol, to assess their cardio-respiratory fitness level. Workload increased by 10 W per minute, starting at 10 W, until exhaustion. A gas-analyzer was used to measure oxygen consumption continuously (Jaeger Benelux BV, Breda, the Netherlands). Maximal oxygen consumption (VO_{2max}) was analyzed as the mean of the last minute of the exercise bout. During the test, heart rate was measured continuously. Two minutes after cessation of the test, capillary blood lactate level (Roche Diagnostics GmbH, Mannheim, Germany) was measured.

Statistical analysis

All statistical analyses were conducted in SPSS 20 (Statistical Package for Social Sciences 20.0, SPSS Inc., Chicago, Illinois, USA). The sample size calculation to achieve statistical power were based on data from previous research published on the effects of exercise training on circulating leptin levels in obese individuals.[23] The estimated sample size was 10 participants in order to detect a difference of 4.1 ng/ml in leptin levels after training (α=0.05, β=0.85). Baseline characteristics of the groups were compared with the use of an unpaired t-test. A Two-way repeated ANOVA was used to examine the impact of exercise training on VO_{2max}, insulin sensitivity and cytokines. Correlations between circulating levels of cytokines and the M-value were assessed with the use of Spearman's correlation coefficient. The level of statistical significance for data except the microarray data was defined at α=0.05. In the microarray analysis, genes were defined as significantly changed at α=0.01. Data are presented as mean ± SD, unless stated otherwise.

RESULTS

Subject characteristics

The physical characteristics of the women with the MetS and the lean sedentary controls are presented in Table 1. In both groups pre-, peri- and post-menopausal women were included (Table 1). None of the included women used hormone replacement therapy, since this is known to directly influence circulating cytokine levels[24]. As a consequence of the selection procedure, women with MetS demonstrated a significantly higher body weight, BMI, blood pressure and triglycerides at baseline (Table 1). Analysis of physical activity data at baseline demonstrated that the women with MetS spent 10 minutes per day in vigorous intensity activities and 0 minutes per day in very vigorous intensity activities. Lean controls spent on average 19 minutes per day in vigorous intensity activities and 0 minutes per day in very vigorous intensity.

Table 1. Physiological characteristics before and after exercise training.

	Women with MetS (n=11)		**Lean control women (n=10)**		**P-value**		
	Pre	**Post**	**Pre**	**Post**	**Time**	**Group**	**Interaction**
Age (years)	53.2±7.2		48.5±10.2		-	0.24	-
Body composition (anthropometry)							
Weight (kg)	96.4±11.3	94.8±9.6	67.0±6.6#	66.9±6.2	0.18	<0.001	0.201
Body mass index (kg/m^2)	34.5±3.2	34.0±2.9	22.8±1.7#	22.7±1.5	0.18	<0.001	0.23
Waist (cm)	108.6±9.4	105.0±8.3	80.4±6.4#	78.7±4.7	0.03	<0.001	0.97
Waist-to-hip ratio	0.91±0.08	0.90±0.07	0.80±0.05*	0.78±0.04	0.57	<0.001	0.22
Body composition (DXA)							
Lean body mass (kg)	56.6±7.2	55.9±6.4	47.2±4.3*	47.4±4.1	0.62	0.002	0.24
Total fat mass (kg)	38.6±6.5	37.6±5.8	19.2±4.3#	18.7±3.9	0.08	<0.001	0.61
Trunk fat mass (kg)	20.2±4.0	19.5±3.1	8.4±2.8#	8.0±2.4	0.09	<0.001	0.54
Trunk fat percentage (%)	52.4±5.1	52.0±4.8	42.9±7.1*	42.6±7.0	0.47	0.002	0.91
Blood markers							
Fasting glucose (mmol/l)	5.5±0.6	5.7±0.8	4.5±0.3#	4.7±0.4	0.10	<0.001	0.85
Insulin (mE/l)	17.4±10.4	15.4±5.2	8.9±3.0#	10.0±3.8	0.76	0.013	0.24

Table 1. Continued

	Women with MetS (n=11)		Lean control women (n=10)		P-value		
	Pre	**Post**	**Pre**	**Post**	**Time**	**Group**	**Interaction**
HDL-cholesterol (mmol/l)	1.21±0.30	1.38±0.29	1.59±0.29	1.58±0.19	0.49	0.007	0.29
Triglycerides (mmol/l)	1.98±0.84	1.83±0.66	0.87±0.27*	0.92±0.22	0.61	<0.001	0.22
Free fatty acids (mmol/l)	0.58±0.15	0.45±0.18	0.52±0.16	0.43±0.23	0.01	0.86	0.69
Blood pressure & heart rate							
Systolic blood pressure (mmHg)	138±11	132±11	120±9*	114±9	0.02	<0.001	0.99
Diastolic blood pressure (mmHg)	84±5	80±7	76±5*	73±6	0.03	0.001	0.90
Resting heart rate (bpm)	68±5	59±7	60±7*	58±5	0.003	0.03	0.06
Physical fitness							
VO_2max (ml/min/kg)	22.8±4.5	25.3±3.8	32.0±4.7#	35.6±5.5	<0.001	<0.01	0.32
VO_2max (ml/min/kg FFM)	38.6±6.5	42.6±5.6	45.5±7.1*	50.4±7.8	<0.001	0.023	0.48
Power (Watt)	158±28	185±23	180±21	205±27	<0.001	0.05	0.83
Menopausal status							
Pre-menopausal (n)	2	-	3	-	-	-	-
Peri-menopausal (n)	4	-	4	-	-	-	-
Post-menopausal (n)	5	-	4	-	-	-	-

Exercise training intervention

Eleven women with MetS and ten lean control women all successfully completed the exercise intervention, with a training compliance of 92%. Cardio-respiratory fitness, determined as the peak oxygen uptake, improved significantly in both MetS and the lean control women, but improvement did not differ between the two groups (Table 1). Waist circumference improved significantly in both groups, whereas other body composition measurements showed no change over time, but remained different between the two groups. Analysis of the dietary records in a subgroup of women (MetS n = 4; lean control women n =5) showed no significant change in caloric intake during the training intervention (pre: 7.6 ± 1.2 MJ/day; post: 7.2 ± 1.2 MJ/day; p = 0.12). Circulating markers of glucose homeostasis (i.e.

fasting glucose and insulin) and lipids showed no change over time in both groups, whilst blood pressure decreased (Table 1). Insulin sensitivity (M-value) improved significantly in both groups, with no differences in the magnitude of improvement between both groups (Figure 1). Three out of eleven women with the metabolic syndrome (27%) did not meet the criteria for the metabolic syndrome anymore after six months of cycling training.

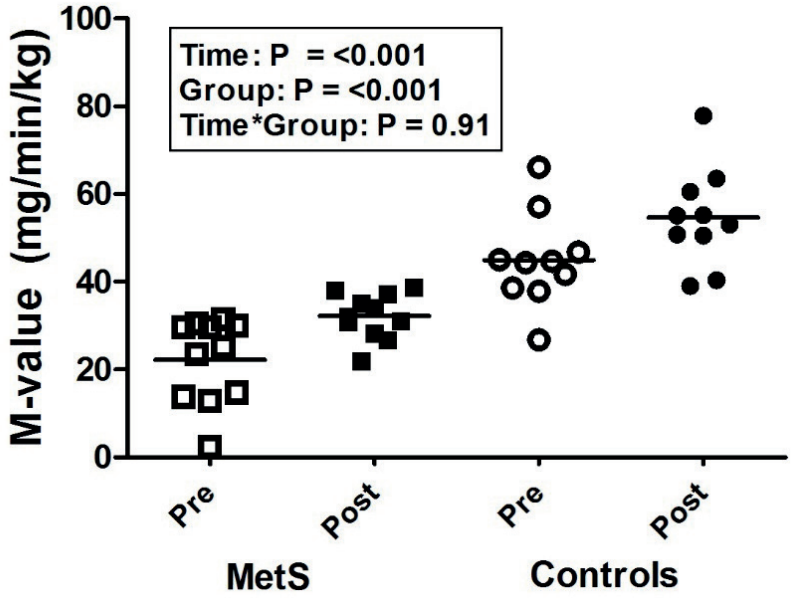

Figure 1. Insulin sensitivity (M-value) in women with the metabolic syndrome (MetS) before (□) and after (■) exercise training in women and in lean control women (Controls) before (○) and after (●) training. * P-value < 0.05

Cytokines

Plasma cytokine levels. At baseline, plasma levels of omentin and adiponectin were significantly higher in healthy control women, whilst leptin levels were lower in healthy women compared to women with MetS. No differences between the groups were found for the other cytokines (Figure 2). Exercise training did not change plasma levels of cytokines in both groups (Figure 2). At baseline, circulating vaspin did not reach the minimum detecting value of 0.16 ng/ml in n=8 women with the metabolic syndrome and in n=6 lean control women. Plasma levels of IL-6 did not meet the minimal detection value of 3 pg/ml at baseline in all lean control women (n=10) and in most women with the metabolic syndrome (n=9). After the training period, levels of both vaspin and IL-6 remained below our minimal detection limit

in these subjects. Since a large proportion of the individuals included in this study did not show detectable circulating IL-6 and vaspin levels, data on both IL-6 and vaspin were excluded from further analysis.

Skeletal muscle mRNA expression. Skeletal muscle gene expression levels of novel and known cytokines were compared before and after the training intervention in five women with the metabolic syndrome and four lean control women. In both groups, no differences were found in gene expression levels of the cytokines before and after exercise training (Table 2).

Table 2. Micro-array results for gene-expression in skeletal muscle.

	Women with MetS (n=5)			**Healthy control women (n=4)**		
	P-value	**Corrected P-value**	**Fold Change**	**P-value**	**Corrected P-value**	**Fold Change**
Fractalkine (ng/ml)	0.492269	0.997875	-1.16	0.544348	0.957122	-1.13
Omentin (ng/ml)	0.970832	0.999025	-1.02	0.930327	0.99287	1.00
Osteopontin (ng/ml)	0.514872	0.997875	1.06	0.40586	0.941683	-1.07
Vaspin (ng/ml)	0.778275	0.997875	1.02	0.727007	0.974637	1.03
Leptin (ng/ml)	0.0950706	0.997875	-1.20	0.485465	0.952995	1.03
Adiponectin (µg/ml)	0.145784	0.997875	-1.91	0.973873	0.996383	1.02

Correlation analysis

At baseline we found a significant inverse correlation between insulin sensitivity (i.e. M-value) and circulating levels of leptin (R=0.65, P=0.002), whereas no correlation was found between the M-value and adiponectin (R=0.35, P=0.124), fractalkine (R=-0.22, P=0.33), omentin (R=0.38, P=0.09), or osteopontin (R=0.14, P=0.55). When exploring the exercise training induced changes in insulin sensitivity (ΔM-value) and the changes in cytokines (ΔFractalkine (R=-0.36, P=0.18); ΔOmentin (R=0.22, P=0.34) ; ΔOsteopontin (R=-0.13, P=0.59), ΔLeptin (R=-0.24, P=0.29), ΔAdiponectin(R=0.37, P-0.10)), no significant correlations were found. Furthermore, no correlation between weight change (Δweight) and changes in ΔFractalkine (R=-0.20, P=0.40) ; ΔOmentin (R=-0.25, P=0.27); ΔOsteopontin (R=0.10, P=0.68), ΔLeptin (R=-0.22, P=0.34) or ΔAdiponectin (R=0.06, P =0.79) was present.

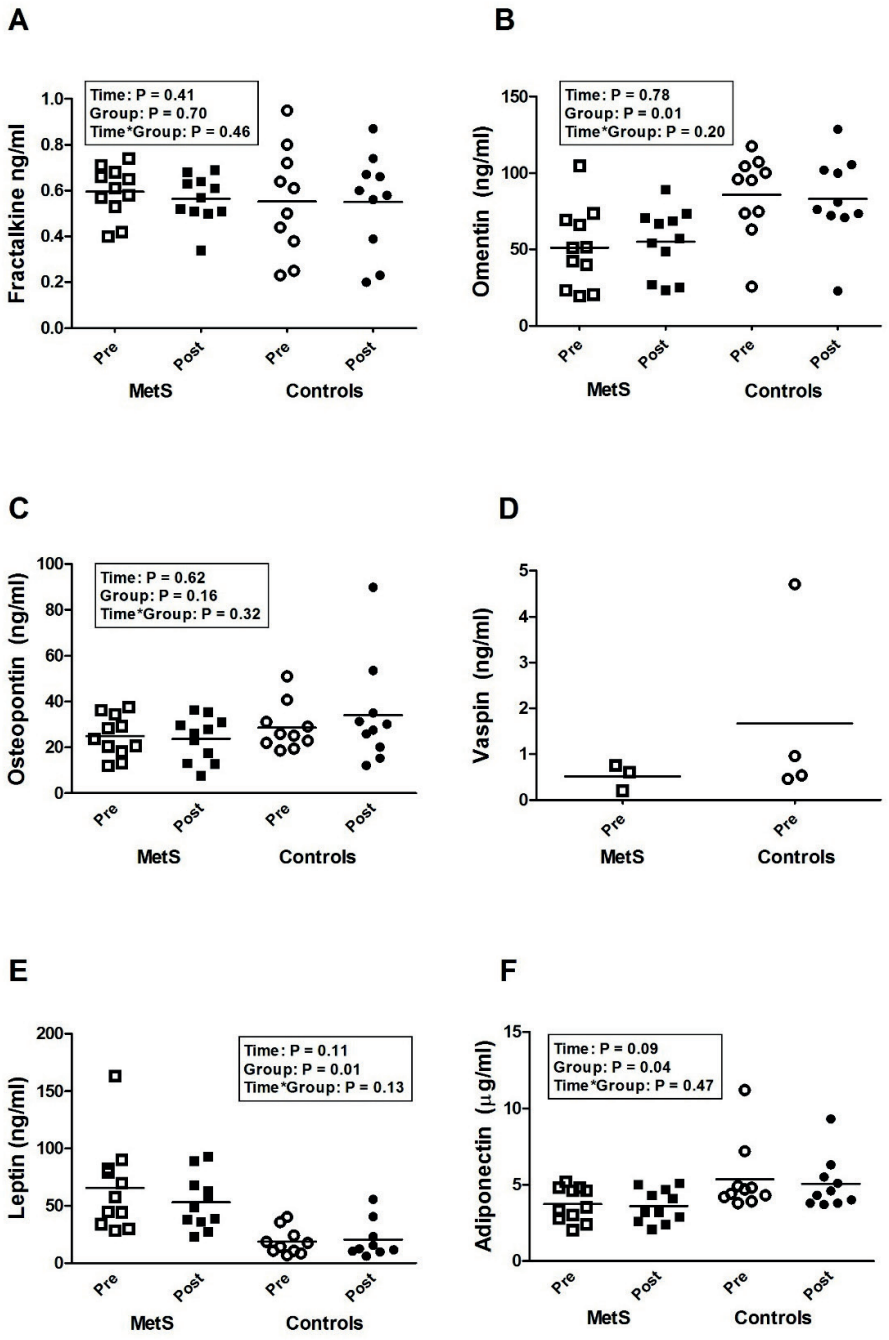

Figure 2. Circulating levels of cytokines before (□) and after (■) exercise training in women with the metabolic syndrome (MetS) and in lean control women (Controls) before (○) and after (●) training. A. Fractalkine; B. Omentin. C. Osteopontin. D. Vaspin (only baseline values shown). E. Leptin. F. Adiponectin. * P-value < 0.05.

DISCUSSION

Our results confirm that exercise training successfully improves insulin sensitivity, in both women with the metabolic syndrome and lean, sedentary controls. The improvement in insulin sensitivity after exercise training was not accompanied by changes in circulating levels of novel (i.e. fractalkine, omentin, osteopontin and vaspin) and traditional (i.e. leptin, adiponectin and IL-6) cytokines. Furthermore, gene expression levels of these cytokines in skeletal muscle did not change after exercise training. Taken together, these results suggest that cytokines are not associated with exercise-induced improvements in insulin sensitivity in women.

Baseline

In this study, we confirmed with use of the M-value as a gold standard technique, that insulin sensitivity in women with the MetS is significantly lower compared to age-matched lean controls. Furthermore, we found that lean controls show lower leptin levels, and higher omentin and adiponectin levels than women with MetS. Whilst previous research showed that omentin is lower in subjects with obesity and type 2 diabetes, this study is the first to show that omentin is also lower in women with MetS compared to controls.[11,25] Previous studies found that fractalkine, osteopontin and vaspin are associated with insulin resistance and obesity.[9,10,14] However, no baseline differences in circulating levels of cytokines were found between women with MetS and controls in our study. Furthermore, we found no correlation between insulin sensitivity and levels of these cytokines. One important difference with previous studies is that we used the gold standard to measure insulin sensitivity rather than the less invasive, but also less reliable oral glucose tolerance test (OGTT). Such measures for insulin sensitivity, that use fasting levels of glucose and insulin have clear limitations,[26] and should therefore be interpreted with caution to examine the relationship between cytokines and insulin sensitivity. With the use of M-value, our study tempers the results of previous work that suggested an association between serum levels of fractalkine, omentin, osteopontin, vaspin with insulin sensitivity.

Training

Six months of aerobic exercise training significantly improved VO_{2max} in both women with MetS and sedentary control women. In both groups, no changes in body mass and waist to hip ratio were observed. Furthermore, circulating levels of cytokines and their gene expression in skeletal muscle did not change after training. The effect of aerobic exercise training on leptin and adiponectin is not clear and conflicting results have been reported.[27,28] Some studies showed a decrease in

leptin levels after aerobic exercise training in obese women,[23,29,30] whilst others demonstrated no change.[29] Some studies have shown that adiponectin increases after exercise training in obese subjects,[31,32] but also an absence of a response has been reported.[29,33] In our study we found no change in leptin and adiponectin both in circulating serum levels and on gene expression level in skeletal muscle, despite the significant improvement in M-value. Furthermore, our correlation analysis was unable to demonstrate an association between changes in leptin and adiponectin and changes in M-value. Taken together, it is unlikely that leptin or adiponectin play a pivotal role in the mechanisms underlying a change in M-value by exercise training.

The response of omentin, fractalkine and osteopontin to exercise training in humans have scarcely been studied and evidence is inconclusive. Omentin has been studied most extensively in relation to exercise training of non-diabetic individuals and in combination with parameters of glucose homeostasis. Saremi et al. reported an increase in circulating omentin levels after 12 weeks of aerobic exercise (5x/week, 50-60 minutes), which was accompanied by a significant decrease in HOMA-IR and significant weight loss.[25] Another study reported no effect of 3 months (3x/week, 30 minutes) of exercise on circulating omentin levels women, whilst BMI and HOMA-IR decreased significantly.[25,34] One previous study by Catoire et al. examined the response of fractalkine to combined exercise training (2x/ week endurance exercise; 1x/week resistance exercise; 45 minutes each) of 12 weeks and found no change in RNA expression levels and circulating levels of fractalkine.[8] Our study confirms these findings after a 6 month endurance training intervention in women. Two previous studies investigated circulating osteopontin levels before and after an aerobic training intervention, without assessing measurements of insulin sensitivity and both found no change. [35] [36] In line with previous work our study demonstrates that omentin, osteopontin and fractalkine do not change after training. An important difference between studies that did find an effect on cytokines versus those who did not is the presence of weight loss. It has been suggested in previous reviews that considerable weight loss (of at least 5%) is needed to achieve a change in cytokine expression,[28,37] and thereby circulating levels. However, in exercise training studies the relation between weight loss and change in cytokines seems less clear. In some exercise studies significant weight loss is present, whilst no change in cytokines occurred.[25,34,35] Furthermore, endurance training does not necessarily result in weight loss. During endurance training adipose tissue depots may decrease, whilst lean body mass and/or circulating blood volume may increase.[38-40] These counteracting processes may result in the absence of weight loss during exercise training. Indeed, in our study no correlation was present between weight change and change in

circulating cytokines. Furthermore, we demonstrated significant and clinically relevant improvements in VO_{2max} and insulin sensitivity in the absence of weight loss and changes in plasma cytokine levels and skeletal muscle RNA expression. This suggests that exercise-induced improvements in glycemic control are not accompanied by a change in cytokines.

Another explanation for the differences between training studies on cytokine responses might be related to training frequency and intensity[41] Whilst we implemented a training frequency of three times per week, Saremi implemented a frequency of five training sessions per week and found a significant increase in omentin levels.[25] Urbanova et al. implemented a 3x/week protocol and found no change in omentin levels.[34] Several studies that investigated leptin and adiponectin applied similar training frequency and found conflicting cytokine responses.[23,30-32] Possibly, a higher training frequency may be needed to cause a change cytokines.

A more plausible explanation for the absence of changes in circulating cytokines and their expression in skeletal muscle in our study relates to a difference in time-dependent responses of cytokines and M-value to exercise training. Each training bout elicits acute changes in energy metabolism and homeostasis, and also in circulating cytokine levels and their expression. Some acute changes in cytokines and their expression will persist for hours, whilst others quickly disappear after cessation of exercise.[42,43] Potentially, the cytokine response to an acute bout of exercise can influence metabolic adaptations that contribute to a change in insulin sensitivity. However, with our study design acute changes in cytokines after exercise were not examined. In the discussed exercise training studies, blood (and tissue) samples were collected at 24 hours,[30,35] 48 hours[25,36] or 72 hours[29] after the last exercise session, whilst the majority of the authors did not report the timing of sampling. Considering the responses of cytokines to an acute exercise bout, this heterogeneity among study designs might be an explanation for differences found in the response of cytokines to training. Very little is known about the specific time course of expression levels and circulating levels of well-investigated and recently discovered cytokines after an exercise bout. This knowledge is needed to interpret each study on its merits.

Limitations. Microarray analysis was performed in a subgroup of nine women, which limited statistical power. Both circulating levels and mRNA expression levels of cytokines in skeletal muscle were analyzed. Since these subgroups were a good representation of the whole group, we do not expect larger numbers would have altered the main outcomes of our study. In this study pre-, peri- and post-

menopausal women were included, which provides a well representation of the entire female population in this age category. Although the menopause affects fat distribution, its effects on cytokines is less clear.[44,45] We therefore do not believe that the main outcome of this study is influenced by menopausal status. In this study we explored cytokine expression levels in skeletal muscle, whilst adiponectin, omentin and vaspin might not be expressed in this tissue. However, gene expression analysis allows us to examine the potential role of skeletal muscle as source for circulating cytokines. Since white adipose tissue is also an important source for circulating cytokines, future work should examine expression changes in this tissue.[28]

CONCLUSION

In conclusion, this study shows that exercise training successfully improves insulin sensitivity and physical fitness in both women with the metabolic syndrome and lean controls. The improvement in insulin sensitivity after exercise training was not accompanied by or correlated with changes in circulating levels or gene expression levels in skeletal muscle of novel (i.e. fractalkine, omentin, osteopontin and vaspin) and traditional (i.e. leptin, adiponectin and IL-6) cytokines. Our data may suggest that exercise-induced improvements in insulin sensitivity are not accompanied by a change in cytokines.

REFERENCES

1. Ford ES, Li C, Sattar N. Metabolic syndrome and incident diabetes: current state of the evidence. *Diabetes care*. 2008;31:1898-1904. doi: 10.2337/dc08-0423
2. Malik S, Wong ND, Franklin SS, Kamath TV, L'Italien GJ, Pio JR, Williams GR. Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation*. 2004;110:1245-1250. doi: 10.1161/01.cir.0000140677.20606.0e
3. Eckel RH, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet*. 2010;375:181-183. doi: 10.1016/s0140-6736(09)61794-3
4. Rockl KS, Witczak CA, Goodyear LJ. Signaling mechanisms in skeletal muscle: acute responses and chronic adaptations to exercise. *IUBMB life*. 2008;60:145-153. doi: 10.1002/iub.21
5. Henriksen EJ. Invited review: Effects of acute exercise and exercise training on insulin resistance. *Journal of applied physiology (Bethesda, Md : 1985)*. 2002;93:788-796. doi: 10.1152/japplphysiol.01219.2001
6. Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006;444:860-867. doi: 10.1038/nature05485
7. Stienstra R, van Diepen JA, Tack CJ, Zaki MH, van de Veerdonk FL, Perera D, Neale GA, Hooiveld GJ, Hijmans A, Vroegrijk I, et al. Inflammasome is a central player in the induction of obesity and insulin resistance. *Proc Natl Acad Sci U S A*. 2011;108:15324-15329. doi: 10.1073/pnas.1100255108
8. Catoire M, Mensink M, Kalkhoven E, Schrauwen P, Kersten S. Identification of human exercise-induced myokines using secretome analysis. *Physiological genomics*. 2014;46:256-267. doi: 10.1152/physiolgenomics.00174.2013
9. Shah R, Hinkle CC, Ferguson JF, Mehta NN, Li M, Qu L, Lu Y, Putt ME, Ahima RS, Reilly MP. Fractalkine is a novel human adipochemokine associated with type 2 diabetes. *Diabetes*. 2011;60:1512-1518. doi: 10.2337/db10-0956
10. Ebert T, Hindricks J, Kralisch S, Lossner U, Jessnitzer B, Richter J, Bluher M, Stumvoll M, Fasshauer M. Serum levels of fractalkine are associated with markers of insulin resistance in gestational diabetes. *Diabetic medicine : a journal of the British Diabetic Association*. 2014;31:1014-1017. doi: 10.1111/dme.12451
11. Tan BK, Adya R, Randeva HS. Omentin: a novel link between inflammation, diabesity, and cardiovascular disease. *Trends in cardiovascular medicine*. 2010;20:143-148. doi: 10.1016/j.tcm.2010.12.002
12. Uaesoontrachoon K, Yoo HJ, Tudor EM, Pike RN, Mackie EJ, Pagel CN. Osteopontin and skeletal muscle myoblasts: association with muscle regeneration and regulation of myoblast function in vitro. *The international journal of biochemistry & cell biology*. 2008;40:2303-2314. doi: 10.1016/j.biocel.2008.03.020
13. Nomiyama T, Perez-Tilve D, Ogawa D, Gizard F, Zhao Y, Heywood EB, Jones KL, Kawamori R, Cassis LA, Tschop MH, et al. Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. *The Journal of clinical investigation*. 2007;117:2877-2888. doi: 10.1172/jci31986
14. Bluher M. Vaspin in obesity and diabetes: pathophysiological and clinical significance. *Endocrine*. 2012;41:176-182. doi: 10.1007/s12020-011-9572-0
15. Wada J. Vaspin: a novel serpin with insulin-sensitizing effects. *Expert opinion on investigational drugs*. 2008;17:327-333. doi: 10.1517/13543784.17.3.327

16. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC, Jr., et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009;120:1640-1645. doi: 10.1161/circulationaha.109.192644

17. Harlow SD, Paramsothy P. Menstruation and the menopausal transition. *Obstetrics and gynecology clinics of North America*. 2011;38:595-607. doi: 10.1016/j.ogc.2011.05.010

18. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*. 1979;237:E214-223. doi: 10.1152/ajpendo.1979.237.3.E214

19. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics (Oxford, England)*. 2003;4:249-264. doi: 10.1093/biostatistics/4.2.249

20. Lin K, Kools H, de Groot PJ, Gavai AK, Basnet RK, Cheng F, Wu J, Wang X, Lommen A, Hooiveld GJ, et al. MADMAX - Management and analysis database for multiple ~omics experiments. *Journal of integrative bioinformatics*. 2011;8:160. doi: 10.2390/biecoll-jib-2011-160

21. Sartor MA, Tomlinson CR, Wesselkamper SC, Sivaganesan S, Leikauf GD, Medvedovic M. Intensity-based hierarchical Bayes method improves testing for differentially expressed genes in microarray experiments. *BMC bioinformatics*. 2006;7:538. doi: 10.1186/1471-2105-7-538

22. Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, Macera CA, Heath GW, Thompson PD, Bauman A. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Medicine and science in sports and exercise*. 2007;39:1423-1434. doi: 10.1249/mss.0b013e3180616b27

23. Kondo T, Kobayashi I, Murakami M. Effect of exercise on circulating adipokine levels in obese young women. *Endocrine journal*. 2006;53:189-195.

24. Straub RH, Hense HW, Andus T, Scholmerich J, Riegger GA, Schunkert H. Hormone replacement therapy and interrelation between serum interleukin-6 and body mass index in postmenopausal women: a population-based study. *The Journal of clinical endocrinology and metabolism*. 2000;85:1340-1344. doi: 10.1210/jcem.85.3.6355

25. Saremi A, Asghari M, Ghorbani A. Effects of aerobic training on serum omentin-1 and cardiometabolic risk factors in overweight and obese men. *Journal of sports sciences*. 2010;28:993-998. doi: 10.1080/02640414.2010.484070

26. Pisprasert V, Ingram KH, Lopez-Davila MF, Munoz AJ, Garvey WT. Limitations in the use of indices using glucose and insulin levels to predict insulin sensitivity: impact of race and gender and superiority of the indices derived from oral glucose tolerance test in African Americans. *Diabetes care*. 2013;36:845-853. doi: 10.2337/dc12-0840

27. Bouassida A, Zalleg D, Bouassida S, Zaouali M, Feki Y, Zbidi A, Tabka Z. Leptin, its implication in physical exercise and training: a short review. *Journal of sports science & medicine*. 2006;5:172-181.

28. Thompson D, Karpe F, Lafontan M, Frayn K. Physical activity and exercise in the regulation of human adipose tissue physiology. *Physiological reviews*. 2012;92:157-191. doi: 10.1152/physrev.00012.2011

29. Polak J, Klimcakova E, Moro C, Viguerie N, Berlan M, Hejnova J, Richterova B, Kraus I, Langin D, Stich V. Effect of aerobic training on plasma levels and subcutaneous abdominal adipose tissue gene expression of adiponectin, leptin, interleukin 6, and tumor necrosis factor alpha in obese women. *Metabolism: clinical and experimental*. 2006;55:1375-1381. doi: 10.1016/j.metabol.2006.06.008

30. Friedenreich CM, Neilson HK, Woolcott CG, McTiernan A, Wang Q, Ballard-Barbash R, Jones CA, Stanczyk FZ, Brant RF, Yasui Y, et al. Changes in insulin resistance indicators, IGFs, and adipokines in a year-long trial of aerobic exercise in postmenopausal women. *Endocrine-related cancer*. 2011;18:357-369. doi: 10.1530/erc-10-0303

31. Bluher M, Bullen JW, Jr., Lee JH, Kralisch S, Fasshauer M, Kloting N, Niebauer J, Schon MR, Williams CJ, Mantzoros CS. Circulating adiponectin and expression of adiponectin receptors in human skeletal muscle: associations with metabolic parameters and insulin resistance and regulation by physical training. *The Journal of clinical endocrinology and metabolism*. 2006;91:2310-2316. doi: 10.1210/jc.2005-2556

32. Christiansen T, Paulsen SK, Bruun JM, Pedersen SB, Richelsen B. Exercise training versus diet-induced weight-loss on metabolic risk factors and inflammatory markers in obese subjects: a 12-week randomized intervention study. *American journal of physiology Endocrinology and metabolism*. 2010;298:E824-831. doi: 10.1152/ajpendo.00574.2009

33. Hayashino Y, Jackson JL, Hirata T, Fukumori N, Nakamura F, Fukuhara S, Tsujii S, Ishii H. Effects of exercise on C-reactive protein, inflammatory cytokine and adipokine in patients with type 2 diabetes: a meta-analysis of randomized controlled trials. *Metabolism: clinical and experimental*. 2014;63:431-440. doi: 10.1016/j.metabol.2013.08.018

34. Urbanova M, Dostalova I, Trachta P, Drapalova J, Kavalkova P, Haluzikova D, Matoulek M, Lacinova Z, Mraz M, Kasalicky M, et al. Serum concentrations and subcutaneous adipose tissue mRNA expression of omentin in morbid obesity and type 2 diabetes mellitus: the effect of very-low-calorie diet, physical activity and laparoscopic sleeve gastrectomy. *Physiological research / Academia Scientiarum Bohemoslovaca*. 2014;63:207-218.

35. Duggan C, Xiao L, Wang CY, McTiernan A. Effect of a 12-month exercise intervention on serum biomarkers of angiogenesis in postmenopausal women: a randomized controlled trial. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2014;23:648-657. doi: 10.1158/1055-9965.epi-13-1155

36. Venojarvi M, Korkmaz A, Wasenius N, Manderoos S, Heinonen OJ, Lindholm H, Aunola S, Eriksson JG, Atalay M. 12 weeks' aerobic and resistance training without dietary intervention did not influence oxidative stress but aerobic training decreased atherogenic index in middle-aged men with impaired glucose regulation. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*. 2013;61:127-135. doi: 10.1016/j.fct.2013.04.015

37. Klimcakova E, Kovacikova M, Stich V, Langin D. Adipokines and dietary interventions in human obesity. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2010;11:446-456. doi: 10.1111/j.1467-789X.2009.00704.x

38. Convertino VA. Blood volume: its adaptation to endurance training. *Med Sci Sports Exerc*. 1991;23:1338-1348.

39. Janssen I, Fortier A, Hudson R, Ross R. Effects of an energy-restrictive diet with or without exercise on abdominal fat, intermuscular fat, and metabolic risk factors in obese women. *Diabetes Care*. 2002;25:431-438.

40. Ross R, Rissanen J, Pedwell H, Clifford J, Shragge P. Influence of diet and exercise on skeletal muscle and visceral adipose tissue in men. *Journal of applied physiology (Bethesda, Md : 1985)*. 1996;81:2445-2455.

41. Umpierre D, Ribeiro PA, Schaan BD, Ribeiro JP. Volume of supervised exercise training impacts glycaemic control in patients with type 2 diabetes: a systematic review with meta-regression analysis. *Diabetologia*. 2013;56:242-251. doi: 10.1007/s00125-012-2774-z

42. Egan B, O'Connor PL, Zierath JR, O'Gorman DJ. Time course analysis reveals gene-specific transcript and protein kinetics of adaptation to short-term aerobic exercise training in human skeletal muscle. *PloS one*. 2013;8:e74098. doi: 10.1371/journal.pone.0074098

43. Mahoney DJ, Parise G, Melov S, Safdar A, Tarnopolsky MA. Analysis of global mRNA expression in human skeletal muscle during recovery from endurance exercise. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2005;19:1498-1500. doi: 10.1096/fj.04-3149fje

44. McKane WR, Khosla S, Peterson JM, Egan K, Riggs BL. Circulating levels of cytokines that modulate bone resorption: effects of age and menopause in women. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 1994;9:1313-1318. doi: 10.1002/jbmr.5650090821

45. Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. *Endocrine reviews*. 2002;23:90-119. doi: 10.1210/edrv.23.1.0456

Chapter 5

Impact of 8-week aerobic exercise training on white adipose tissue gene expression in obese men and women

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Submitted

ABSTRACT

Background. Exercise training is known to improve insulin sensitivity in obese individuals. White adipose tissue (WAT) plays a central role in glucose homeostasis. Few studies examined whether the beneficial effects of regular exercise training on insulin resistance in obesity are associated with changes in molecular pathways in WAT.

Objective. To examine the impact of 8-week aerobic exercise training on WAT gene expression and compare these effects between obese men and women.

Methods. Twenty subjects, 9 men and 11 women, performed an 8-week aerobic exercise intervention. Before and after training, we examined insulin sensitivity (hyperinsulemic euglycemic clamp), cardiorespiratory fitness (peak oxygen uptake), and visceral adiposity (DXA-scan). WAT whole genome gene expression levels were examined using microarray analysis based on subcutaneous WAT biopsies.

Results. 8-week exercise training improved insulin sensitivity, cardiorespiratory fitness and visceral adiposity (all $P<0.05$). Exercise training induced significant changes in 1,475 out of 12,306 genes expressed in WAT. Fourteen pathways were significantly upregulated, mostly related to RNA metabolism (n=3) and extracellular matrix remodeling (n=3). At individual transcript level, 20-25% overlap in genes between sexes was found. Of these n=176 overlapping genes, 104 encode for small nucleolar (sno) and small Cajal-body specific (sca)RNAs.

Conclusion. 8-week exercise training results in a significant enrichment of sno- and scaRNAs in WAT that show a remarkable overlap between obese men and women. This suggests a potential role for these molecules in metabolic adaptations in WAT to exercise training that has not yet been described in response to exercise training.

INTRODUCTION

Obesity is a major independent risk factor for the development of type 2 diabetes mellitus.[1,2] Central in the etiology of type 2 diabetes is the progressive development of impaired regulation of blood glucose levels through insulin resistance.[3] An important, reversible factor contributing to the development of insulin resistance in obese individuals relates to physical inactivity.[4] Indeed, a large number of studies have demonstrated that exercise training represents an excellent non-pharmacological tool to decrease insulin resistance[5] and is found to decrease morbidity and all-cause mortality in obese individuals.[6] These effects of exercise training cannot be simply explained through its effects on reducing body weight. Even in absence of weight loss, exercise training is able to reduce insulin resistance and thereby prevents the development of type 2 diabetes.[7,8] This relates to the effects of exercise training on skeletal muscle. Indeed, a large number of studies examined adaptations in skeletal muscle that contribute to exercise induced improvements in insulin sensitivity.[9] The effects of exercise training on molecular processes in adipose tissue have been investigated to a lesser extent.

White adipose tissue (WAT) is increasingly recognized to play a central role in the etiology of insulin resistance through its function as energy storage organ and as endocrine organ reflected by its role in maintaining whole body glucose homeostasis.[10] Storage of the excess energy in obesity leads to accumulation of WAT, subsequently causing hypertrophy of adipocytes. As a direct result of adipocyte hypertrophy, local hypoxia develops and, eventually, necrosis of WAT.[11] These processes induce a pro-inflammatory reaction, creating a chronic inflammatory state that accelerates the development of insulin resistance.[12] Indeed, studies have linked WAT inflammation processes to the development of insulin resistance.[11,12] This suggests that beneficial effects of regular exercise training on insulin resistance in obesity might be explained through activating molecular pathways in WAT.

The prevalence of type 2 diabetes mellitus differs between sexes: more men than women suffer from diabetes worldwide and men tend to be diagnosed at a younger age than women.[13] These epidemiological differences between men and women are not merely caused by differences in sex hormones, but also relate to fat distribution and energy homeostasis[13]. Interestingly, sex differences seem also present in metabolic adaptations to exercise training [14]. Since sex differences are present in relation to gene expression profiles in WAT [15], exercise training may also cause sex-related changes in WAT gene expression levels. The sex-specific differences in energy metabolism, WAT function and glucose homeostasis highlight

the relevance of exploring sex differences in response to exercise training with gene expression analyses in WAT. Therefore, the aim of this study is to examine the impact of 8-week aerobic exercise training on gene expression levels in WAT. The secondary, explorative aim is to investigate sex differences in exercise-induced gene expression changes in WAT.

MATERIALS AND METHODS

Subjects

Twenty inactive subjects with obesity (BMI > 30kg/m^2) were included in this study. Baseline physical activity levels were assessed with the use of the Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH), a validated tool to assess physical activity levels in the Dutch population. Subjects were eligible for inclusion when their SQUASH score was 6400 or lower.[16] Exclusion criteria included inflammatory bowel disease and usage of pro- or antibiotics since these conditions are associated with an altered gut microbiota [17,18], as well as having a medical history of diabetes mellitus. Written informed consent was obtained from all participants prior to the start of the study. This study was approved by the Medical Ethical Committee of the Radboud University Nijmegen Medical Center, and was conducted in accordance with the declaration of Helsinki. This study was registered as NTR5737 in the Dutch Clinical Trial Register.

Study design

All subjects who participated in this study were engaged in an 8-week supervised aerobic exercise training intervention. Subjects were instructed not to change dietary habits during the participation in this study. Before and after the intervention, a subcutaneous adipose tissue biopsy, a hyperinsulinemic, euglycemic clamp, a maximal cycling test and a DXA-scan as gold standard techniques to examine insulin sensitivity, physical fitness level and body composition, respectively, were performed.

Exercise training. During this training study, all subjects trained 2-4 times a week under the supervision of an experienced researcher. Training consisted of cycling exercise on an ergometer (Lode, Groningen, the Netherlands) starting with a 5 minute warming-up, followed by 50 minutes of exercise at 65%-85% of the individual heart rate reserve (HRR) and ending with a cooling-down of 5 minutes. Training frequency and percentage of HRR were gradually increased during the first two weeks of the intervention period. The HRR was calculated based on individual

maximal heart rate assessed during the maximal cycling test. Exercise intensity was continuously monitored and documented with the use of heart rate monitors (Polar®) and workload was adjusted accordingly on an individual basis. Subjects had to attend at least 95% of the training sessions during the 8 week intervention period to be eligible for inclusion in the statistical analysis.

Measurements

Anthropometry. At baseline and after the 8 weeks intervention (except for height), height and body weight (Seca 888 scale, Hamburg, Germany) were measured to calculate body mass index (BMI). Waist and abdominal circumference were measured with a measuring tape (Seca 201, Chino, USA) to calculate waist-to-hip ratio. Resting heart rate and blood pressure were measured in supine position, after a 5 minute rest period. Before and after the training period a total body Dual-X-ray Absorptiometry (DXA) scan was performed to determine lean body mass and total fat (QDR 4500 densitometer, Hologic Inc. Waltham, MA). Visceral adipose tissue (VAT) mass, VAT volume and VAT area were calculated with standardized Hologic Software with results that correlate excellent with gold standard techniques for the measurement of VAT.[19]

Gene expression levels

After an overnight fast, subcutaneous adipose tissue biopsies were obtained under local anesthesia by needle biopsies performed 6-10cm lateral to the umbilicus in the right lower quadrant.

Microarray processing

Total RNA was extracted from frozen adipose tissue specimens using TRIzol reagent (Invitrogen, Breda, The Netherlands) and purified on columns using the Qiagen RNeasy Micro Kit (Qiagen, Venlo, The Netherlands). Total RNA (100 ng per sample) was labeled by Whole-Transcript Sense Target Assay and hybridized to human whole-genome Affymetrix Gene 1.1 ST arrays targeting 19 793 unique genes (Affymetrix, Santa Clara, CA, USA).

Microarray data analysis

Quality control and data analysis have been described in detail previously.[20] Individual genes were defined as changed when comparison of the normalized signal intensities showed a P-value < 0.05 in a two-tailed paired intensity-based moderated t-statistics.[21] These analyses were performed within MADMAX system.[22] Further functional data analysis was performed on the filtered data set with MetaCore Pathway Analysis (MetaCore, xxxx, USA) and Reactome analysis based

on Benjamini-Hochberg false-discovery rate-adjusted p-values of pre- versus post-intervention using paired Students' t-test per gene. Array data have been submitted to the Gene Expression Omnibus.

Insulin sensitivity. Peripheral tissue sensitivity to exogenous insulin was measured using a hyperinsulinemic euglycemic clamp as previously described.[23] After an overnight fast (10 hours), the subject was placed in the supine position in a quiet, temperature controlled (22 – 24 ºC) room. Insulin (Novorapid, Novo-Nordisk, Copenhagen, Denmark) was infused intravenously in a dose of 430 $pmol \cdot m^{-2} \cdot min^{-1}$ ($60\ mU \cdot m^{-2} \cdot min^{-1}$) for 120 minutes. Insulin $50\ U \cdot ml^{-1}$ was diluted in 47.5 ml NaCl 0.9% with the addition of 2 ml blood from the subject to a concentration of $1\ U \cdot ml^{-1}$. Venous plasma glucose concentrations were clamped at 5.0 $mmol \cdot L^{-1}$ by a variable glucose 20% infusion rate, adjusted depending on venous plasma glucose level measured at 5-minute intervals. Serum glucose levels were determined using a Biosen C-Line Glucose and Lactate Analyser (Biosen C-line GP+, EKF-diagnostic GmbH, Barleben, Germany).Fullerton, CA 92634, USA). Whole body glucose disposal was calculated as the mean glucose infusion rate per kilogram body weight ($mg \cdot kg^{-1} \cdot min^{-1}$) during the last 30 minutes of the clamp (M-Value).

Dietary intake. During the training intervention, subjects were instructed not to change their dietary habits. To assess potential changes in daily food intake, subjects were asked to record dietary intake records before and in the last week of the training intervention in a detailed food journal. Subjects were individually instructed how to record food items and were provided with example diaries. Dietary records of the 24 hours prior to stool collection were analyzed with Eetmeter Software (Voedingscentrum, the Hague, Netherlands), based on the Dutch Food Composition Database of 2016.[24] Furthermore, an online validated 180–item semi–quantitative Food Frequency Questionnaire (FFQ) was used to assess habitual daily energy intake, and macronutrient intake.[25,26] The FFQ reference period was one month, and portion sizes were estimated using standard portions.[27] Intake of total energy and nutrients was calculated using the Dutch Food Composition Database. [24]

Cardio-respiratory fitness level. Subjects performed a maximal exercise test on an electrically braked leg-cycling ergometer (Lode Excalibur, Groningen, the Netherlands) using an incremental protocol, to assess their cardio-respiratory fitness level. Workload increased by 10-30 W per minute, starting at 0 W, until exhaustion. A calibrated gas-analyzer was used to measure oxygen consumption continuously (COSMED Pulmonary Function Equipment, Chicago, US). During the

test, an electrocardiogram (ECG) was continuously recorded and checked by a physician. The maximal exercise test was terminated by adhering to the guidelines of the American Heart Association.[28] Maximal oxygen consumption (VO_{2max}) was defined as the highest oxygen uptake (30 second average).

Statistical analysis

All statistical analyses were conducted in SPSS 22 (Statistical Package for Social Sciences 22.0, SPSS Inc., Chicago, Illinois, USA). Data was checked for normality with use of the Shapiro-Wilk test. Subject characteristics were normally distributed and therefore assessed with use of a paired Students' t-test to examine the impact of exercise training. The level of statistical significance was defined at α=0.05. Data are presented as mean±SD, unless stated otherwise. For microarray data analysis: see above.

RESULTS

Subject characteristics before and after training are presented in Table 1. Twenty subjects (n=9 men, n=11 women) completed the exercise intervention with adherence to the training sessions of 97%. Assessment of dietary intake with the use of food journals showed no significant change in daily energy intake (Pre: 2028±622 kcal/day; post: 1906±390 kcal/day; p=0.18), nor in macronutrient composition before and at the end of the intervention period. Training was associated with significant increases in cardio-respiratory fitness levels and insulin sensitivity (M-value), and caused a significant decrease in measures of (visceral) adiposity (Table 1). When examining men and women separately, no significant differences in the changes in cardio-respiratory fitness levels, insulin sensitivity (M-value) and measures of adiposity were found between both sexes.

Impact of exercise training on WAT gene expression and pathways

Exercise training induced significant changes in 1,475 out of 12,306 expressed transcripts in WAT, with an average fold change between -1.56 and 3.56. In men, 980 genes were significantly altered and in women 874 genes showed a significant change. Pathway analysis showed that 14 distinct pathways were significantly affected, with most pathways being (in)directly related to RNA metabolism (3 out of 14) and extracellular matrix remodeling (3 out of 14) (Figure 1). When analyzed separately for men and women, we found 12 pathways being significantly altered after exercise training in men, whilst only 2 pathways were altered in women. Strikingly, we found no overlap in pathways between men and women.

Table 1. Physiological characteristics before and after the exercise intervention of the n=20 subjects and subgroups (n=9 men and n=11 women).

	Entire group (n=20)		***P-value***
	Pre	***Post***	
Age (years)	*48±11*	*-*	*-*
Body composition			
Weight (kg)	*106.4±15.8*	*104.3±16.3*	*0.03*
Body mass index (kg/m²)	*35.6±4.6*	*34.9±4.9*	*0.03*
Waist-to-hip ratio	*0.98±0.11*	*0.96±0.07*	*0.36*
VAT mass (g)	*865±293*	*827±297*	*0.04*
VAT volume (cm^3)	*935±2167*	*879±334*	*0.02*
Insulin sensitivity			
M-value (mg/min/kg)	*3.6±1.7*	*4.4±1.7*	*0.007*
Blood pressure			
Systolic blood pressure (mmHg)	*132±16*	*131±14*	*0.6*
Diastolic blood pressure (mmHg)	*87±11*	*80±9*	*0.003*
Resting heart rate (bpm)	*68±10*	*69±12*	*0.5*
Physical fitness			
VO_2max (ml/min/kg)	*27.1±5.0*	*30.4±7.1*	*<0.0001*
VO_2max (ml/min/kg FFM)	*46.4±6.4*	*50.6±8.1*	*0.001*
Power (Watt)	*200±46*	*245±56*	*<0.001*
Daily dietary composition			
Energy intake (kcal)	*2028±622*	*1905±390*	*0.18*
Carbohydrates (g)	*211±39*	*210±58*	*0.94*
Fat (g)	*75±42*	*66±22*	*0.28*
Unsaturated fat (g)	*28±18*	*26±9*	*0.74*
Protein (g)	*98±26*	*93±18*	*0.52*

Subgroup men *(n=9)*		Subgroup women (n=11)	
Pre	***Post***	***Pre***	***Post***
51±13		46±11	
110.2±17.0	108.7±16.6	103.3±14.7	100.7±15.8
33.7±3.8	33.3±3.8	37.1±4.8	36.2±5.5
1.05±0.1	1.02±0.04	0.9±0.08	0.9±0.07
1026±321	981±326	733±195	701±209
1109±347	1060±352	792±210	730±243
3.3±2.0	4.2±1.7	3.7±1.6	4.4±1.4
139±16	137±16	126±14	126±12
88±11	85±6	87±10	80±8
65±10	67±10	67±10	69±11
29.8±5.7	34.8±7.2	25.1±5.6	27.2±5.1
44.4±6.3	51.0 ±8.8	46.4±6.7	50.6 ±8.5
232±45	286±45	174±26	210±39
2045±366	1936±264	2004±761	1863±548
213±38	199±45	209±35	226±65
73±22	69±16	79±50	61±26
26±9	27±7	31±21	26±12
103±25	96±18	92±21	90±24

Pathway identifier	Pathway name
R-HSA-6785470	tRNA processing in the mitochondrion
R-HSA-1442490	Collagen degradation
R-HSA-8957275	Post-translational protein phosphorylation
R-HSA-8948216	Collagen chain trimerization
R-HSA-5083635	Defective B3GALTL causes Peters-plus syndrome (PpS)
R-HSA-8868766	rRNA processing in the mitochondrion
R-HSA-5173214	O-glycosylation of TSR domain-containing proteins
R-HSA-6798695	Neutrophil degranulation
R-HSA-5576894	Phase 1 - inactivation of fast Na+ channels
R-HSA-202670	ERKs are inactivated
R-HSA-5339717	Misspliced LRP5 mutants have enhanced beta-catenin-dependent signaling
R-HSA-212300	PRC2 methylates histones and DNA
R-HSA-73728	RNA Polymerase I Promoter Opening
R-HSA-1474228	Degradation of the extracellular matrix

Figure 1 Pathway Analysis: significantly altered pathways in WAT after 8 weeks of aerobic exercise in n=20 obese subjects.

Impact of exercise training on WAT gene expression: overlap between men and women

As no overlap at pathway level was observed, we next examined exercise training induced gene expression changes at individual transcript level. Of these individuals genes, 176 genes showed overlap between men and women (Figure 2A), with 170 of these 176 showing remarkably similar regulation patterns (up-up or down-down). Out of the 170 genes, 117 genes demonstrated an upregulation, of which 96 encode for small nucleolar RNAs (snoRNA; n=88) and small Cajal body-specific RNAs (scaRNA; n=8) (Figure 2B). Out of the 264 transcripts that relate to "small nucleolar" or "small Cajal" in our dataset, 171 (63%) are significantly altered after exercise training in our cohort. Of the significantly ($p<0.01$) altered transcripts, all are upregulated in both men and women (Figure 3A) and show large overlap between sexes (Figure 3B).

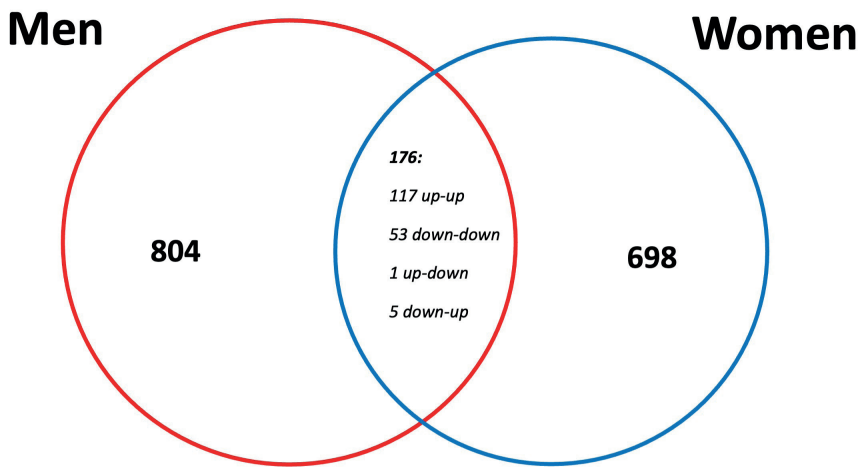

Figure 2A. Analysis on single transcript level of WAT after exercise training shows n=176 (20-25%) overlapping differentially expressed genes after exercise training between men and women.

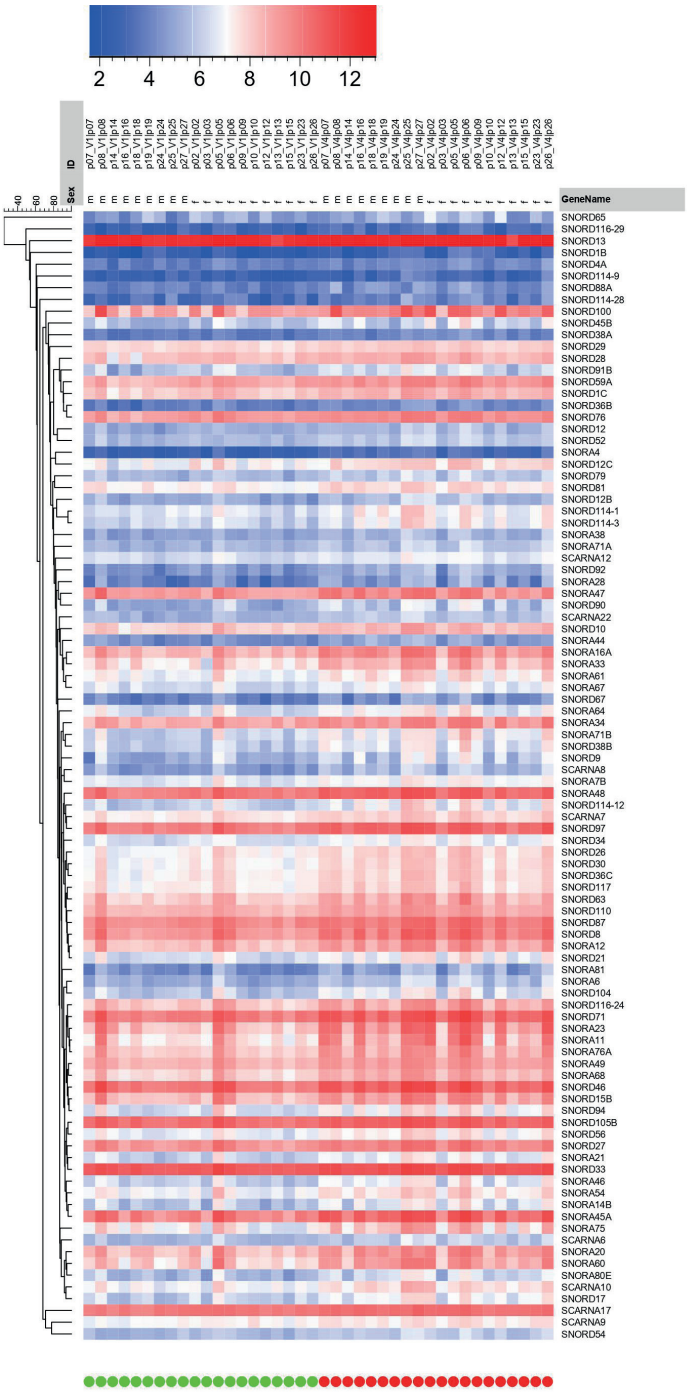

Figure 2B. Heatmap with cluster analysis of differentially expressed snoRNAs and scaRNAs before and after exercise training in obese men and women.

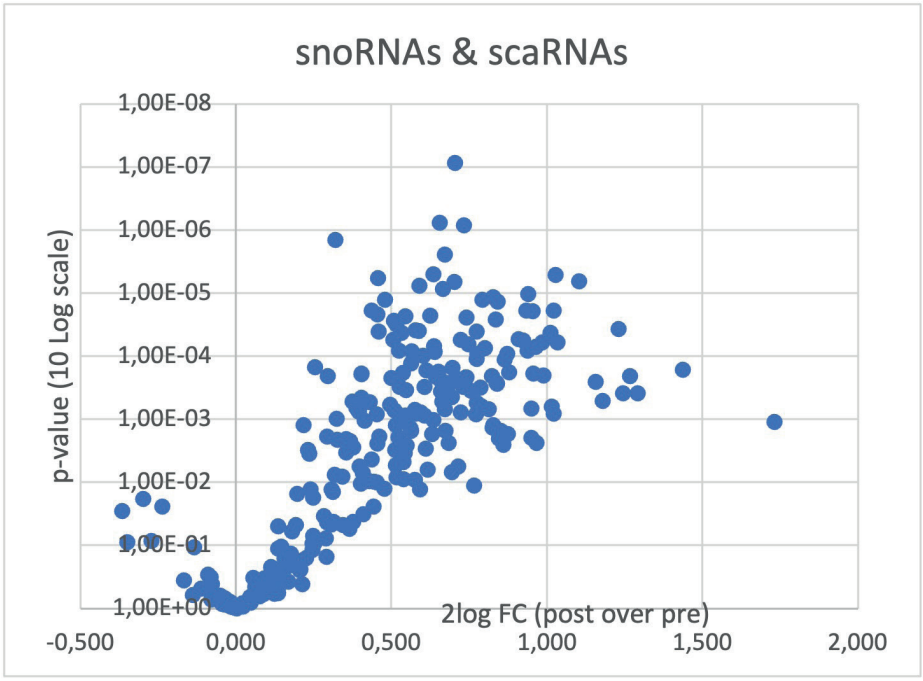

Figure 3A. Volcano plot of all (n=272) transcripts with "small nucleolar" or "small Cajal" in their Gene Ontology (GO) description. Plotted -10log p-value (y-axis) vs signal log ratio (SLR, x-axis). For p<0.01: all transcripts are upregulated.

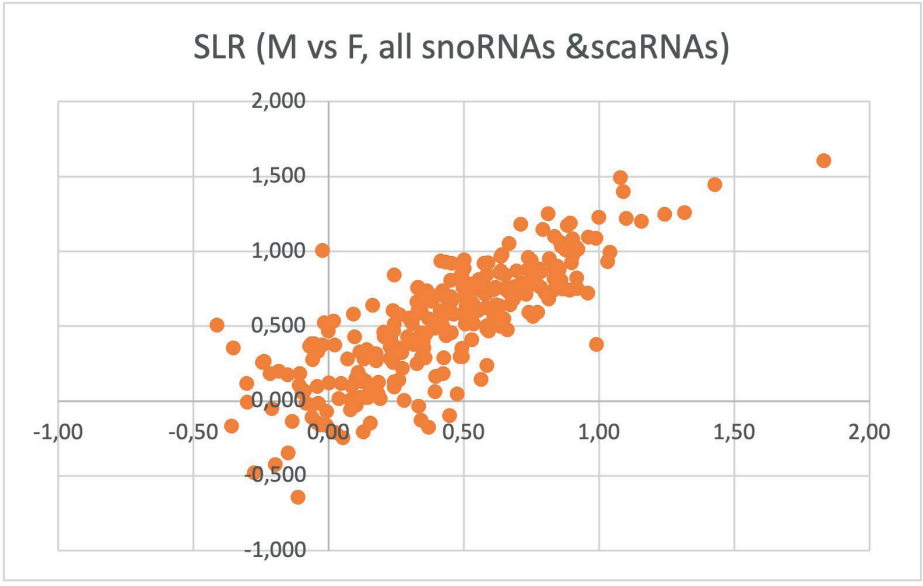

Figure 3B. Plot of all (n=272) transcripts with "small nucleolar" or "small Cajal" in description: men (y-axis) vs. women (x-axis). Data shown as SLR (2Log(fold change): all significantly upregulated genes are up-regulated in both men and women.

DISCUSSION

In this study, the effects of an 8-week exercise intervention on gene expression levels in white adipose tissue in both men and women were examined and compared. First, reinforcing previous work and proving the successfulness of this training intervention, we found that 8-week exercise training resulted in improvements in cardiorespiratory fitness and insulin sensitivity, and in a loss of visceral adipose tissue and modest bodyweight. Secondly, we found that exercise-training resulted in changes in ~12% of the genes evaluated in WAT, an effect that was found in both men and women. Despite a comparable number of genes altered in men and women, pathway analysis revealed distinct pathway activation between men and women. At an individual transcript level, 20-25% overlap was found between men and women in differentially expressed genes that were altered upon exercise training, mainly caused by a significant upregulation of small nucleolar RNAs (snoRNAs) and small Cajal body-specific RNAs (scaRNAs). In total, 61% of the total amount of genes encoding for snoRNAs and scaRNAs was upregulated in WAT after exercise training in obese subjects, resulting in a remarkable enrichment of these small non-coding RNAs. This suggests a potential role for these non-coding RNAs in adaptation of WAT in response to exercise training, which may subsequently have contributed to the benefits of exercise on insulin sensitivity.

In accordance with previous work, a significant improvement in insulin sensitivity, cardiorespiratory fitness and (visceral) adipose tissue after exercise training was observed in this study. This proves that, even with modest weight loss, exercise training is successful in improving metabolic health. When examining adaptations in WAT in response to exercise training, we found a relatively small number of pathways (n=14) being significantly altered, with most pathways being related to RNA metabolism (n=3) and extracellular matrix remodeling (n=3). The extracellular matrix (ECM) in adipose tissue plays a significant role in the pathogenesis of metabolic complications in obesity. In obese humans, collagen deposits cause fibrosis of WAT and contribute to focal necrosis which in turn causes chronic inflammation and insulin resistance.[29] Furthermore, in our study two pathways were significantly upregulated ("Collagen degradation" and "Degradation of the extracellular matrix"), which both contribute to the breakdown of collagen deposits. This is in accordance with previous work, that also demonstrated exercise-induced changes in extracellular matrix remodeling in WAT.[30] The upregulation of pathways involved in extracellular matrix remodeling suggests that exercise training contributes to a more 'healthy' ECM, which potentially could contribute – at least in part – to the beneficial effects of exercise on insulin sensitivity in obese humans.

The modest change in pathways we observed in our study might relate to the modest weight loss that occurred (i.e. an average of 1.6% of body weight). Previous work suggested that greater weight loss causes larger gene expression in WAT after a lifestyle intervention.[31,32] Another potential explanation for the relatively modest change in pathways might relate to depot specific changes in adipose tissue that occur after exercise training. Indeed, it was found that after exercise training, gluteal adipose tissue depots show different changes in gene expression levels than abdominal subcutaneous WAT.[33] In addition to the potential differences in qualitative changes between different adipose tissue, exercise training also seems to favorably affect a larger drop in visceral adipose tissue (VAT) compared to subcutaneous WAT.[34] Therefore, exercise might induce site-specific changes in adipose tissue, with relatively modest changes in subcutaneous WAT. Taken together, 8 weeks of exercise training caused a significant improvement in metabolic health outcomes accompanied by a modest alteration in pathways in subcutaneous abdominal WAT.

On individual transcript level, exercise training resulted in a significant change of 12% of total genes. A remarkably large overlap in differentially expressed genes between men and women was found in our study: 176 identical genes were similarly regulated in both groups (20-25%). This is in contrast to previous work, suggesting a sex-specific response to exercise in different tissues with marginal overlap in up- or downregulation in genes following exercise training between men and women.[35,36] The overlap between men and women we found was largely explained by specific changes in expression of snoRNAs and scaRNAs (89% of the total differentially upregulated genes). These individual transcripts comprise 61% of the total number of transcripts that relate to "small nucleolar" or "small Cajal" in our dataset, demonstrating a significant enrichment of these type of molecules. This observation fits with our pathway analysis, since 3 pathways specifically related to RNA metabolism were upregulated. In these pathways, nor in others in the Reactome library we used for the pathway analysis, snoRNAs and scaRNAs are included. This observation suggests a potential role for these small non-coding RNAs. However, relatively little is known about these snoRNAs and scRNAs.

Over the last decade, studies have revealed a role for non-coding RNAs in regulating gene expression at the (post-)transcriptional level. Small nucleolar RNAs (snoRNAs) play a role as housekeeping molecules for ribosomal maturation and protein translation and are located in the nucleolus.[37,38] The physiological role of these small non-coding RNAs in WAT, especially in response to exercise training remains largely unknown and evidence of their relationship to exercise is very

limited. In subcutaneous WAT of humans, a total number of 173 different snoRNAs has been found in a previous study, of which some were linked to an obese phenotype.[38] In relation to exercise, one previous study found upregulation of one snoRNA (SNORD114.1) after an exercise bout in elite athletes[39] whilst another study demonstrated a marked upregulation of snoRNAs and scaRNAs in circulating white blood cells following strenuous exercise in athletes.[40] Whilst exercise intensity may importantly contribute to the difference between these previous studies, it is important that both only examined the impact of a single bout of exercise. Finally, small Cajal body specific RNAs (scaRNAs) also play a role in posttranscriptional modifications of rRNA. They display an overlap with SnoRNAs, both in localization and in function.[41] However, we were unable to find a single study mentioning scaRNA in relationship to adipose tissue in humans in the regular scientific databases. These observations highlight the uniqueness of our study, as we are the first to describe a significant enrichment of small RNAs in WAT in response to exercise training, suggesting a potential role for these small non-coding RNAs in exercise induced adaptations in WAT.

Limitations. Our study has a number of limitations that must be kept into account. First of all, we did not perform quantitative real-time PCR (qPCR) in adipose tissue to validate the microarray data. Secondly, since snoRNAs can be detected in circulation and show high stability,[42] a further validation step would be to measure scoRNAs in serum samples before and after the exercise intervention, to confirm our hypothesis that WAT is capable of secreting snoRNAs. Finally, our data – although robust – was obtained in relatively small subgroups of men and women.

In conclusion, 8 weeks of aerobic exercise training results in significant improvements in cardiorespiratory fitness and insulin sensitivity, which is accompanied by upregulation of individual genes in WAT. Interestingly, a relatively large overlap was present between men and women in differentially upregulated individual genes in WAT, which mostly belong to the classes of small nucleolar RNA and small Cajal body specific RNA. This is the first study that reports a significant enrichment of snoRNAs and scaRNAs in WAT in response to exercise training in humans. Our data suggest that these small non-coding RNAs play a role in the molecular response of adipose tissue to exercise training, by alternating post-transcriptional processes. Therefore, this study provides novel insight into a potential role of specific changes in WAT-related gene expression levels that ultimately may contribute, at least partly, to the impact of exercise training on improvements in metabolic function in subjects with obesity.

REFERENCES

1. Kopelman P. Health risks associated with overweight and obesity. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2007;8 Suppl 1:13-17. doi: 10.1111/j.1467-789X.2007.00311.x
2. NIH. Health risks of being overweight. 2017.
3. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *The Lancet*. 2005;365:1415-1428. doi: https://doi.org/10.1016/S0140-6736(05)66378-7
4. Eckardt K, Taube A, Eckel J. Obesity-associated insulin resistance in skeletal muscle: Role of lipid accumulation and physical inactivity. *Reviews in Endocrine and Metabolic Disorders*. 2011;12:163-172. doi: 10.1007/s11154-011-9168-2
5. Thomas D, Elliott EJ, Naughton GA. Exercise for type 2 diabetes mellitus. *Cochrane Database of Systematic Reviews*. 2006. doi: 10.1002/14651858.CD002968.pub2
6. Posadzki P, Pieper D, Bajpai R, Makaruk H, Könsgen N, Neuhaus AL, Semwal M. Exercise/physical activity and health outcomes: an overview of Cochrane systematic reviews. *BMC Public Health*. 2020;20:1724. doi: 10.1186/s12889-020-09855-3
7. Ross R, Bradshaw AJ. The future of obesity reduction: beyond weight loss. *Nat Rev Endocrinol*. 2009;5:319-325. doi: 10.1038/nrendo.2009.78
8. Shaw KA, Gennat HC, O'Rourke P, Del Mar C. Exercise for overweight or obesity. *Cochrane Database of Systematic Reviews*. 2006. doi: 10.1002/14651858.CD003817.pub3
9. Bird SR, Hawley JA. Update on the effects of physical activity on insulin sensitivity in humans. *BMJ Open Sport Exerc Med*. 2016;2:e000143. doi: 10.1136/bmjsem-2016-000143
10. Luo L, Liu M. Adipose tissue in control of metabolism. *J Endocrinol*. 2016;231:R77-r99. doi: 10.1530/joe-16-0211
11. Zatterale F, Longo M, Naderi J, Raciti GA, Desiderio A, Miele C, Beguinot F. Chronic Adipose Tissue Inflammation Linking Obesity to Insulin Resistance and Type 2 Diabetes. *Frontiers in Physiology*. 2020;10. doi: 10.3389/fphys.2019.01607
12. Stienstra R, van Diepen JA, Tack CJ, Zaki MH, van de Veerdonk FL, Perera D, Neale GA, Hooiveld GJ, Hijmans A, Vroegrijk I, et al. Inflammasome is a central player in the induction of obesity and insulin resistance. *Proc Natl Acad Sci U S A*. 2011;108:15324-15329. doi: 10.1073/pnas.1100255108
13. Tramunt B, Smati S, Grandgeorge N, Lenfant F, Arnal J-F, Montagner A, Gourdy P. Sex differences in metabolic regulation and diabetes susceptibility. *Diabetologia*. 2020;63:453-461. doi: 10.1007/s00125-019-05040-3
14. Kautzky-Willer A, Harreiter J, Pacini G. Sex and Gender Differences in Risk, Pathophysiology and Complications of Type 2 Diabetes Mellitus. *Endocrine Reviews*. 2016;37:278-316. doi: 10.1210/er.2015-1137
15. Karastergiou K, Fried SK, Xie H, Lee M-J, Divoux A, Rosencrantz MA, Chang RJ, Smith SR. Distinct Developmental Signatures of Human Abdominal and Gluteal Subcutaneous Adipose Tissue Depots. *The Journal of Clinical Endocrinology & Metabolism*. 2013;98:362-371. doi: 10.1210/jc.2012-2953
16. Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. *Journal of clinical epidemiology*. 2003;56:1163-1169.

17. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clinical journal of gastroenterology*. 2017. doi: 10.1007/s12328-017-0813-5

18. Angelakis E, Merhej V, Raoult D. Related actions of probiotics and antibiotics on gut microbiota and weight modification. *The Lancet Infectious diseases*. 2013;13:889-899. doi: 10.1016/s1473-3099(13)70179-8

19. Micklesfield LK, Goedecke JH, Punyanitya M, Wilson KE, Kelly TL. Dual-energy X-ray performs as well as clinical computed tomography for the measurement of visceral fat. *Obesity (Silver Spring)*. 2012;20:1109-1114. doi: 10.1038/oby.2011.367

20. Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH, Hoeks J, van der Krieken S, Ryu D, Kersten S, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell metabolism*. 2011;14:612-622. doi: 10.1016/j.cmet.2011.10.002

21. Sartor MA, Tomlinson CR, Wesselkamper SC, Sivaganesan S, Leikauf GD, Medvedovic M. Intensity-based hierarchical Bayes method improves testing for differentially expressed genes in microarray experiments. *BMC Bioinformatics*. 2006;7:538. doi: 10.1186/1471-2105-7-538

22. Lin K, Kools H, de Groot PJ, Gavai AK, Basnet RK, Cheng F, Wu J, Wang X, Lommen A, Hooiveld GJ, et al. MADMAX - Management and analysis database for multiple ~omics experiments. *J Integr Bioinform*. 2011;8:160. doi: 10.2390/biecoll-jib-2011-160

23. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*. 1979;237:E214-223. doi: 10.1152/ajpendo.1979.237.3.E214

24. RIVM/Voedingscentrum. NEVO-tabel 2016. http://nevo-online.rivm.nl/. 2017.

25. Streppel MT, de Vries JH, Meijboom S, Beekman M, de Craen AJ, Slagboom PE, Feskens EJ. Relative validity of the food frequency questionnaire used to assess dietary intake in the Leiden Longevity Study. *Nutrition journal*. 2013;12:75. doi: 10.1186/1475-2891-12-75

26. Siebelink E, Geelen A, de Vries JH. Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults. *The British journal of nutrition*. 2011;106:274-281. doi: 10.1017/s0007114511000067

27. van der Heijden L. *Maten, gewichten en codenummer 2003 in Informatorium VOeding en Diëtitiek - Voedingsleer*. 2013.

28. Fletcher GF, Ades PA, Kligfield P, Arena R, Balady GJ, Bittner VA, Coke LA, Fleg JL, Forman DE, Gerber TC, et al. Exercise standards for testing and training: a scientific statement from the American Heart Association. *Circulation*. 2013;128:873-934. doi: 10.1161/CIR.0b013e31829b5b44

29. Ruiz-Ojeda FJ, Méndez-Gutiérrez A, Aguilera CM, Plaza-Díaz J. Extracellular Matrix Remodeling of Adipose Tissue in Obesity and Metabolic Diseases. *Int J Mol Sci*. 2019;20. doi: 10.3390/ijms20194888

30. Nigro P, Vamvini M, Yang J, Caputo T, Ho LL, Carbone NP, Papadopoulos D, Conlin R, He J, Hirshman MF, et al. Exercise training remodels inguinal white adipose tissue through adaptations in innervation, vascularization, and the extracellular matrix. *Cell Rep*. 2023;42:112392. doi: 10.1016/j.celrep.2023.112392

31. Vink RG, Roumans NJ, Fazelzadeh P, Tareen SH, Boekschoten MV, van Baak MA, Mariman EC. Adipose tissue gene expression is differentially regulated with different rates of weight loss in overweight and obese humans. *Int J Obes (Lond)*. 2017;41:309-316. doi: 10.1038/ijo.2016.201

32. Campbell KL, Foster-Schubert KE, Makar KW, Kratz M, Hagman D, Schur EA, Habermann N, Horton M, Abbenhardt C, Kuan LY, et al. Gene expression changes in adipose tissue with diet- and/or exercise-induced weight loss. *Cancer Prev Res (Phila)*. 2013;6:217-231. doi: 10.1158/1940-6207.Capr-12-0212

33. Nono Nankam PA, Blüher M, Kehr S, Klöting N, Krohn K, Adams K, Stadler PF, Mendham AE, Goedecke JH. Distinct abdominal and gluteal adipose tissue transcriptome signatures are altered by exercise training in African women with obesity. *Scientific Reports*. 2020;10:10240. doi: 10.1038/s41598-020-66868-z

34. Ronquillo MD, Mellnyk A, Cárdenas-Rodríguez N, Martínez E, Comoto DA, Carmona-Aparicio L, Herrera NE, Lara E, Pereyra A, Floriano-Sánchez E. Different gene expression profiles in subcutaneous & visceral adipose tissues from Mexican patients with obesity. *Indian J Med Res*. 2019;149:616-626. doi: 10.4103/ijmr.IJMR_1165_17

35. Northoff H, Symons S, Zieker D, Schaible EV, Schäfer K, Thoma S, Löffler M, Abbasi A, Simon P, Niess AM, et al. Gender- and menstrual phase dependent regulation of inflammatory gene expression in response to aerobic exercise. *Exerc Immunol Rev*. 2008;14:86-103.

36. Abbasi A, de Paula Vieira R, Bischof F, Walter M, Movassaghi M, Berchtold NC, Niess AM, Cotman CW, Northoff H. Sex-specific variation in signaling pathways and gene expression patterns in human leukocytes in response to endotoxin and exercise. *Journal of Neuroinflammation*. 2016;13:289. doi: 10.1186/s12974-016-0758-5

37. Esteller M. Non-coding RNAs in human disease. *Nat Rev Genet*. 2011;12:861-874. doi: 10.1038/nrg3074

38. Parts L, Hedman Å K, Keildson S, Knights AJ, Abreu-Goodger C, van de Bunt M, Guerra-Assunção JA, Bartonicek N, van Dongen S, Mägi R, et al. Extent, causes, and consequences of small RNA expression variation in human adipose tissue. *PLoS Genet*. 2012;8:e1002704. doi: 10.1371/journal.pgen.1002704

39. Håkansson KEJ, Sollie O, Simons KH, Quax PHA, Jensen J, Nossent AY. Circulating Small Non-coding RNAs as Biomarkers for Recovery After Exhaustive or Repetitive Exercise. *Frontiers in Physiology*. 2018;9. doi: 10.3389/fphys.2018.01136

40. Sakharov DA, Maltseva DV, Riabenko EA, Shkurnikov MU, Northoff H, Tonevitsky AG, Grigoriev AI. Passing the anaerobic threshold is associated with substantial changes in the gene expression profile in white blood cells. *Eur J Appl Physiol*. 2012;112:963-972. doi: 10.1007/s00421-011-2048-3

41. Deryusheva S, Gall JG. scaRNAs and snoRNAs: Are they limited to specific classes of substrate RNAs? *Rna*. 2019;25:17-22. doi: 10.1261/rna.068593.118

42. Liao J, Yu L, Mei Y, Guarnera M, Shen J, Li R, Liu Z, Jiang F. Small nucleolar RNA signatures as biomarkers for non-small-cell lung cancer. *Mol Cancer*. 2010;9:198. doi: 10.1186/1476-4598-9-198

Chapter 6

Eight-week exercise training in humans with obesity: marked improvements in insulin sensitivity, modest changes in gut microbiome

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ABSTRACT

Objective. Obesity is associated with impaired gut microbiota diversity, which has been linked to development of type 2 diabetes. This study aims to examine effects of an 8-week aerobic exercise intervention on insulin sensitivity, visceral adiposity and gut microbiota diversity and composition in subjects with obesity.

Methods. Fourteen subjects (age 51±11 years; BMI 34.9±4.9kg/m^2) performed an 8-week exercise intervention (2-4 times /week on 65-85% of HRR). Insulin sensitivity (hyperinsulemic euglycemic clamp), cardiorespiratory fitness (maximal oxygen uptake), visceral adiposity (DXA-scan) and gut microbiota composition (16-s rRNA gene sequencing) were measured before and after the intervention.

Results. Insulin sensitivity showed a significant increase (pre: 3.8±1.9 mg/min/kg; post: 4.5±1.7; P-value: 0.007) after training whilst visceral adiposity decreased (pre: 959±361 cm^3; post: 897±364; P-value: 0.02). No change in gut microbiota α- or β-diversity was found. On genus level, the abundance of Ruminococcus gauvreauii (P=0.02); Lachnospiraceae_FCS020group (P=0.04) and Anaerostipes (P=0.04) significantly increased after exercise training. Significant positive correlations were present for M-value (R. gauvreauii) and VO2max (R. gauvreauii and Anaerostipes).

Conclusions. 8-week exercise training in humans with obesity leads to marked improvements in insulin sensitivity and body composition and is accompanied by modest changes in three gut microbiome genera, all belonging to the Firmicutes phylum.

INTRODUCTION

Over the last decade, the gut microbiota has emerged as an important modulator of the immune system and energy homeostasis.[1] An imbalance (i.e. dysbiosis) in gut microbiota composition in humans has been associated with various metabolic diseases, such as type 2 diabetes mellitus.[2,3] Obesity, a major risk factor for type 2 diabetes mellitus and cardiovascular disease, is associated with gut microbiota dysbiosis that is characterized by decreased diversity and altered composition.[4] This highlights the clinical relevance of targeting and improving the gut microbiota in obesity.

In the absence of widely accepted pharmacological therapeutic strategies to improve metabolic health by altering gut microbiota, exercise training may represent a potent therapy. This is supported by the strong and independent health effects of (regular) exercise training in reducing the risk of type 2 diabetes mellitus, by improving insulin sensitivity, especially in untrained individuals with obesity.[5-7] Based on the previously identified link between the gut microbiota and insulin sensitivity, benefits of exercise training on insulin sensitivity may be accompanied by alterations in gut microbiota composition. To support this notion, data from rodent studies reveal that exercise training in obese mice/rat models improves gut microbiota diversity and composition.[8,9] Voluntary exercise caused an increase in gut microbiota diversity whilst these improvements in gut microbiota were linked to enhanced glucose homeostasis (using an oral glucose tolerance test) in mice.[8]

In humans, cross-sectional data suggest that athletes display larger gut microbiota diversity than inactive controls,[10] which might be linked to differences in fitness level.[11] However, to date, prospective data on the direct effects of exercise training on gut microbiota and metabolic health (ie. insulin sensitivity) in humans with obesity are scarce and display heterogenous results.[12-14] Therefore, we examined the impact of an 8-week aerobic exercise intervention on insulin sensitivity and gut microbiota diversity and composition in individuals with obesity. We hypothesize that exercise training will improve insulin sensitivity, whilst these changes are correlated with alterations in gut microbiota.

MATERIALS AND METHODS

Subjects

Twenty inactive subjects with obesity (BMI > 30kg/m^2) were included in this study. Physical activity levels were assessed with the use of the Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH), a validated tool to assess physical activity levels in the Dutch population. Subjects were eligible for inclusion when their SQUASH score was 6400 or lower.[15] Subjects with inflammatory bowel disease and subjects who used pro- or antibiotics were excluded from participation since these conditions are associated with an altered gut microbiota.[16,17] Subjects with a medical history of diabetes mellitus were excluded from participation. Written informed consent was obtained from all participants prior to the start of the study. This study was approved by the Medical Ethical Committee of the Radboud University Nijmegen Medical Center, and was conducted in accordance with the declaration of Helsinki. This study was registered as NTR5737 in the Dutch Clinical Trial Register. Data represented in this manuscript are a result of a secondary analysis of the primary aim for which the trial was registered. Ethical approval for this specific analysis was obtained.

Study design

All subjects who participated in this study were engaged in an 8-week supervised aerobic exercise training intervention. Subjects were instructed not to change dietary habits during the participation in this study. Before and after the intervention, a fresh stool sample was collected by the participants and a hyperinsulinemic, euglycemic clamp, a maximal cycling test and a DXA-scan to examine insulin sensitivity, physical fitness level and body composition, respectively, were performed.

Exercise training. During this training study, all subjects trained 2-4 times a week under the supervision of an experienced researcher. Training consisted of cycling exercise on an ergometer (Lode, Groningen, the Netherlands) starting with a 5 minute warming-up, followed by 50 minutes of exercise at 65%-85% of the individual heart rate reserve (HRR) and ending with a cooling-down of 5 minutes. Training frequency and percentage of HRR were gradually increased during the first two weeks of the intervention period. The HRR was calculated based on individual maximal heart rate assessed during the maximal cycling test. Exercise intensity was continuously monitored and documented with the use of heart rate monitors (Polar®) and workload was adjusted accordingly on an individual basis. Subjects

had to attend at least 95% of the training sessions during the 8 week intervention period to be eligible for inclusion in the statistical analysis.

Measurements

Anthropometry. At baseline, height and body weight (Seca 888 scale, Hamburg, Germany) were measured to calculate body mass index (BMI). Waist and abdominal circumference were measured with a measuring tape (Seca 201, Chino, USA) to calculate waist-to-hip ratio. Resting heart rate and blood pressure were measured in supine position, after a 5 minute rest period. Before and after the training period a total body Dual-X-ray Absorptiometry (DXA) scan was performed to determine lean body mass and total fat (QDR 4500 densitometer, Hologic Inc. Waltham, MA). Visceral adipose tissue (VAT) mass, VAT volume and VAT area were calculated with standardized Hologic Software with results that correlate excellent with gold standard techniques for the measurement of VAT.[18]

Gut microbiota. Subjects were provided with a plastic device to collect stool samples, which were stored at -80ºC until DNA-extraction. Each subject was instructed to collect a stool sample 48-72 hours after cessation of the exercise bout, preferably on a week day between 6 and 11.30 AM in order to quickly store the sample in -80ºC at the research facility. Subjects collected their stool sample at home and were asked to hand in the sample as soon as possible. When a subject was not able to travel to the research facility immediately, the stool sample was stored in a fridge at 7ºC at the subjects home. Since most of the participants lived nearby the research facility and collection time was during working hours, all samples were stored at -80ºC within 4 hours after collection. Microbial DNA was isolated from feces using the Maxwell 16 Total RNA system (Promega, Leiden, The Netherlands). Fecal samples were homogenized with two times bead beating followed by incubation at 95°C at 100rpm. Each time, samples were centrifuged for 5 min at 4 °C and 14,000 g to collect the supernatant which was placed in a new sterile eppendorf tube. Following, 250ul from the obtained supernatant was loaded to Maxwell 16 Tissue LEV Total RNA Purification Kit (Promega) instrument for DNA extraction. DNA was eluted in 50ul of nuclease free water and its concentration was quantified using Nanodrop (ThermoScientific. Landsmeer, the Netherlands). For the amplification of the bacterial 16S rRNA gene fragment, primers targeting the V5-V6 region were selected (F784-R1061). PCR reaction for each sample were performed in triplicates in a total reaction volume of 35 µl. The master mix contained 0.7 µl of the barcoded primer(10 µM each per reaction), 0.7 µl dNTPs mixture, 0.35 µl Phusion Green Hot Start II High-Fidelity DNA Polymerase (2 U/µl; ThermoScientific, Landsmeer, The Netherlands), 7 µl 5× Phusion Green HF Buffer, and 25.55 µl DNAse-

RNAse-free water. The amplification program included 30 s of initial denaturation step at 98°C, followed by 25 cycles of denaturation at 98°C for 10 s, annealing at 42°C for 10 s, elongation at 72°C for 10 s, and a final extension step at 72°C for 7 min. The PCR product was visualized in 1% agarose gel (~290 bp) and purified with CleanPCR kit (CleanNA, Alphen aan den Rijn, The Netherlands). The concentration of the purified PCR products was measured with Qubit dsDNA BR Assay Kit (Invitrogen, California, USA) and 200 ng of microbial DNA from each sample was pooled for the generation of the sequencing library. Data filtering and taxonomy assignment were performed using the NG-Tax pipeline using the default.[19] Two distinct in-house assembled mock communities were included in the library and were compared with their theoretical composition for quality control.

Insulin sensitivity. Peripheral tissue sensitivity to exogenous insulin was measured using a hyperinsulinemic euglycemic clamp as previously described.[20] After an overnight fast (10 hours), the subject was placed in the supine position in a quiet, temperature controlled (22 – 24 °C) room. Insulin (Novorapid, Novo-Nordisk, Copenhagen, Denmark) was infused intravenously in a dose of 430 $pmol \cdot m^{-2} \cdot min^{-1}$ ($60\ mU \cdot m^{-2} \cdot min^{-1}$) for 120 minutes. Insulin $50\ U \cdot ml^{-1}$ was diluted in 47.5 ml NaCl 0.9% with the addition of 2 ml blood from the subject to a concentration of $1\ U \cdot ml^{-1}$. Venous plasma glucose concentrations were clamped at 5.0 $mmol \cdot L^{-1}$ by a variable glucose 20% infusion rate, adjusted depending on venous plasma glucose level measured at 5-minute intervals. Serum glucose levels were determined using a Biosen C-Line Glucose and Lactate Analyser (Biosen C-line GP+, EKF-diagnostic GmbH, Barleben, Germany).Fullerton, CA 92634, USA). Whole body glucose disposal was calculated as the mean glucose infusion rate per kilogram body weight ($mg \cdot kg^{-1} \cdot min^{-1}$) during the last 30 minutes of the clamp (M-Value). The hyperinsulemic euglycemic clamp after the training period was performed at least 72 hours after the last exercise bout.

Dietary intake. During the training intervention, subjects were instructed not to change their dietary habits. To assess potential changes in daily food intake, subjects were asked to record dietary intake records before and in the last week of the training intervention in a detailed food journal. Subjects were individually instructed how to record food items and were provided with example diaries. Dietary records of the 24 hours prior to stool collection were analyzed with Eetmeter Software (Voedingscentrum, the Hague, Netherlands), based on the Dutch Food Composition Database of 2016.[21] Furthermore, an online validated 180–item semi–quantitative Food Frequency Questionnaire (FFQ) was used to assess habitual daily energy intake, and macronutrient intake.[22,23] The FFQ reference period was

one month, and portion sizes were estimated using standard portions.[24] Intake of total energy and nutrients was calculated using the Dutch Food Composition Database. [21]

Cardio-respiratory fitness level. Subjects performed a maximal exercise test on an electrically braked leg-cycling ergometer (Lode Excalibur, Groningen, the Netherlands) using an incremental protocol, to assess their cardio-respiratory fitness level. Workload increased by 10-30 W per minute, starting at 0 W, until exhaustion. A calibrated gas-analyzer was used to measure oxygen consumption continuously (COSMED Pulmonary Function Equipment, Chicago, US). During the test, an electrocardiogram (ECG) was continuously recorded and checked by a physician. The maximal exercise test was terminated by adhering to the guidelines of the American Heart Association.[25] Maximal oxygen consumption (VO_{2max}) was defined as the highest oxygen uptake (30 second average).

Statistical analysis

All statistical analyses were conducted in SPSS 22 (Statistical Package for Social Sciences 22.0, SPSS Inc., Chicago, Illinois, USA). Data was checked for normality with use of the Shapiro-Wilk test. Subject characteristics were normally distributed and therefore assessed with use of a paired t-test to examine the impact of exercise training. Correlations between measures of alpha diversity and abundance of gut microbiota versus subject characteristics were assessed with use of Repeated measures correlation (Rmcorr).[26] The level of statistical significance was defined at $\alpha=0.05$. Data are presented as mean±SD, unless stated otherwise.

Microbial data analysis

Alpha and beta diversity analyses were performed and visualized using the publicly available Microbiome R package (version 1.2.1).[27] Alpha diversity analyses provide information about richness (number of species) and/or evenness (relative abundance of those species) within a sample.[28] Alpha diversity as determined by Chao index (non-parametric estimation of species richness)[29], Shannon index (measuring richness and evenness by taking relative abundance into account)[30] and Faiths index (PD, phylogenetic diversity: the sum of the branch lengths of the phylogenetic tree, a measurement of diversity in taxon subsets)[31]. Beta diversity analyses provide information about variation between samples.[28] Beta diversity was calculated using the Bray-Curtis dissimilarity index and visualized through a Principal Coordinates analysis. The envfit function from the Vegan package that fits environmental vectors or factors onto an ordination, was used to evaluate if age, sex, body mass (kg), insulin sensitivity (M-value), BMI, cardiorespiratory fitness

(VO2max), VATvolume, and dietary measures (daily intake of Kcal, fat, saturated fat, carbohydrate, protein) were associated with the NMDS ordinations; ie. could explain the variance observed in the data set. The significance of the fitted factors was estimated using 999 permutations. Repeated measures correlations, designed for paired samples, were performed using the Rmcorr package, to assess correlations between environmental variables and bacterial taxa.[26] The Wilcoxon signed rank test was used to examine whether significant changes in gut microbiota occurred on genus / family / order / class level.

RESULTS

Effect of training intervention

Subject characteristics before and after training are presented in Table 1. Twenty subjects (11 women, 9 men) completed the exercise intervention. Since two participants were unable to collect a stool sample before start of the intervention and at four additional subjects were unable to collect a stool sample in the given timeframe after the intervention, these were excluded from analysis, leading to a sample size of n=14 subjects (7 women, 7 men) with samples collected before and after training. Data was analyzed for this subgroup. Training compliance for this subgroup was 98%. (Figure 1) Characteristics from this subgroup (n=14) were not different from the entire cohort (data not shown).

Assessment of dietary intake by food journals showed no significant change in daily energy intake (Pre: 2028±622 kcal/day; post: 1906±390 kcal/day; p=0.18), nor in macronutrient composition before and at the end of the intervention period (Table 1). Cardio-respiratory fitness levels, insulin sensitivity (M-value) and body composition improved significantly. (Table 1).

Table 1. Physiological characteristics before and after the exercise intervention of the subgroup (n=14) with available microbiota data on both time points. P-value represents the level of significance for post *versus* pre-values. Data represents mean ± standard deviation.

	Subgroup analysis (n=14)		
Pre	**Post**	**P-value**	**Age (years)**
Age (years)	51±11	-	-
Female sex (%)	50%		
Body composition			
Weight (kg)	105.4±16.8	102.6±17.4	0.03
Body mass index (kg/m^2)	34.9±4.9	33.9±5.2	0.03
Waist-to-hip ratio	1.00±0.10	0.99±0.07	0.36
VAT mass (g)	887±334	830±337	0.04
VAT volume (cm3)	959±361	897±364	0.02
Insulin sensitivity			
M-value (mg/min/kg)	3.8±1.9	4.5±1.7	0.007
Blood pressure			
Systolic blood pressure (mmHg)	132±16	131±14	0.6
Diastolic blood pressure (mmHg)	87±11	80±9	0.003
Resting heart rate (bpm)	67±10	72±12	0.5
Physical fitness			
VO2max (ml/min/kg)	27.7±5.5	31.9±7.0	<0.0001
VO2max (ml/min/kg FFM)	46.0±6.6	51.2±8.0	0.001
Power (Watt)	204±42	250±47	<0.001
Daily dietary composition			
Energy intake (kcal)	2028±622	1905±389	0.18
Carbohydrates (g)	211±39	210±58	0.94
Fat (g)	75±42	66±22	0.28
Unsaturated fat (g)	28±18	26±9	0.74
Protein (g)	98±26	93±18	0.52
Lipid Profile			
Cholesterol (mmol/L)	5.6±1.5	5.2±1.1	0.03
HDL (mmol/L)	1.4±0.3	1.4±0.3	0.35
LDL (mmol/L)	3.7±1.3	3.4±1.2	0.05
Triglycerides (mmol/L)	1.8±0.7	1.8±0.	0.99

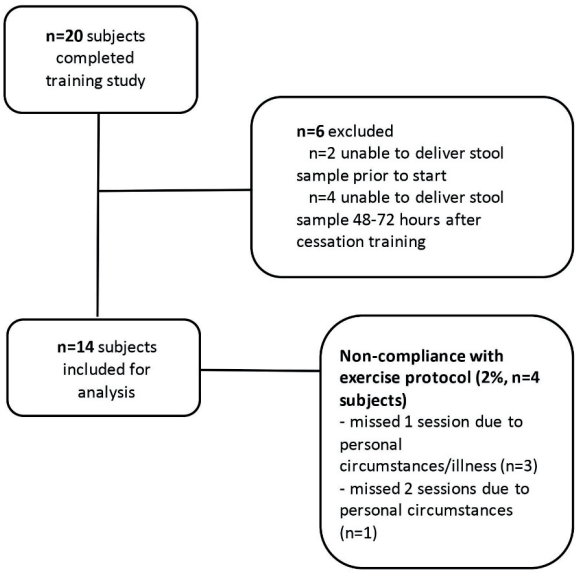

Figure 1. Consort diagram of excluded subjects and exercise training compliance

Gut microbiota

Alpha Diversity. No change in alpha diversity of the gut microbiota was observed after training, as assessed by Shannon Index, Phylogenetic diversity index and Chao 1 (Figure 2a).

Beta Diversity. Bray-Curtis analysis showed that samples did not cluster by time (pre- versus post training) and that, consistently, β-diversity did not change post- versus pretraining (Figure 2b).

Composition. On genus level, a total number of 3 taxa showed a significant change after the exercise intervention: Ruminococcus gauvreauii (P=0.02); uncultured genus from the Lachnospiraceae (P=0.04) and Anaerostipes (P=0.04) (Figure 3). On family, phylum, class and order level no significant change in taxa was found after the 8-week exercise training intervention.

Envfit analysis showed that only body mass showed borderline significance explaining the total variation in gut microbiota composition ($p = 0.05$), while the improvement in VO2max (i.e. effect size of the exercise training intervention) and the other potential explanatory variables (ie. sex, age, M-Value, BMI, VO2max, VATvolume and dietary intake measures) did not explain significantly the differences in microbial composition of the subjects before and after the intervention.

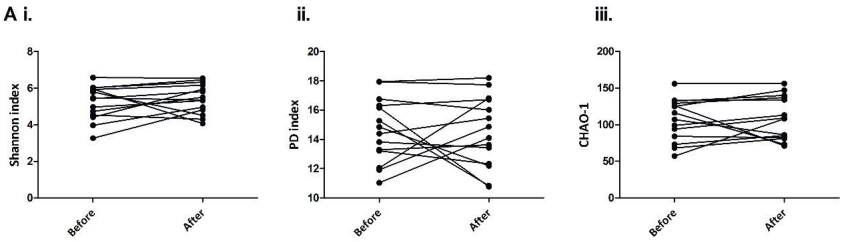

Figure 2A. Alpha diversity before and after the exercise intervention i. Shannon Index; ii. Phylogenetic diversity; iii. Chao1

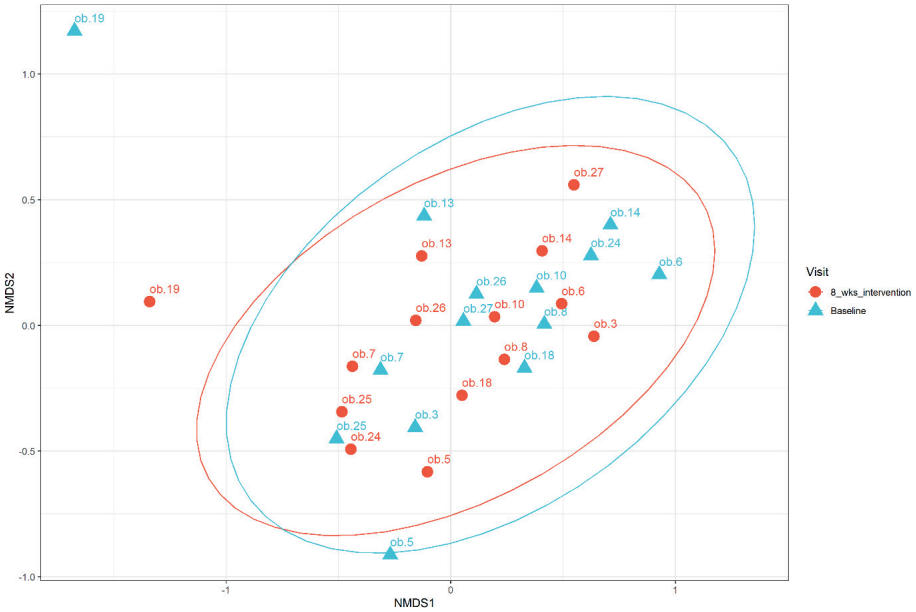

Figure 2B. Beta diversity (Bray-Curtis) before (▲) and after (●) the exercise intervention

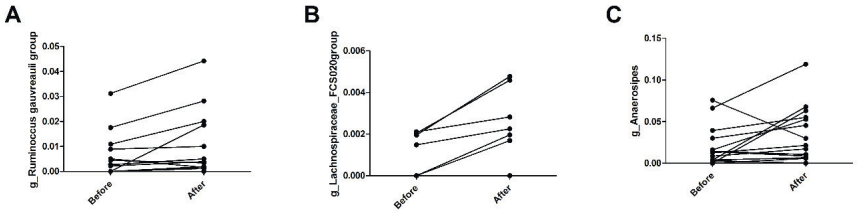

Figure 3. Significant changes in genus A. Ruminococcus gauvreauii B Lachnospiraceae and C. Anaerostipes before and after the exercise intervention. Y-axis represents relative abundance of each genus

Correlation analysis

For this analysis, both samples collected prior to and after the exercise intervention were used. To examine the relationship between gut microbiota composition and subject characteristics (insulin sensitivity, cardiorespiratory fitness, body composition measures and dietary intake measures) further, the top 30 most abundant bacterial genera were correlated with M-value, VO2max, body mass, BMI, VATvolume and caloric intake, intake of fat, carbohydrate and protein. The abundance of Anaerostipes is strongly positively correlated with VO2max (R=0.64, p-value=0.015). The abundance of Ruminococcus2 is positively correlated with VATvolume (R=0.51, p-value=0.0048). The abundance of Ruminococcus gauvreauiigroup is positively correlated with M-value (R=0.60, p-value=0.023) and VO2max (R^2=0.61, p-value=0.0018) and negatively with VATvolume (R=-0.54, p-value=0.028) (Figure 4). The abundance of Subdoligranulum is negatively correlated with intake of fat (R=-0.59, p-value=0.035) and caloric intake (R=-0.62, p-value=0.021). The abundance of Roseburia and Eubacterium halliigroup are both negatively correlated with carbohydrate intake (R=-0.61, p-value=0.026 and R=-0.58, p-value=0.04, respectively).

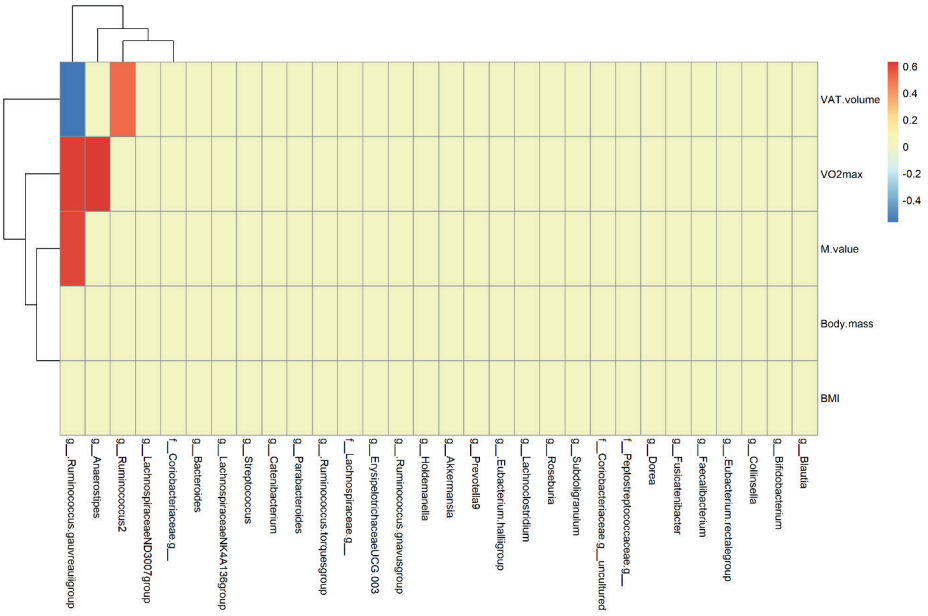

Figure 4. Correlations between subject characteristics (M-value, body mass, BMI, VAT volume and VO2max) and the 30 most abundant genera of gut microbiota in fecal samples (data from before and after the exercise intervention)

No significant correlations between the different measures of alpha diversity (ie. Shannon Index, Phylogenetic diversity index and Chao 1) and changes in body composition (body weight, BMI, VAT mass), insulin sensitivity (M-value) or cardiorespiratory fitness (VO_2 max) were found (data not shown).

DISCUSSION

This study presents the following findings. First, an 8-week aerobic exercise intervention in humans with obesity leads to marked improvements in insulin sensitivity and body composition, whilst this is not accompanied by improvements in gut microbiota alpha(α)- and beta(β)-diversity. Modest but significant changes in 3 genera (Ruminococcus gauvreauii, uncultured Lachnospiraceae and Anaerostipes) after the exercise intervention were found. Of these genera, Ruminococcus gauvreaii and Anaerostipes both showed a significantly positive correlation with VO2max. Ruminococcus gauvreaii also correlated positively with M-value and negatively with VAT volume. This suggests that gut microbiota exerts adaptability in response to exercise training which might be associated with improvements in metabolic and cardiovascular health.

To demonstrate the impact of exercise training, precise, high quality techniques were used for the measurement of insulin sensitivity,[20] visceral adiposity (VAT),[18] and cardiorespiratory fitness.[25] In line with our hypothesis, and reinforced by several previous studies,[5] large beneficial effects of exercise training on M-value, visceral adipose tissue and fitness levels were observed. This proves that the 8-week exercise intervention subjects performed in this study is a successful tool in improving risk factors for the development of metabolic and cardiovascular disease. After 8 weeks of effective exercise training, no change in gut microbiota diversity was found in our cohort of individuals with obesity. The lack of exercise-induced alterations in α- and β-diversity is in accordance with previous human exercise intervention studies of both shorter (3 weeks) and longer (12 weeks) duration and similar exercise intensities.[12,14] This is in contrast to cross-sectional work in athletes that demonstrated marked differences in the gut microbiota diversity when compared to inactive controls, suggesting a role for exercise as an influencer of gut microbiota diversity.[32] Indeed, two exercise studies found alterations in β-diversity of the gut microbiota.[33,34] In one study by Allen et al., these alterations were dependent on obesity status: in lean subjects, exercise induced shifts in bacterial taxa were more pronounced than in individuals with obesity.[33] This suggests that gut microbiota diversity in humans with obesity might be more rigid and unable to response to

an exercise stimulus. As in our study, participants in Allen et al. were instructed to maintain their regular dietary intake to discard the influence of a change in diet on gut microbiota. Moreover, the exercise intervention was of similar intensity (60-75% HRR) and duration (6 weeks).[33] Therefore, the differences between our study and tat of others are unlikely the result of a different exercise design. More likely, other factors might play a role, such as lifelong training status and childhood dietary regimen, which could also explain the large differences observed in cross-sectional comparison of elite athletes to sedentary controls. At least, this suggests that exercise-mediated improvements in insulin sensitivity occur independently of changes in gut microbiota diversity.

In this study, modest but significant changes in gut microbiota composition occurred. On genus level, abundance of Ruminococcus gauvreauii group, Lachnospiraceae FCS020group and Anaerostipes was increased after exercise training. Interestingly, R. gauvreauii was also positively correlated with insulin sensitivity (M-value), cardiorespiratory fitness levels (VO2max) and inversely correlated with visceral adiposity. R. gauvreauii is a genus derived from the order of Clostridium in the Phylum of Firmicutes.[35] Its abundance is decreased in patients with coronary artery disease when compared to controls.[36] VO2max is a strong, independent risk factor for the development of cardiovascular disease. The exercise induced increase in R. gauvreauii and its positive correlation with VO2max we found in our study, together with the observation that its abundance is lower in CAD patients, suggests that exercise might be capable of improving cardiovascular risk mediated by altering gut microbiota in individuals with obesity. R. gauvreauii produces acetate as end-product of fermentation.[35] Acetate is a short chain fatty acid (SCFA), that elicits various beneficial effects on other tissues in the body, ultimately improving body weight control and insulin sensitivity.[37] This is in accordance with our results that demonstrate a positive correlation between R. gauvreauii and gold standard measurement of insulin sensitivity and a negative correlation with visceral adipose tissue mass. Taken together, our results suggest that exercise-induced improvements in glucose homeostasis might be associated with an increase in acetate-producing R. gauvreauii.

This study also demonstrated a modest increase in Anaerostipes derived from the family of Lachnospiraeceae in the phylum Firmicutes, in the presence of a positive correlation with VO2max. Anaerostipes is a butyrate producer by lactate utilization.[38] Its abundance has not been described to be altered by exercise interventions in humans with obesity in previous studies. However, Rettedal et al. found that its abundance is higher in lean subjects compared to subjects with obesity.[37]

The correlation with VO2max we found in our study suggests it adaptability to an exercise stimulus towards a more favorable "lean" phenotype. However, it can also be a direct consequence of the lactate shifts that result from multiple strenuous exercise interventions. Lastly, we also found an increase in the genus Lachnospiraceae FCS020group, also derived from the family of Lachnospiraeceae in the phylum Firmicutes. Data on Lachnospiraceae FCS020group in humans is scarce. It has been associated with circulating VLDL and small HDL particles and plasma trimethylamine N-oxide, both potential risk factors for coronary artery disease.[39,40] In our study, its abundance was not correlated to any of the established cardiovascular risk factors (ie. insulin resistance or visceral adiposity). Therefore, it remains unknown what the clinical significance of this change is. Taken together, our study demonstrates that exercise training increases the abundance of two SCFA-producing genera belonging to the Firmicutes phylum that are associated with improvements in cardiorespiratory fitness levels and insulin sensitivity. This suggests that exercise induced improvements in cardio-metabolic health might be mediated by SCFA-producing gut microbiota. Future work is required to directly study this hypothesis.

Some methodological considerations must be taken into account in our study. First, although based on previous work, short chain fatty acids (SCFAs), produced by bacterial taxa from the Firmicutes phylum, might play a role in exercise-induced improvements in insulin sensitivity, we were unable to measure these in the stool samples of our subjects. Unfortunately, this was not part of the original research design. This should be incorporated in future studies examining this topic. Second, the gut microbiota data in our cohort show a large inter-individual variance which is in accordance with large cohort microbiota studies in humans[41,42] and also smaller human intervention studies.[12] Nonetheless, our primary comparison involves intra-individual changes, which adds strength to our observation that exercise training did not alter the gut microbiota. Third, the timing of gut microbiota measurement (i.e. collection of the stool sample) is an important factor potentially affecting results as a temporarily dysbiosis in gut microbiota after strenuous exercise can occur.[43] Since all participants collected a stool 48-72 hours after cessation of the last exercise bout to rule out acute effects of the last exercise bout, this has minimized the potential impact of the last exercise bout on gut microbiota measures. Lastly, a change in diet is known to cause an alteration in gut microbiota.[44] Therefore, participants were carefully instructed not to change caloric and macronutrient intake which was objectified with the use of food diaries. Since our data demonstrated that diet had not changed, we can exclude changes in diet as a potential factor influencing our results. This is further supported by the Envfit

analysis which showed that macronutrient and caloric intake do not influence the variation in change of gut microbiota composition and the correlation analysis in which no significant correlation was found between the three significantly altered genera after exercise training and dietary intake measures.

This study demonstrated that an 8-week exercise intervention in humans with obesity causes significant improvements in cardiovascular and metabolic health in the presence of modest changes in three gut microbiome genera, all belonging to the SCFA producing Firmicutes phylum. Of these genera, R. gauvreauii is positively correlated with insulin sensitivity and cardiorespiratory fitness, which suggests a potential role for this acetate producer to cause improvement in insulin sensitivity in response to exercise.

REFERENCES

1. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature.* 2006;444(7122):1027-1031.
2. Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature.* 2012;490(7418):55-60.
3. Kostic AD, Gevers D, Siljander H, et al. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell host & microbe.* 2015;17(2):260-273.
4. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. *Nature.* 2009;457(7228):480-484.
5. Boule NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *Jama.* 2001;286(10):1218-1227.
6. Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *The New England journal of medicine.* 2001;344(18):1343-1350.
7. Shaw KA, Gennat HC, O'Rourke P, Del Mar C. Exercise for overweight or obesity. *Cochrane Database of Systematic Reviews.* 2006(4).
8. Evans CC, LePard KJ, Kwak JW, et al. Exercise prevents weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. *PloS one.* 2014;9(3):e92193.
9. Petriz BA, Castro AP, Almeida JA, et al. Exercise induction of gut microbiota modifications in obese, non-obese and hypertensive rats. *BMC genomics.* 2014;15:511.
10. Clarke SF, Murphy EF, O'Sullivan O, et al. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut.* 2014;63(12):1913-1920.
11. Estaki M, Pither J, Baumeister P, et al. Cardiorespiratory fitness as a predictor of intestinal microbial diversity and distinct metagenomic functions. *Microbiome.* 2016;4(1):42.
12. Liu Y, Wang Y, Ni Y, et al. Gut Microbiome Fermentation Determines the Efficacy of Exercise for Diabetes Prevention. *Cell metabolism.* 2020;31(1):77-91.e75.
13. Munukka E, Ahtiainen JP, Puigbó P, et al. Six-Week Endurance Exercise Alters Gut Metagenome That Is not Reflected in Systemic Metabolism in Over-weight Women. *Front Microbiol.* 2018;9:2323.
14. Rettedal EA, Cree JME, Adams SE, et al. Short-term high intensity interval training (HIIT) exercise does not affect gut bacterial community diversity or composition of lean and overweight men. *Exp Physiol.* 2020.
15. Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. *Journal of clinical epidemiology.* 2003;56(12):1163-1169.
16. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clinical journal of gastroenterology.* 2017.
17. Angelakis E, Merhej V, Raoult D. Related actions of probiotics and antibiotics on gut microbiota and weight modification. *The Lancet Infectious diseases.* 2013;13(10):889-899.
18. Micklesfield LK, Goedecke JH, Punyanitya M, Wilson KE, Kelly TL. Dual-energy X-ray performs as well as clinical computed tomography for the measurement of visceral fat. *Obesity (Silver Spring).* 2012;20(5):1109-1114.

19. Ramiro-Garcia J, Hermes G, Giatsis C, et al. *NG-Tax, a highly accurate and validated pipeline for analysis of 16S rRNA amplicons from complex biomes [version 1; referees: 2 approved with reservations, 1 not approved]*. Vol 52016.
20. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* 1979;237(3):E214-223.
21. RIVM/Voedingscentrum. NEVO-tabel 2016. http://nevo-online.rivm.nl/. Published 2017. Accessed.
22. Streppel MT, de Vries JH, Meijboom S, et al. Relative validity of the food frequency questionnaire used to assess dietary intake in the Leiden Longevity Study. *Nutrition journal.* 2013;12:75.
23. Siebelink E, Geelen A, de Vries JH. Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults. *The British journal of nutrition.* 2011;106(2):274-281.
24. van der Heijden L. *Maten, gewichten en codenummer 2003 in Informatorium VOeding en Diëtitiek - Voedingsleer.* 2013.
25. Fletcher GF, Ades PA, Kligfield P, et al. Exercise standards for testing and training: a scientific statement from the American Heart Association. *Circulation.* 2013;128(8):873-934.
26. Bakdash JZ, Marusich LR. Repeated Measures Correlation. *Frontiers in Psychology.* 2017;8(456).
27. Shetty SA, Lahti L. Microbiome data science. *Journal of Biosciences.* 2019;44(5):115.
28. Kim BR, Shin J, Guevarra R, et al. Deciphering Diversity Indices for a Better Understanding of Microbial Communities. *J Microbiol Biotechnol.* 2017;27(12):2089-2093.
29. Chao A. Nonparametric Estimation of the Number of Classes in a Population. *Scandinavian Journal of Statistics.* 1984;11(4):265-270.
30. Lemos LN, Fulthorpe RR, Triplett EW, Roesch LF. Rethinking microbial diversity analysis in the high throughput sequencing era. *J Microbiol Methods.* 2011;86(1):42-51.
31. Faith DP. Conservation evaluation and phylogenetic diversity. *Biological Conservation.* 1992;61(1):1-10.
32. Barton W, Penney NC, Cronin O, et al. The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level. *Gut.* 2018;67(4):625-633.
33. Allen JM, Mailing LJ, Niemiro GM, et al. Exercise Alters Gut Microbiota Composition and Function in Lean and Obese Humans. *Medicine and science in sports and exercise.* 2018;50(4):747-757.
34. Cronin O, Barton W, Skuse P, et al. A Prospective Metagenomic and Metabolomic Analysis of the Impact of Exercise and/or Whey Protein Supplementation on the Gut Microbiome of Sedentary Adults. *mSystems.* 2018;3(3).
35. Domingo MC, Huletsky A, Boissinot M, Bernard KA, Picard FJ, Bergeron MG. Ruminococcus gauvreauii sp. nov., a glycopeptide-resistant species isolated from a human faecal specimen. *Int J Syst Evol Microbiol.* 2008;58(Pt 6):1393-1397.
36. Toya T, Corban MT, Marrietta E, et al. Coronary artery disease is associated with an altered gut microbiome composition. *PloS one.* 2020;15(1):e0227147.
37. Hernández MAG, Canfora EE, Jocken JWE, Blaak EE. The Short-Chain Fatty Acid Acetate in Body Weight Control and Insulin Sensitivity. *Nutrients.* 2019;11(8).
38. Schwiertz A, Hold GL, Duncan SH, et al. Anaerostipes caccae gen. nov., sp. nov., a new saccharolytic, acetate-utilising, butyrate-producing bacterium from human faeces. *Syst Appl Microbiol.* 2002;25(1):46-51.
39. Diling C, Longkai Q, Yinrui G, et al. CircNF1-419 improves the gut microbiome structure and function in AD-like mice. *Aging (Albany NY).* 2020;12(1):260-287.

40. Gao J, Yan KT, Wang JX, et al. Gut microbial taxa as potential predictive biomarkers for acute coronary syndrome and post-STEMI cardiovascular events. *Sci Rep.* 2020;10(1):2639.

41. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature.* 2010;464:59.

42. Structure, function and diversity of the healthy human microbiome. *Nature.* 2012;486(7402):207-214.

43. Peters HP, De Vries WR, Vanberge-Henegouwen GP, Akkermans LM. Potential benefits and hazards of physical activity and exercise on the gastrointestinal tract. *Gut.* 2001;48(3):435-439.

44. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature.* 2014;505(7484):559-563.

Chapter 7

General Discussion and future perspectives
‘How to activate your lazy fat’

Exercise is medicine

The 2020s: how three pandemics work together to impair health

Anno 2025, the prevalence of (morbid) obesity is still increasing and has risen to pandemic numbers. Despite both worldwide and national initiatives to put more emphasis on prevention of the detrimental health effects of obesity, the effects of obesity on public health are larger than ever. Currently, 50% of the adult population in the Netherlands is overweight whereas 14% suffers from obesity. These numbers are still increasing. The harmful effects of obesity were highlighted during the recent COVID-19 pandemic. Within months of the start of the COVID-19 pandemic, studies demonstrated that obesity is an independent risk factor to develop severe complications of a COVID-19 infection for which hospitalization and intubation are needed.[1,2] Specifically, 30% of hospital admissions due to COVID19 in the US could be attributed to obesity.[3] The third pandemic present in the 2020s, which is closely linked to both the obesity- and COVID-19 pandemics, relates to physical inactivity. The proportion of both children and adults that adheres to guidelines of minimal physical activity per week is gradually decreasing. Physical inactivity contributes to a decrease in fitness and thereby to a large number of diseases, such as diabetes mellitus and cardiovascular disease and ultimately to mortality.[4,5] Finally, it cannot be ignored that these three pandemics are closely linked, especially given the increase in body weight and decrease in physical activity that was present during the COVID-19 lockdowns.[6,7] This highlights the need to specifically target lifestyle as a preventive and treatable target to decrease body weight and increase physical activity.

In the Netherlands in 2025, more than 1 million humans suffer from type 2 diabetes mellitus. Based on demographic development, the number of individuals living with diabetes is estimated to be 1.4 million in 2040.[8] The World Health Organization advocates lifestyle strategies to prevent overweight and obesity in order to prevent other negative health effects, such as the development of type 2 diabetes mellitus.[9] Lifestyle interventions are aimed at restoring the energy imbalance that forms the basis for becoming obese. Caloric restriction reduces energy intake whereas exercise training increases energy expenditure. Indeed, both a hypocaloric diet and increasing physical activity by a training program are associated with beneficial effects on insulin resistance that underlie the pathogenesis of type 2 diabetes mellitus. Health effects of lifestyle interventions in general, and exercise training in particular, seem to be explained by more than simply decreasing body weight. Indeed, the effects of exercise training on insulin sensitivity are multifactorial since exercise training:

- Improves specific adipose tissue depots, such as visceral adipose tissue
- Enhances the inflammasome (ie. circulating cytokines, molecular pathways in skeletal muscle and adipose tissue)
- May alter the gut microbiome.

In this thesis, we have explored various mechanisms that contribute to exercise-induced increases in insulin sensitivity in obese humans. In this final chapter, we will discuss and integrate the results of this thesis with scientific literature in order to examine the concept why *'exercise is medicine'*, and how to integrate the novel information and insights into clinical practice.

Prevention of type 2 diabetes mellitus in obesity – increasing fitness or decreasing fatness?

When improving metabolic health, two concepts play a central role: fitness and fatness. High fitness levels and low fatness are associated with health benefits as they reduce cardiovascular and metabolic risk. Their mutual relationship, however, is matter for ongoing debate. Consensus about which of the two is most important to improve general health is lacking in scientific literature. Below, a definition of both factors is given to provide better insight into the role of both fitness and fatness.

Fitness – different domains of health

Fitness is an important measure to assess a person's health. Fitness is defined as the condition of being physically fit and healthy, comprising different domains such as mental acuity, cardiorespiratory endurance and muscular strength. More specifically, both in this thesis and in the scientific literature, 'fitness' refers to cardiorespiratory fitness (CRF); the capacity of the circulatory and respiratory systems to warrant sufficient oxygen supply to the mitochondria in skeletal muscle during sustained (exhaustive) exercise. The gold standard to measure CRF is by performance of a maximal exercise test, during which the peak oxygen uptake level (VO2max)can be assessed. The duration and need for specialized equipment makes it difficult to assess CRF in the consulting room of a medical doctor, despite the current advise of the American Heart Association to implement this as a routine clinical test.[10]

CRF is a very strong and independent marker of a person's health, with better CRF being associated with decreased all-cause mortality and morbidity across all ages and both sexes.[10-13] A decline in CRF results in health risks, both cardiovascular and metabolic (*ie*. cardiometabolic): it is associated with an increased risk of developing

type 2 diabetes [12,13] as well as cardiovascular events and heart failure.[10,11] This is specifically relevant for individuals who suffer from overweight or obesity since these also serve as important risk factors of cardiometabolic health. Ideally, life style interventions should therefore aim for an increase in CRF as well as a decrease in the amount of body weight.

Fatness – different shapes and sizes

The second concept to be taken into account when assessing the success of lifestyle interventions is 'fatness'. 'Fatness' is most commonly measured by body weight or Body Mass Index (BMI), which allows routinely assessment during medical consultation. Although BMI is a known risk factor for cardiovascular and metabolic disease, BMI as a measure of fatness has a number of limitations. First, BMI does not distinguish between fat mass and lean body mass, making it possible that a muscular, fit, elite athlete is classified as obese if the BMI is >30 kg/m2. Second, BMI does not take fat distribution into account.[14,15] The way adipose tissue is distributed across different depots in the human body is known to be a stronger marker for cardiovascular and metabolic risk than the quantity of adipose tissue *per se*. More specifically, central or abdominal obesity as a measurement for visceral adipose tissue (VAT) outperforms BMI in the prediction for relative risk for morbidity and mortality.[16] Although the gold standard to evaluate body fat distribution (the use of radiographic scans, such as a CT or MRI scan)[17] is not feasible during daily practice, measuring waist circumference (WC) or calculating the waist-to-hip-ratio (WHR) are both useful, practical, and easy-to-perform measurements to assess a persons' (abdominal) fat distribution. Nonetheless, also these markers have their limitations based on depot-specific functions of adipose tissue. For instance, subcutaneous adipose tissue (SCAT) can be distinguished from VAT. VAT is localized within the intra-abdominal cavity and surrounds the abdominal organs. An excess of VAT is strongly correlated with all cause morbidity and mortality.[16,18,19] This relationship is not merely based on its quantitative abundance, but also relates to the endocrine effects linked to the presence of VAT. Excessive volumes of VAT are linked to the secretion of bioactive molecules (cytokines) that enter the blood flow and are able to impair function of organs that are pivotal for glucose homeostasis, such as the liver and skeletal muscle.[18,19] Specific measurements of VAT are challenging, since expensive and time-consuming imaging techniques are needed to accurately assess its quantity.[17] Unfortunately, measures of body weight, WC and WHR have been reported to be inaccurate in estimating VAT.[17] Taken together, evaluation of fatness comes in different 'shapes and sizes', and is far more than the result of your weighing scale or tapeline.

The fitness vs. fatness debate

In a landmark paper, published more than three decades ago, it became clear that the explanation for obesity being a risk factor for disease and mortality goes beyond the amount of 'fatness'.[11] In this prospective, longitudinal study (n=10224 men and n=3120 women, median follow-up 8 years) higher cardiorespiratory fitness was associated with lower all-cause mortality in both sexes, even when corrected for age, smoking, cholesterol, blood pressure, glucose level and BMI.[11] The observation that obesity *per se* may not be a risk factor is confirmed in a meta-analysis from 2014. This study compared the impact of BMI and CRF on all cause mortality (10 studies, 92986 participants) and found that 'unfit' individuals, regardless of BMI category, had an increased risk of all-cause mortality. In contrast, 'fit' individuals who were classified as overweight/obese had a similar mortality risk when compared to 'fit' individuals with normal weight.[20] Both studies suggest to put an emphasis on strategies that increase physical activity levels and thereby CRF, rather than focus on weight loss alone in humans with obesity. This data contributed to the notion of a 'metabolically healthy obese' (MHO) phenotype which is defined as being obese (BMI > 30 kg/m^2) in the absence of other cardiovascular/metabolic risk factors (e.g., hypertension, insulin resistance, dyslipidemia). A number of studies suggested that metabolically healthy obesity is still associated with an increased mortality risk when compared to normal weight individuals. However, these studies did not take CRF into account to understand the role of fitness in these observations. Especially in the metabolically healthy obese group, CRF seems to be higher when compared to other obese individuals that already suffer from the complications of obesity.[21] These data suggest a protective role of a high CRF against complications of obesity. Furthermore, when taking CRF into account and considering other confounders, it has been established that individuals with metabolically healthy obesity have a similar life expectancy as normal weight individuals.

Taken together, there is a large body of evidence, involving cross-sectional and longitudinal data, that suggests that a low CRF is a more accurate and powerful risk factor for all cause mortality and morbidity than BMI or body weight. First, it may therefore be pivotal to examine interventions aimed at increasing fitness, rather than aiming at decreasing fatness (alone). Second, this raises questions on the reasons why a high CRF and/or performing exercise training, even in the presence of overweight or obesity, exerts its positive health effects.

Exercise training: increasing fitness but also influencing fatness

A robust body of evidence demonstrates that being physically active, resulting in higher CRF, is associated with longevity by influencing cardiovascular and

metabolic risk factors.[4,11,22] For example, aerobic exercise training aims at improving CRF, and is the most powerful tool to do so. In adults, 16-20 weeks of training 2-3 times a week will improve CRF by 16.3%.[23] The benefits of exercise training go beyond improvement in CRF. Indeed, regular aerobic exercise training exerts other effects on the body, especially in obese humans. Exercise training is also able to alter fat mass, by inducing a negative energy balance as a result of increased energy expenditure. The potential effects of exercise on fatness, take place on three levels: 1.quantity adipose tissue, 2.quality adipose tissue, and 3. other processes. In this thesis, the effects of exercise training on all of these levels were examined and results will be discussed below.

Exercise training and quantity of adipose tissue: move your muscles vs constrain your calories

A number of meta-analyses demonstrates that aerobic exercise training is a less powerful tool to decrease bodyweight than a hypocaloric diet.[24,25] However, it would be more relevant to examine exercise-induced effects on relevant adipose tissue depots, such as visceral AT (VAT), since VAT is more strongly correlated with metabolic and cardiovascular health risks than body weight.[16] In *Chapter 2* we examined the effects of exercise training *versus* hypocaloric diet on visceral adipose tissue (VAT). With use of a meta-analysis we demonstrated that hypocaloric diet induces more weight loss, but aerobic exercise training is more strongly correlated with a decrease in VAT. Our data demonstrated that exercise training causes a specific reduction in VAT, an effect that occurs largely independent of weight loss. This suggests that aerobic exercise training is a more powerful tool to reduce visceral adipose tissue than is caloric restriction.

This meta-analysis contributes to the notion that merely measuring body weight when evaluating the successfulness of a life style intervention, may lead to an underestimation of the amount of VAT that is lost. This notion is supported by data from others: in a longitudinal study in Japan with a follow-up time of 50 months, investigators analyzed the effects of lifestyle interventions on both body weight and VAT. Changes in VAT were more strongly correlated to improvements in cardiometabolic risk factors than changes in body weight. Even in the absence of weight loss, an improvement in VAT and risk factors was observed. Furthermore, individuals with the highest amount of VAT at baseline (regardless of their BMI) experienced the greatest benefit of an exercise training program in terms of losing VAT mass and improving risk factors.[26]

The results of our meta-analysis, together with a high number of prospective studies such as the Japanese cohort, has resulted in a high impact position paper of the International Atherosclerosis Society and International Chair on Cardiometabolic Risk Working Group on Visceral Obesity.[27] This position paper highlighted the urgent need to: 1) quantitatively measure VAT in individuals with cardiometabolic health risks, and 2) implement exercise interventions as the most powerful tool to reduce VAT. Interestingly, the authors of this paper examined dose-response relationships between amount and intensity of exercise and its effects on VAT by reviewing the available randomized controlled trials, but were unable to find a clear relationship.[27] This has an important practical implication, as this observation suggests that physically inactive, obese individuals can already loose significant amounts of VAT by starting to exercise at low intensity and low frequency. This further emphasizes the importance of implementing exercise as a 'first choice' prescription in the worldwide battle against the obesity pandemic and associated cardiometabolic risk factors.

The different effects of exercise training and caloric restriction on VAT suggests the presence of physiological mechanisms to cause this difference. The physiologic response might relate to the effects of exercise training on the *quality* rather than the *quantity* of adipose tissue. In a landmark paper by Wedel-Neergaard*et al.,* it was hypothesized that pro-inflammatory cytokines play a role in exercise induced reductions of VAT. They examined whether interleukin-6 (IL-6), a cytokine secreted by adipose tissue that regulates energy metabolism and that is increased in obese individuals and rises following exercise, is a mediator in the effects of exercise training. In a prospective study, exercise training or control was combined with IL-6 receptor blockade in centrally obese individuals. Interestingly, 12 weeks of exercise training successfully decreased VAT mass when compared to control. However, blocking IL-6 signaling abolished the effects of exercise training on VAT, demonstrating that IL-6 signaling plays a pivotal role in the effects of exercise on VAT.[28] This observation contributes to the paradigm that exercise training is able to improve the quality of adipose tissue, *i.e.* its secretory capacity of cytokines which play a central role in organ-cross talk and improving cardiometabolic health.

The effects of exercise training on quality of adipose tissue: circulating cytokines and White adipose tissue (WAT) gene expression

In contrast to the old dogma that focussed on the 'quantity' of fat mass, in the past decade studies have revealed the importance of adipose tissue 'quality'. Adipose tissue serves as an endocrine organ, secreting numerous factors that influence processes throughout the body.[29] Obesity is characterized by a chronic, low-level,

pro-inflammatory milieu, in which adipose tissue has been shown to secrete pro-inflammatory cytokines, that enter the circulation and influence glucose homeostasis in other organs, thereby contributing to the pathogenesis of insulin resistance.[29-31] It has been postulated that exercise training might beneficially affect the production and secretion of cytokines in adipose tissue. This effect of exercise training may result in an improvement in insulin resistance. It is therefore somewhat counterintuitive that an acute bout of exercise has been shown to cause an increase in pro-inflammatory cytokines as it acts as a stressor to the body's immune status.[32,33] In this thesis we examined effects of both acute and chronic exercise on cytokines in different sites in the body. After a brief summary of our findings (per chapter), the implications will be discussed below.

First, in *chapter 3* we examined the impact of acute exercise bouts of repeated prolonged walking on circulating cytokine responses in both overweight and lean individuals, i.e., all participants of the Nijmegen 4 Day Marches. After the first bout of exercise (*i.e.,* the first walking day), all cytokines showed a significant increase compared to baseline. From the second exercise bout onwards, most cytokines returned to baseline values, except for IL-6 that remained elevated compared to baseline. Interestingly, the return to baseline levels occurred slower in the overweight cohort when compared to lean participants. This suggests the presence of early adaptive responses in which the body alters its exercise induced inflammatory response, which occur delayed in overweight individuals.

In *chapter 4*, we examined the effects of exercise training on circulating cytokines, as well as gene expression levels of cytokines in skeletal muscle. Next to adipose tissue, skeletal muscle is able to secrete cytokines to the circulation. Our data showed that in response to a 6-month aerobic exercise training program, significant improvements in insulin sensitivity (M-value) occurred in lean and obese women. However, this improvement in insulin sensitivity was not accompanied by a change in circulating levels or RNA-expression values in skeletal muscle of cytokines. Similarly, a correlation between M-value and cytokine levels was absent. The absence of a response in circulating cytokines and expression levels in skeletal muscle in *chapter 4*, contributes to the hypothesis that exercise induced adaptations might not occur in skeletal muscle alone, but also in other tissues, such as adipose tissue, another source of (exercise induced) cytokines.

Therefore, in *chapter 5* we examined changes in gene expression levels in subcutaneous WAT in response to an 8-week aerobic exercise intervention in obese individuals. In this study, we further examined differences in men versus women.

~12% of known genes were altered after the exercise intervention, in both men and women. None of these genes encoded for known cytokines. In the pathway analysis, no overlap between men and women was found. Interestingly, when examining individual transcripts, a significant and robust alteration was found in a large number of small non-coding RNAs which we will discuss below.

Exercise and cytokines: an ambiguous relationship

The exact role of cytokines in exercise-induced responses is matter for ongoing debate. Data from exercise training studies is heterogenous. In a recent review, a total of 90 studies that examined the effect of acute exercise on levels of circulating cytokines were included. After an acute bout of exercise, an increase in individual cytokines was reported in 46% of studies, whereas 19% reported a decrease and 35% reported no change. After exercise training, the percentage of studies that showed a decrease in cytokines was higher (46%), but a comparable number of studies demonstrated no significant change in circulating cytokines after exercise training (42%).[34](Figure 1) The authors reported a potential confounding role for the degree of intensity, frequency and duration of the exercise intervention that might influence the magnitude of response in cytokines levels. The exact influence of these factors was not reported and remains to be studied, but differences in these characteristics of the exercise bout or training could explain the heterogenous results between studies and the lack of a rise in cytokines in response to a 6-month exercise intervention we found in the study in chapter 4. Interestingly, these data from Saeidi *et al.* demonstrate that the ratio between an increasing *versus* decreasing effect on cytokines is different between acute *versus* training exercise studies. Acute exercise results in an increase in cytokines rather than a decrease (ratio ~3:1) whilst exercise training much more frequently causes a decrease than an increase in cytokines (ratio ~3:1). This further underlines the importance of distinguishing between the effects of acute versus chronic (ie. training exercise) when physiologically examining exercise induced changes in cytokines.

Of all known cytokines that play a role in exercise-induced physiological responses, IL-6 (Interleukin-6) has been most extensively studied. IL-6 functions as an 'exercise factor'; a signalling marker that initiates and maintains the adaptive response of the body when exposed to an exercise stimulus, and was already identified in 2003 as a potential candidate for this role.[35] At that time, it was believed that IL-6 exerts a pro-inflammatory role, being somewhat contra-intuitive since exercise is believed to decrease inflammation. More recently, it has become clear that IL-6 has anti-inflammatory effects in the context of acute exercise, since IL-6 induces other anti-inflammatory cytokines (IL1-Ra and IL-10) and inhibits pro-inflammatory TNF-α.

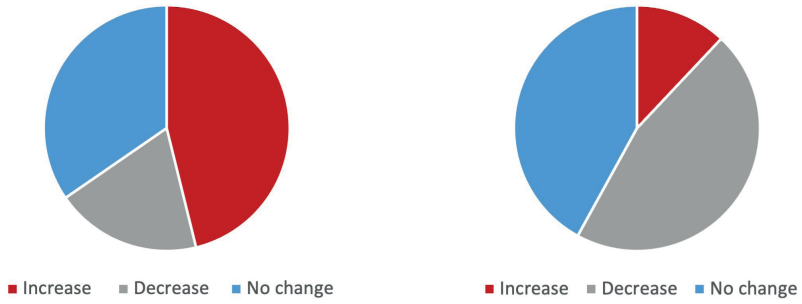

Figure 1. Results of studies examining levels of circulating cytokines in response to A acute exercise and B exercise training. Figure adapted from Saeidi *et al*.[34]

Its function and effects seem to be directed towards a protective immunological response against the stressor of an acute exercise bout.[36] This is supported by our data of *chapter 3*, in which IL-6 is the only cytokine that remains elevated, whilst other cytokines show a blunted response to an extended exercise bout on consecutive days. Next to its immunomodulating role in response to exercise, IL-6 has an important role in glucose metabolism during exercise. It is upregulated in response to low glycogen levels and subsequently increases glucose production in the liver and increases lipolysis in adipose tissue, thereby restoring glucose homeostasis. In the skeletal muscles, IL-6 enhances glucose transporter 4 (GLUT4 expression), thereby contributing to maintain glucose handling in skeletal muscle during exercise.[37] IL-6 therefore may be a potential mediator of beneficial effects on glucose metabolism caused by both acute exercise and exercise training. However, in both our training studies (*chapters 4 and 5*) we failed to identify changes in IL-6 in the circulation and expression levels in both skeletal muscle and subcutaneous WAT. This is, at least in part, in accordance with previous work reporting varying results in cytokine response in response to exercise.[34,38]

The absent of a clear, uniform effect of exercise training on IL-6 and other cytokines might be partly due to methodological reasons: it remains a challenge to establish a window in which cytokine levels can be measured in resting metabolic conditions, completely eliminating the influence of the last exercise bout. Furthermore, as is the case with all cytokines: their presence in the circulation is of a transient nature and each cytokine has another pattern of rise and fall in the circulation in response to stressors, which is demonstrated by data from us and others.

Interestingly, the response of dietary (ie. caloric restriction) interventions shows similar heterogenous results as exercise intervention studies. In an extensive review, Klimcakova *et al.* concluded that significant weight loss (ie. 5-10%) is

needed to cause a significant change in cytokines and subsequent improvement in metabolic health.[39] In contrast to this observation, significant changes in cytokines in the absence of weight loss have been described.[40] This observation contributes to the notion that exercise training is able to alter cytokine secretion irrespective of altering the quantity of adipose tissue.

Taken together, our data and those of others have failed to establish a clear role for cytokines as mediators for lifestyle-related improvements in insulin sensitivity, when measured in the circulation or by examining gene expression levels. However, there is little debate of the functional role of cytokines in decreasing inflammation in response to exercise training based on mechanistic work. Data from interleukin-blockade studies allows for a better understanding of the mechanistic implications of altered cytokine release in response to exercise than merely measuring cytokines in tissue or in the bloodstream. This is impressively demonstrated by IL-6 blockade studies, such as the study by Wedel-Neergaard *et al.* that demonstrated that the presence of IL-6 is pivotal to cause a decline in VAT with exercise training in humans.[28] Indeed, in very recent years an increasing number of studies in obese humans using IL-6 receptor blockade by drugs (tocilizumab) in combination with both acute and chronic exercise has been published.[41,42] Trinh *et al.* demonstrated that IL-6 receptor blockade changes fat turn over, resulting in fat storage rather than mobilization in response to both acute and chronic exercise, suggesting a beneficial (and crucial role) for IL-6 in fat metabolism in response to exercise.[41] Wueest *et al.* examined the effect of IL-6 receptor blockade *versus* placebo on other cytokines in response to exercise training and found an enhancing effect of IL-6 receptor blockade on the increase of beneficial adiponectin in response to training, which was correlated to a decrease in insulin resistance.[42] Again, these two studies together with the landmark paper from Wedel-Neergaard further contribute to the notion that exercise and cytokines show an ambiguous relationship, with some studies demonstrating beneficial effects[28,41] in contrast to others who demonstrate detrimental effects of IL-6 in response to exercise.[42] Furthermore, the use of pharmacological IL-blockade has some practical (and ethical) considerations making the execution of these studies in humans more challenging. Examining direct effects of exercise on cytokine secreting organs, such as skeletal muscle and adipose tissue therefore remains relevant. Instead of examining circulating levels of cytokines, gene expression analysis provides the opportunity to examine the source of endogenous cytokines at the site of the secretory organs.

Small nucleolar RNAs; a novel exercise factor?

Gene expression analysis of WAT offers a chance to exploratively examine adaptive pathways and changes in individual gene expression levels in response to exercise training. Potentially, new exercise-mediated factors can be discovered. In **chapter 5** of this thesis, an exercise intervention of 8 weeks of aerobic exercise training in obese men and women was performed. A significant increase in insulin sensitivity and cardiorespiratory fitness was found, in the presence of modest weight loss. When comparing altered gene expression levels between men and women, a remarkable large overlap was found in upregulated small nucleolar RNAs (snoRNAs) and small Cajal body RNAs (scaRNAs) in response to exercise training. This was accompanied by a significant change in three pathways related to RNA metabolism. SnoRNAs and scaRNAs genes encode for transcripts that are essential for ribosomal function. They act as housekeeping molecules that play a role in ribosomal maturation and protein translation within the nucleolus.[43] In recent years, it was first described that snoRNAs play a role in glucose metabolism and obesity. First, a genetic disorder called the Prader-Willi syndrome, which is characterized by morbid obesity and insulin resistance, is characterized by a deletion of chromosome 15 on which the units coding for SNORD115 and SNORD116 are located.[44,45] SNORD116 knockout mice (a mouse model for Prader-Willi syndrome) display defects in pancreatic island cells leading to metabolic disturbances in glucose handling.[45] This suggests that a lack in these specific snoRNAs is associated with metabolic disturbances that might be counteracted when these snoRNAs are increased, suggesting a beneficial role for snoRNAs on glucose metabolism. However, other studies examining other snoRNAs demonstrated that knock-out mice for four snoRNAs (SNORD32a, -33, -34 and -35) display improved insulin secretion and glucose tolerance, suggesting a damaging role for these types of snoRNAs.[46] The exact role of snoRNAs in glucose metabolism therefore remains unclear.

The effect of exercise on snoRNAs and scaRNAs has scarcely been investigated. In accordance with our study, one exercise study in sedentary lean men (n=47) found a number of 55 genes encoding for snoRNAs and 9 genes encoding for scaRNAs in adipose tissue being significantly upregulated after a 6 month exercise intervention.[47] To date no other studies examined the impact of exercise training on snoRNAs. A few studies examined the effect of an acute exercise bout, demonstrated increases in circulating snoRNAs. For example, one study demonstrated SNORD114.1 to be upregulated in plasma in response to an exhaustive exercise bout in elite athletes. In this study, six other snoRNAs were examined and showed no change.[48] Another study examined gene expression levels of peripheral white blood cells in response to an exhaustive exercise bout in

well trained athletes and found 57 genes (18% of all upregulated genes) encoding for snoRNAs and scaRNAs being upregulated.[49] In accordance with our data, this at least suggests that both acute exercise and exercise training result in a rise in snoRNAs and scaRNAs, which are – at least in part - derived from adipose tissue and can be measured in the circulation. Their specific role and function, especially in relation to obesity and glucose metabolism, should be further examined in humans. It can be hypothesized, that small nucleolar RNAs serve as exercise factors, mitigating the body's response to an exercise stimulus. Our study is one of the first that demonstrated that snoRNAs are upregulated in WAT in response to exercise training, in the presence of modest but significant weight loss. This observation further contributes to the notion that exercise is able to significantly improve the quality of adipose tissue affecting cardiometabolic health. The exact physiological implications of this observation and the potential role of snoRNAs in improving metabolic health by exercise should be subject of future research in humans with obesity.

The effects of exercise training on gut microbiome - Governing your gut

Another route through which lifestyle interventions improves cardiometabolic health in obese humans is by influencing the gut microbiome. In recent years, the gut microbiome has received increasing scientific attention since the gut microbiome plays a pivotal role in maintaining the energy homeostasis in the body. Humans with obesity demonstrate an imbalance in gut microbiota, which has been associated with decreased insulin sensitivity.[50,51] The effect of lifestyle interventions on glucose handling, therefore, may be in part related through changing the gut microbiome. Therefore, in **chapter 6** of this thesis, the effect of an 8 week exercise training intervention was examined on the gut microbiota and insulin sensitivity in obese humans. Despite demonstrating marked improvements in cardiometabolic health (insulin sensitivity and cardiorespiratory fitness levels), we found no effect of training on gut microbiota diversity and only modest increases in abundance of 3 genera (*Ruminucoccus gauvreaii*, *Lachnospiraceae FCS020 group* and *Anaerostipes*).

Data from prospective studies examining effects of exercise training on the gut microbiota of obese humans remain scarce and show heterogeneous results (table 1). For example, two studies reported significant changes in diversity of the gut microbiome compared to three studies that demonstrated no change after a training intervention in obese humans.[52] Interestingly, in these 5 studies with conflicting results, exercise training duration, frequency and intensity was comparable to our study. Also the effects of exercise training on gut microbiota composition show a large variety between studies, resulting in different microbiota

species/genera being altered after exercise training, whilst some studies report no change (table 1).

Table 1. Results of supervised exercise intervention studies in obese humans and effects on gut microbiota (in part adopted from Aya *et al.*[52]) and further updated in 2024

Reference	Subjects	Study Design	Results - Diversity	Results - Composition
Allen *et al.*[53]	Obese humans (n=41)	Six weeks aerobic exercise, 30-60 min, 60-75% HR	NR	↓Faecalibacterium spp. ↑Bacteroides ↑Colinsella
Munukka *et al.*[54]	Sedentary women, BMI > 27.5 kg/ m2 (n=17)	Six weeks aerobic exercise	NR	↑Dorea ↑Anearofilum ↑Akkermansia ↓Porphyromonadaceae ↓Odoribacter ↓Desulfovibrionaceae ↓Enterobacteriaeceae
Cronin *et al.*[55]	Sedentary, overweight/ obese humans (n=25 in exercise only arm)	8 weeks aerobic exercise, moderate intensity (BORG 5-7/10)	Increase in α diversity	NR
Kern *et al.*[56]	Overweight/ obese humans (n=88)	Different exercise groups with one supervised exercise group	Increase in α diversity	NR
Rettedal *et al.*[57]	Overweight men (n=15)	HIIT, 9 session	No differences in α-, and β-diversity	No change
Liu et al.[58]	Pre-diabetic men (n=39)	12 weeks supervised aerobic exercise	No differences in α-, and β-diversity	No change
Verheggen et al.[59]	Overweight/ obese individuals (n=14)	8 weeks aerobic exercise, 60 min, 65-85% VO2max	No differences in α-, and β-diversity	↑Ruminucoccus gauvreaii ↑Lachnospiraceae FCS020 group ↑Anaerostipes

Abbrevations: NR = not reported

Some of these heterogeneous results may be related to other factors, such as lifelong training status and dietary regimens. Indeed, previous work suggested that these factors influence the adaptability of the gut microbiome to an exercise stimulus.[60] Another explanation for the mixed results, is that the gut microbiome in the obesity state might be more rigid in displaying change after exercise training. This was underlined by a study by Allen et al. that demonstrated that exercise training-induced changes in gut microbiota composition occurred to a larger extent in lean individuals than in those with obesity, when exposed to the exact

same exercise intervention.[53] Perhaps a longer or more intense period of exercise training is required in the obese state, explaining part of the mixed results of previous studies.

When examining the effects of exercise training on gut microbiome, other clinical relevant endpoints need to be taken into account. Since the aim of altering gut microbiome by lifestyle interventions essentially is to alter metabolic health (ie. reduce chronic inflammation, improve insulin sensitivity), studies examining the gut microbiome should also correlate microbiota findings with markers for metabolic health. More specifically, there is no ''normal value" for the gut microbiome as is the case for markers of insulin sensitivity or its counterpart; insulin resistance (such as M-value, HOMA-IR or fasting glucose levels). Since the gut microbiota shows large interindividual variation, which is dependent on numerous factors that can be influenced throughout the life span, future gut microbiota studies should always relate their findings to clinically relevant endpoints to assess the meaning of the observed change in gut microbiota composition and diversity. Taken together, despite its sound effects on insulin sensitivity, further studies are needed to better understand if and how exercise training alters an obese person's gut microbiome (and whether this then translates to the improved insulin sensitivity following exercise training).

Conclusion and future perspectives – Exercise is Medicine: prescription needed?

In this thesis, a number of findings were reported that contribute to the scientific evidence that exercise training is a powerful tool to improve cardiometabolic health in overweight and obese humans. We demonstrated that the success of an exercise intervention cannot be simply derived from its effects on the display of your weighing scale. Next to its well-established and robust positive effects on cardiorespiratory fitness levels, exercise training has also important effect on adipose tissue quantity and quality.

In this thesis, we demonstrated that exercise training results in a larger reduction in visceral adipose tissue than caloric restriction does (Chapter 2). Supported by the scientific evidence as presented in this Chapter, the importance of targeting VAT to improve metabolic health in obese individuals has become widely established, resulting in official guidelines that suggest measuring VAT as a standard marker for health risk and to incorporate exercise training in strategies to improve VAT in patients.

In addition to effect on the quantity of fat, studies have also explored how exercise training alters the quality of fat. However, robust results from exercise training studies are harder to find. Despite these heterogenous results, our thesis contributed to the scientific evidence for beneficial health effects of exercise. In contrast to but also in accordance with other studies, data from our exercise (training) studies:

- Demonstrated a transient rise in cytokines in response to repeated prolonged exercise, suggesting quick adaptability of the body's cytokine responses. These rapid adaptive responses may explain why chronic exercise has anti-inflammatory effects rather than the pro-inflammatory effects of acute exercise
- Was unable to show effects of exercise training (6 months and 8 weeks) on circulating cytokines and gene expression levels of cytokines in skeletal muscle of obese humans
- Revealed a potential new group of exercise factors, derived from adipose tissue: snoRNAs and scaRNAs

This thesis further contributed to the notion that Exercise is Medicine. Ideally, a prescription for the exact amount, intensity and frequency of exercise training that is needed to target different domains of cardiometabolic health, fitness and fatness in humans with obesity could be derived from these data. This thesis shows however that a one size fits all – recipe for exercise training does not exist. For decreasing visceral fat mass and improving cardiorespiratory fitness, aerobic exercise training has been established being a suitable 'recipe'. However, exercise training is reported to have heterogeneous effects on changing cytokine or gut microbiome profiles. Therefore, the specific recipe for an exercise training remains subject for further research. The study in chapter 6 is a good example of how gene expression analyses allow novel exercise factors to be discovered that might play a pivotal role in further illuminating the body's adaptability to exercise stimuli. Further research into these novel factors seems a logical next step.

Taken together, this thesis emphasizes the importance of exercise training studies examining physiological responses in humans in vivo. However, we still do not fully understand *why* exercise training leads to the protective effects. To better understand this area of research, it is important to select the right outcome variables, including measurement of visceral fat as well as a marker for cardiorespiratory fitness. This seems even relevant when another domain of metabolic health (such as cytokines, inflammasome, microbiome) serves as the primary outcome, to learn more about the relationship between them.

Last but not least, when counseling your overweight/obese patient as a medical doctor it is of utmost importance to look beyond the weighing scale. Measurements of quality of adipose tissue, such as visceral adiposity as well as markers for cardiorespiratory fitness, need to find their way into the consulting room and should be targets for assessing the successfulness of a lifestyle intervention. Overall, large steps still are required to incorporate the increased attention for and emphasis on prevention of disease, that is part of international and national health guidelines, into daily medical practice. Rather than prescribing a recipe, asking your patient about his or her physical activity habits and comparing them to the 'Norm Gezond Bewegen'[61] is a feasible first step for every MD to provide insight and advice regarding the importance of exercising for almost every patient.

REFERENCES

1. Gao F, Zheng KI, Wang X-B, Sun Q-F, Pan K-H, Wang T-Y, Chen Y-P, Targher G, Byrne CD, George J. Obesity is a risk factor for greater COVID-19 severity. *Diabetes care*. 2020;43:e72-e74.
2. Sattar N, McInnes IB, McMurray JJ. Obesity is a risk factor for severe COVID-19 infection: multiple potential mechanisms. *Circulation*. 2020;142:4-6.
3. Kompaniyets L GA, Belay B, et al. Body Mass Index and Risk for COVID-19–Related Hospitalization, Intensive Care Unit Admission, Invasive Mechanical Ventilation, and Death — United States, March–December 2020. *MMWR Morb Mortal Wkly Rep*. 2021;70:355-361.
4. Blair SN, Brodney S. Effects of physical inactivity and obesity on morbidity and mortality: current evidence and research issues. *Medicine and science in sports and exercise*. 1999;31:S646-662.
5. Katzmarzyk P, Janssen I, Ardern C. Physical inactivity, excess adiposity and premature mortality. *Obesity reviews*. 2003;4:257-290.
6. Deschasaux-Tanguy M, Druesne-Pecollo N, Esseddik Y, de Edelenyi FS, Allès B, Andreeva VA, Baudry J, Charreire H, Deschamps V, Egnell M, et al. Diet and physical activity during the coronavirus disease 2019 (COVID-19) lockdown (March-May 2020): results from the French NutriNet-Santé cohort study. *Am J Clin Nutr*. 2021;113:924-938. doi: 10.1093/ajcn/nqaa336
7. Ruissen MM, Regeer H, Landstra CP, Schroijen M, Jazet I, Nijhoff MF, Pijl H, Ballieux B, Dekkers O, Huisman SD, et al. Increased stress, weight gain and less exercise in relation to glycemic control in people with type 1 and type 2 diabetes during the COVID-19 pandemic. *BMJ Open Diabetes Res Care*. 2021;9. doi: 10.1136/bmjdrc-2020-002035
8. Nielen MP, R.; Korevaar, J. Diabetes mellitus in Nelderland. Prevalentie en incidentie: heden verleden en toekomst. *Nivel*.
9. Organization WH. Diabetes Key Facts. 2022.
10. Ross R, Blair SN, Arena R, Church TS, Després JP, Franklin BA, Haskell WL, Kaminsky LA, Levine BD, Lavie CJ, et al. Importance of Assessing Cardiorespiratory Fitness in Clinical Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement From the American Heart Association. *Circulation*. 2016;134:e653-e699. doi: 10.1161/cir.0000000000000461
11. Blair SN, Kohl HW, 3rd, Paffenbarger RS, Jr., Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality. A prospective study of healthy men and women. *Jama*. 1989;262:2395-2401. doi: 10.1001/jama.262.17.2395
12. Katzmarzyk PT, Church TS, Janssen I, Ross R, Blair SN. Metabolic syndrome, obesity, and mortality: impact of cardiorespiratory fitness. *Diabetes Care*. 2005;28:391-397. doi: 10.2337/diacare.28.2.391
13. Sui X, Hooker SP, Lee IM, Church TS, Colabianchi N, Lee CD, Blair SN. A prospective study of cardiorespiratory fitness and risk of type 2 diabetes in women. *Diabetes Care*. 2008;31:550-555. doi: 10.2337/dc07-1870
14. Nevill AM, Stewart AD, Olds T, Holder R. Relationship between adiposity and body size reveals limitations of BMI. *Am J Phys Anthropol*. 2006;129:151-156. doi: 10.1002/ajpa.20262
15. Romero-Corral A, Somers VK, Sierra-Johnson J, Thomas RJ, Collazo-Clavell ML, Korinek J, Allison TG, Batsis JA, Sert-Kuniyoshi FH, Lopez-Jimenez F. Accuracy of body mass index in diagnosing obesity in the adult general population. *Int J Obes (Lond)*. 2008;32:959-966. doi: 10.1038/ijo.2008.11
16. Ross R, Bradshaw AJ. The future of obesity reduction: beyond weight loss. *Nat Rev Endocrinol*. 2009;5:319-325. doi: 10.1038/nrendo.2009.78

17. Shuster A, Patlas M, Pinthus JH, Mourtzakis M. The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. *Br J Radiol*. 2012;85:1-10. doi: 10.1259/bjr/38447238

18. Jensen MD. Role of body fat distribution and the metabolic complications of obesity. *J Clin Endocrinol Metab*. 2008;93:S57-63. doi: 10.1210/jc.2008-1585

19. Mathieu P, Poirier P, Pibarot P, Lemieux I, Després JP. Visceral obesity: the link among inflammation, hypertension, and cardiovascular disease. *Hypertension*. 2009;53:577-584. doi: 10.1161/hypertensionaha.108.110320

20. Barry VW, Baruth M, Beets MW, Durstine JL, Liu J, Blair SN. Fitness vs. fatness on all-cause mortality: a meta-analysis. *Prog Cardiovasc Dis*. 2014;56:382-390. doi: 10.1016/j.pcad.2013.09.002

21. Ortega FB, Cadenas-Sánchez C, Sui X, Blair SN, Lavie CJ. Role of Fitness in the Metabolically Healthy but Obese Phenotype: A Review and Update. *Prog Cardiovasc Dis*. 2015;58:76-86. doi: 10.1016/j.pcad.2015.05.001

22. Blair SN, Kohl HW, Barlow CE, Paffenbarger RS, Gibbons LW, Macera CA. Changes in physical fitness and all-cause mortality. *Jama*. 1995;273:1093-1098.

23. Huang G, Gibson CA, Tran ZV, Osness WH. Controlled endurance exercise training and VO2max changes in older adults: a meta-analysis. *Prev Cardiol*. 2005;8:217-225. doi: 10.1111/j.0197-3118.2005.04324.x

24. Franz MJ, VanWormer JJ, Crain AL, Boucher JL, Histon T, Caplan W, Bowman JD, Pronk NP. Weight-loss outcomes: a systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up. *J Am Diet Assoc*. 2007;107:1755-1767. doi: 10.1016/j.jada.2007.07.017

25. Miller WC, Koceja DM, Hamilton EJ. A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. *Int J Obes Relat Metab Disord*. 1997;21:941-947. doi: 10.1038/sj.ijo.0800499

26. Okauchi Y, Kishida K, Funahashi T, Noguchi M, Morita S, Ogawa T, Imagawa A, Nakamura T, Matsuzawa Y, Shimomura I. 4-year follow-up of cardiovascular events and changes in visceral fat accumulation after health promotion program in the Amagasaki Visceral Fat Study. *Atherosclerosis*. 2010;212:698-700. doi: 10.1016/j.atherosclerosis.2010.06.011

27. Neeland IJ, Ross R, Després JP, Matsuzawa Y, Yamashita S, Shai I, Seidell J, Magni P, Santos RD, Arsenault B, et al. Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. *Lancet Diabetes Endocrinol*. 2019;7:715-725. doi: 10.1016/s2213-8587(19)30084-1

28. Wedell-Neergaard AS, Lang Lehrskov L, Christensen RH, Legaard GE, Dorph E, Larsen MK, Launbo N, Fagerlind SR, Seide SK, Nymand S, et al. Exercise-Induced Changes in Visceral Adipose Tissue Mass Are Regulated by IL-6 Signaling: A Randomized Controlled Trial. *Cell metabolism*. 2019;29:844-855.e843. doi: 10.1016/j.cmet.2018.12.007

29. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab*. 2004;89:2548-2556. doi: 10.1210/jc.2004-0395

30. Eckardt K, Taube A, Eckel J. Obesity-associated insulin resistance in skeletal muscle: Role of lipid accumulation and physical inactivity. *Reviews in Endocrine and Metabolic Disorders*. 2011;12:163-172. doi: 10.1007/s11154-011-9168-2

31. Hardy OT, Czech MP, Corvera S. What causes the insulin resistance underlying obesity? *Current opinion in endocrinology, diabetes, and obesity*. 2012;19:81-87. doi: 10.1097/MED.0b013e3283514e13

32. Niemela M, Kangastupa P, Niemela O, Bloigu R, Juvonen T. Acute Changes in Inflammatory Biomarker Levels in Recreational Runners Participating in a Marathon or Half-Marathon. *Sports medicine - open*. 2016;2:21. doi: 10.1186/s40798-016-0045-0

33. Ostrowski K, Hermann C, Bangash A, Schjerling P, Nielsen JN, Pedersen BK. A trauma-like elevation of plasma cytokines in humans in response to treadmill running. *The Journal of physiology*. 1998;513 (Pt 3):889-894.

34. Saeidi A, Haghighi MM, Kolahdouzi S, Daraei A, Abderrahmane AB, Essop MF, Laher I, Hackney AC, Zouhal H. The effects of physical activity on adipokines in individuals with overweight/obesity across the lifespan: A narrative review. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. 2021;22:e13090. doi: 10.1111/obr.13090

35. Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, Febbraio M, Saltin B. Searching for the exercise factor: is IL-6 a candidate? *J Muscle Res Cell Motil*. 2003;24:113-119. doi: 10.1023/a:1026070911202

36. Docherty S, Harley R, McAuley JJ, Crowe LAN, Pedret C, Kirwan PD, Siebert S, Millar NL. The effect of exercise on cytokines: implications for musculoskeletal health: a narrative review. *BMC Sports Sci Med Rehabil*. 2022;14:5. doi: 10.1186/s13102-022-00397-2

37. Docherty S, Harley R, McAuley JJ, Crowe LAN, Pedret C, Kirwan PD, Siebert S, Millar NL. The effect of exercise on cytokines: implications for musculoskeletal health: a narrative review. *BMC Sports Science, Medicine and Rehabilitation*. 2022;14:5. doi: 10.1186/s13102-022-00397-2

38. Pal M, Febbraio MA, Whitham M. From cytokine to myokine: the emerging role of interleukin-6 in metabolic regulation. *Immunol Cell Biol*. 2014;92:331-339. doi: 10.1038/icb.2014.16

39. Klimcakova E, Kovacikova M, Stich V, Langin D. Adipokines and dietary interventions in human obesity. *Obes Rev*. 2010;11:446-456. doi: 10.1111/j.1467-789X.2009.00704.x

40. Koh Y, Park K-S. Responses of inflammatory cytokines following moderate intensity walking exercise in overweight or obese individuals. *J Exerc Rehabil*. 2017;13:472-476. doi: 10.12965/jer.1735066.533

41. Trinh B, Peletier M, Simonsen C, Plomgaard P, Karstoft K, Klarlund Pedersen B, van Hall G, Ellingsgaard H. Blocking endogenous IL-6 impairs mobilization of free fatty acids during rest and exercise in lean and obese men. *Cell Rep Med*. 2021;2:100396. doi: 10.1016/j.xcrm.2021.100396

42. Wueest S, Seelig E, Timper K, Lyngbaek MP, Karstoft K, Donath MY, Ellingsgaard H, Konrad D. IL-6 Receptor Blockade Increases Circulating Adiponectin Levels in People with Obesity: An Explanatory Analysis. *Metabolites*. 2021;11. doi: 10.3390/metabo11020079

43. Amri EZ, Scheideler M. Small non coding RNAs in adipocyte biology and obesity. *Mol Cell Endocrinol*. 2017;456:87-94. doi: 10.1016/j.mce.2017.04.009

44. Burnett LC, Hubner G, LeDuc CA, Morabito MV, Carli JFM, Leibel RL. Loss of the imprinted, non-coding Snord116 gene cluster in the interval deleted in the Prader Willi syndrome results in murine neuronal and endocrine pancreatic developmental phenotypes. *Human Molecular Genetics*. 2017;26:4606-4616. doi: 10.1093/hmg/ddx342

45. Jacovetti C, Bayazit MB, Regazzi R. Emerging Classes of Small Non-Coding RNAs With Potential Implications in Diabetes and Associated Metabolic Disorders. *Frontiers in Endocrinology*. 2021;12. doi: 10.3389/fendo.2021.670719

46. Lee J, Harris AN, Holley CL, Mahadevan J, Pyles KD, Lavagnino Z, Scherrer DE, Fujiwara H, Sidhu R, Zhang J, et al. Rpl13a small nucleolar RNAs regulate systemic glucose metabolism. *J Clin Invest*. 2016;126:4616-4625. doi: 10.1172/jci88069

47. Rönn T, Volkov P, Tornberg A, Elgzyri T, Hansson O, Eriksson KF, Groop L, Ling C. Extensive changes in the transcriptional profile of human adipose tissue including genes involved in oxidative phosphorylation after a 6-month exercise intervention. *Acta Physiol (Oxf)*. 2014;211:188-200. doi: 10.1111/apha.12247

48. Håkansson KEJ, Sollie O, Simons KH, Quax PHA, Jensen J, Nossent AY. Circulating Small Non-coding RNAs as Biomarkers for Recovery After Exhaustive or Repetitive Exercise. *Frontiers in Physiology*. 2018;9. doi: 10.3389/fphys.2018.01136

49. Sakharov DA, Maltseva DV, Riabenko EA, Shkurnikov MU, Northoff H, Tonevitsky AG, Grigoriev AI. Passing the anaerobic threshold is associated with substantial changes in the gene expression profile in white blood cells. *Eur J Appl Physiol*. 2012;112:963-972. doi: 10.1007/s00421-011-2048-3

50. Bermon S, Petriz B, Kajeniene A, Prestes J, Castell L, Franco OL. The microbiota: an exercise immunology perspective. *Exerc Immunol Rev*. 2015;21:70-79.

51. Kallus SJ, Brandt LJ. The intestinal microbiota and obesity. *J Clin Gastroenterol*. 2012;46:16-24. doi: 10.1097/MCG.0b013e31823711fd

52. Aya V, Flórez A, Perez L, Ramírez JD. Association between physical activity and changes in intestinal microbiota composition: A systematic review. *PloS one*. 2021;16:e0247039. doi: 10.1371/journal.pone.0247039

53. Allen JM, Mailing LJ, Niemiro GM, Moore R, Cook MD, White BA, Holscher HD, Woods JA. Exercise Alters Gut Microbiota Composition and Function in Lean and Obese Humans. *Med Sci Sports Exerc*. 2018;50:747-757. doi: 10.1249/mss.0000000000001495

54. Munukka E, Ahtiainen JP, Puigbó P, Jalkanen S, Pahkala K, Keskitalo A, Kujala UM, Pietilä S, Hollmén M, Elo L, et al. Six-Week Endurance Exercise Alters Gut Metagenome That Is not Reflected in Systemic Metabolism in Over-weight Women. *Front Microbiol*. 2018;9:2323. doi: 10.3389/fmicb.2018.02323

55. Cronin O, Barton W, Skuse P, Penney NC, Garcia-Perez I, Murphy EF, Woods T, Nugent H, Fanning A, Melgar S, et al. A Prospective Metagenomic and Metabolomic Analysis of the Impact of Exercise and/or Whey Protein Supplementation on the Gut Microbiome of Sedentary Adults. *mSystems*. 2018;3. doi: 10.1128/mSystems.00044-18

56. Kern T, Blond MB, Hansen TH, Rosenkilde M, Quist JS, Gram AS, Ekstrøm CT, Hansen T, Stallknecht B. Structured exercise alters the gut microbiota in humans with overweight and obesity-A randomized controlled trial. *Int J Obes (Lond)*. 2020;44:125-135. doi: 10.1038/s41366-019-0440-y

57. Rettedal EA, Cree JME, Adams SE, MacRae C, Skidmore PML, Cameron-Smith D, Gant N, Blenkiron C, Merry TL. Short-term high-intensity interval training exercise does not affect gut bacterial community diversity or composition of lean and overweight men. *Exp Physiol*. 2020;105:1268-1279. doi: 10.1113/ep088744

58. Liu Y, Wang Y, Ni Y, Cheung CKY, Lam KSL, Wang Y, Xia Z, Ye D, Guo J, Tse MA, et al. Gut Microbiome Fermentation Determines the Efficacy of Exercise for Diabetes Prevention. *Cell metabolism*. 2020;31:77-91.e75. doi: 10.1016/j.cmet.2019.11.001

59. Verheggen R, Konstanti P, Smidt H, Hermus A, Thijssen DHJ, Hopman MTE. Eight-week exercise training in humans with obesity: Marked improvements in insulin sensitivity and modest changes in gut microbiome. *Obesity (Silver Spring)*. 2021;29:1615-1624. doi: 10.1002/oby.23252

60. Aya V, Jimenez P, Muñoz E, Ramírez JD. Effects of exercise and physical activity on gut microbiota composition and function in older adults: a systematic review. *BMC Geriatr*. 2023;23:364. doi: 10.1186/s12877-023-04066-y

61. Gezondheidsraad. Kernadvies beweegrichtlijnen 2017. 2017.

Chapter 8

Nederlandse Samenvatting (Summary in Dutch)

Exercise is medicine

In 2025 heeft meer dan de helft van de Nederlanders overgewicht of obesitas. Deze mensen lopen een verhoogd risico op het ontwikkelen van verschillende ziekten, zoals type 2 diabetes mellitus (suikerziekte). Van alle mensen met diabetes mellitus, leidt 80% aan overgewicht/obesitas. Het aantal mensen dat diabetes mellitus heeft, neemt gestaag toe. Obesitas verhoogt het risico op diabetes mellitus door het optreden van insulineresistentie: het onvermogen van het lichaam om adequaat te reageren op de aanwezigheid van insuline op cellulair niveau. Een aantal mechanismen draagt hieraan bij:

1. Een overschot aan visceraal vetweefsel
2. Vetweefsel als endocrien orgaan: veranderingen in afgifte van cytokines
3. Veranderd microbioom van de darm

Exercise is medicine: bewegen is een succesvolle manier om gezondheid te bevorderen. Met name duurtraining is in staat cardiorespiratoire fitheid te vergroten en daarmee het risico op hart- vaatziekten en diabetes mellitus (*cardiometabole* risico) te verkleinen. Eerder onderzoek heeft ook aangetoond dat duurtraining insulinegevoeligheid vergroot. Hiermee wordt het risico op het ontwikkelen van diabetes mellitus verkleind, en kan het beginstadium zelfs genezen worden. Het doel van dit proefschrift is om bovenstaande mechanismen die bijdragen aan verbetering van insulinegevoeligheid door training in mensen met obesitas verder bloot te leggen. Hieronder volgt een samenvatting van de belangrijkste bevindingen van dit proefschrift.

Obesitas wordt gekenmerkt door een overschot aan vetweefsel. De lokalisatie van het vetweefsel in het lichaam heeft verschillende functies. Met name een overschot aan visceraal vet (“buikvet”), gelokaliseerd rondom de buikorganen, is sterk geassocieerd met een hoog risico op gezondheidscomplicaties in het algemeen en het ontwikkelen van diabetes mellitus in het bijzonder. Een aantal meta-analyses heeft aangetoond dat het volgen van een calorie-arm (hypocalorisch) dieet tot meer gewichtsverlies leidt dan het volgen van een trainingsinterventie. Omdat een overschot aan visceraal vet sterker geassocieerd is met het risico op metabole ziekten dan lichaamsgewicht alleen, is het echter ook relevant om de effecten van een trainingsinterventie *versus* een hypocalorisch dieet op de hoeveelheid visceraal vet te onderzoeken. In *hoofdstuk 2* hebben we aan de hand van een systematische review deze onderzoeksvraag onderzocht. In deze studie van de op dat moment beschikbare literatuur vonden wij dat een hypocalorisch dieet inderdaad leidt

tot meer gewichtsverlies. Echter, het volgen van een trainingsprogramma dat gebruik maakt van duurinspanning leidt tot een grotere reductie van visceraal vet. Bovendien vermindert het visceraal vet tijdens een trainingsinterventie *onafhankelijk* van de mate van gewichtsverlies.

Deze observatie heeft belangrijke implicaties voor de klinische praktijk. Om het effect van een leefstijlinterventie op gezondheid te evalueren, zou men verder moeten kijken dan naar de weegschaal alleen. Zeker het effect van een training kan onderschat worden wanneer alleen het gewicht voor en na de interventie vergeleken wordt. Onze meta-analyse heeft bijgedragen aan nieuwe internationale richtlijnen die stellen dat

1) het viscerale vet vaker gemeten moet worden bij mensen die cardiometabool risico lopen *en*

2) trainingsinterventies de meest krachtige tool zijn om visceraal vet te reduceren.

Zeer recent (juli 2023) is ook de Nederlandse Richtlijn Obesitas herzien waarin benadrukt wordt dat het meten van BMI alleen onvoldoende is om gezondheidsrisico's te meten en te monitoren. Het gebruik van de buikomvang neemt vanaf heden een belangrijke plaats in in de spreekkamers.

Het succes van leefstijlinterventies gaat verder dan de effecten op de kwantiteit van vetmassa. Ook de kwaliteit van vetweefsel kan veranderen door training. Vetweefsel is een endocrien orgaan, in staat om verschillende factoren (cytokines of adipokines) af te scheiden die via het bloed ook elders in het lichaam processen beïnvloeden. Bij mensen met obesitas is er sprake van een chronische, laaggradige ontsteking gekenmerkt door secretie van pro-inflammatoire cytokines door vetweefsel die effect hebben op de glucosehomeostase en bijdragen aan het ontstaan van insulineresistentie en uiteindelijk type 2 diabetes mellitus. Eerdere studies hebben aangetoond dat lichamelijke inspanning invloed heeft op de afgifte van (pro-)inflammatoire cytokines. In dit proefschrift (*hoofdstukken 3 t/m 5*) hebben we de effecten van zowel acute als chronische inspanning onderzocht op de secretie van cytokines in verschillende plekken in het lichaam.

In *hoofdstuk 3* onderzochten we de invloed van het lopen van een lange afstand op vier achtereenvolgende dagen (tijdens de Nijmeegse Vierdaagse) op cytokines in het bloed van mensen met en mensen zonder overgewicht/obesitas. Na de eerste wandeldag lieten alle cytokines een stijging zien. De daaropvolgende dagen

zagen we dat meeste cytokines na een langdurige wandeling niet meer stegen ten opzichte van het uitgangsniveau, uitgezonderd interleukine-6 (IL-6). Daarnaast vonden we dat de terugkeer naar baseline van de andere cytokines trager optrad in mensen met overgewicht/obesitas dan in mensen met een normaal gewicht. Dit veronderstelt de aanwezigheid van vroege adaptieve responsen van het lichaam op (herhaalde) blootstelling aan training, die trager optreden in mensen met overgewicht/obesitas.

In *hoofdstuk 4* hebben we de effecten van duurtraining gedurende een langere periode op cytokines in het bloed en in skeletspierweefsel onderzocht in vrouwen met en zonder overgewicht. Naast vetweefsel, is ook spierweefsel in staat om cytokines te secreteren aan de circulatie. Na 6 maanden training vond er een significante verbetering plaats in insulinegevoeligheid. Deze ging echter niet gepaard met veranderingen in circulerende cytokines, noch een verandering in secretie van deze cytokines in skeletspierweefsel.

Gezien de afwezigheid van een respons van cytokines in zowel skeletspierweefsel als in de circulatie op een succesvolle trainingsinterventie, richtten we ons in *hoofdstuk 5* op de effecten van een trainingsinterventie op genexpressie levels in vetweefsel. Hierin onderzochten we vetweefsel voorafgaand en na een duurtrainingsinterventie van in totaal 8 weken in mensen met obesitas en vergeleken vervolgens mannen met vrouwen. In dit hoofdstuk maakten we gebruik van genexpressie- en pathway analyse om processen bloot te leggen die bijdragen aan het verbeteren van insulinegevoeligheid. Na 8 weken training vonden we inderdaad een significante verbetering in cardiorespiratoire fitheid en insulinegevoeligheid, zowel bij vrouwen als bij mannen. Daarnaast vonden we dat ongeveer 12% van alle genen veranderde na 8 weken duurtraining. Deze genen codeerden niet voor bekende cytokines, in tegenstelling tot onze hypothese. In de pathway analyse vonden we geen overlap tussen mannen en vrouwen in veranderde pathways. Op het niveau van individuele transcripten, vonden we een significante upregulatie in een groot aantal kleine non-coderende RNAs (small nucleolar RNAs (snoRNAs) en small Cajal body RNAs (scaRNAs)) die bovendien zowel in mannen als vrouwen optrad. Dit ging samen met upregulatie van 3 pathways die gerelateerd zijn aan RNA metabolisme. Deze genen coderen voor transcripten die cruciaal zijn voor de ribosomale functie. Over hun rol in obesitas en glucosemetabolisme is weinig bekend en ook data over de invloed van training op sno- en scaRNAs is zeer zeldzaam. Twee andere studies in mensen toonden een upregulatie in genen die hiervoor coderen, net zoals wij vonden. Dit resulteert in de hypothese dat small nucleair RNAs wellicht een exercise factor zijn, die de respons van het menselijk lichaam op een trainingsinterventie reguleert.

Hun exacte rol in (trainingsgeïnduceerde veranderingen in) glucosemetabolisme zal in de toekomst verder moeten worden onderzocht.

In de afgelopen jaren is er steeds meer duidelijkheid gekomen over de rol van microbioom van de darm in het ontstaan van ziektes. In de menselijke darm wonen minsten 100 triljoen verschillende micro-organismen: het microbioom. Het microbioom van de darm speelt een significante rol in energiehomeostase omdat de opname van energie uit voedingsmiddelen hierdoor in grote mate beïnvloed wordt. Daarnaast speelt het microbioom van de darm een belangrijke rol in het menselijk immuunsysteem en de manier waarop het lichaam omgaat met infectie en inflammatie. Obesitas is geassocieerd met een veranderd microbioom. Ten opzichte van mensen met een normaal BMI, wordt bij mensen met overgewicht/ obesitas een minder gevarieerd microbioom gezien, dat geassocieerd is met het ontstaan van insulineresistentie. Data over de effecten van duurtraining op de samenstelling van het microbioom van de darm in mensen met obesitas is beperkt. Daarom onderzochten we in *hoofdstuk 6* de effecten van 8 weken durende duurtraining op de diversiteit en samenstelling van het microbioom van de darm in mensen met obesitas. Ondanks significante veranderingen in insulinegevoeligheid en cardiorespiratoire fitheid, vonden we geen effect op de diversiteit van het microbioom van de darm. We vonden een bescheiden stijging in de aanwezigheid van 3 genera (*Ruminucoccus gauvreaii*, *Lachnospiraceae FCS020 group* and *Anaerostipes*), waarvan alleen R. gauvreaii geassocieerd met insulinegevoelheidheid.

De resultaten van dit hoofdstuk dragen bij aan de beschikbare literatuur over de relatie tussen duurinspanning en het darm microbioom in mensen met obesitas die tot dusverre sterk heterogene data laat zien. Sommige studies rapporteren een duidelijke verandering in diversiteit en samenstelling van het microbioom na training, terwijl andere (waaronder de onze) slechts minimale veranderingen laten zien. Dit veronderstelt dat er andere factoren dan alleen het veranderen van inspanningsniveau, een rol spelen in het veranderen van het microbioom van de darm. Hoewel de deelnemers aan onze studie hun dieet niet veranderden, heeft hun levenslange dieet wellicht invloed op het aanpassingsvermogen van het microbioom. Hoewel significant, was het gewichtsverlies in onze groep deelnemers relatief bescheiden. Ook dit zou het uitblijven van grote veranderingen kunnen beïnvloeden. Samengevat is het op basis van deze studie, de vraag of de significante effecten van een trainingsinterventie op het verbeteren van insulinegevoeligheid wel gemedieerd worden door het veranderen van het microbioom van de darm.

Conclusie en aanbevelingen

Dit proefschrift heeft verder bijgedragen aan het concept "Exercise is Medicine". Idealiter, zou deze data kunnen leiden tot een doktersvoorschrift voor de exacte soort en dosis training die nodig is om insulinegevoeligheid te verbeteren in mensen met obesitas. Dit proefschrift toont echter aan dat een *one size fits all* recept voor training niet bestaat. Het is wel evident dat duurtraining het beste recept is voor het vergroten van cardiorespiratoire fitheid en het reduceren van visceraal vetweefsel. Voor het verbeteren van cytokineprofielen en het microbioom van de darm blijkt training echter niet altijd geschikt. Toekomstig onderzoek zal zich blijven richten op het identificeren van nieuwe *exercise factoren*; moleculen die bijdragen aan de respons van het menselijk lichaam op duurtraining – en daardoor verbetering van gezondheid in het algemeen en insulinegevoeligheid in het bijzonder.

Om het succes van leefstijlinterventies in de spreekkamer te kunnen beoordelen, is het belangrijk om de goede variabelen te meten. Dit proefschrift heeft aangetoond dat alleen de weegschaal daartoe onvoldoende informatie biedt. Alleen het meten van gewicht leidt tot een onderschatting van het effect van training op het viscerale vetweefsel. Het blijven meten van de buikomvang zal in de nabije toekomst een meer standaard plek in de spreekkamers innemen. Tenslotte blijft het concept van preventie van ziekten door leefstijlinterventies in mensen met obesitas een belangrijk onderdeel van de dagelijkse praktijk van (para)medici. In plaats van het voorschrijven van een recept is het voor elke arts in Nederland raadzaam om eens met patiënten in gesprek te gaan over het naleven van de "Norm Gezond Bewegen" – en hoeveel gezondheidswinst daarmee te behalen valt.

Chapter 9

Research Data Management

Ethics and privacy

This thesis is based on the results of medical-scientific research with human participants. The studies described in chapters 3, 4, 5 and 6 were subject to the Medical Research Involving Human Subjects Act (WMO) and were conducted in accordance with the ICH-GCP guidelines (Good Clinical Practice). The medical ethical review committee 'METC Radboudumc' has given approval to conduct these studies (file numbers: CMO 2005/281; CMO 2007/148 and CMO 2014/1336 (NTR L50995.092.14). Informed consent was obtained from research participants. Technical and organizational measures were followed to safeguard the availability, integrity and confidentiality of the data (these measures include the use of independent monitoring, pseudonymization, access authorization and secure data storage).

Data collection and storage

Data for chapter 5 and 6 was collected through electronic Case Report Forms (eCRF) using CASTOR EDC. From Castor EDC data were exported to SPSS (SPSS Inc., Chicago, Illinois, USA). Pseudonymized data were stored and analyzed on the department server and in Castor EDC and are only accessible by project members working at the Radboudumc. Paper (hardcopy) data is stored in cabinets on the department.

Availability of data

All studies are published open access. The data will be archived for 15 years after termination of the study. Reusing the data for future research is only possible after a renewed permission by the participants. The anonymous datasets that were used for analysis are available from the corresponding author upon reasonable request.

Chapter 10

List of Publications

1. Verheggen RJHM, van Schothorst E, Keijer J, Hermus ARMM, Thijssen DHJ, Hopman MTE. Impact of 8-week aerobic exercise training on white tissue gene expression in obese men and women. *Submitted*
2. Y. van Meeuwen, A.S.M Dofferhoff, **RJHM Verheggen**. Nachtzweten, een veelvoorkomend symptoom. Ned Tijdschr Geneeskd. 2024;168:D8039
3. Bavinck AP, **Verheggen RJHM**, van der Velden WJFM, Munnix ICA. De Kunst van het kijken: uw diagnose? Ned Tijdsch v Hematologie 2023; 20:180-2.
4. **Verheggen RJHM**, De Kort E, Boerrigter E, Brüggemann R, Blijlevens NMA. Antifungale profylaxe bij nieuwe hematologische behandelingen: uitdagingen en interacties. Ned Tijdsch v Hematologie 2022;19:14-23
5. Brüggeman R, **Verheggen RJHM**, Boerrigter E, Lewis R, Blijlevens NMA. Drug interactions between azoles and novel target therapies in hematology. Lancet Hematology 2022 Jan;9(1):e58-e72.
6. **Verheggen RJHM**, Konstanti P, Smidt H, Hermus, ARMM, Thijssen D, Hopman M. Effects of 8-week aerobic exercise training on gut microbiota and insulin sensitivity. Obesity 2021 Oct;29(10):1615-1624
7. De Kort E, **Verheggen RJHM**, Brüggemann R, Blijlevens NMA. Antifungale profylaxe bij nieuwe hematologische behandelingen: een klinisch noodzakelijke keuze. Ned Tijdschr Hematol 2021;18:153-61.
8. Dimopoulos G, de Mast Q, Markou N, Theodorakopoulou M, Komnos A, Mouktaroudi M, Netea MG, Spyridopoulos T, **Verheggen RJ**, Hoogerwerf J, Lachana A, van de Veerdonk FL, Giamarellos-Bourboulis EJ. Favorable Anakinra Responses in Severe Covid-19 Patients with secondary Hemophagocytic Lymphohistiocytosis. Cell Host Microbe 2020 Jul 8;28(1):117-123.e1.
9. Lafeber M, Schoffelen R, **Verheggen RJ**, van Herwaarden N, ten Oever J, Kramers K. Geneesmiddelen: wat leverde het afgelopen decennium ons op en wat mogen we voor de komende 10 jaar verwachten? Ned Tijdschr Geneeskunde 2020 Apr 23
10. Maas ML, Heuts M, van den Heuvel-Bens SPWH, Hendriks SM, **Verheggen RJHM**, Ligthart-Naber AF, Elving LD, van Gurp PJM. Proactieve klinische diabeteszorg met het D-team. Ned Tijdschrift Diab 2019.
11. Drenthen LCA, **Verheggen RJHM**, de Galan BE. Clinical impact of artifactual hypoglycaemia an its diagnosis at the bedside. Rheumatology (Oxford) 2019 Sep 1;58(9):1691-1692.
12. **Verheggen RJHM**, Eijsvogels TMH, Catoire M, Terink R, Ramakers R, Bongers CCWG, Mensink M, Hermus ARMM, Thijssen DHJ, Hopman, MTE. Cytokine responses to repeated prolonged walking in lean versus overweight/obese individuals. J Sci Med Sport. 2018 Jul 31.
13. Allard NAE, Schirris TJJ, **Verheggen RJHM**, Russel FFM, Rodenburg RJ, Smeitink JAM, Thompson PD, Hopman MTE, Timmers S. Statins affect skeletal muscle

performance: evidence for disturbances in energy metabolism. J Clin Endocrinol Metab. 2018 Jan. 103(1): 75-84.

14. Hartman Y, Hopman M, Schreuder T, **Verheggen RJHM**, Scholten R, Oudegeest M, Poelkens F, Maiorana A, Naylor L, Willems P, Tack C, Thijssen D, Green D. Improvements in fitness are not obligatory for exercise training-induced improvements in CV risk factors. Physiological Reports. 2018 Feb;6(4):e13595
15. Maessen MFH, Schalkwijk CG, **Verheggen RJHM**, Aengevaeren VL, Hopman MTE, Eijsvogels TMH. A comparison of dicarbonyl stess and advanced glycation endproducts in lifelong endurance athletes vs. sedentary controls. 2017. J Sci Med Sport 2017. Okt; 20(10): 931-926.
16. **Verheggen RJHM**, Poelkens F, Roerink S, Catoire M, Hermus ARMM, Hopman MTE, Thijssen DHJ. Exercise improves insulin sensitivity in the absence of changes in cytokines. Med Sci Sports exerc. 2016 Dec; 48(12): 2378-2386.
17. **Verheggen RJHM,** Maessen MFH, Green DJ, Hermus ARMM, Hopman MTE, Thijssen DHJ. Distinct effect of exercise training versus caloric restriction on visceral adipose tissue: a systematic review and meta-analysis. Obes Rev 2016 Aug;17(8):664-90.
18. Greyling A, Schreuder THA, Landman T, Draijer R, **Verheggen RJHM**, Hopman MTE, Thijssen DHJ. Elevation in blood flow and shear rate prevents hyperglycemia-induced endothelial dysfunction in healthy and type 2 Diabetic subjects. J Appl Physiol (1985). 2015 Mar 1;118(5):579-85.
19. Maessen MFH, Eijsvogels TMH, **Verheggen RJHM**, Hopman MTE, Verbeek A, de Vegt F. Entering a new era of body indices: the feasability of A Body Shape Index and Body Roundness Index to assess cardiovascular disease prevalence. PLoS One. 2014 Sep 17;9(9):e107212
20. Poelkens F, Eijsvogels TH, Brussee P, **Verheggen RJHM**, Tack CJ, Hopman MTE. Physical fitness can partly explain the metabolically healthy obese phenotype in women. Exp Clin Endocrinol Diabetes. 2014 Feb;122(2):87-91.
21. Jones H, Eijsvogels TH, Nyakayiru J, **Verheggen RJHM,** Thompson A, Groothuis JT, van Someren EJW, Atkinson G, Hopman M, Thijssen DHJ Within-subject correlations between evening-related changes in body temperature and melatonin in the spinal cord injured. Chronobiol Int. 2014 Mar;31(2):157-65.
22. Oudegeest-Sander MH, Eijsvogels TH, **Verheggen RJHM,** Poelkens F, Hopman MTE, Jones H, Thijssen DHJ. The impact of physical fitness and daily energy expenditure on sleep efficiency in young and older humans. Gerontology. 2013;59(1):8-16.
23. **Verheggen RJHM**, Jones H, Nyakayiru J, Thompson A, Groothuis JT, Atkinson G, Hopman MTE, Thijssen DHJ. Complete absence of evening melatonin increase in tetraplegics. FASEB J. 2012 Jul;26(7):3059-64.

Chapter 11

PhD portfolio

Department: **Department of Physiology**

PhD period: 01/12/2013 – 01/01/2019 (combined with residency Internal Medicine)

PhD Supervisor(s): Prof. Dr. M.T.E. Hopman, Prof. Dr. A.R.M.M. Hermus

PhD Co-supervisor(s): Prof. Dr. D.H.T. Thijssen

Training activities	**Hours**
Courses	
• Introduction days Radboudumc (2013)	8
• RIHS (graduate school) introduction course (2014)	45
• BROK course (2014)	26
• Opfriscursus Statistiek voor Promovendi (2014)	26
• Evidence Based Medicine (2014)	8
• Scientific Integrity (2015)	20
• Librarian: individual sessions how to perform a systemic review (2014)	4
• Scientific writing workshops (2015-2016)	8
• Clinical Epidemiology for AIOS (2015)	8
• Biometrics Course (2016)	60
• Teach the Teacher (2017)	8
Seminars	
• RIHS research meeting 2014 (poster)	8
• PhD Retreat RIHS (2014 and 2015)	32
Conferences	
• European Congress on Obesity 2015 (mini oral session)	26
• NASO (Netherlands Association Study Obesity) meeting 2015 (oral session)	8
• European Congress on Obesity 2016 (poster session)	8
• Internistendagen 2015	16
Other	
• Monthly Research Meeting Department of Physiology	16
• Weekly research meeting Integrative Physiology research group	40
• Member PhD council RIHS (2014-2016; 1.5 years as vice chair)	102
• PhD student Member Training and Supervision committee RIHS (2014-2016)	108
Teaching activities	
Lecturing	
• Welvaartsziekten (introductie BMW Open Dag RUMC 2014 en 2015)	
• Wet- en Regelgeving / WMO (regulier onderwijs afdeling Fysiologie) 2014 en 2015	4
• Various work groups, practica and occasional "responsie" colleges bachelors Biomedical Sciences and Medicine 2014-2017 (estimation of total time spent)	4 20
Supervision of internships / other	
• Rob Ramakers (Master Thesis internship Biomedical Sciences)	45
• Wesley Tangerink (Master Thesis Research Geneeskunde)	30
• Paul van Dun (Master Thesis Research Geneeskunde)	30
• Ihsane Mokadem (Master Thesis Research Geneeskunde)	30
Total	**748**

Chapter 12

Curriculum Vitae

Curriculum Vitae

Rebecca Verheggen werd geboren op 5 juni 1986 in Geleen en groeide op in Dieteren. In 2004 behaalde zij haar Gymnasium diploma aan het Trevianum, te Sittard. Aansluitend begon zij met de studie geneeskunde in Nijmegen, die zij combineerde met een aantal vakken bij de studie Filosofie. Tijdens haar wetenschappelijke stage op de afdeling Fysiologie onder begeleiding van dr. D. Thijssen en Prof. Dr. M. Hopman ontstond het idee om samen subsidie aan te vragen voor een volledig PhD traject. Die subsidie (IGMD Grant Junior Researcher) werd in 2012 gehonoreerd en stond aan de basis van dit proefschrift. De scriptie die voortkwam uit de wetenschappelijke stage werd bekroond met de Masterscriptieprijs Geneeskunde 2011.

Alvorens te starten met het fulltime doen van onderzoek, begon Rebecca aan de opleiding tot internist, in de eerste paar jaar in het Tweesteden Ziekenhuis, Tilburg – onder begeleiding van dr. T. Wierema. In december 2013 keerde zij terug naar Nijmegen om te starten aan haar PhD traject. Dit traject werd afgewisseld met het vervolg van de opleiding tot internist, en later tot internist-hematoloog in het Radboudumc onder begeleiding van Prof. Dr. J. de Graaf, dr. G. Vervoort en Prof. Dr. N. Blijlevens.

Tijdens haar opleidingen was Rebecca bestuurlijk actief in meerdere gremia, van de PhD council van de RIHS, tot aan het dagelijks bestuur van de JNIV en was zij lid van het Concilium Medicinae Internae. Tijdens haar onderzoek won zij verschillende prijzen, waaronder een award voor Best Poster Pitch (mini oral) session op het European Congress on Obesity (2015), het Christine Mohrmann stipendium (2016) en de Boston prijs van de opleiding interne geneeskunde (2017).

Na afronding van de opleiding tot internist begon ze met haar eerste baan als internist-hematoloog in het Canisius Wilhelmina Ziekenhuis te Nijmegen. Daar werkt zij nog altijd, inmiddels ook als medisch manager van de vakgroep interne geneeskunde.

Rebecca woont samen met haar partner Rik en twee dochters Hannah en Suze in Weurt.

Chapter 13

Dankwoord

Het voltooien van dit proefschrift was niet gelukt zonder hulp, steun en vertrouwen van heel wat mensen. Het was niet altijd even makkelijk voor me om dit proces tot een goede afronding te brengen. Gelukkig kwam dat grotendeels doordat mijn leven zowel professioneel als privé gevuld is met heel veel ander leuks. Dus voor een ieder die dit al die jaren met me vol heeft gehouden: bedankt voor de volharding!

Allereerst wil ik de **deelnemers aan de onderzoeken** waarop dit proefschrift gebaseerd is van harte bedanken voor hun inzet, geduld en humor tijdens de metingen en de trainingssessies. Het mooie van de afdeling Fysiologie blijft de basis waarop al het onderzoek gefundeerd is: onderzoek doen in en voor mensen. Het is een groot verschil met mijn alledaagse praktijk als arts. In de eerste plaats omdat ik gewend ben te behandelen, wanneer de ziekte reeds aanwezig is. Dit project bestond uit handelen, met het doel ziektes te voorkomen. In plaats van een korte ontmoeting in de spreekkamer, kreeg ik de kans om (letterlijk) naast iemand te staan – of liever: op de fiets te zitten. We beleefden daardoor de successen en soms ook de uitdagingen van de '(be)handeling' veel meer samen dan je als patiënt en arts normaliter kunt doen. Een deel van jullie is na afronding van de trainingsstudie, heel fanatiek doorgegaan met sporten. Daar mogen jullie heel trots op zijn. Het stemt me nederig dat ik een kleine rol in de kick-off van een gezonder leven heb mogen spelen.

Maria, ik ken weinig mensen met zo'n oog voor gedrevenheid en talent. Met jouw tomeloze enthousiasme heb je een prachtige afdeling vormgegeven. De jaren dat ik daar deel van uit mocht maken, behoren tot de gezelligste van mijn carrière. De manier waarop je je belangeloos inzet om te helpen bij de opstart van carrières van jonge collega's (ik kan me je aanbevelingsbrief voor toelating tot de opleiding tot internist nog goed herinneren) is vrij uniek in de academische wereld. Dankjewel voor je positiviteit, je geduld en je relativeringsvermogen!

Dick, mijn eerste onzekere stappen in de wereld van wetenschappelijk onderzoek mocht ik zetten onder jouw vleugels. Wat betreft het ballen in de lucht houden, doe je niet onder voor Maria. Ik vind het grenzen aan het onmogelijke dat je al die jaren nooit je geduld met of vertrouwen in me bent verloren. Dankzij jouw vertrouwen werd ik zo nu en dan overvallen door een felicitatie voor een prijs waarvan ik niet eens wist dat je me aangemeld had. Dat vertrouwen was echt de basis voor het succesvol afronden van dit traject. Dankjewel!

Ad, ik wil je van harte bedanken voor je continu positieve bijdrage aan dit werk. Hoewel fysiek weliswaar op afstand, voelde dat eigenlijk nooit zo. Versies van de

artikelen kwamen altijd even snel terug, met gedegen maar positief geformuleerde feedback. Hoewel ik uiteindelijk niet gekozen heb voor de endocrinologie, heeft dat niet gelegen aan jouw fantastische begeleiding. Dankjewel.

Collega's van andere afdelingen. Alleen ben je nergens, zeker niet in het grote academische bos waardoor je soms de bomen niet meer ziet. Daarom wil ik een aantal mensen bedanken die in de loop der jaren hun steentje hebben bijgedragen aan dit proefschrift, waarbij ik niet de illusie koester hierin volledig te zijn en niet iedereen bij naam zal noemen. **Prof. Dr. M. Netea, Prof. Dr. J. van der Meer**, bedankt voor jullie advies in de vroege dagen van mijn onderzoekstijd. **Prof. Dr. H. Smidt and Prokopis Konstanti**: thanks for introducing me in the wonderous world of the gut microbiome and for sculpting the paper with me. **Prof. dr. ir. J. Keijer en Evert van Schothorst**, bedankt voor jullie bijdragen aan Hoofdstuk 5. **Inge en Sean:** bedankt voor het me aanleren van de vet- en spierbiopten. **Hanne en Elsemieke**: bedankt voor het me aanleren van de clamp. Voor een bijdrage aan de metingen en de analyse daarvan: alle betrokken collega's van de nucleaire geneeskunde RUMC, van het laboratorium interne geneeskunde RUMC, van de verschillende labs in Maastricht UMC+ en Wageningen Universiteit; bedankt! En last but not least, dankjewel voor het Universitair Sportcentrum van de RU, voor het belangeloos verstrekken van de sportabonnementen voor de deelnemers en het onderzoeksteam.

Op de afdeling Fysiologie geldt dat een trainingsstudie eigenlijk niet tot een succes te brengen valt zonder de inzet van **de vele studenten.** Zelfs buiten kantoortijd zaten jullie soms nog een uurtje met de deelnemers op de fiets. Heel erg bedankt allemaal en in het bijzonder uiteraard mijn stagiaires: **Wesley, Paul en Ihsan.**

Rob, jij verdient een bijzonder dankwoord. Jouw maandenlange inzet als masterstudent heeft een deel van dit boekje echt naar een hoger plan getild. Je ICT-skills doen niet onder voor je excellente communicatieve vaardigheden. Je bijbaan als kok in het familierestaurant en de daaruit voortkomende verhalen hebben ervoor gezorgd dat dit de eerste trainingsstudie moet zijn geweest waarover het vooral heel veel over lekker eten is gegaan tijdens het sporten. Dankjewel voor je inzet.

Collega's fysiologie. **Bregina**: waar een leger studenten nodig is om de trainingsstudies uit te voeren ben jij in een je eentje een *force of nature* die jarenlang aan de basis stond van zo'n beetje alle studies die liepen op de Integratieve

Fysiologie. Als ik de afdeling opliep was weinig meer verwelkomend dan jouw bulderlach die over de gangen schalde. Dankjewel, voor alles!

De roomies. Het lot van een PhD student is om door de jaren heen heel wat kamergenoten te slijten. Op de een of andere manier had ik het geluk dat dit altijd collega's bleken die perfect aansloten op mijn manier van werken, of kletsen. **Nathalie**, op het oog leken we soms wat *random* of *negative*, maar jij en ik weten wel beter. Jij was het die mij (en vele anderen) op de been hield met slechte woordgrappen, leuke anekdotes, of gewoon "vloeken als een bootwerker". Ik kan nog altijd hardop lachen als ik daaraan terugdenk. Dankjewel! **Joost,** samen met Nathalie was jij een soort natuurlijke mentor voor me toen ik startte als groen blaadje in onderzoeksland. Het leven is veel te zwaar voor je geweest op veel te jonge leeftijd. Je veerkracht is ongeëvenaard. **Coen,** toch wel het hoofd van de *2nd generation* roomies. Jij deed alles snel, in ieder geval heel wat sneller dan ik (al is dat, toegegeven, niet al te moeilijk). Ik heb het in dit dankwoord veel over positieve mensen maar jij spant toch wel de kroon. Brabantse nuchterheid, maar nooit afstandelijk en altijd behulpzaam. Dankjewel voor de leuke tijd!

Dominique, Do ook al zo'n Brabantse – of is het Limburgse? – positivo, met je eigen quirks en eigenaardigheden. Maar altijd goed gekleed! (en met een maandvoorraad pepermuntjes op zak) Dankjewel voor alle lachbuien samen! **Lando**, nogmaals: ik heb het in dit dankwoord veel over positieve mensen. Dan heb ik het nadrukkelijk niet over jou. Neemt niet weg dat je in cynische humor en eerlijkheid wel een koploper bent en daar kan ik enorm van genieten. Ik koester nog steeds de stiekeme hoop ooit collega hematologen te worden in hetzelfde centrum. Tot die tijd: tot op de internistendagen voor de laatste roddels!

Martijn: Jij hebt een significant stempel gedrukt op dit proefschrift en mijn tijd bij de fysiologie. Weloverwogen, kritisch maar tegelijkertijd hartelijk en altijd ad hoc tijd maken voor al mijn Endnote/SPSS/bureaustoel vragen. Dat monnikenwerk voor de meta-analyse schiep een band. Dank voor je geduld! En voor de occasionele lift naar Limburg! **Silvie**: in heel veel een voorbeeld voor ons allemaal, wetenschappelijk gezien maar minstens zoveel als mens. Het meest bewonderenswaardig vind ik hoe jij altijd je interne morele kompas je weg hebt laten bepalen, soms tegen de stroming in. Daar mag je echt trots op zijn! **Femke, F**: Heel leuk dat we elkaar soms weer spreken tijdens een dinertje en dat je me zo nu en dan over het hoofd ziet in mijn witte jas. Dankjewel voor de gezelligheid op het secretariaat! **Tim:** ik las ooit in een dankwoord, misschien wel dat van jou zelf, iets over jouw rol als troubadour van de afdeling. Een soort hofnar van de fysiologie was je. Elke afdeling kan wel

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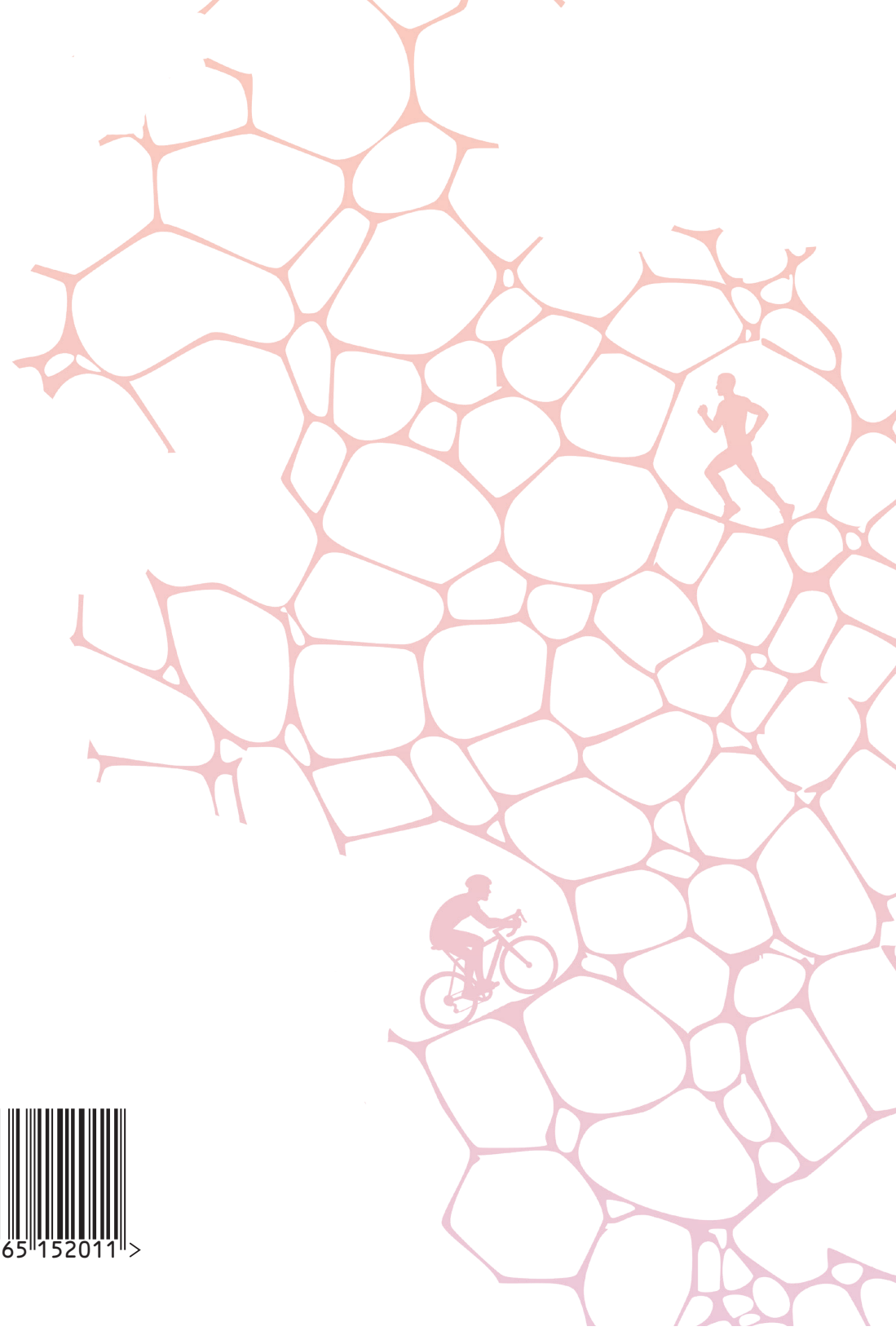

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