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CONTACT
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FROM THE EDITORIAL BOARD

Dear reader,

A new year has begun; we have left 2016 behind us. We worked hard for you to be able to read this new edition, filled with all sorts of articles covering a wide range of medical subjects. Each time we aim to improve the journal and spread more enthusiasm about writing and publishing among the students. In the spirit of a new year I could say that this is one of our New Year's resolutions. While writing this, one question comes to mind: where do New Year's resolutions come from?

The Babylonians are known to be the first to celebrate the beginning of a new year, during a twelve day festival that was held in March. For the start of the new year, they made their gods promises of paying debts or returning borrowed objects. This could be where the tradition of New Year's resolutions began. After Julius Caesar established a calendar where a new year began on January 1st, this day had a special meaning to the Romans. They offered sacrifices and made promises to Janus, the two-faced god, who was believed to look back into the past year and into the coming year. Nowadays, New Year's resolutions have little to do with any god and mainly consist of intentions to lose weight or to quit smoking and drinking. According to research in the USA, only about 9% of the people who make these resolutions feel like they actually achieved them at the end of the year.

Making resolutions for the new year, if they are not promises to any deity, doesn't seem to get us very far. This leaves me to conclude that progress and improvement are things to be sought after at all times, not just after ending another year on the calendar. In the coming pages, it will be confirmed that you do not need New Year's resolutions to stay young and healthy. You will read about the science behind eternal youth, battling diabetes, diagnosing metastases and why you should or shouldn't drink that extra glass of wine. A much better way of approaching your goals for 2017, if you ask me.

Anne Kokke
Editorial Editor-in-Chief

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CAN'T WE STAY YOUNG FOREVER?

Vera M. Kho[1]

[1]Bachelor Biomedical Science student, Radboud University Medical Center, Nijmegen, the Netherlands

Introduction

Editorial

For centuries, explorers and travellers have devoted years of their lives in search of the Fountain of Youth: a source which, if bathed in or drunk from, would provide you with eternal youth and longevity. There are some indications of the existence of such a source, but they have never been confirmed; the fountain is widely seen as a myth. The wish for eternal life, however, remains. Could it, with the current or future technology and knowledge, be possible to fulfill this wish?

Death is inevitable. Right? This is not necessarily true, according to biomedical gerontologist and Chief Science Officer and Co-founder of the SENS Research Foundation Aubrey de Grey. He perceives ageing as a disease that can be cured and believes that we will be able to defeat ageing and thus prolong life - indefinitely. His main argument for why we should cure ageing is because it kills people. Before being able to find possible therapies that could prevent or reverse ageing and age associated diseases, we need to know what exactly ageing is.

Ageing is often defined as "the process of growing old" or "relating to getting older" [1, 2]. However, it can also be defined as all changes that occur with the passage of time in a (human) body including growth and differentiation, which can result in an increased probability of death as someone grows older [3]. It is suspected that an accumulation of damage is responsible for the symptoms of ageing and there are several theories about this [3, 4]. The process of ageing results in a loss of cells and therefore loss of (organ) function and increased vulnerability [3]. Some of the theories on the process of ageing are more plausible than others. Nonetheless, all of them are considered possibilities and no consensus on which one is true has been reached. The Programmed theory exists of three subcategories: the Programmed Longevity, the Endocrine theory and the Immunological theory. The Programmed Longevity states that ageing is programmed genetically, which means that it is essentially the last step in development. The Endocrine theory suggests that hormones are responsible for the pace of ageing. Finally, the Immunological theory proposes that the immune system is programmed to decline over time, which leads to increased susceptibility to infectious diseases and ageing and death. Other theories, e.g. the War and Tear theory, which says that cells and tissues wear out due to repeated use, or the Free Radicals theory, which hypothesises the fact that radicals such as reactive oxygen species (ROS) cause cellular and DNA damage, exist as well. [3, 4] The Somatic DNA Damage theory, in which DNA damage is responsible for deterioration and malfunction of cells due to DNA repair defects is also imaginable. Perhaps the best known is the Telomere theory. The shortening of the telomeres with each cell division can cause genomic instability and is associated with ageing. As mentioned before, there is no consensus on which theory is the correct one, and it is highly probable that the truth consists of a combination of the many theories.

Beside this, Aubrey de Grey proposes seven types of damage [5, 6] that have been established that lead to ageing: (i) cell loss or atrophy; (ii) cell senescence, which is the irreversible cell cycle arrest [7]; (iii) nuclear (epi) mutations or cancerous cells; (iv) mitochondrial mutations; (v) protein crosslinks; (vi) extracellular aggregates; and (vii) intracellular aggregates [5, 6]. As you grow older, tissue-specific stem cells become less effective in replacing damaged or dead cells, meaning that long-lived tissues will lose cells and the function of a damaged organ will be compromised. This results in weakening of muscles, loss of neurons, leading to cognitive decline [8]. Senescent cells are cells that have lost the ability to divide, e.g. when abnormal changes in DNA expression are observed. However, these senescent cells are alive and can still produce proteins and secrete them, eventually causing inflammation, which causes damage. Mutations and epimutations also induce damage by causing abnormal gene expression, changing the conditions in which the protein is expressed or by altering the protein structure and thus the function. These can all lead to cancerous cells and uncontrolled growth.

Premature ageing syndromes

It is also possible to gain insight into the ageing process by looking at premature ageing syndromes, which are diseases that cause ageing symptoms at a very early age. Examples of these are the Werner syndrome (WS) and the Hutchinson-Gilford progeria syndrome (HGPS). WS is a rare autosomal recessive disorder and patients have a life expectancy of 47 to 54 years. The ageing symptoms can start to occur when the patients are in their teenage years and resemble many signs of normal ageing, including grey hair, cataracts, ischemic heart disease and osteoporosis [9, 10]. The cause is often a mutation in the *WRN* gene that codes for the Werner protein, which is important for DNA replication and repair and telomere maintenance. The mutation often leads to a shortened protein that is unable to be transported to the nucleus, where it normally executes its function [9-11]. Cells from individuals with WS present themselves with increased chromosomal aberrations, premature cell senescence in vitro and accelerated telomere shortening. HPGS is a slightly more severe disease. Children seem healthy at birth, but develop clinical symptoms soon thereafter, including for example growth failure. A remarkable aspect is that patients retain normal cognitive function and development. Life expectancy ranges from 8 to 21 years and death is often caused by a stroke or cardiovascular disease [9, 10]. Since patients often do not live to a reproductive age, the mutation responsible for HGPS is rarely inherited. Often the cause is an autosomal dominant de novo point mutation in the *LMNA* gene [9, 10], leading to a mutant lamin protein, normally situated in the inner nuclear lamina that is involved in many nuclear activities as DNA replication, nuclear migration, cell development and apoptosis [12]. There is no cure available for either WS or HPGS and the only option is to treat symptoms [9]. Based on these observations, one may conclude that genetic components are involved in ageing. In the case of these two syndromes, a mutation is responsible for accelerated ageing, but could the opposite be true as well?

Genetic components in longevity

Researchers from the University of California have found mutations in the *daf-2* gene of the *C. elegans*, causing them to live an active life for twice

as long as as the wild type [13]. This gene codes for a hormone receptor. Due to the mutation, the receptor's function is reduced. The same effect has been observed in flies and mice [14, 15]. The human equivalent of the *daf-2* receptor is the insulin and insulin-like growth factor 1 (IGF-1) receptor. The IGF signaling pathway has also been suggested to have an effect on the human lifespan [16, 17]. In research of Suh et al. (2008) [16], mutations in the IGF-1 receptor (IGF1R) gene were more prevalent in female centenarians compared to controls. These mutations were associated with reduced IGF1R activity and higher serum IGF-1 level as a compensatory method. Later, an association between IGF-1 serum levels and survival was observed [17]. Here, low IGF-1 levels were correlated with a higher survival in females, especially in individuals with a history of malignancy. Overall, some individuals may experience benefits from certain genetic variations, resulting in a prolonged life.

Slowing or reversing ageing

Other researchers are working on unraveling possible solutions for ageing. At the SENS Research Foundation, researchers are exploring several possible therapies for slowing ageing, like cell therapy and tissue engineering. However, what may be even more interesting, is that some researchers are looking at a way to reverse ageing and actually believe the Fountain of Youth may be within us. In an animal study, a young mouse's blood circulation was connected to that of an aged mouse through parabiosis and it was found that the exposure of the old mouse to the young mouse's blood improved stem cell function in muscles, liver and the brain. In another study, aged mice (18 months old) were either given an intravenous injection of "young" plasma, from 3 month old mice, "aged" plasma, from 18 month old mice, or no injection. Aged mice given young plasma showed enhanced learning and memory compared to the aged mice given old plasma or untreated mice [18]. This study shows that exposure to young blood may enhance cognitive function in aged mice, but also counteract ageing at a molecular and structural level. This suggests that young blood may contain factors that can reverse ageing or that old blood contains factors that are pro-ageing. Abolishing these pro-ageing factors might counteract ageing. Following these results, a clinical trial was started in Alzheimer patients [19] to assess the effect of regular plasma injections, voluntarily obtained from young men. Even though another study suggests that not the young blood is rejuvenative but perhaps the old blood toxic [20], the implications of certain factors in the blood that can influence ageing are considerable.

Conclusion

Researchers have come a long way in deciphering the mysteries of ageing, but they still have a long way to go. Ageing is a multifaceted issue which means that finding a simple solution is not that straightforward. Besides this, much of the research has been done in vitro or in animals and the techniques that are being used, like genetic engineering of certain age-associated genes, are not ready yet to be applied in humans. Therefore, with the current status of research, we are not yet able to slow, stop or reverse ageing. However, a crucial question remains unanswered: do we want to prolong our lives? Perhaps we should embrace ageing and death as a part of life and consider to stop looking for the Fountain of Youth, because once we have found it, there is no turning back.

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CERVICAL CANCER: DIAGNOSTIC ACCURACY OF NANO-MRI

Daniëlle J. van Dis[1], Janoe J.A.W. van Hemert[1], Vera E. Mekers[1], Renee M.W. Smets[1], Maaike ten Westenend[1]

Corresponding Author: Janoe J.A.W. van Hemert (janoevanhemert@hotmail.nl)

[1]Bachelor Biomedical Science student, Department of Health Evidence, Radboud University Medical Center, Nijmegen, the Netherlands

ABSTRACT

Systematic Review

BACKGROUND: The current technique (PET/CT) to detect lymphatic metastases in cervical cancer does not have the desired accuracy. This means not all patients with lymphatic metastases are correctly diagnosed. A lack in accuracy leads to unnecessary false negatives and false positives. Therefore a new technique is needed, nano-MRI can be the solution.
OBJECTIVE: Study sensitivity and specificity of nano-MRI compared to PET/CT in the diagnosis of pelvic lymph node metastases in women with cervical carcinoma.
METHODS: PubMed and Embase were searched to find relevant articles. After selection based on title and abstract, 23 articles were included. A study was selected when the following criteria were met: domain, determinant and outcome were present; sensitivity and specificity were calculated. Articles were evaluated for the critical appraisal using the QUADAS-2 tool and the relevant data was extracted and pooled.
RESULTS: The overall sensitivity and specificity of PET/CT including all the studies were 56% (95% CI: 50%-63%) and 60% (95% CI: 56%-64%), respectively. The overall sensitivity and specificity for nano-MRI including all of the studies were 83% (95% CI: 78%-87%) and 87% (95% CI: 84%-89%), respectively.
CONCLUSION: Nano-MRI is expected to be a better alternative to detect metastases in pelvic lymph nodes in women with cervical cancer in comparison to PET/CT. But no solid conclusion can be drawn and therefore further research is needed.

WHAT'S KNOWN: To date, detecting lymphatic metastasis in cervical cancer is done with PET/CT, but this lacks accuracy. A low accuracy causes unwanted errors in lymph node metastases diagnostics.

WHAT'S NEW: According to our results, the specificity of PET/CT is 56% (95% CI: 50%-63%) compared to 83% (95% CI: 78%-87%) of nano-MRI. The sensitivity of PET/CT is 60% (95% CI: 56%-64%) compared to 87% (95% CI: 84%-89%) of nano-MRI. This indicates that nano-MRI would be a better alternative to detect metastases in lymph nodes in women with cervical cancer than PET/CT.

KEYWORDS: Nano-MRI, PET/CT scan, Cervical Cancer, Pelvic Lymphatic Metastasis, Sensitivity and Specificity

*Supplementary material has been marked with * and can be found online at www.ramsresearch.nl*

Introduction

Cervical cancer is a type of cancer arising from the cervix. It can develop after infection of cervix cells with human papilloma virus (HPV), which causes changes in the transition area from columnar epithelium to squamous epithelium. These changes in the transition area can lead to the precursor stage of cervical cancer [1, 2]. When a gynecologist diagnoses cervical cancer, the stage of the tumor is determined by FIGO guidelines [3, 4]. FIGO guidelines score according to size and invasiveness of the tumor. There are four main stages, which are divided into two overall stages namely the early and late stage. In an early stage the tumor is relatively small and has not grown into surrounding tissues. This in contrast to the late stage tumors, which are relatively large and have grown into surrounding tissues [4]. The risk of metastases is higher in a late stage (20%-92%), but the risk in an early stage cannot be ignored (<20%)[1].Treatment of tumors in early stages consists of surgically removing the uterus and all the pelvic lymph nodes [5]. Late stage tumors are mainly treated with radiotherapy because these tumors cause too many complications and difficulties when surgically removed. These difficulties include cells getting loose which can result in metastases, when the uterus is surgically removed. Removing the whole tumor will cause even more difficulties because it is inevitable to surgically remove the tumor without damaging the surrounding tissues. This can lead to major complications, e.g peritonitis.

Cervical cancer metastasizes mainly through lymphatic vessels. The pelvic nodes are in 98 to 100% of the cases affected first. It is important to detect possible metastases, because these patients should be treated with chemotherapy in addition to their usual intervention to improve their prognosis [6]. To determine metastases in early stages, removed lymph nodes are histological evaluated. In case of a late stage tumor there is no surgical intervention and metastases are detected with a PET/CT scan. Recent studies show a low accuracy of the PET/CT [6-15]. This means that not all patients with metastases are found with this diagnostic tool or patients are over treated [9, 10, 15]. False negatives, which include patients with metastases that are not found and so do not get treatment against these metastases, have major implications for the prognosis of these patients [16]. The false negatives in the diagnosis of lymph node metastases in women with cervical carcinoma are usually the cause of an adverse prognosis.

For this reason we studied PET/CT in comparison to a new method to detect metastases namely nano-MRI. With this procedure a contrast agent called ferumoxtran-10 (which is an ultra-small particle of iron oxide, also called USPIO) is injected intravenously. After approximately 24 hours, the iron particles have had enough time to spread through the whole body. They have been taken up by macrophages and these transport the iron oxide particles to the lymph nodes. In normal lymph nodes, iron loaded macrophages can spread through the whole node. However, if the lymph node contains metastases, this is not possible. Iron particles are

less present in metastases in comparison to normal lymph node tissue, because macrophages cannot penetrate the tumorous tissue. A low signal will be detected when many iron particles are present in the lymph node. A low signal correlates to no present metastases. A high signal means there are a low amount of iron particles present, which suggests there is a metastasis in the lymph node [17].

In men with prostate cancer, research proved that nano-MRI can detect metastases at 2 mm, while PET/CT detects metastases at a minimal of 8 mm according to Radboud University Medical Center [18]. Because metastases are found earlier with the use of nano-MRI, more effective and personalized treatment can be given, such as chemotherapy, pelvic lymph node dissection or radiotherapy. The use of USPIO for the diagnosis of lymphatic metastases in prostate cancer can also be applied for the diagnosis of lymphatic metastases in cervical cancer. This means fewer side effects for the patient and a greater chance to cure from cervical cancer. This is why this study focuses on the sensitivity and specificity of nano-MRI compared to PET/ CT to diagnose pelvic lymph node metastases in women with cervical carcinoma [12], writing a systematic literature review. The review will focus on the following question: what is the accuracy of nano-MRI in comparison to PET/CT in detecting lymph node metastases in women with cervix carcinoma?

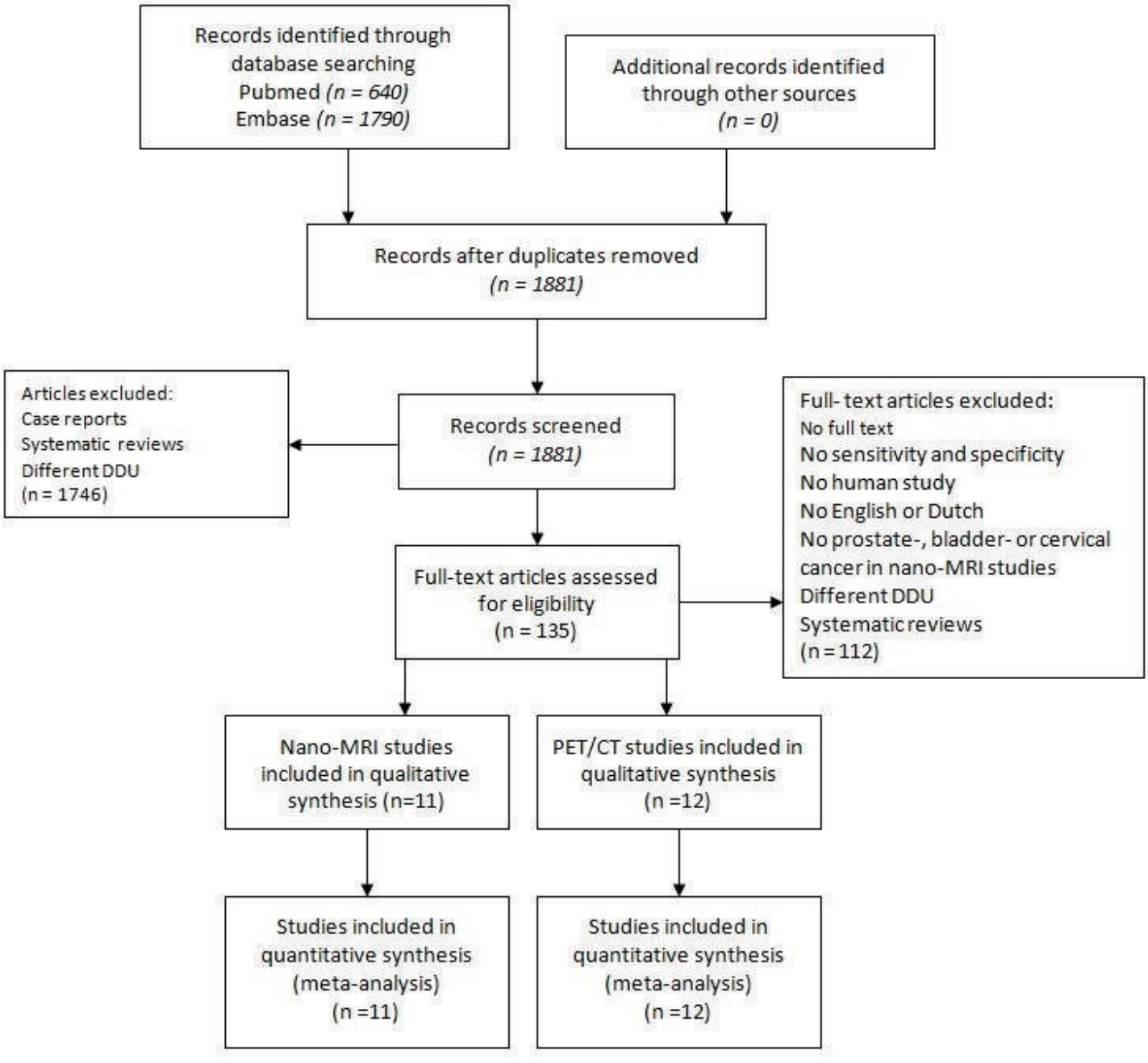

Figure 1: *Flow chart of the research strategy. We retrieved the first selection of articles in PubMed and Embase with the search strategy shown in table 1*. A study was selected when the following criteria were met: domain, determinant and outcome were present; sensitivity and specificity were calculated.*

Methods

Search and selection
Firstly, we formulated a diagnostic research question on the predictive value of nano-MRI in comparison to PET/CT, which is stated in the introduction. Due to the fact that little research has been done on the comparison between nano-MRI and PET/CT in women with cervical cancer we decided to divide our clinical question into two separate questions.

The first sub-question states: what is the accuracy of nano-MRI in the diagnosis of lymph node metastases in women with cervix carcinoma? And the second sub-question states: what is the accuracy of PET/CT in the diagnosis of lymph node metastases in women with cervix carcinoma?

A search filter was designed for both of the sub-questions separately by using relevant synonyms for the domain, cervical cancer and other types of cancers for nano-MRI, determinant, nano-MRI or PET/CT, and for the outcome, pelvic lymph node metastases (Table 1*).

By using title and abstract terms, MeSH and EMTREE terms in the search strategy we found relevant publications in Embase and PubMed. The titles and abstracts of all of these articles were screened for selection. For a more in depth selection the full-text of eligible studies were screened. A study was selected when the following criteria were met: domain, determinant and outcome were present; sensitivity and specificity were calculated or could be calculated using the true positives, true negatives, false positives and false negatives.

Systematic reviews, congress abstracts, animal studies, studies with no full text available and studies with languages other than Dutch or English were excluded.

The search yielded 640 articles in PubMed and 1790 articles in Embase (Figure 1). In total 1881 unique studies were retrieved. Screening the titles and abstracts and the full-text of the remaining studies resulted in 112 articles being excluded. So in conclusion 23 articles were adequate.

Critical appraisal
The quality of methods and reporting of results of the remaining 23 articles were critically appraised according to the criteria in the QUADAS guideline [19] and are presented in Table 2. Articles which scored 2 times high at risk of bias for nano-MRI and 3 times high at risk of bias for PET/CT were seen as low quality. The reason for this is that studies regarding PET/CT are more frequently done and therefore were more critically looked upon.

Statistical analysis
Sensitivity and specificity were used as the principal summary measures. The sensitivity and specificity data were extracted from the articles. The true positives, true negatives, false positives and false negatives were calculated by hand or extracted from the article, if possible. This data was analyzed with a program called Review Manager (RevMan). RevMan provides analytic methods to summarize your results in for example a forest plot. Since a pooled estimator is not possible in RevMan for a diagnostic study, the studies were combined into one overall study and sensitivity and specificity were calculated by RevMan for PET/CT and nano-MRI separately.

The final analysis of the results were executed both with and without the articles which scored high at risk of bias according to the critical appraisal to see if they affect the overall outcome. No additional analyses were done.

Results

In total 23 articles were included in this study including twelve studies regarding PET/CT and eleven studies regarding nano-MRI. Study characteristics are shown in Table 3. All 23 articles were critically appraised according to the QUADAS guidelines (Table 2). The quality of most of the articles was high enough to be included in the report. However six articles scored high at risk of bias, which we stated as two or more insufficient categories. Because of this high risk, the results of these articles cannot be trustworthy. These six articles included Cetina et al.[20], Halpenny et al.[10], Nogami et al.[14], Birkhauser et al.[21], Hong et al.[22], and Keller et al.[23].

The overall sensitivity and specificity of PET/CT including all the studies, even the studies which scored high at risk of bias, were 56% (95% CI: 50%-63%) and 60% (95% CI: 56%-64%), respectively (Figure 2a). The overall sensitivity and specificity for nano-MRI including all of the studies were 83% (95% CI: 78%-87%) and 87% (95% CI: 84%-89%), respectively (Figure 2b).

The overall sensitivity and specificity of PET/CT excluding the articles scoring high at risk of bias according to the critical appraisal were 60% (95% CI: 53%-66%) and 53% (95% CI: 49%-58%), respectively. The overall sensitivity and specificity of nano-MRI excluding the articles scoring high at risk of bias were 84% (95% CI: 80%-88%) and 86% (95% CI: 83%-89%), respectively.

The studies regarding nano-MRI and cervical cancer were Hong et al., Keller et al., and Rockall et al. After taking only these studies into account we found an overall sensitivity and specificity of 75% (95% CI: 51%-91%) and 88% (95% CI: 75%-95%), respectively.

Discussion

The purpose of this study was to compare nano-MRI with the current technique PET/CT to find a more accurate way to diagnose lymph node metastases, since the presence of lymph node metastases can radically modify the prognoses in cervical cancer [16]. Finding the metastases is important to prescribe the right treatment for the patient. A more sensitive and specific technique will track metastases at an earlier stage and so will improve the prognoses.

The current technique PET/CT has a sensitivity and specificity of 56% (95% CI: 50%-63%) and 60% (95% CI: 56% - 64%) respectively, which is not accurate enough. Therefore a better technique is needed to diagnose lymphatic metastases. Promising is the introduction of the new method nano-MRI which uses a contrast agent, ferumoxtran-10. Because of the more accurate approach of this method smaller lymph node metastases will be detected. The iron-oxide particles are transported from the interstitial space to lymphatic vessels via macrophages. Iron loaded macrophages are not present in tumorous tissue and therefore lymphatic metastases will light up on a MR image.

To our knowledge, we are one of the first studies to compare the efficacy of PET/CT and nano-MRI in lymph node metastases in women with cervix carcinoma. In our study we found very promising results for the nano-MRI, to be precise a sensitivity of 83% (95% CI: 0.78-0.87) and a specificity of 87% (95% CI: 0.84-0.89).

However, these promising results, some limitations are present. Firstly, both the selection of the items as the critical appraisal of the articles is done by just one person, due to time constraints. This could have led to selection bias. Selection bias may also arise because we only included

Table 2: *Critical appraisal according to QUADAS guidelines. Risk of bias is judged as "low", "high", or "unclear", shown as +, – and ?, respectively. Concerns regarding applicability are rated as "low", "high" or "unclear", shown as +, – and ?, respectively.*

References				PET/CT scan					
Authors	**Year**	**No. of patients**	**Region**	**TP**	**FP**	**FN**	**TN**	**Sensitivity (%)**	**Specificity (%)**
Cetina et al. [20]	2011	16	Cervical	12	2	0	2	100	50
Choi et al. [7]	2006	22	Cervical	7	1	6	8	57,6	92,6
Chung et al. [8]	2009	34	Cervical	7	1	10	16	41,2	94,1
Goyal et al. [9]	2010	80	Cervical	14	4	10	52	58,3	92,8
Halpenny et al. [10]	2015	47	Cervical	0	0	2	45	0	100
Kim et al. [11]	2009	79	Cervical	14	14	16	35	44,1	93,9
Leblanc et al. [6]	2011	125	Cervical	7	6	14	98	33,3	94,2
Loft et al. [12]	2007	27	Cervical	3	1	1	22	75	96
Moller et al. [13]	2012	136	Cervical	38	30	18	49	57,6	71
Nogami et al. [14]	2015	70	Cervical	5	4	10	51	33,3	92,7
Signorelli et al. [15]	2011	159	Cervical	8	127	20	4	32,1	96,9
Suzuki et al. [24]	2010	100	Cervical	27	67	3	3	90	97
				Nano-MRI					
Anzai et al. [25]	2003	147	All body	99	35	18	68	85	66
Birkhauser et al. [21]	2013	75	Bladder/Prostate	14	3	6	52	70	94
Harisinghani et al. [26]	2003	80	Pelvis	33	2	0	45	100	95,7
Heesakkers at al. [17]	2008	375	Prostate	50	25	11	291	82	93
Hong et al. [22]	2012	28	Cervical	4	1	1	22	80	95,7
Keller et al. [23]	2004	13	Pelvis	1	1	3	4	25	80
Pandharipande et al. [27]	2009	230	All body regions	28	5	0	9	100	64
Rockall et al. [28]	2005	44	Endometrial/Cervical	10	4	1	27	91	87
Thoeny et al. [30]	2009	20	Bladder/Prostate	4	2	1	13	80	87
Thoeny et al. [29]	2014	120	Pelvis	24	12	9	75	72,7	86,2
Triantafyllou et al. [31]	2013	75	Bladder/Prostate	12	9	8	46	58.3	83

Table 3: *Study characteristics of the selected articles. For each article true positives (TP), false positives (FP), false negatives (FN), true negatives (TN) and sensitivity and specificity were extracted and/or calculated. Also the region of the lymphatic metastasis, which were tested, were registered.*

Study	Risk of bias				Applicability concerns		
	Patient Selection	**Index Test**	**Reference Standard**	**Flow and Timing**	**Patient Selection**	**Index Test**	**Reference Standard**
Anzai et al. [25]	+	+	–	+	+	+	–
Birkhauser et al. [21]	–	+	–	+	+	+	+
Cetina et al. [20]	–	+	?	–	–	+	?
Choi et al. [7]	+	+	+	+	+	+	+
Chung et al. [8]	+	+	+	+	+	+	+
Goyal et al. [9]	+	+	?	+	+	+	+
Halpenny et al. [10]	–	–	+	–	–	+	+
Harisinghani et al. [26]	+	+	+	+	+	+	+
Heesakkers et al. [17]	+	+	–	+	+	+	+
Hong et al. [22]	+	+	?	–	+	+	+
Keller et al. [23]	+	+	–	–	–	+	+
Kim et al. [11]	+	+	–	+	+	+	+
Leblanc et al. [6]	+	–	–	+	+	+	+
Loft et al. [12]	+	+	–	–	+	+	–
Møller et al. [13]	+	+	+	+	+	+	+
Nogami et al. [14]	+	–	?	?	+	+	+
Pandharipande et al. [27]	+	+	+	+	+	+	–
Rockall et al. [28]	+	+	+	?	+	+	+
Signorelli et al. [15]	+	?	+	?	+	+	+
Suzuki et al. [24]	+	+	?	?	+	+	+
Thoeny et al. [30]	+	+	+	+	+	–	+
Thoeny et al. [29]	+	+	+	?	+	+	+
Triantafyllou et al. [31]	+	+	+	+	+	+	+

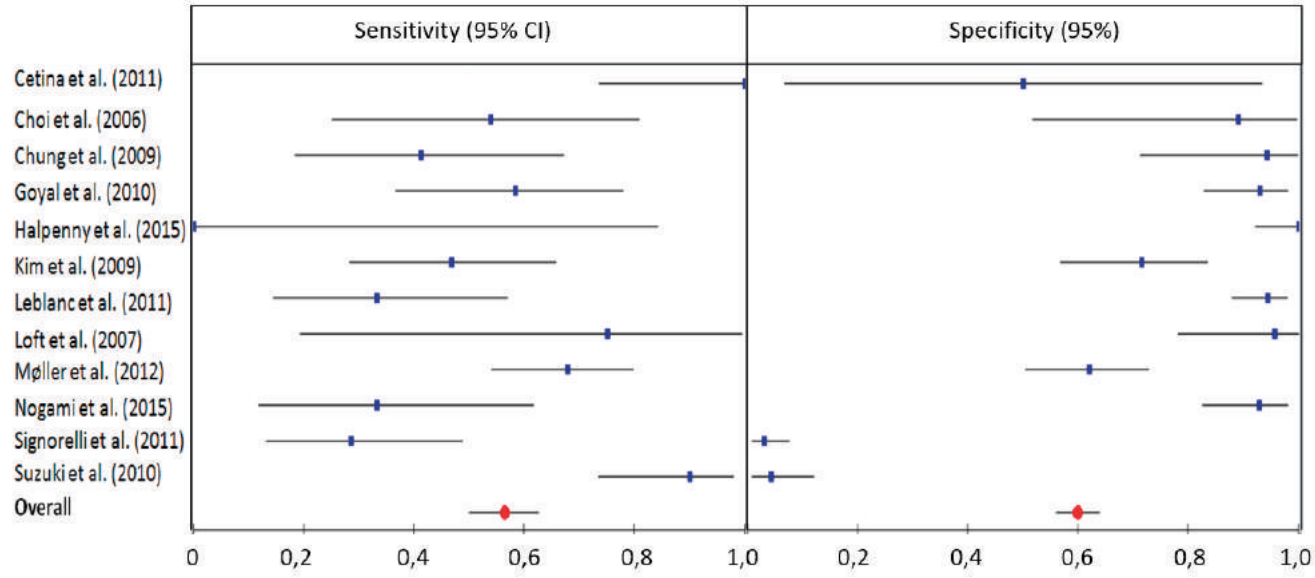

Figure 2a: *Forest plot of studies regarding PET/CT [6-15, 20, 24]. All studies included: Sensitivity = 56% (95% CI: 50%-63%); Specificity = 60% (95% CI: 56%-64%). Cetina et al., Halpenny et al., Nogami et al. excluded: Sensitivity = 60% (95% CI: 53%-66%); Specificity = 53% (95% CI: 49% - 58%).*

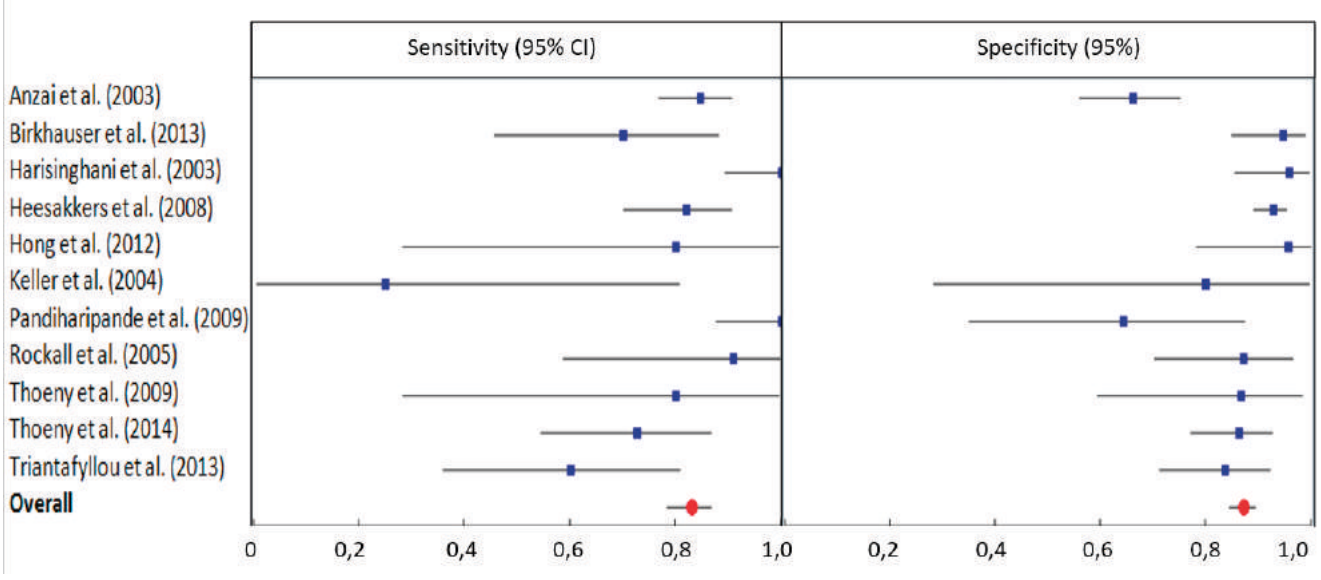

Figure 2b: *Forest plot of studies regarding nano-MRI [17, 21-23, 25-31]. All studies included: Sensitivity = 83% (95% CI: 78%-87%); Specificity = 87% (95% CI: 84%-89%). Birkhauser et al, Hong et al., Keller et al. excluded: Sensitivity = 84% (95% CI: 80%-88%); Specificity = 86% (95% CI: 83%-89%).*

articles in Dutch or English. Secondly, the studies viewing nano-MRI were not only linked to cervical cancer, but also to bladder cancer and prostate cancer. This because of the lack of researches that consider nano-MRI combined with cervical cancer. This could have had an effect on the extent to which our outcome can be generalized.

The difference in overall sensitivity and specificity of PET/CT after including all studies or excluding the articles with a high risk of bias were negligible. This also applies to the difference in overall sensitivity and specificity of nano-MRI. So per definition, excluded articles do not necessarily represent the articles with the most extreme outcomes.

The studies regarding nano-MRI and cervical cancer included two articles with a low quality and therefore the overall outcome of this analysis was not trustworthy.

A further issue is that all studies have been combined into one overall study because with our dataset it was not possible to calculate a pooled estimator in RevMan. Not every study is equally good and heterogeneity was not considered. Besides, each study is weighted equally because the population size no longer counts. We tried to solve this problem in a statistical program called SAS but we were not capable to do this in our timeframe.

A final point of discussion that occurred in some studies was the differences in use of readers. A reader is someone who assesses the outcome of the nano-MRI scan. If possible we calculated the mean outcome of the readers but if not we decided to go for the safest option and take the outcome of the worst reader. This could lead to an overestimation as well as an underestimation of the final result.

Conclusion

The sensitivity and specificity of nano-MRI are considerably more accurate than the sensitivity and specificity of PET/CT. However this research shows no hard evidence for this and more research is needed to make a solid conclusion.

It is expected that nano-MRI could be a better alternative to detect metastases in pelvic lymph nodes in women with cervical cancer.

Acknowledgments

Casper Tax* has given his consent for publication.

*Casper Tax, PhD Candidate Evidence Based Surgery, Radboudumc.

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EXAM QUESTIONS

As RAMS aims to enlighten both students and professionals, we would like to present you two exam questions. Find out if you can remember what you learned during the bachelor! The right answers can be found further on in this journal.

We challenge you!

Question 1
After finishing a marathon, many runners immediately cease walking. It frequently happens that a participant faints behind the finish line. What is the primary cause of this? This is primarily caused by a decrease in:

A. blood pressure
B. venous return
C. cardiac output

Question 2
In case of chronic alcoholism, the VLDL concentration in the blood is:

A. decreased
B. normal
C. elevated

The answers to these questions can be found on page 15 in this journal.

INJECTING BEFORE EJECTING

Jasper M. Maters[1], Wendy Schreurs[2]

[1]Master Medical student, Radboud University Medical Center, Nijmegen, the Netherlands
[2]Bachelor Medical student, Radboud University Medical Center, Nijmegen, the Netherlands

Introduction

Editorial

"Birth control linked to depression" [1]; "Combined contraceptive pills increase risk of blood clots"[2]; "Birth control may alter the structure of a woman's brain" [3]. The headlines do not lie, there is room for improvement concerning contraception. Women face many side-effects like mood swings, painful breasts and weight gain when taking oral contraceptives. The question rises: why do mostly women have to experience these side-effects?

Let us talk about a subject that would make every female heart beat faster: contraceptives for men. The possibilities for birth control for men stretch beyond condoms and vasectomy. In this article, we will discuss current developments, in particular three upcoming methods of contraception for men: Vasalgel, hormonal injections and anti-Eppin. For better understanding, let us first go back to the basics: How does spermatogenesis work? How can you interfere with this process in order to develop effective contraceptives?

Spermatogenesis

Each day millions of sperm cells are produced in human testicles. This is the process of the gradual transformation of germ cells into spermatozoa, called spermatogenesis (see figure 1). The spermatogenesis starts with stem cells called type A spermatogonia. These cells undergo mitosis several times and become type B spermatogonia. Type B spermatogonia eventually turn into spermatids through meiotic divisions. During the final stage of spermatogenesis, called the spermiogenesis, spermatids undergo differentiation into mature, motile spermatozoa (sperm cells). Non-motile spermatozoa are transported to the epididymis in testicular fluid secreted by the Sertoli cells. There they acquire motility [4].

Postcoital, human sperm cells can survive within the female reproductive tract for more than 5 days because of seminal plasma. This is produced in the seminal vesicles, prostate gland and urethral glands and secreted together with the spermatozoa during ejaculation. This seminal plasma provides a nutritive and protective medium for the spermatozoa during their journey through the female reproductive tract.

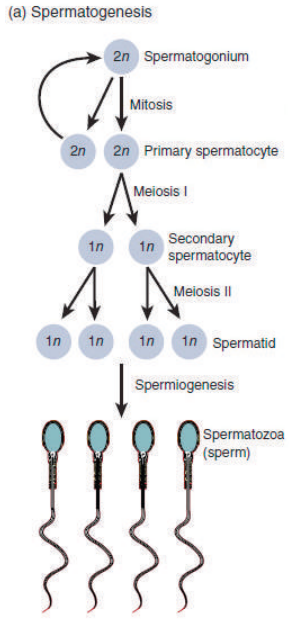

Figure 1: *Adapted from OpenStax College. Anatomy & Physiology, Connexions Web site. June 19, 2013. http://cnx.org/content/col11496/1.6/.*

Hormonal control

Spermatogenesis is controlled by a feedback mechanism involving the hypothalamus, anterior pituitary and testes. Gonadotrophic releasing hormone (GnRH) released by the hypothalamus travels through portal vessels to the anterior pituitary (see figure 2). Here, gonadotrophic cells respond by producing luteinising hormone (LH) and follicle stimulating hormone (FSH). LH stimulates the Leydig cells to convert steroids to testosterone. Together with FSH, testosterone contributes to the activation of the Sertoli cells. As mentioned before, Sertoli cells provide factors necessary for the successful progression of spermatogonia into sperm cells.

How to intervene

The question is: how can we intervene in this process of spermatogenesis? Non-hormonal and hormonal targets could be the solution.

Non-hormonal targets include sperm production at the testicular level, sperm maturation and sperm motility. On hormonal level, you could think of hormones which suppresses the anterior pituitary and hypothalamus like inhibin and testosterone. A different option would be substances which counteract LH and FSH.

We will now review three of the most promising methods of contraception which are under development.

Vasalgel

This non-hormonal method targets the tubes carrying sperm from the epididymis to the ejaculatory duct (vas deferens). Vasalgel, developed in the USA, is a high molecular styrene maleic anhydride (SMA) that is injected into the man's scrotum under local anesthetic. After injection, the SMA acid in Vasalgel forms a hydrogel and fills the lumen of the vas deferens, creating a mechanical barrier for sperm, while other fluid can still pass through. A second injection would undo the effect by dissolving the polymer [5].

A great advantage of Vasalgel is that only one trip to the policlinic is needed to put a halt to fertility as long as desirable. Moreover, it might be

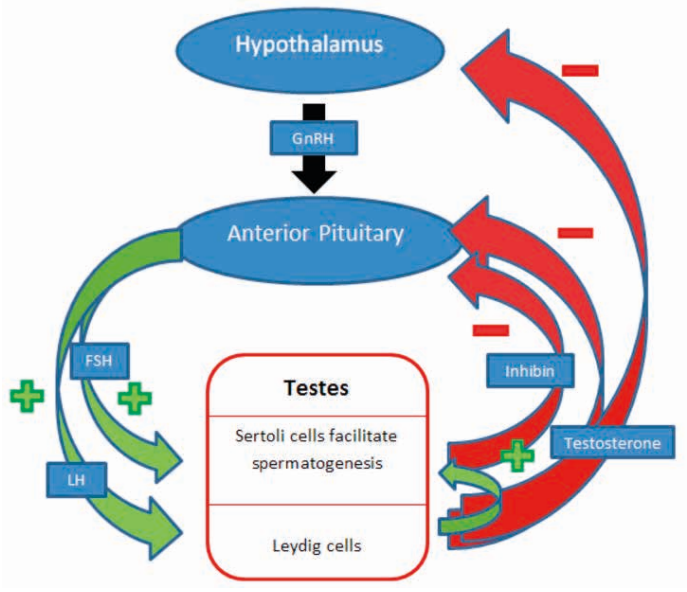

Figure 2: *Adapted from OpenStax College. Hormonal Control of Human Reproduction. October 17, 2013. http://cnx.org/content/m44841/latest/Figure_43_04_01.jpg*

reversible without decreasing the fertility of the man. The possibility of reversing the effect of Vasalgel by a second injection makes it a good alternative for the temporary methods of contraception.

Vasalgel could also be a good replacement for vasectomies. Back pressure caused by remaining ejaculation is an occasional issue with vasectomy. The back pressure occurs because a vasectomy fully close the tubes while Vasalgel does not. Sperms cannot pass the polymer because they are too big, but other fluids can find their way out through the body. This means the back pressure is no issue with Vasalgel and makes it a good alternative.

But what are the disadvantages? Other than that it requires a needle to be inserted in the scrotum, Vasalgel does not seem to have that many. A study with rabbits showed that 12 months after being injected with Vasalgel, the mucosal epithelium in the tube of the animals demonstrated some degree of attenuation. However, this tissue response only appeared to be minor and were no different from any other reaction of the body against a foreign body. These histological observations did not appear to be dependent upon the duration of Vasalgel. Further studies have to reveal if there are any more disadvantages [6].

Unfortunately, Vasalgel has not been tested on humans yet. The latest study of Vasalgel was performed in rabbits. The researchers' conclusion was that the onset of azoospermia was rapid and durable over the 12 month period by using Vasalgel. And as mentioned before, the only disadvantage was the mucosal epithelium attenuation.
So far Vasalgel sounds promising, since it can even stop rabbits from reproducing. However, further investigation is needed to look into the effects on humans.

Hormonal injections
Hormonal contraceptives are used very often by women. It won't be a surprise that the endocrine system can also be a target for male contraceptives.

As shown in figure 2, intratesticular testosterone stimulates spermatogenesis, but exogenous testosterone gives negative feedback to the anterior pituitary and hypothalamus. It thereby decreases secretion of gonadotrophic hormones: FSH and LH. That is how testosterone injections can reduce sperm production. A testosterone-progestin combination regimen was shown to be even more effective than testosterone only in suppressing spermatogenesis [7]. This is attributed to the fact that progestins can also inhibit gonadotropin secretion from the pituitary.

So testosterone in combination with progestins could serve as an effective contraceptive. The testosterone has to be injected into muscle tissue every four weeks, making it a very invasive method. That is not the only disadvantage. Unlike Vasalgel, hormonal injections have already been tested on humans. Several studies show many side-effects, as can be expected when using a hormonal target. One study showed a reversible reduction in testicular volume, an increase in haemoglobin and a reduction in HDL cholesterol [8]. A more recent study revealed side-effects like acne, injection site pain, increased libido and mood disorders [9]. These side-effects have a lot in common with the side-effects reported by women using hormonal contraception.

Healthy men were given injections every four weeks. The efficacy was determined after the last injection. Reversibility was determined during the recovery phase which began 8 weeks after the final injection and could last for up to 56 weeks. Around 96 percent of the continuing users had decreased sperm concentration within 24 weeks. Only four pregnancies occurred with the continuing users. In 94.8 percent of the cases, the suppression of spermatogenesis was reversible.

To summarize, testosterone injections resulted into near-complete and reversible suppression of spermatogenesis. The frequencies of side effects were relatively high, causing six percent of the participants to discontinue the injections. Furthermore, the usability of weekly to monthly intramuscular injections could be doubtful.

Anti-Eppin
Another promising target for contraception is the epididymal protein inhibitor (Eppin). Eppin is secreted by Sertoli cells and is found on the human sperm surface complexed with other proteins [10]. During ejaculation, the Eppin protein complex is activated. Eppin's functions include providing antimicrobial protection and sperm motility regulation [11].

Inhibition of Eppin could provide infertility by decreasing the motility of the sperm cells. Therefore, researchers have developed an antibody that binds to Eppin and blocks its function. The efficacy of this Eppin-based therapy has been tested in a study on monkeys. Nine male monkeys (Macaca radiata) were immunised with recombinant human Eppin [12].

None of the immunised monkeys with high anti-Eppin titers were able to impregnate females, indicating that these seven males were infertile. After the completion of fertility testing, immunisations were stopped. During this recovery time period, five monkeys recovered their fertility.

Although this represented a success, a number of concerns for further clinical development of this approach was raised. Firstly, immunisations were required frequently (every 3 weeks) throughout the treatment to maintain high titer anti-Eppin antibodies. Secondly, not all animals mounted a sufficient immune response. Thirdly and finally, two out of nine animals failed to reproduce after ending the injections, even after more than a year. A vaccine intended for men could also have such inconsistent responses among individuals. Reversibility could be unpredictable as well.

Researchers are now working to find organic compounds that mimic the effect of anti-Eppin antibodies binding to the sperm surface [13].

These compounds are hoped to produce a more consistent response among individuals than the immunisations.

Conclusion

As presented, not only the woman's body can be used to prevent pregnancy. We have looked at 3 different targets for male contraception: Vasalgel, Hormone therapy and anti-Eppin. These methods are very different and so are the advantages and disadvantages. The one thing in common is that they are all in experimentation phase. It will still take some time before these contraceptives could be on the market. For now, women will have to be the ones to deal with side effects as long as they are not ready to deal with a baby.

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CORRECT ANSWERS TO THE EXAM QUESTIONS

Answer question 1
B. venous return

While running or walking, your leg muscles contract and contribute to the return of blood from your legs to your heart (skeletal-muscle pump). Your heart rate remains elevated after you stop running, but your leg muscles are no longer contracting. Blood pools in the legs, consequently decreasing blood pressure and cardiac output, causing lightheadedness and possibly passing out.

Bloktoets Circulatie en Respiratie I, maart 2015

During the exam, 65% of the participants answered this question correctly.

Answer question 2
A. decreased

Long-term moderate alcohol intake causes an increase in LPL activity. LPL hydrolyses the triglycerides in VLDL, turning them into LDL particles thus decreasing VLDL concentration. N.B. Acute alcohol intake and heavy alcohol abuse often show the opposite effect.

Bloktoets Stofwisseling, water- en zouthuishouding I, april 2015

During the exam, 20% of the participants answered this question correctly. 52% answered C: elevated.

The exam questions can be found back on page 12 in this journal.

THE EFFECT OF THE MENSTRUAL CYCLE ON EXERCISE PERFORMANCE

Janneke Elzinga[1]

[1]Master Student Molecular Mechanisms of Disease, Nijmegen, the Netherlands

Introduction

Editorial

Imagine the daily life of a professional male athlete – regardless of his discipline – whose diet, training schedule and sleeping patterns are fine-tuned to optimize their performance. To make it even more complicated: how about the female athlete, subject to her menstrual cycle? Unfortunately, evidence on the effect of hormonal levels on exercise performance is very scarce as women are still significantly underrepresented in studies on sport and exercise medicine [1]. Bruinvels et al. recently reported a negative impact of the menstrual cycle on exercise training and performance, as experienced by both elite and non-elite female athletes [2]. Moreover, the need for more research into the hormonal effects on athletic performance to address female physiology and avoid further sex misrepresentation is emphasised [3]. What is the current evidence on the hormonal influences on exercise performance and what are potential obstacles in this research field?

The menstrual cycle

Based on the levels of ovarian hormones – oestrogen and progesterone – the ovulatory menstrual cycle can be divided into three phases. During the early-follicular phase (day 2-7), levels of both hormones are low. During the late-follicular phase, oestrogen levels increase just before ovulation, which is followed by the mid-luteal phase with increasing levels of both hormones. Apart from their reproductive function, oestrogen and progesterone are known to influence other physiological systems, such as the thermoregulatory, respiratory, cardiovascular and metabolic system. Consequently, these hormones influence exercise performance and, moreover, interact with each other, which further complicates the system. It is not surprising that most research on female exercise performance has focused on the early follicular phase, e.g. to minimise the impact of hormone levels on study outcome [4].

Effects on exercise performance

In exercise medicine, different physiological parameters are studied, depending on the discipline of interest. A review by Janse de Jonge looked at the effects of hormone fluctuations on three different exercise-related outcomes: skeletal muscle contractile characteristics, maximal oxygen consumption (VO2max) as indicator of aerobic exercise performance and prolonged exercise performance. Based on the available evidence, the author concluded that muscle contractile characteristics and VO-2max are not affected by the menstrual cycle. In maximising prolonged exercise performance on the other hand, female endurance athletes may have to adjust for their menstrual cycle. This is especially true when competition is expected to take place in hot, humid conditions due to increased body temperature and potentially increased cardiovascular strain during the (mid-)luteal phase. Included studies, however, showed conflicting results and several limitations in menstrual cycle research are put forward, as will be discussed later on [5].

Effects on exercise metabolism

Oosthuyse and Bosch acknowledge the inconsistency in studies investigating the effect of the menstrual cycle on exercise performance and reviewed the alterations in metabolism associated with ovarian hormone levels [4]. They suggest that menstrual phase variations in exercise performance could be largely due to changes in exercise metabolism driven by fluctuations in ovarian hormone levels. In endurance performance, for example, oestrogen might promote performance by influencing carbohydrate, fat and protein metabolism, whereas progesterone often acts antagonistically. Consequently, endurance performance might only be higher in the mid-luteal phase compared to the early follicular phase when the E/P (oestrogen-to-progesterone) ratio is high. Metabolic perturbations were often found to be dependent on the extent to which hormone levels increase between the different phases and the E/P ratio, but energy demand and nutritional status could be confounding. The authors state that the effect of hormone levels on exercise metabolism and performance occurs in a highly complex and often tissue-specific manner [4].

Limitations

Several limitations are encountered when studying the menstrual cycle. This is partly due to the biologically complex effects of hormones, as discussed previously. Next to the large variations in hormone levels between different phases, there is a high intra- and inter- individual variability in oestrogen and progesterone levels [6]. Due to the pulsatile secretion of hormones, levels might differ even within a day, for instance with higher progesterone in the morning [7,8]. Additionally, exercise is known to temporarily increase hormone levels, which makes the timing of measurement critical [9, 10]. Furthermore, a majority of the women does not have a natural cycle due to the use of oral contraceptive. Some female athletes, in particular endurance athletes, no longer menstruate due to intensive athleticism [11].

Menstrual phase comparative research also shows limitations with respect to methodology. Difficulties arise when determining the cycle phase of the female subject. Counting the number of days from the onset of menstruation is not sufficient, as there is a high incidence of anovulation and luteal phase deficiency (LPD) in active women with regular bleeding. Both are characterised by low progesterone levels during the second half of the cycle [12]. Basal body temperature or urinary luteinising hormone are two other phase indicators, but the golden standard is the actual measurement of oestrogen and progesterone. This method comprises the only way to identify the three phases. However, the most accurate determination – in serum – is quite invasive. The use of the E/P-ratio, instead of only looking at absolute values, may provide information about opposing effects of oestrogen and progesterone. Next to the complications in determining the actual phase, there has been inconsistency in phase terminology and subsequent protocols used by previous studies, which further impedes comparison of existing evidence [5].

Apart from hormonal influences, exercise performance is already a complex combination of multiple parameters. A remarkable example is the

VO2max, which is determined by hormone-associated variables and additional factors varying per individual [5]. Confounding by these additional factors are hard to exclude in determining the effect of hormonal levels alone.

Furthermore, exercise trials investigating hormonal effects have not always been methodologically representative. For instance, Oosthuyse and Bosch suggested to investigate the effect of oestrogen in ultra-endurance events, considering its (long-term) metabolic influence on fat use and sparing glycogen stores, whereas exercise trials to date were limited to less than 2 hours [4]. In other cases, good methodology is almost impossible: a time-to-exhaustion test at submaximal intensity is not very reproducible, but this is not a hormone-specific issue [5].

Conclusion

Although research on hormonal effects on exercise performance has been scarce and inconsistent, it should be clear that it comprises a highly complex subject that definitely deserves more attention. Apparently, the effect of hormones on exercise performance varies at least per discipline, between women and even within one individual. It is likely that female athletes can optimize exercise performance according to their menstrual cycle. Georgie Bruinvels (see introduction) recently launched a campaign with Libresse (the Red.fit programme [13]), in which advice is given on exercise and nutrition based on the cycle phase. This initiative is a step forward in creating awareness of the hormonal effects on exercise performance. Increasing attention to female physiology as a clearly distinct aspect of exercise medicine can possibly help uncover and overcome potential obstacles currently faced, to further advance this field of research and support female athletes in their exercise goals.

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EFFECT OF ALPHA-GLUCOSIDASE INHIBITORS ON PREDIABETES

Julie Verhoef[1], Martin Flipsen[1], Anouk Lamers[1], Suzanne Moelands[1], Monse Wieland[1]

Corresponding Author: (julieverhoef@gmail.com)

[1]Bachelor Biomedical Science student, Radboud University Medical Center, Nijmegen, the Netherlands

ABSTRACT

Systematic Review

BACKGROUND: Prediabetes is a precursor stage of type 2 diabetes mellitus (T2DM). T2DM is an increasing global problem, leading to many clinical complications. Alpha-glucosidase inhibitors slow down uptake of glucose in the small intestine resulting in decreased blood sugar fluctuations, which makes it a treatment for T2DM. It is not yet known whether it is also beneficial for people with prediabetes.
OBJECTIVE: The purpose of this evidence-based systematic review is to research the effect of alpha-glucosidase inhibitors on the development of prediabetes to type 2 diabetes mellitus, glucose levels, macro vascular morbidity, mortality and side effects in prediabetic people.
METHODS: The PubMed and Embase databases were used to search for articles, using synonyms of the determinant, alpha-glucosidase inhibitors, and the domain, prediabetic people. All investigators abstracted data and evaluated study quality independently. Each article was evaluated by at least two researchers. The critical appraisals were based on the Cochrane risk of bias tool. The results were evaluated by meta-analyses.
RESULTS: The search strategy resulted in a total of 917 articles, of which eight were relevant to our question and were included in this systematic review.. There was a significant reduction in development to T2DM and in HbA1c values in the intervention group compared to the placebo group. There was no significant difference in fasting glucose. One study reported a reduction in cardiovascular events in the intervention group. The intervention had no association with mortality.
CONCLUSION: The risk of development to T2DM decreases with alpha-glucosidase inhibitors treatment. Furthermore, alpha-glucosidase inhibitors lower the blood glucose levels and decrease macrovascular morbidity. It is not associated with mortality. However, there are side effects and no account is taken of the complications other than macrovascular morbidity that accompany T2DM. Even though alpha-glucosidase inhibitors have positive effects, we cannot yet recommend to prescribe alpha-glucosidase inhibitors to prediabetic people.

KEY WORDS: Prediabetes, Alpha-glucosidase Inhibitors, Acarbose, Glucose Levels, Macrovascular Morbidity

*Supplementary material is marked with * and can be found online at www.ramsresearch.nl*

Introduction

Type 2 diabetes mellitus is a major global health problem. There were 382 million people with diabetes in 2013, and this number is expected to rise to 592 million by 2035. The incidence is rising rapidly, due to obesity, decreased physical activity and aging of the population [1]. T2DM increases the risk of cardiovascular diseases, diabetic nephropathy and blindness. Prediabetes is a precursor stage of T2DM and increases the risk of hypertension and other cardiovascular diseases [2]. It is therefore important that prediabetes is treated, so that T2DM can be prevented [3,4].

Prediabetes is an intermediate metabolic state between normoglycaemia and diabetes. Prediabetic patients have impaired glucose tolerance (IGT) and impaired fasting glucose (IFG). IGT is characterized as 2-hour plasma glucose of 7,8-11,0 mmol/L after ingestion of 75 g of oral glucose. If this value gets above 11 mmol/L it indicates T2DM. IFG is a similar condition, defined as fasting plasma glucose of 5,6 - 6,9 mmol/L, according to American Diabetes Association (ADA) [5]. A value higher than this is an indication for T2DM. Additionally, glycated hemoglobin (HbA1c) values reflect the average blood glucose levels over a period of twelve weeks [6,7].

In 2015 the prevalence of IGT was estimated to be 318 million worldwide. The International Diabetes Federation predicts that this number would increase to 481 million worldwide by 2040 [8]. The only current treatment for prediabetes is a lifestyle change. People have to lose weight by exercising and a healthy eating habit [9]. Therefore it is important that more research is done to prevent the development of T2DM.

Acarbose, voglibose and miglitol are prescribed to patients with T2DM. These drugs are alpha-glucosidase inhibitors, which slow down the uptake of glucose in the small intestine. This causes the postprandial blood sugar to increase less rapidly, which decreases the fluctuations in blood sugar. Alpha-glucosidase inhibitors can cause gastrointestinal side effects and it has not yet been proven whether they can prevent the progression of prediabetes to T2DM [6]. Hence, the goal of this systematic review is to investigate the effect of alpha-glucosidase inhibitors on the development of T2DM, glucose levels, macro vascular morbidity, mortality and side effects in prediabetic people.

Research question

What is the effect of alpha-glucosidase inhibitors on the development of prediabetes to type 2 diabetes mellitus, glucose levels, macro vascular morbidity, mortality and side effects in prediabetic people?

Methods

Search strategy and selection

We used the PubMed and Embase databases to search for relevant articles. A search filter was designed by using all relevant synonyms of the determinant, alpha-glucosidase inhibitors, and the domain, prediabetic people (Table 1*). In the PubMed search alpha-glucosidase was used as a term for the determinant instead of alpha-glucosidase inhibitors. The

latter is a relatively new term and is classified under glycoside hydrolase inhibitors, which is a MeSH term that includes many other, irrelevant enzymes that were not applicable for our research.

Articles were included if they met the inclusion criteria shown in the flow chart (Figure 1). We excluded all non-clinical studies and studies not written in English. Furthermore, articles that were not available online were excluded due to insufficient resources.

Further relevant articles were found using the snowball method, which entails going through the citations of an article to find more relevant studies.

Critical appraisal

Two researchers independently judged the trustworthiness, value and relevance of each article. Bias was examined by looking at randomization, allocation concealment, blinding of participants, researchers and analysts, incomplete outcome data selective outcome reporting and other sources of bias. These appraisals were combined to form a final evaluation. One article was deemed not valid enough for inclusion, because it was a very small pilot study and had a very high risk of bias. The critical appraisal of the remaining eight articles has been summarized in a Cochrane risk of bias summary and a risk of bias graph (Figure 2).

Analysis

First, the characteristics of the included studies were defined and summarized (Table 2). The relative risk (RR) and mean differences were compared between the studies. The significance was defined as $\alpha<0,05$. The outcomes of development to T2DM, fasting plasma glucose and HbA1c were analyzed in a meta-analysis in Review Manager. Secondly, we summarized the outcomes macrovascular morbidity and the side effects.

Results

Using the aforementioned search strategy, 917 publications were obtained. After the removal of duplicates, 807 articles remained. In the first selection step these articles were screened based on title and abstract, which led to a selection of 131 articles. The references of the reviews were checked to make sure no relevant publications were missed. In the second selection step, abstracts and full articles were screened and the critical appraisal was executed, which narrowed the number of publications down to eight. These eight articles are summarized in Table 2.

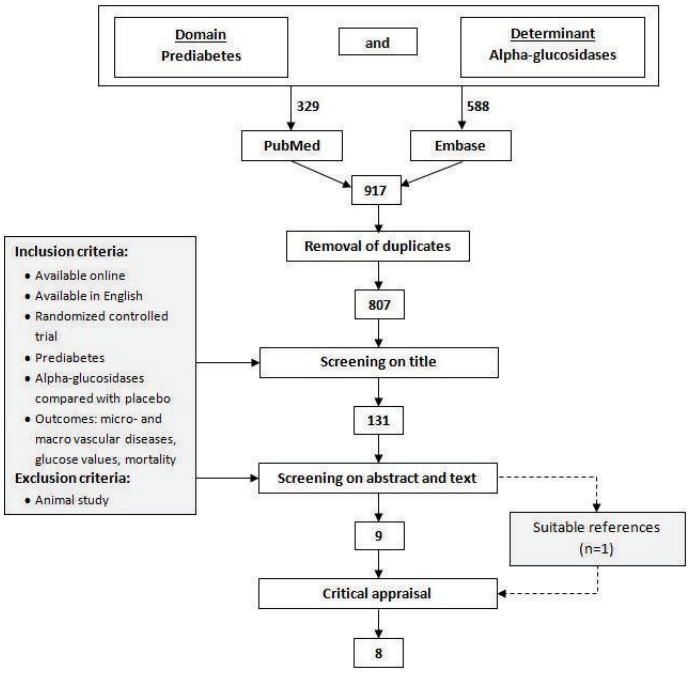

Figure 1: *Flowchart of the search process.*

Table 2: **three times a day, ** STOP-NIDDM trial, ***IOC country codes*

Author (publication year)	Study design	Setting	Study period (weeks)	Amount of included patients (n)	Alpha-glucosidase (n)	Placebo (n)	Female (%)	Mean age (years)	Mean BMI (kg/m²)	Baseline HbA1c (%)	Baseline glucose values (mmol/L)	Dose
Kawamori et al. (2007) [10]	Randomized controlled trial	Japanese institutions	208	1505	786	737	*Voglibose:* 40 *Placebo:* 40	55,7	*Voglibose:* 25,76 *Placebo:* 25,89		*Voglibose:* 5,80 (0,55) *Placebo:* 5,85 (0,56)	0,2 mg t.i.d.*
Kirkman et al. (2006) [11]	Randomized controlled trial	IU School of Medicine, WUSM	343	219	109	110	*Acarbose:* 67,0 *Placebo:* 65,4	53,7	*Acarbose:* 35,1 *Placebo:* 35,2	*Acarbose:* 6,35 (0,65) *Placebo:* 6,33(0,63)	*Acarbose:* 6,78 (0,77) *Placebo:* 6,70 (0,74)	100 mg t.i.d.
Nijpels et al. (2008) [12]	Randomized controlled trial	Residents of Hoorn	182	66	30	36	*Acarbose:* 49,2 *Placebo:* 50	*Acarbose:* 58,5 *Placebo:* 56,5	*Acarbose:* 28,4 *Placebo:* 29,5	*Acarbose:* 5,9 (0,5) *Placebo:* 5,6 (0,6)	*Acarbose:* 6,6 (0,5) *Placebo:* 6,5 (0,6)	50 mg t.i.d.
Rudovich et al. (2011) [13]	Randomized controlled cross-over study		12	63	31 (acarbose-placebo)	32 (placebo-acarbose)	44,4	58,2	31,6	5,7 (0,5)	*Acarbose:* 5,3 (0,6) *Placebo:* 5,3 (0,6)	100 mg t.i.d.
Pan et al. (2002) [14]	Randomized controlled trial	Five centers in the mainland of China	16	252	125	127	*Acarbose:* 60,8 *Placebo:* 59,1	*Acarbose:* 53,4 *Placebo:* 55,6	*Acarbose:* 25,6 *Placebo:* 25,8	*Acarbose:* 6,51 (0,72) *Placebo:* 6,61 (0,62)		50 mg t.i.d.
Hanefeld et al. (2004) [15]**	Randomized controlled trial	Hospitals in CAN, GER, AUT, NOR, DEN, SWE, FIN, ISR, ESP***	160	115	56	59	*Acarbose:* 44,6 *Placebo:* 33,9	*Acarbose:* 54,8 *Placebo:* 55,6	*Acarbose:* 29,5 *Placebo:* 28,6	*Acarbose:* 5,92 (0,50) *Placebo:* 5,73 (0,55)	*Acarbose:* 6,44 (0,50) *Placebo:* 6,34 (0,58)	100 mg t.i.d.
Chiassion et al. (2002, 2003) [16, 17]**	Randomized controlled trial		160	115	682	686	*Acarbose:* 52 *Placebo:* 50	*Acarbose:* 54,3 *Placebo:* 54,6	*Acarbose:* 31,0 *Placebo:* 30,9		*Acarbose:* 6,23 (0,50) *Placebo:* 6,24 (0,53)	100 mg t.i.d.

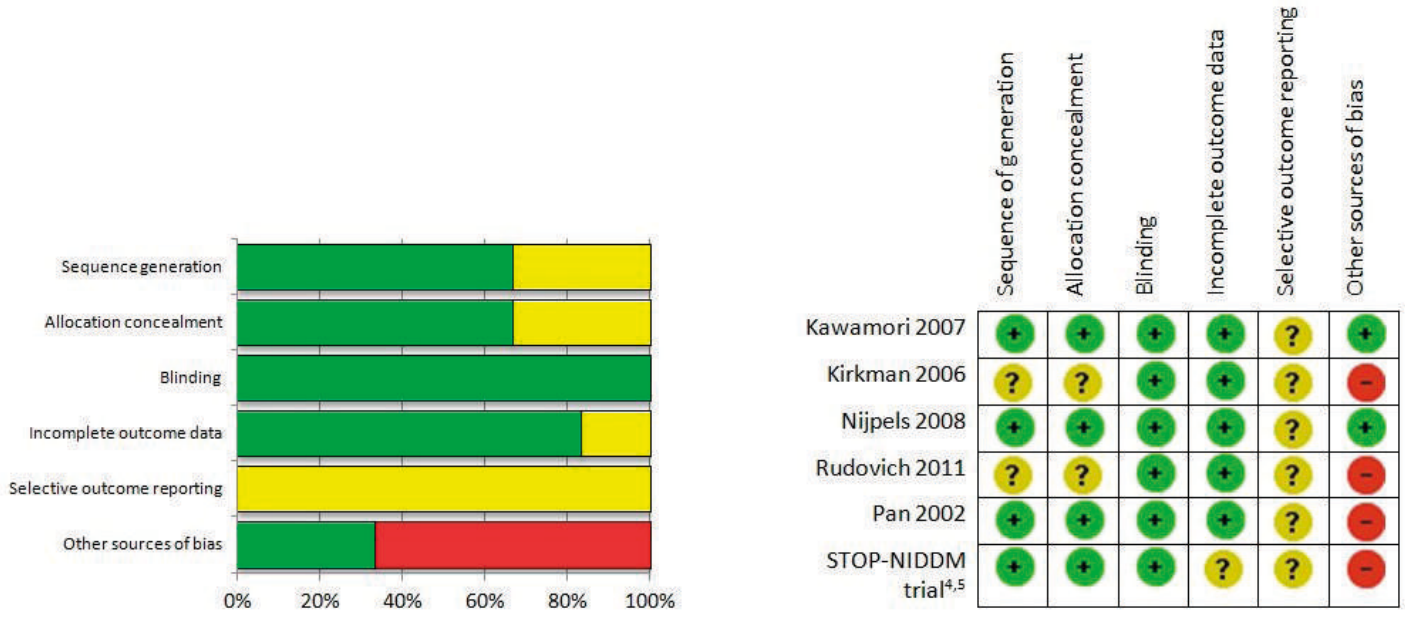

Figure 2: *Risk of bias graph and summary for studies included in the systematic review of the effect of alpha-glucosidase inhibitors on the prevention of T2DM in prediabetic people. Green = low chance of bias; yellow = unclear; red = high chance of bias. +, adequate; –, inadequate; ?, unknown, no information given. a Standardized criteria for validation in therapeutic research as stated in the Cochrane collaboration.*

Four studies reported the number of patients that developed T2DM during follow-up. A meta-analysis was performed in which the number of events was compared to the total number of patients in the intervention or placebo group (Figure 3A). In the study of Nijpels et al. (2008) [12], only the percentage of events and the total number of patients in the group were given. The number of events was calculated by multiplying the number of patients in the group with the percentages of events.After pooling the results, there was a significant effect of alpha-glucosidase inhibitors compared to placebo on development to T2DM (Relative Risk (RR): 0,80 [0,70; 0,91]). The risk difference (0,08 [0,03; 0,13]) was used to calculate the number needed to treat (NNT) [5, 12]. The results of the studies were homogeneous (I2=0%).

Three of the remaining studies reported the fasting plasma glucose levels of the baseline and endpoint. The difference between baseline and endpoint was calculated and reported, in order to take the, differences in baseline values into account. The standard deviation of the difference was calculated by pooling the standard deviations of the baseline and endpoint levels. A meta-analysis of the difference in fasting plasma glucose levels of the three studies was performed (Figure 3B). After pooling the results, no significant difference was observed between the alpha-glucosidase inhibitors and placebo (mean difference: -0,25 [-0,50; 0,01]). The results of the studies were homogeneous (I2= 0%).

The baseline and endpoint levels of HbA1c were given by three of the included studies (Figure 3C). The difference between the baseline and endpoint levels was calculated and reported. This was to correct for the variation between baseline levels. The standard deviation was calculated as mentioned before. There was a significant effect of alpha-glucosidase inhibitors compared to placebo after pooling the results (mean difference: -0,51 [-0,76;-0,26]). The results of the studies were homogeneous (I2=24%).

In the STOP-NIDDM Trial the development of major cardiovascular events (coronary heart disease, cardiovascular death, congestive heart failure, cerebrovascular event and peripheral vascular disease) was described [15,17]. At least one cardiovascular event was experienced by 32 patients from the placebo group and 15 patients from the intervention group. The cumulative annual incidence was 4.7% in the placebo group versus 1.4% in the intervention group. Myocardial infarction occurred significantly less often in the intervention group compared to the placebo group [17].

In the study of Kawamori et al. (2007) [10], six deaths occurred in the intervention group and none in the placebo group. These include accidents, suicide and lung cancer. None of these deaths were considered to be related to the use of voglibose [10]. In the study of Nijpels et al. (2008) [12], one death occurred in the intervention group eight months after the last treatment due to colon carcinoma, but this death was not considered related to the drug [12]. In the STOP-NIDMM Trial three deaths occurred in the placebo group and six deaths occurred in the intervention group [16, 17].

Besides the glucose inhibiting effect of alpha-glucosidase inhibitors, some side-effects were noticed. The STOP-NIDDM trial noted that the most common side-effects are gastrointestinal symptoms, for example gastrointestinal adverse events (RR: 1,4) flatulence (RR: 2,5), diarrhea (RR: 1,9) and abdominal pain (RR: 1,4) [16]. These effects were considered as mild or moderate in severity by the data safety and quality review committee and decrease over time after the last treatment.

The study of Kawamori et al. (2007) [10] found similar results, with flatulence occurring more often in the intervention group than in the placebo group (RR: 2,5). There were more patients with diarrhea in the intervention group (RR: 2,6) [10].

The study of Nijpels et al. (2008) [12] found that the intervention group also had more side effects than the placebo group: abdominal pain (relative risk of 4,0), diarrhea (RR: 11,6) and flatulence (RR: 13,5) [12].

Discussion

The aim of this study was to investigate the effect of alpha-glucosidase inhibitors on macrovascular morbidity, glucose levels and mortality in prediabetic people. A literature search was performed to find relevant articles. There was a significant reduction in development to T2DM and in HbA1c values in the intervention group compared to the placebo group. There was no significant difference in fasting glucose. One study reported a reduction in cardiovascular events in the intervention group. The intervention had no association with mortality.

Remarkable was that the studies where the population had lower baseline glucose values have significance, whereas the studies with higher baseline glucose values have no significance. This could suggest that alpha-glucosidase inhibitors are more effective in patients who have less developed prediabetes.

Our study contains a few drawbacks. Potentially interesting articles could have been missed, due to the exclusion of articles that were not available online or not written in English.

Furthermore, it was not possible to evaluate potential selective outcome reporting, because we were not able to find the protocols of the included studies'. Because we could not find the protocols, we cannot verify if the researchers left out data. This could potentially damage the credibility of this review, as the data that disrepute alpha-glucosidase inhibitors could be left out.

Bayer, a pharmaceutical company, funded four of the included studies. In Europe this company sells acarbose under the brand name Glucobay and has an interest in selling this drug [19]. Bayer would therefore benefit from studies recommending acarbose as a treatment for prediabetic patients.

Unfortunately, not all articles reported the outcome data that were required for our meta-analyses. For example, some studies reported the baseline HbA1c values, but not the HbA1c values after intervention. This made it difficult to include articles in the meta-analyses.

Table 3: *Summary of findings.*

Outcomes and studies	Alpha-glucosidase inhibitors	Placebo	Association measures
Development to T2DM	*Incidence*	*Incidence*	RR *[95% CI]*
- **Chiasson et al. (2002, 2003) [16, 17]**	32,4 %	41,5 %	0,78 [0,68; 0,90]
- **Kawamori et al. (2007) [10]**	5,6 %	12,0 %	0,46 [0,34; 0,64]
- **Kirkman et al. (2006) [11]**	34,4 %	34,0 %	1,01 [0,69; 1,49]
- **Nijpels et al. (2008) [12]**	16,7 %	25,0 %	0,67 [0,25; 1,78]
Fasting blood glucose	*Mean*	*Mean*	Mean difference *[95% CI]*
- **Hanefeld et al. (2004) [15]**	0,29 (SD 1,06)	-0,17 (SD 1,26)	-0,12 [-0,55; 0,31]
- **Nijpels et al. (20080 [12]**	-0,2 (SD 0,95)	0,08 (SD 1,21)	-0,28 [-0,67; 0,11]
- **Rudovich et al. (2011) [13]**	-0,1 (SD 1,00)	0,3 (SD 1,00)	-0,40 [-0,97; 0,17]
HbA1c	*Mean*	*Mean*	*Mean difference [95% CI]*
- **Hanefeld et al. (2004) [15]**	-0,39 (SD 0,87)	0,27 (SD 1,05)	-0,66 [-1,01; -0,31]
- **Kirkman et al. (2006) [11]**	-0,32 (SD 0,96)	0,08 (SD 1,09)	-0,40 [-0,76; -0,13]
- **Pan et al. (2002) [14]**	-0,38 (SD 0,86)	-0,38 (SD 0,80)	0,00 [-0,21; 0,21]
Macrovascular events	*Cumulative incidence*	*Cumulative incidence*	*RR [(95% CI])*
- **Chiasson et al. (2002, 2003) [16, 17]**	1,4 %	4,7 %	0,47 [0,26; 0,86]
Mortality	*Incidence*	*Incidence*	*RR [95% CI]*
- **Kawamori et al. (2007) [10]**	0,7 %	0 %	-
- **Nijpels et al. (2008) [12]**	1,7 %	0 %	-
- **Chiasson et al. (2002, 2003) [16, 17]**	0,9 %	0,4 %	2,01 [0,51; 8,01]
Side effects	*Incidence*	*Incidence*	*RR*
- **Flatulence**			
o **Chiasson et al. (2002, 2003) [16, 17]**	68,0 %	27,0 %	2,5
o **Kawamori et al. (2007) [10]**	17,0 %	7,0 %	2,5
o **Nijpels et al. (2008) [12]**	44,3 %	3,3 %	13,5
- **Diarrhea**			
o **Chiasson et al. (2002, 2003) [16, 17]**	32,0 %	17,0 %	1,9
o **Kawamori et al. (2007) [10]**	13,0 %	5,0 %	2,6
o **Nijpels et al. (2008) [12]**	19,7 %	1, 7 %	11,6
- **Abdominal pain**			
o **Chiasson et al. (2002, 2003) [16, 17]**	17,0 %	12,0 %	1,4
o **Nijpels et al. (2008) [12]**	13,1 %	3,3 %	4,0

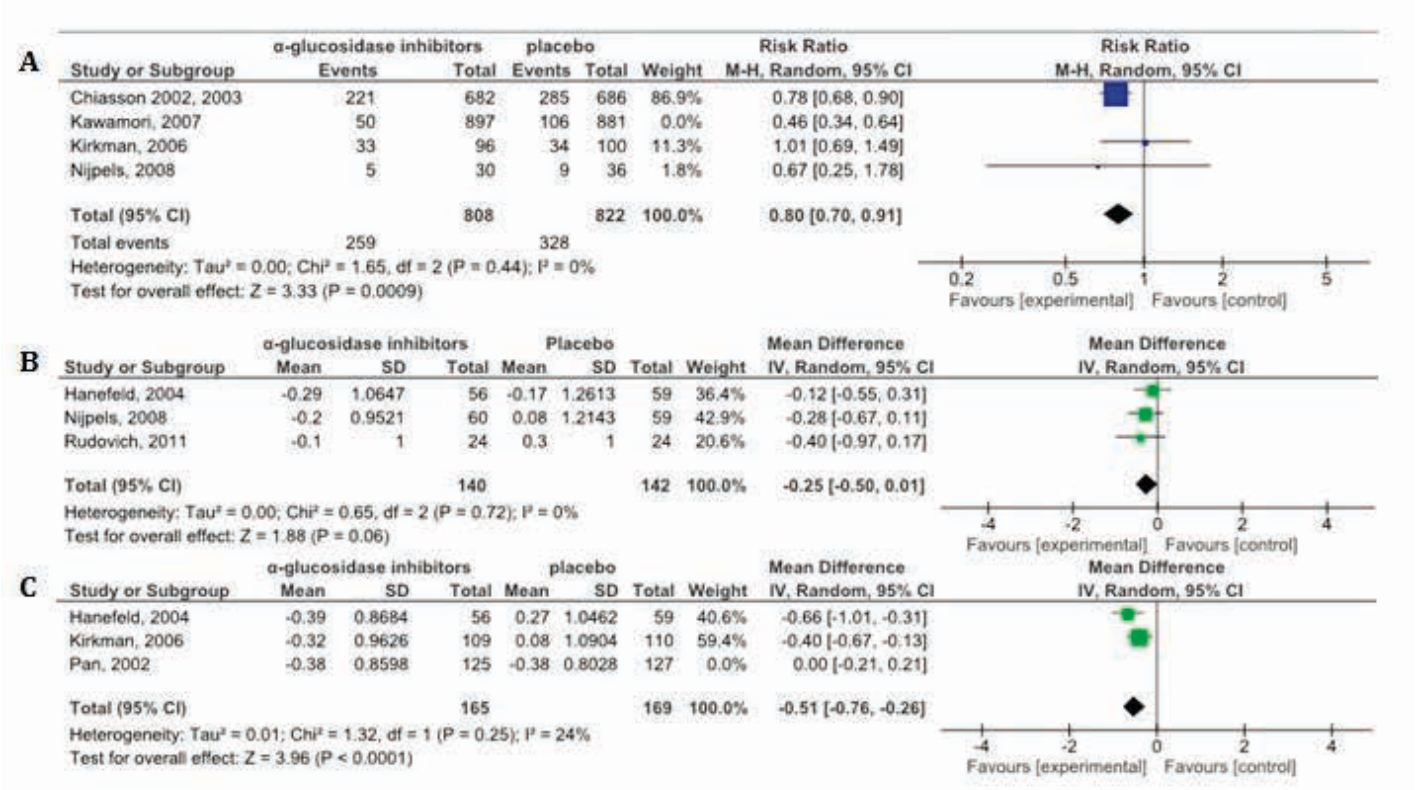

Figure 2: *Forest plots. A) Development to T2DM with risk ratio. B) Fasting glucose with mean difference. C) HbA1c with mean difference. Square size = study population. Diamond = average value with 95%-CI.*

Cardiovascular events were only reported in the STOP-NIDDM trail. In this trial, alpha-glucosidase inhibitors decrease the risk of cardiovascular events suggesting that alpha-glucosidase inhibitors could be beneficial. This conclusion is based on the results of one trial, thus this is not substantial evidence. This study did have many participants and the effect that was found was strong. We recommend further research, since alpha-glucosidase inhibitors have a potential to prevent macrovascular events.

We did not look at microvascular morbidity, because it was not studied in any article. Therefore, we cannot draw a conclusion about the benefit of alpha-glucosidase inhibitors on micro morbidities.

We excluded the study of Kawamori in the meta-analysis on the development to T2DM (Figure 3A), because this was the only study that used voglibose as an intervention instead of acarbose. In the study of Nijpels, we calculated the number of events, because only the percentage of events and the total number of patients in the group was given. This is questionable, because we did not get a rounded number of events after calculation. The study of Chiasson weighed a lot more than the other two studies, so the result of the meta-analysis is predominantly based on this study. The result is significant, but not convincingly. On the other hand, the NNT was low, therefore a small amount of people needs to be treated to prevent one person from developing T2DM.

The difference in fasting glucose between the intervention group and the placebo group (Figure 3B) was not significant. A reason for this could be that after a period of fasting there is only a small amount of glucose in the intestines, so alpha-glucosidase inhibitors have less of an effect on the blood glucose. Another reason could be that the results of the Rudovich study are less reliable, as it is a cross-over trial. The effect of the intervention could potentially contaminate the outcome of the placebo.

The study of Pan was excluded in the meta-analysis on HbA1c (Figure 3C) because the study had a follow-up of 16 weeks, whereas the studies of Hanefeld and Kirkman had a follow-up of respectively 160 and 343 weeks. Moreover, Pan used a dose of 50 mg three times a day, while the other two studies used a dose of 100 mg three times a day. The fact that this meta-analysis now consists of only two studies, makes the conclusion less reliable.

By definition, alpha-glucosidase inhibitors lower the blood glucose level in people, which is also what we found in our review. As the diagnosis of T2DM is based on blood glucose levels, this leads to fewer diagnoses in the intervention group compared to the placebo group. However, whether this effect decreases the morbidity associated with T2DM is unclear. In none of the studies mortality correlated with the treatment of alpha-glucosidase inhibitors. Hence, we can conclude that alpha-glucosidase inhibitors are safe to use and do not cause a higher risk of mortality.

Treatment with alpha-glucosidase inhibitors can cause side effects, but these are considered mild. Even so, these could lead to drug non-compliance. However, not all included articles mentioned the drug compliance. In the studies that did mention compliance, it was shown to be quite high [11,14,16].

This treatment has a lot of implications for the life of prediabetic people. They have to use the medication three times a day, with a meal. It is unclear if this treatment needs to be taken life-long. These implications could also lead to drug non-compliance. People may find taking a drug three times a day too much or they forget to take the drug.

The costs of treatment with alpha-glucosidase inhibitors are not included in this review.

A study has shown that there is a difference in blood glucose levels, depending on whether you measure the blood glucose from capillary or venous sources [20]. This was not taken into account in this analysis, as

the included studies did not mention from which source the blood glucose was measured.

We noted that the studies with an asian population had, on average, a lower BMI than the studies with a western population, which influences the comparability between the included articles. Therefore, studies with an asian population are less applicable when it comes to drawing a conclusion intended for a western population.

Conclusion

Alpha-glucosidase inhibitors decrease the risk of major cardiovascular events, decrease the blood glucose values, and there is no association with mortality. The risk of development to T2DM decreases with alpha-glucosidase inhibitors treatment. This would help tackle a major health problem. However, there are side effects that need to be considered and the effect of alpha-glucosidase inhibitors on complications that are associated with T2DM have not all been studied.

The current recommendation for prediabetic people in the Netherlands is to change their lifestyle by healthy dietary changes and more exercise in order to reduce the risk of developing prediabetes into T2DM [21]. Two large prevention studies have shown a beneficial effect in the reduction of the development of prediabetes into diabetes due to lifestyle changes [22,23]. Alpha-glucosidase inhibitors could perhaps be prescribed when lifestyle changes are not effective enough.

More research needs to be done on the effects of alpha-glucosidase inhibitors on T2DM related morbidity. Alpha-glucosidase inhibitors decrease the chance of T2DM diagnosis. However, there are health effects associated with T2DM that could still be problematic, even without a T2DM diagnosis, which is based on blood glucose levels.

Further research should be done on the cost-effectiveness of the treatment and the implications it has on a patient's life.

Even though alpha-glucosidase inhibitors have positive effects, we cannot yet recommend to prescribe alpha-glucosidase inhibitors to prediabetic people.

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A GLASS OF RED WINE A DAY KEEPS THE DOCTOR AWAY: MYTH OR SCIENCE?

Yalda Alam[1]

[1]Bachelor Medical student, Radboud University Medical Center, Nijmegen, the Netherlands

Introduction

Editorial

Alcohol takes up an important role in the Dutch cultural and social environment in every layer of society. Who doesn't long for a glass of red wine on the couch after spending hours at work? Or a cold beer during the third half of a soccer game? Be it at student parties, summer festivals or business meetings, alcohol is the one guest that is often present. While it is commonly known that excessive alcohol drinking can induce the risk of stroke, coronary heart disease and several cancers, the effects of moderate drinking have been very controversial. The discussion around moderate alcohol use flared up when the 'Guidelines for a Healthy Diet' report, released in November 2015 by the Health Council of the Netherlands, clearly stated that people should actually not be drinking alcohol at all instead of the two alcoholic beverages a day for men and one for women allowed by the previous guidelines from 2006. The new guidelines have been widely criticized by both national and international scientists and nutrition experts for not being based on recent scientific studies and not taking the health benefits that moderate alcohol intake provides into account. However, is there any scientific foundation for these so-called 'positive effects' of alcohol? And does this apply for both men and women?

The Dutch' relationship with alcohol has not always been a healthy one. Although due to stricter legislation and more awareness campaigns like the 'Bob' and the ' Geniet, maar drink met mate', The Netherlands is following in the footsteps of both France and Germany where alcohol consumption has decreased dramatically over the past twenty years. According to the Organization of Economic Cooperation and Development (OESO), the Netherlands is, with a slight decrease of 7% by no means the country with the greatest dependence on alcohol. Still, OESO's report concluded that the average Dutch citizen aged 15 or over consumes almost 10 litres of 'pure' alcohol on a yearly basis. This can be illustrated by 100 bottles of wine or even over 750 litres of beer! Even though these numbers may be lower than before, alcohol consumption is still rising under several groups e.g. young adults, highly educated women, and affluent people [1,2]. Since alcohol has such a prominent social aspect and affects all ages and genders, the recommendations made by the Health Council concern a large section of the population. Therefore, the justification of the new guidelines proposed by the Health Council has been examined thoroughly by the international community, which apparently has a different view on the matter.

The WHO defines moderate alcohol intake as up to one drink per day for women and up to two drinks per day for men. The Health Council stated in the report that moderate alcohol consumption in general is correlated with a reduced risk of cardiovascular disease, diabetes and dementia, but causes a greater risk of breast cancer.

Moreover, they claim that moderate use of beer in men is associated with a higher mortality rate regardless of cause of death, but wine is related to a lower mortality rate. These findings would indicate that limited drinking is both favourable and adversely associated with the risk of chronic disease [3]. According to the International Scientific Forum on Alcohol Research (ISFAR), a forum from Boston University that consists of an international group of invited physicians and scientists who are specialists in their fields and committed to well -researched analysis regarding alcohol and health, the Health Council is contradicting itself in these statements. On the one hand it delivers on the basis of scientific research that mild alcohol use has health benefits, while on the other hand it sets the norm that people should not drink at all [4].

According to ISFAR there is indeed substantial scientific evidence supporting the protective effects of light-to-moderate drinking on coronary heart disease, ischemic stroke, dementia and diabetes in middle-aged and older adults in comparison with abstainers [5]. On these fronts it is important to take into account possible gender differences. Although men are more likely to drink alcohol and drink in larger quantities, gender variations in body structure and metabolism cause women to absorb more alcohol, and take longer to eliminate it. In other words, upon drinking equal amounts, women have higher alcohol levels in their blood than men, and the immediate effects of alcohol occur more quickly and last longer. These contrasts make it plausible that drinking will cause more long-term health problems in women than in men. It could also explain why there is so little evidence that light-to-moderate alcohol intake appreciably increases the risk of cancer, with the exception of breast cancer [6]. ISFAR criticizes this declaration as well by pointing out that among young women the risk of breast cancer is higher than the risk of cardiovascular disease (CVD). Thus, even a small accumulation of cancer risk by moderate alcohol consumption should be taken into careful consideration, especially in view of the accompanying negligible reduction of CVD risk. As opposed to women after menopause where the small increase in the risk of breast cancer is counteracted by a much greater decrease in risk of cardiovascular disease: moderate alcohol intake decreases CVD risk by 20%, with only a very slight extra breast cancer risk. The overall effect for a post-menopausal woman would therefore be a lower mortality risk [4].

When looking at these assumptions, it would seem quite silly to declare that all drinking is bad for one's health and should be banned entirely. So why did the Health Council adjust the 2006 guidelines in the first place? As a matter of fact, the Dutch Health committee does not stand alone in their assessment of moderate drinking. According to the Centers of Disease Control and Prevention (CDC), recent studies show that the health benefits cited by ISFAR on e.g. cardiovascular disease might not be true [7,8].

For instance, the most common proclamation around moderate alcohol consumption is its advantageous effect on CVD. However, histologic markers for the assessment of vascular health show that alcohol consumption is associated with worse vascular health. Alcohol consumption, coronary heart disease risk factors and coronary calcification (a marker of atherosclerosis) were measured during 15 years of follow-up in the Coronary Artery Risk Development in Young Adults (CARDIA) within a sample size of more than 3,000 U.S. participants aged 33–45 years. For

those consuming less than 7 drinks per week, the risk was increased 10 % compared to abstainers and was 50% higher among those drinking on average 7 to 14 drinks per week, which is still considered moderate drinking. The lowest fraction of participants with coronary calcification was found among the lifetime abstainers [9].

Similarly, a study on Finnish young healthy adults found that alcohol consumption has a direct positive relationship with carotid intima–media thickness which is a marker of subclinical atherosclerosis. It revealed a significant increase starting from a consumption of less than two drinks per day as to non-drinkers [10]. In addition, a Mendelian randomisation analysis established that individuals with a genetic variant correlated with non-drinking and lower alcohol consumption had a more favourable cardiovascular profile and a reduced risk of coronary heart disease than those without the genetic variant. This suggests that reduction of alcohol intake, even for light to moderate drinkers, is advantageous for cardiovascular health [11].

These findings contradict the notion of moderate drinking having health benefits regarding CVD as claimed by ISFAR.

Furthermore, the positive effects of alcohol have not been confirmed by controlled studies or RCTs, but by epidemiological studies which are now being challenged on a number of aspects. Recent meta-analyses have shown that many of these studies systematically exclude unhealthy drinkers or misclassify unhealthy ex-drinkers as abstainers. In doing so, this artificially creates the appearance of positive effects. Furthermore, the ability of respondents to accurately recall their own alcohol consumption is highly doubtful and, moreover, very few individuals maintain one standard drinking level or style throughout life. In addition, it is impossible to conclude whether the improved health outcomes are due to moderate alcohol consumption or differences in behavioural factors, genetics or other unknown factors between moderate drinkers and non-drinkers. The relationship between alcohol and some conditions might be a function of drinking patterns but few studies have addressed this issue [12-14].

Another argument CDC mentions repeatedly is that moderate drinking often does not stay 'moderate'. To profit from the alleged health benefits this level of consumption should not be exceeded on any day. This has also been supported by ISFAR itself declaring: 'when limited to 1 drink/day for women or 2 drinks/day for men, with no binge drinking (>4/5 drinks during a single occasion for women/men respectively), and especially when consumed with meals, there are potential health benefits and few risks of such drinking'. However, a recent large-scale review in the United States concluded two in three adult drinkers report drinking above moderate levels at least once a month [15]. This 'gray area' of consumption between moderate and more than moderate drinking was associated with small but significantly increased risks of prevalent and incident alcohol dependence, incident alcohol related interpersonal problems and prevalent job loss. Due to the large proportion of drinkers in this gray area, the impact of this level of consumption cannot be negligible [16].

Although there has been much controversy around the decision of the Health Council to alter the guidelines on alcohol intake based on the health risks, it cannot be denied that other factors probably affected this decision as well. Alcohol in general causes more deaths worldwide than HIV/AIDS, violence and tuberculosis combined due to traffic accidents, domestic violence, sexual aggressive behaviour and physical or mental health issues [17,18]. With a rising number of 'binge drinkers' and other excessive drinkers among adolescents and young adults, the new recommendations could be perceived as a preventive measure in order to lower the alcohol intake and thereby alcohol related deaths in The Netherlands in general [1]. For now, while we are waiting for the international community to reach consensus on the topic, we might as well enjoy some Fristi instead!

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RECENT HIGH-IMPACT PAPERS FROM RADBOUDUMC RESEARCHERS

Janneke Elzinga[1]

With over 3000 publications per year [1], scientific research is a cornerstone of the Radboud University Medical Centre. In this section, recent high-impact papers – published by researchers from Radboudumc – will be discussed.

[1]Master Student Molecular Mechanisms of Disease, Nijmegen, the Netherlands

Worldwide spread of a life-threatening mycobacterium

An infection with *Mycobacterium abscessus*, an intrinsically multidrug-resistant nontuberculous species, can be life-threatening for patients with Cystic Fibrosis (CF) and other lung diseases. Researchers from the department of Medical Microbiology, in a collaboration led by the University of Cambridge, performed a population-level, multinational, whole-genome sequencing analysis of over a thousand *M. abscessus* isolates from infected CF patients. Whereas it was previously thought that the mycobacterium was acquired from the environment in an independent manner, the results revealed the emergence of global dominant circulating clones which have probably been transmitted patient-to-patient via fomites and aerosols. Moreover these clones demonstrated to become more virulent during spreading in both cell and mouse models of infection. To be able to interfere in the worldwide spread of the *M. abscessus*, the mode of transmission needs further clarification. [2]

Additional protective effects of the BCG-vaccine

The efficacy of the BCG vaccine (*Bacillus Calmette-Guerin*) extends beyond protection against infection with *Mycobacterium tuberculosis* by also protecting against, for instance, non-related infections and bladder cancer. These effects are known to be mediated by epigenetic reprogramming of immune cells ('trained immunity'), which can be accompanied by long-term metabolic changes. Researchers from the Department of Internal Medicine investigated the changes in cellular metabolism pathways underlying BCG-induced trained immunity. In both human and mice it was found that BCG-vaccination induces enhanced glycolysis and glutamine metabolism. These were proven to be important for the induction of BCG-induced trained immunity. The research shows unexpectedly, that the innate immune system can adapt after infection or vaccination. By investigating the role of the modulated metabolic pathway, current vaccines could be further improved. [3]

The mesoscale organisation of podosomes

Podosomes are dynamic, actin-based cytoskeletal structures needed for cell protrusion and matrix remodeling in, among others, osteoclasts and dendritic cells (DCs). Previously, it was shown that podosomes are clustered in well-defined compartmentalized zones in DCs, which seem to be coordinated on mesoscale. Researchers from the Department of Tumor Immunology and Cell Biology studied the dynamic and structural mechanisms of podosome clusters in human DCs. Using newly developed microscopy techniques, they demonstrated the interconnectedness of multiple proximal podosomes and the molecular mechanisms underlying the spatiotemporal organization of this network. Increased insight into the architecture and dynamic behaviour of podosomes in DCs could facilitate research in podosome-like structures, such as invadopodia in cancer cells, and lead to the development of effective anti-cancer therapies. [4]

The BLUEPRINT-project: A retrospective overview

The recently concluded BLUEPRINT-project, associated with the International Human Epigenome Consortium (IHEC) and coordinated by Henk Stunnenberg from the Department of Molecular Biology, has led to the publication of multiple papers in Cell, other Cell Press journals and elsewhere. The main goals of this project were to generate reference maps of human epigenomes for key cellular states relevant to health and disease, to facilitate rapid distribution of the data to the research community, and to accelerate translation of the generated knowledge to improve human health. To this aim, a series of molecular and computational methods have been developed to determine and analyze the so-called epigenomic signatures from heterogenous cell populations. Consequently, insight has been gained into the epigenetic and transcriptional basis of the differentiation capabilities of primary human cell types. Additionally, their responses to specific (environmental) stimuli and how these are modulated in pathological conditions have been investigated. According to the authors, the epigenomic analysis will improve the understanding of the molecular control of cell phenotypes, and the identification of novel biomarkers or therapeutic targets will probably improve disease management. A comprehensive overview of all the 24 papers published in the context of this project can be found in the Cell Press IHEC web portal (http://www.cell.com.ru.idm.oclc.org/consortium/IHEC). [5]

The Human Functional Genomics Project: The first papers

The Human Functional Genomics project comprises a large-scale project with the goal to identify the effect of genetic variation in humans and of the human microbiome on physiological processes in the human body. The project is run by, among others, two professors from the Radboudumc (Mihai Netea and Leo Joosten) and the first three papers have been published recently in Cell, Cell Host & Microbe and Cell Reports. One of these articles describes how differences in cytokine production by human leukocytes during an infection with Borrelia bacterium - associated with Lyme disease - can be explained. It was found that IL-22 and IFN-γ production was dependent on age and genetic factors. HIF-1α-mediated glycolysis was shown to play an important role in the cytokine response to Borrelia. An increase in HIF-1α elevates the level of lactic acid, resulting in an energy deficiency in immune cells and reduced IL-22 production. The shift in cellular glucose metabolism pathways could provide a new therapeutic target. [6]

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Dear reader,

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On behalf of the Board of RAMS,

Ferhat Beyaz
Vice-Chair RAMS

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