The metabolic impact of hypoxia exposure in human obesity

IMPACT

This thesis describes the effects of mild hypoxia exposure as a potential strategy for improving metabolic health in obesity. We investigated the effects of mild intermittent hypoxia (MIH) exposure on metabolic health, in particular tissue-specific insulin sensitivity and glucose homeostasis, in overweight and obese men. In addition, we determined whether hypoxic exercise improved glucose homeostasis in overweight and obese men with impaired glucose metabolism. Finally, we investigated the effects of hypoxia exposure in at the molecular level by using adipose (AT) and skeletal muscle (SM) biopsies, as well as primary AT and SM *in vitro* cell models. In this section, the impact of the work described in this thesis will be discussed in terms of scientific and societal relevance, and further implications and applications of hypoxia exposure as a potential therapeutic strategy to improve metabolic health in obesity will be highlighted.

Societal relevance

Obesity is a major cause of morbidity and mortality worldwide, with more than 1.9 billion and 650 million people being overweight and obese, respectively (1, 2). In addition, the global prevalence of obesity will increase drastically in the near future (3). For instance, it is estimated that the prevalence of obesity in the USA will increase to 51% of the total population (4). Since obesity is often accompanied with complications such as cardiovascular diseases, type 2 diabetes mellitus, cognitive decline, depression, obstructive sleep apnea syndrome, skin problems, asthma and several types of cancer (5-10), a forecasted further increase in obesity prevalence will pose a major public health challenge. In addition, improving metabolic health in obesity may also be an interesting approach from a socioeconomic perspective. Indeed, in European countries, it has been stated that on average 7% of their national healthcare budgets is spent on obesity-related complications, which substantially burdens national economies (11). Furthermore, in 2012, €1.6 billion, i.e. 2.2% of total healthcare budget, was spend on overweight-related complications in the Netherlands (12), thereby hindering containment of healthcare costs. Therefore, alternative strategies to prevent or reverse obesity and related complications are urgently required.

Scientific impact

In the past decade, it has been postulated that tissue oxygen tension (pO_2) may be a key determinant in cardiometabolic health in obesity (13, 14). Indeed, it had been found that abdominal adipose tissue (AT) pO_2 was increased in obese (15), and appeared to be inversely correlated with peripheral insulin sensitivity, independent of adiposity and gender (16). Moreover, diet-induced weight loss

decreased AT pO_2 in humans with overweight/obesity, which was accompanied by improved insulin sensitivity (17). Lowering of pO_2 by mild hypoxia exposure may therefore be an alternative and effective approach for improving metabolic health in human obesity.

This thesis focused on the application of MIH exposure as an intervention to study the effects of lowering AT and SM pO_2 on various metabolic parameters like substrate utilization and insulin sensitivity. The outcomes of this study gained insight in the metabolic adaptations induced by MIH exposure in obesity. In addition, this thesis provides knowledge for the scientific community on the molecular adaptations induced by hypoxia exposure in AT, SM and the gut, as well as in vitro AT and SM models.

In addition, this thesis provides insight on the effects of hypoxic exercise in overweight and obese metabolically comprised individuals. Noteworthy, 4-day HE at lower absolute workload intensity had similar effects on mean glucose homeostasis as compared to normoxic exercise (NE). However, the reduced absolute workload results in less mechanical strain, and therefore may be a valuable approach for improving metabolic health in obese individuals with orthopedic comorbidities, such as elderly (18).

Furthermore, the findings in this thesis provide knowledge regarding the effects of different oxygen levels in mechanistic experiments using *in vitro* cell models. Indeed, hypoxia exposure greatly influenced glucose uptake in primary human myotubes experiments. Interestingly, exposure to physiologically relevant oxygen levels (*in situ* hypoxia or normoxia) substantially altered myotube and adipocyte function, and gene/protein expression as compared to standard laboratory conditions (21% O_2). This knowledge further underlines the importance of taking the physiological relevant oxygen levels of tissues from which cells originate into account, which may be implemented in a wide variety of *in vitro* cell culture models in different research areas.

The findings in this thesis are relevant for the scientific community studying the effects of hypoxia in different metabolically active tissues, such as AT, SM, gut and the liver. In addition, we provide molecular insight in the effects of hypoxia exposure on regulation of gene expression, and found a wide variety of pathways being affected by hypoxia, which may be relevant for scientists outside our field of study. The findings of this thesis were presented and discussed at (interdepartmental) research meetings and symposia (*Annual NUTRIM symposia*, 2016-2019, Maastricht) and national (*The Netherlands Association for the Study of Obesity, NASO spring meeting*, 2018, Utrecht, *Annual Dutch Diabetes Research Meeting, ADDRM*, 2017-2019, Oosterbeek) and international conferences (*European/International Congress on Obesity, ECOICO2020*, 2020, virtual meeting; *Diabetes and Metabolism Research Symposium*, 2018, Maastricht). The aim of presenting and discussing our findings

at these conferences was to increase knowledge transfer to both the scientific community and medical specialists (i.e. clinicians and dietitians) on the potential role of tissue oxygenation in metabolic health in human obesity. In addition, the outcomes of this thesis will be communicated with the scientific community by publication in international peer-reviewed journals.

The studies performed in this thesis are conducted in collaboration with several other research groups and institutes. For the *in vitro* cell culture experiments, we used the Roxybot technology developed by the Department of Radiotherapy (MAASTRO) of Maastricht University. In addition, we collaborated with Dr. Henrike Sell and Prof. Hadi Al-Hasani at the Institute for Clinical Biochemistry and Pathobiochemistry of the German Diabetes Center (Dusseldorf, Germany) to determine adipokine/myokine secretion in human and *in vitro* samples. Furthermore, we performed microarray and gene set enrichment analyses in collaboration with Prof. Sander Kersten (Division of Human Nutrition and Health, Wageningen University and Research). Lastly, we collaborated with Prof. Koen Venema, Centre for Healthy Eating & Food Innovation, Campus Venlo of Maastricht University, to determine the effects of hypoxia exposure on gut microbiota composition.

Further research is warranted to investigate variations in hypoxia exposure regimen, since it has been found that severity, frequency and duration of hypoxic exposure determine metabolic adaptations. In addition, a personalized strategy may be optimal for investigating the effects of hypoxia exposure on metabolism in obesity. In fact, we demonstrate that hypoxic exercise has the most pronounced effect on 24-h glucose levels in peole with obesity who demonstrated the greatest reduction in systemic saturation induced by hypoxia. Due to the inter-individual difference in tolerability of hypoxia, it would be interesting to personalize the intervention by adjusting FiO_2 according to the change in systemic oxygen saturation, thereby optimizing metabolic adaptations. Furthermore, it has previously been suggested that the metabolic phenotype may play a key role in the response to hypoxia, since obese individuals with worst baseline insulin sensitivity improved most upon mild hypoxia exposure (19).

Commercial exploitation

The present results may be of interest to pharmaceutical companies in the field of type 2 diabetes, cardiovascular and liver disease, since these chronic diseases are strongly associated with impairments in glucose homeostasis and inflammation. For example, a pharmacological approach using prolyl-hydroxylase dehydrogenase (PHD) inhibitors may be used to improve metabolic health, since both environmental hypoxia and PHD inhibitors stabilize hypoxia-inducible factor-1α (HIF-1α). Interestingly, several PHD inhibitors are currently used in clinical trials for

treatment of anemia in chronic kidney disease (CKD). The metabolic and molecular adaptations induced by MIH may therefore be interesting for the scientific community studying the use of PHD inhibitors in other clinical fields such as CKD.

In addition, a non-pharmacological approach may be of interest for commercial exploitation. For example, the commercialization of an air-tight room, with an airlock system, in which nitrogen dilution can regulate the oxygen content, thereby potentially influencing metabolic health, may be worthwhile to explore. However, this might be a costly innovation. Nevertheless, continuous positive airway pressure (CPAP) therapy, currently mainly used in the treatment of obstructive sleep apnea syndrome (OSAS), may provide a valuable alternative. OSAS is characterized by frequent hypoxic episodes during sleep, resulting in systemic oxygen desaturation. The positive airway pressure in CPAP prevents the upper airways from collapsing, thereby eliminating apnea and desaturation. Interestingly, if CPAP devices may be developed with nitrogen dilution capabilities, CPAP therapy may expose the individual to the desired oxygen content for inducing metabolic adaptations. In that way, hypoxia may be applied at home during the night.

To conclude, this thesis provides information about the metabolic and molecular effects of mild hypoxia exposure on metabolic health in obesity, based on human studies and human primary cell culture experiments. In addition, this thesis generates knowledge on the combined effects of hypoxia and moderate-intensity exercise on glucose homeostasis in obesity. The results of this thesis may also be used to further investigate variable hypoxic exposure regimens, as well as a personalized intervention using variable FiO_2. Therefore, the overall findings of this thesis may contribute to the development of alternative approaches for combatting obesity-related complications, other than traditional lifestyle interventions, and as such may be of interest for both academic and industrial scientists, as well as healthcare professionals.

References

1. Kopelman PG. Obesity as a medical problem. Nature. 2000;404(6778):635-43.

2. Organization WH. Obesity and Overweight: Fact sheet: World Health Organization Media Centre; [

3. Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M. Health and economic burden of the projected obesity trends in the USA and the UK. Lancet. 2011;378(9793): 815-25.

4. Finkelstein EA, Khavjou OA, Thompson H, Trogdon JG, Pan L, Sherry B, et al. Obesity and severe obesity forecasts through 2030. Am J Prev Med. 2012;42(6):563-70.

5. Nguyen JC, Killcross AS, Jenkins TA. Obesity and cognitive decline: role of inflammation and vascular changes. Front Neurosci. 2014;8:375.

6. Luppino FS, de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx BW, et al. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. Arch Gen Psychiatry. 2010;67(3):220-9.

7. De Pergola G, Silvestris F. Obesity as a major risk factor for cancer. J Obes. 2013;2013:291546.

8. Schwartz AR, Patil SP, Laffan AM, Polotsky V, Schneider H, Smith PL. Obesity and obstructive sleep apnea: pathogenic mechanisms and therapeutic approaches. Proc Am Thorac Soc. 2008;5(2):185-92.

9. Yosipovitch G, DeVore A, Dawn A. Obesity and the skin: skin physiology and skin manifestations of obesity. J Am Acad Dermatol. 2007;56(6):901-16; quiz 17-20.

10. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation. 2006;113(6):898-918.

11. Commission E. European Union EU research Leads Battle against Obesity epidemic: European Commission, 2015 2015 [Available from: http://ec.europa.eu/programmes/horizon2020/en/news/eu-research-leads-battle-against-obesity-epidemic.

12. Panhuis-Plasmans M, Luijben G, Hoogenveen R. Zorgkosten van ongezond gedrag - Kosten van ziekten notities 2012-2. Centrum voor Volksgezondheid Toekomstverkenningen, RIVM; 2012.

13. Lempesis IG, van Meijel RLJ, Manolopoulos KN, Goossens GH. Oxygenation of adipose tissue: A human perspective. Acta Physiol (Oxf). 2020;228(1):e13298.

14. Trayhurn P. Hypoxia and adipose tissue function and dysfunction in obesity. Physiol Rev. 2013;93(1):1-21.

15. Goossens GH, Bizzarri A, Venteclef N, Essers Y, Cleutjens JP, Konings E, et al. Increased adipose tissue oxygen tension in obese compared with lean men is accompanied by insulin resistance, impaired adipose tissue capillarization, and inflammation. Circulation. 2011;124(1):67-76.

16. Goossens GH, Vogel MAA, Vink RG, Mariman EC, van Baak MA, Blaak EE. Adipose tissue oxygenation is associated with insulin sensitivity independently of adiposity in obese men and women. Diabetes Obes Metab. 2018;20(9):2286-90.

17. Vink RG, Roumans NJ, Cajlakovic M, Cleutjens JPM, Boekschoten MV, Fazelzadeh P, et al. Diet-induced weight loss decreases adipose tissue oxygen tension with parallel changes in adipose tissue phenotype and insulin sensitivity in overweight humans. Int J Obes (Lond). 2017;41(5):722-8.

18. Wiesner S, Haufe S, Engeli S, Mutschler H, Haas U, Luft FC, et al. Influences of Normobaric Hypoxia Training on Physical Fitness and Metabolic Risk Markers in Overweight to Obese Subjects. Obesity. 2010;18(1):116-20.

19. Lecoultre V, Peterson CM, Covington JD, Ebenezer PJ, Frost EA, Schwarz JM, et al. Ten nights of moderate hypoxia improves insulin sensitivity in obese humans. Diabetes Care. 2013;36(12):e197-8.