META-ANALYSIS IN

CANCER EPIDEMIOLOGY

by

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Abstract

A published meta-analysis on breast cancer and vegetables and fruit consumption was described to present a methodology used on meta-analysis in Epidemiology. Meta-analysis confirmed the association between intake of vegetables (RR=0.74; 95%CI 0.65-0.84) and, to a lesser extent, fruit and breast cancer risk (RR=0.93; 95%CI 0.79-1.09). Using this methodology, present in a peer-reviewed journal, a systematic meta-analysis on melanoma was conducted extracting RRs from published studies. Fully adjusted estimates were obtained from those studies, when available; RRs adjusted for confounders not related to sun exposure, such as naevi, were considered for sun exposure and sunburns pooled estimates. Pooled estimates were obtained for all main risk factors for melanoma: sun exposure (total, intermittent and chronic), sunburns (in childhood and in adulthood), indicators of actinic damage, family history of melanoma and phenotype characteristics. Investigation of biases and inconsistencies among studies was one of the key phases of the meta-analysis to look for patterns among studies that might explain discrepant findings. The analyses on pigmented lesions and sun exposure showed that the choice of sources of cases and controls influenced significantly the estimate. An indication of a protective effect of chronic sun exposure came from studies that did not include subjects with dermatological problems (significantly different from the other studies: p=0.01). Publication year was an important factor for total sun exposure (p=0.005). Latitude of the study seemed to be an important factor for sunburns (p=0.002) and for high density of freckles (p=0.04). Estimates for hair colour and eye colour adjusted for phenotype and/or photo-type were significantly lower than unadjusted ones (p=0.06 and p=0.06, respectively). This study highlighted how several features of study design, type of analysis, categorization of exposures, study location and populations significantly explained between-study heterogeneity.

To my beloved

Mum, Sergio and Eleonora

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Table of Contents

Table of Contents v

List of Figures xi

List of Tables xiv

Abbreviations xvi

Chapter 1. Meta-analysis in Epidemiology

1.1 Introduction Pag. 1

1.2 Meta-analysis of observational studies Pag. 3

1.3 Main aims of the thesis Pag. 6

1.4 Previous works Pag. 8

1.5 Conclusions Pag. 10

Chapter 2. Heterogeneity and bias: Statistical methods

2.1 Introduction Pag. 13

2.2 Bias in meta-analysis Pag. 14

2.2.1 Funnel plots and graphical tests to detect bias Pag. 16

2.3 Meta-analysis dilemma: heterogeneity Pag. 19

2.3.1 Fixed effects models Pag. 22

2.3.2 Random effects models Pag. 23

2.3.3 Dose-response models Pag. 24

2.4 Multiple endpoints Pag. 26

2.5 Bayesian approach Pag. 27

2.6 Conclusions Pag. 28

Chapter 3. Breast cancer and fruit and vegetables consumption

3.1 Introduction Pag. 31

3.2 Material and methods

3.2.1 Selection of studies Pag. 31

3.2.2 Extraction and unification of the data Pag. 35

3.2.3 Analysis Pag. 38

3.2.3.1 Heterogeneity analysis Pag. 40
3.2.3.2 Sensitivity analysis Pag. 40
3.3 Results
3.3.1 Characteristics of studies Pag. 41
3.3.2 Pooled Relative Risk estimates Pag. 41
3.3.3 Results from heterogeneity analysis Pag. 42
2.3.4 Results from sensitivity analysis Pag. 43
3.3.5 Publication bias Pag. 44
3.4 Discussion Pag. 44
3.5 Tables Pag. 51
3.4 Figures Pag. 54

Chapter 4: **Melanoma and pigmented lesions**
4.1 Introduction Pag. 57
4.2 Materials and Methods
4.2.1 Data sources and search strategy Pag. 60
4.2.2 Selection of studies Pag. 61
4.2.3 Extraction and unification of the data Pag. 62
4.2.4 Data analysis strategy Pag. 65
4.3 Results
4.3.1 Literature search and selection Pag. 66
4.3.2 Studies characteristics Pag. 67
4.3.3 Pooled RR estimates Pag. 68
4.3.4 Heterogeneity analysis Pag. 69
4.3.5 Sensitivity Analysis Pag. 72
4.3.6 Publication bias Pag. 74
4.4 Discussion Pag. 75
4.5 Appendix: Reasons for exclusion Pag. 79
4.6 Tables Pag. 82
4.7 Figures Pag. 88

Chapter 5: **Melanoma and the other risk factors**

5.1 Introduction Pag. 94

5.2 Methods Pag. 97

5.3 Ultraviolet radiations exposure: Introduction Pag. 99

5.3.1 Extraction and homogenisation of the data on sun exposure Pag.103

5.3.2 Total sun exposure

5.3.2.1 Materials and methods Pag. 105

5.3.2.2 Results Pag. 106

5.3.2.3 Heterogeneity analysis Pag. 106

5.3.2.4 Sensitivity analysis Pag. 108

5.3.3 Intermittent sun exposure

5.3.3.1 Materials and methods Pag. 109

5.3.3.2 Results Pag. 111

5.3.3.3 Heterogeneity analysis Pag. 111

5.3.3.4 Sensitivity analysis Pag. 113

5.3.4 Chronic sun exposure

5.3.4.1 Materials and methods Pag. 114

5.3.4.2 Results Pag. 115

5.3.4.3 Heterogeneity analysis Pag. 115

5.3.4.4 Sensitivity analysis Pag. 119

5.3.5 Discussion on intermittent and chronic sun exposure Pag. 120

5.3.6 Sunburns history: Introduction Pag. 124

5.3.7 Sunburn in childhood

5.3.7.1 Materials and methods Pag. 126

5.3.7.2 Results Pag. 127

5.3.7.3 Heterogeneity analysis Pag. 127

5.3.7.4 Sensitivity analysis Pag. 128

5.3.8 Sunburn in adulthood

5.3.8.1 Materials and methods Pag. 129

5.3.8.2 Results Pag. 131

5.3.8.3 Heterogeneity analysis Pag. 131

5.3.8.4 Sensitivity analysis Pag. 134
5.3.9 Discussion on sunburns Pag. 135
5.3.10 Conclusions on sunburns and sun exposure Pag. 138
5.4 Family history of melanoma
5.4.1 Introduction Pag. 139
5.4.2 Materials and methods Pag. 140
5.4.3 Results Pag. 140
5.4.4 Sensitivity analysis Pag. 141
5.4.5 Discussion on family history Pag. 141
5.5 Indicators of actinic damage
5.5.1 Introduction Pag. 142
5.5.2 Materials and methods Pag. 143
5.5.3 Results Pag. 144
5.5.6 Heterogeneity analysis Pag. 144
5.5.5 Sensitivity analysis Pag. 146
5.5.6 Discussion on indicators of photodamage Pag. 146
5.6 Host factors
5.6.1 Freckles
5.6.1.1 Materials and methods Pag. 147
5.6.1.2 Results Pag. 148
5.6.1.3 Heterogeneity analysis Pag. 149
5.6.1.4 Sensitivity analysis Pag. 151
5.6.2 Eye colour
5.6.2.1 Materials and methods Pag. 152
5.6.2.2 Results Pag. 153
5.6.2.3 Heterogeneity analysis Pag. 154
5.6.2.4 Sensitivity analysis Pag. 154
5.6.3 Hair colour
5.6.3.1 Materials and methods Pag. 156
5.6.3.2 Results Pag. 157
5.6.3.3 Heterogeneity analysis Pag. 157

5.6.3.4 Sensitivity analysis Pag. 159
5.6.4 Skin colour
5.6.4.1 Materials and methods Pag. 160
5.6.4.2 Results Pag. 161
5.6.4.3 Heterogeneity analysis Pag. 161
5.6.4.4 Sensitivity analysis Pag. 161
5.6.5 Skin phototype
5.6.5.1 Materials and methods Pag. 163
5.6.5.3 Results Pag. 164
5.6.5.4 Heterogeneity analysis Pag. 165
5.6.5.5 Sensitivity analysis Pag. 166
5.6.6 Discussion on host factors Pag. 167
5.7 Conclusions Pag. 167
5.8 Tables Pag. 170
5.8.1 Total sun exposure Tables Pag. 170
5.8.2 Intermittent sun exposure Tables Pag. 171
5.8.3 Chronic sun exposure Tables Pag. 175
5.8.4 Sunburns in childhood Tables Pag. 177
5.8.5 Sunburns in adulthood Tables Pag. 179
5.8.6 Family history Tables Pag. 180
5.8.7 Indicators of photodamage Tables Pag. 181
5.8.8 Freckles Tables Pag. 182
5.8.9 Eye colour Tables Pag. 183
5.8.10 Hair colour Tables Pag. 186
5.8.11 Skin colour Tables Pag. 190
5.8.12 Skin phototype Tables Pag. 192
5.9 Figures Pag. 194
5.9.1 Total sun exposure Figures Pag. 195
5.9.2 Intermittent sun exposure Figures Pag. 198
5.9.3 Chronic sun exposure Figures Pag. 199
5.9.4 Sunburns in childhood Figures Pag. 203

5.9.5 Sunburns in adulthood Figures Pag. 205
5.9.6 Family history Figures Pag. 208
5.9.7 Indicators of photodamage Figures Pag. 209
5.9.8 Freckles density Figures Pag. 211
5.9.9 Eye colour Figures Pag. 214
5.9.10 Hair colour Figures Pag. 216
5.9.11 Skin colour Figures Pag. 218
5.9.12 Skin phototype Figures Pag. 220

Chapter 6:**Conclusions**
6.1 Introduction Pag. 222
6.2 Breast cancer Pag. 223
6.3 Melanoma Pag. 225
6.3.1 Need for screening on melanoma Pag. 232
6.4 Future studies Pag. 234

Reference List Pag. 238

List of Figures

Chapter 2

Figure 2.1: Symmetrical funnel plot Pag. 17

Figure 2.2: Asymmetrical funnel plot Pag. 17

Chapter 3.

Figure 3.1: RR estimates and CI of breast cancer and vegetables Pag. 55

Figure 3.2: RR estimates and CI of breast cancer and vegetables Pag. 55

Figure 3.3: RR estimates and CI of breast cancer and beta-carotene Pag. 56

Figure 3.4: RR estimates and CI of breast cancer and Vitamin C Pag. 56

Chapter 4

Figure 4.1: RR and CI for the increase of one common nevus on whole body Pag. 88

Figure 4.2: RR and CI for the increase of one common nevus on arms Pag. 88

Figure 4.3a: RR and CI for one atypical nevus in case-controls studies Pag. 89

Figure 4.3b: RR and CI for one atypical nevus in cohort studies Pag. 89

Figure 4.4: RR and CI for one common nevus on arms by source of cases Pag. 90

Figure 4.5: RR and CI for one atypical nevus by source of controls Pag. 90

Figure 4.6: RR and CI for one atypical nevus by type of study Pag. 91

Figure 4.7: RR and CI for one atypical nevus by type of categorization Pag. 91

Figure 4.8: Funnel plot for common naevi on whole body Pag. 92

Figure 4.9: Funnel plot for atypical naevi Pag. 92

Figure 4.10: Funnel plot for common naevi on arms Pag. 93

Figure 4.11: Log Relative Risk (y) vs its Standard Error (s) for common naevi Pag. 93

Chapter 5

Figure 5.1: Scheme summarising some hypotheses for the disease model Pag. 194

Figure 5.2: RR and CI for total sun exposure Pag. 195

Figure 5.3: RR and CI for total sun exposure by type of controls Pag. 195

Figure 5.4: RR and CI for total sun exposure by publication year Pag. 196

Figure 5.5: RR and CI for total sun exposure by adjustment for phenotype Pag. 196
Figure 5.6: Funnel plot on total sun exposure Pag. 197
Figure 5.7: RR and CI on intermittent sun exposure Pag. 198
Figure 5.8: Funnel plot on intermittent sun exposure Pag. 199
Figure 5.9: RR estimates and CI on chronic UV exposure Pag. 199
Figure 5.10: RR and CI for chronic sun exposure by type of controls Pag. 200
Figure 5.11: RR and CI for chronic sun exposure
by inclusion of subjects with dermatological problems Pag. 201
Figure 5.12: RR and CI for chronic sun exposure by type of countries Pag. 201
Figure 5.13: Funnel display chronic UV exposure Pag. 202
Figure 5.14: RR and CI for Chronic and Intermittent sun exposure Pag. 202
Figure 5.15: RR and CI on sunburns in childhood Pag. 203
Figure 5.16: Funnel display of sunburns in childhood Pag. 204
Figure 5.17: RR and CI for sunburns in adulthood Pag. 205
Figure 5.18: RR and CI for sunburns in adulthood by latitude Pag. 206
Figure 5.19: Funnel plot for adulthood sunburns Pag. 206
Figure 5.20: Log Relative Risk (y) vs its Standard error (s) Pag. 207
Figure 5.21: RR and CI for sunburns in Adulthood and Childhood Pag. 207
Figure 5.22: RR estimates and CI for family history Pag. 208
Figure 5.23: Funnel plot for family history Pag. 209
Figure 5.24: RR estimates and CI on pre-malignant cancer lesions Pag. 209
Figure 5.25: RR estimates and CI on other indicators of photodamage Pag. 210
Figure 5.26: Funnel plot on indicators of photodamage Pag. 211
Figure 5.27: RR estimates and CI for high freckles density Pag. 211
Figure 5.28: RR and CI for freckles density by type of countries Pag. 212
Figure 5.29: RR and CI for freckles density by latitude Pag. 213
Figure 5.30: Funnel plot for high freckle density Pag. 213
Figure 5.31: RR estimates and CI for blue versus dark eye colour Pag. 214
Figure 5.32: RR and CI for blue eye by adjustment for phenotype Pag. 215
Figure 5.33: Funnel plot for blue eyes Pag. 215
Figure 5.34: RR estimates and CI for red versus dark hair colour Pag. 216

Figure 5.35: RR and CI for red hair by adjustment for phenotype or phototype Pag. 217
Figure 5.36: Funnel plot for red hair Pag. 218
Figure 5.37: RR estimates and CI for “light” versus “dark” skin colour Pag. 219
Figure 5.38: RR and CI for light skin colour by type of cases Pag. 219
Figure 5.39: Funnel plot for light skin colour Pag. 220
Figure 5.40: RR estimates and CI for skin phototype Pag. 220
Figure 5.41: Funnel plot for skin phototype Pag. 221

List of Tables

Chapter 2

Table 2.1: Table 2x2 with cases and controls by exposure categories Pag. 23

Chapter 3

Table 3.1: List of studies with indication of which estimates were excluded from meta-analysis and reasons for exclusion Pag. 47

Table 3.2: List of studies with indication of which estimates were included and excluded and reasons for exclusion Pag. 47

Table 3.3: Estimates from meta-analysis of the RR of getting breast cancer Pag. 48

Table 3.4: Dose-response estimates from meta-analysis Pag. 49

Table 3.5: Estimates from meta-analysis, for breast cancer risk and vegetable consumption for sub-groups of studies Pag. 49

Table 3.6: Estimates from meta-analysis, from random effects models, when all possible studies are included Pag. 50

Chapter 4

Table 4.1: Characteristics of the studies on pigmented lesions Pag. 76

Table 4.2: Estimates from meta-analysis for common naevi Pag. 77

Table 4.3: Estimates from meta-analysis for atypical naevi Pag. 77

Table 4.4: Subgroup analysis on common naevi Pag. 78

Table 4.5: Subgroup analysis on atypical naevi Pag. 79

Table 4.6: Estimates from studies that used dichotomization of atypical naevi number, by type of study Pag. 80

Table 4.7: Estimates from meta-analysis for atypical naevi on case-controls studies Pag. 80

Table 4.8: Estimates from meta-analysis for common naevi on whole body. Sensitivity analysis. Pag. 80

Table 4.9: List of studies excluded with reasons for exclusion Pag. 81

Chapter 5

Table 5.1: Characteristics of the studies on sun total exposure Pag. 170
Table 5.2: Definitions of total sun exposure Pag. 170
Table 5.3: Characteristics of the studies on intermittent sun exposure Pag. 171
Table 5.4: Definitions of intermittent sun exposure Pag. 172
Table 5.5: Characteristics of the studies on chronic sun exposure Pag. 175
Table 5.6: Definitions of chronic sun exposure Pag. 176
Table 5.7: Characteristics of the studies on sunburns in childhood Pag. 177
Table 5.8: Definitions for sunburns in childhood Pag. 178
Table 5.9: Characteristics of the studies on sunburns in adulthood Pag. 179
Table 5.10 Definitions of sunburns in adulthood Pag. 179
Table 5.11: Characteristics of the studies on family history Pag. 180
Table 5.12: Characteristics of the studies on indicators of photodamage Pag. 181
Table 5.13: Definitions and classification for indicators of photodamage Pag. 181
Table 5.14: Characteristics of the studies on freckles Pag. 182
Table 5.15: Definitions and categories for freckles Pag. 183
Table 5.16: Characteristics of the studies on eye colour Pag. 184
Table 5.17: Categories and methods of assessment for eye colour Pag. 185
Table 5.18: Characteristics of the studies on hair colour Pag. 186
Table 5.19: Definitions and methods of assessment of hair colour Pag. 187
Table 5.20: RR estimates from meta-analysis Pag. 190
Table 5.21: Characteristics of studies on skin colour Pag. 190
Table 5.22: Methods of assessment for skin colour Pag. 191
Table 5.23: Characteristics of the studies on skin phototype Pag. 192
Table 5.24: Definitions and methods of assessment for skin phototype Pag. 192
Table 5.25: RR estimates from meta-analysis for skin phototype Pag. 193

Abbreviations

RR: Relative Risk;
OR: Odds Ratio;
SIR: Standard Incidence Rates;
SE: Standard Error;
C.I.: Confidence Intervals;
Chi: Chi-squared test for heterogeneity;
p: p-value showing statistical significance;
w: weight of the study;
d.f.: degrees of freedom;
CC: Case-control study;
Co. or Cohort: Cohort study;
FFQ: Food Frequency Questionnaire.

Studies have been usually identified by the name of the first author of the original publication and, for simplification, in the text they are referred to as if there were only one author.

CHAPTER 1. META-ANALYSIS IN EPIDEMIOLOGY

1.1 Introduction

The New York Times, 7 January 1994, stated "A meta-analysis aims at gleaning more information from existing data by pooling the results of smaller studies and applying one or more statistical techniques. The benefits or hazards that may not be detected in small studies can be found in meta-analysis that uses data from thousands of subjects." Eysenck,[1] on the contrary, described meta-analysis as an exercise in "mega-silliness" and Feinsten defined meta-analysis of non-randomised observational studies “as the attempt of a quadriplegic person to climb Mount Everest unaided”.[2]

The term “meta-analysis” was formulated for the first time by Glass in 1976 and its meaning is related to an analysis of several other analyses with a process that includes a search of the results of independent studies as well as a quantifiable combination of effect sizes.[3] It provides a systematic approach to selecting and integrating findings across studies and to control for chance and potential bias. It is a methodology used for contrasting and combining results of different studies, where the individual unit of the statistical analysis is the study result. Study characteristics are first carefully coded, then mean effect sizes are examined according to different study characteristics, in order to look for patterns among studies that might explain discrepant findings. This approach allows hypothesis testing regarding sources of heterogeneity and quantification of biases. Meta-analysis can also help to identify gaps in knowledge found in the published literature and thus can help provide guidance for future research.

Meta-analysis differs from qualitative or narrative review because conclusions from publications are not only discussed qualitatively but also involves a quantitative manipulation of the available information. Narrative reviews may present several problems because they are

influenced by several biases and do not have tools to analyse them. They may be simple catalogues, without integration, affected by publication bias; or they may be based upon a subset of possible studies, which may lead to reviewer bias in selecting studies to be included.[4] Without the obligation to clearly state inclusion criteria, it is likely that researchers include studies that support their own opinion and ignore those that do not. Cooper and Rosenthal[5] showed that even with only seven studies, narrative and quantitative reviews led to different results. However in a research area with only two or three similar studies, there is no need to integrate the data. In these situations, a narrative review of the studies is perhaps the more suitable approach.

A number of definitions and synonyms of meta-analysis exists: quantitative review, combined analysis, pooled analysis, literature synthesis and quantitative synthesis. Some of them define substantially different methods and the main differences are to be found among the meta-analysis of literature (quantitative synthesis of published data), the re-analysis of individual primary data (pooled analysis) and finally the prospectively planed pooled analysis, where pooling is already part of the protocol and which offers the highest degree of comparability between studies.

Pooled meta-analysis considers primary data obtained from authors of the papers and it is characterized by numerous advantages. It allows analyses among exposures and confounders, not investigated in the original studies, and it permits variables to be recoded across studies to make them more compatible and to make adjustments to deal more extensively with heterogeneity. A major impediment to this kind of meta-analysis is the fact that it is very time–consuming because it requires several years just to obtain the data and demands close cooperation between the authors of the studies.[6] Therefore meta-analysis of published studies can be considered a sound approach when resources and time are limited and when original study data are not available. In public health epidemiology, data from

original studies are often accessible only to limited numbers of research groups and few opportunities for pooled analysis exist.[7;8]

Prospective meta-analyses, in which studies are designed jointly so they may be combined later via meta-analysis, are becoming an interesting area of research. However in contrast to multicenter clinical trials, more heterogeneity in the individual study centres will still exist, arising from differences among populations or study designs. Furthermore the costs for this meta-analysis are very high and the planning, which is substantial, may be not easy.[9]

The use of meta-analysis for published observational studies is less accepted than in the area of clinical trials for their intrinsic biases and differences in study designs. However in many situations randomised controlled designs are not feasible, and only data from observational studies are available.

In this Chapter, I introduce the purposes and features of meta-analysis of published observational studies and I present the aims of the meta-analyses of epidemiological studies on breast cancer and melanoma that I carried out: This will be described in detail in Chapters 3, 4 and 5.

1.2 Meta-analysis of observational studies

Although significant progress was made in the systematic approach to clinical studies with meta-analytic procedures being widely employed since the early 1970s, the inherent challenges of published observational studies have meant that only recently these methods have become more and more important in epidemiology. However a continuous increase in the number of published meta-analyses, concerning observational studies, was observed during the past four decades.[10] The need to assess risks that are small, but that may have large public interest or have important implications for public health, has amplified their use in summarizing the evidence. Because of pressure for appropriate informed decisions in public

health and the explosion of information in the scientific literature, research results must be synthesized to face urgent problems. Thus, in order to be able to cope with the current information explosion, meta-analysis has now become essential.

Epidemiological studies are traditionally classified as either observational or experimental. In practice, primarily the ethical problems in human experimentation usually preclude extensive use of the experimental design. Most studies, therefore, are observational and the investigator measures association between changes in outcomes of interest. Two aspects characterize an observational study: the aim is to study relationships among certain elements and controlled experimentation cannot be employed. Since a meta-analysis appears to embrace both of these features, it may not be illogical to also think of a meta-analysis as an observational study. One of the main problems with meta-analysis, working at the individual study level, is that it has limited control over the availability of studies and the information collected and reported in the individual studies. Thus researchers are dealing with associations rather than causation and should be aware that the hazards in meta-analysis on observational studies are much more numerous than in meta-analysis of randomised clinical trials, because of their intrinsic biases and differences in study designs.[11;12]

Conflicting results among studies may arise when sample sizes of individual studies are too small to find stable results. Actually most epidemiological studies are too small to detect anything but a comparatively large Relative Risk (RR) associated with a fairly common exposure. Thus meta-analyses may become a useful tool to evaluate weak risk factors that have large public health impact. An increase of risk of only 20% of certain cancers, for example, may involve millions of people and to detect such small increases in risk, huge studies are necessary.[13] If, however, many studies produce modest relative risks, those estimates may well be due to same biases in all the studies. If the same systematic biases are present across a range of studies, the only effect of meta-analysis is to reinforce them, to

produce spurious statistical stability. Thus meta-analysis can lead to insights when study design, exposure assessment or exposure levels, study populations, etc., are found to relate to study outcome. An important function of meta-analysis is the investigation of between-study heterogeneity which is an opportunity to understand study variation. Investigation of heterogeneity can provide interesting hypotheses for future analyses and should be viewed as strength of meta-analysis, not a barrier to its use. Actually if all of the studies show same results, meta-analysis would not be very useful because it would not provide much more information than the original studies.

Longnecker[14] in a meta-analysis assessing the association between alcohol consumption and risk of breast cancer noted that the strength of the relation varied by study design. The association was stronger in prospective follow-up studies compared to case-control studies. Accumulating evidence suggested that retrospective assessment of diet and alcohol of case-control studies may be biased by differential recall among those who have been diagnosed and treated for cancer.

Discrepancies in study design were found in several meta-analysis on diet and breast cancer. Several meta-analyses[15-17] showed a considerable association with saturated fat intake in case-control studies but much lower in cohort studies The most likely explanation for this finding is that biases in the recall of dietary items and in the selection of study participants have created a spurious association in the case-control comparisons.

The importance of the methods used for assessing exposure is illustrated by a meta-analysis[18] of cross-sectional data of dietary calcium intake and blood pressure from 23 different studies. It was found that the approach used for assessing the amount of calcium consumed strongly modified the change in systolic blood pressure per 100 mg of calcium intake. The association was small and only slightly significant when diet histories were used but large and highly significant when food frequency questionnaires were used. In fact, diet

histories and food frequency questionnaires are very different methods to assess food consumption. Diet histories are conducted with a nutritionist and determine patterns of usual intake over long periods of time, whereas food frequency questionnaires are simpler methods that reflect current food consumption. It is likely that differing precision in the evaluation of current calcium intake may explain discrepancies in the strength of the associations found.

1.3 Main aims of the thesis

This work discusses some typical issues, involved with meta-analysis of published data in cancer epidemiology, describing two studies on breast cancer and melanoma. Statistical methods, useful to calculate summary estimates and to investigate between-study heterogeneity and potential sources of bias, are presented in Chapter 2.

There are several indications that a diet rich in green vegetables and/or fresh fruit can provide some protection against a number of cancers. Several studies have also suggested a relationship linking vegetable and fruit consumption to hormone related cancers. A meta-analysis was carried out to investigate results from published epidemiological studies on breast cancer and fruit and vegetable consumption.

Breast cancer was chosen because it is a major public health problem in industrialized countries. However in cancer studies incidence and mortality are relatively rare and meta-analysis effectively provides a gain in statistical power for average estimates. Meta-analysis may offer an opportunity to observe more events of interest in the groups followed. Thus, when incidence or mortality is rare, combined estimates are likely to be more precise.

Published data on vegetables, fruit and some micronutrients, were evaluated to estimate their potential protective effect on breast cancer. Variation in study results was addressed, adopting some inclusion criteria and investigating the potential sources of heterogeneity. Results from this work are published in the European Journal of Cancer with

the title “Meta-analysis of studies on breast cancer risk and diet: the role of fruit and vegetables consumption and the intake of associated micronutrients”.[19] Thus, Chapter 3 is extracted from this paper, which I completed in 1999. This section was included in the thesis to present an approved methodology in meta-analysis of epidemiological published studies, which was later applied to meta-analyses on melanoma, described in Chapter 4 and Chapter 5.

Melanoma presents an increase in the incidence rate that in men has exceeded that for any cancer. Several publications have investigated risk factors for melanoma, producing results that appeared conflicting. In point of fact they used different methods of information ascertainment and statistical analyses, and considered completely different populations. Thus a systematic revision of all literature for a comprehensive meta-analysis of all main risk factors on melanoma allowed a deep exploration of associations and interactions among risk factors and provided some clues in epidemiology of melanoma looking extensively at inconsistencies and variability in the estimates. Meta-analysis permitted questions to be debated on whether the association of melanoma with some risk factors may depend on the composition of the population under study, the level of exposure in the study population, the definition of disease employed in the studies, or methodological quality of the studies. Therefore prior to embarking on any research study, a meta-analysis should be attempted in order to establish reliably of what is already known.

Pooled estimates, which summarize results of all the literature, became essential to quantify the risk associated to all risk factors and thus identify subsets of the population at high risk of its development. It is hoped in the future to evaluate the possibility to combine the summary relative risk estimates of all main risk factors, calculated with meta-analytic techniques, to build an individual probability score. A combination of the estimates, obtained for all risk factors, could be useful for discriminating high-risk subjects who can be targeted for prevention. A case-control study is carried out by IDI (Istituto Dermopatico

dell'Immacolata, IRCCS), in Rome, to validate a possible score based on them. (Personal Communication)

Meta-analysis on melanoma and pigmented lesions is presented in Chapter 4. The procedure adopted for this analysis, extensively described in this Chapter, was used for investigation of the other risk factors, presented in Chapter 5. Heterogeneity and potential sources of bias were widely explored in these two chapters, because a considerable number of studies was available. The possibility of exploring all the literature on all main risk factors together allowed investigation of methodological correspondences and symmetries among types of studies and among risk factors, providing interesting considerations on study variability.

Conclusions and suggestions for future works are illustrated in Chapter 6.

1.4 Previous meta-analyses on breast cancer and melanoma

No meta-analysis looking at the association between breast cancer and vegetable and fruit consumption had been published at the time of the meta-analysis presented in Chapter 3 and published in 2000.[19] One of the reasons may be related to the fact that it is not easy to deal with heterogeneity of coding, categorizations, definitions and quantification of portions of foods.

An individual data meta-analysis,[16] which included twelve case-control studies completed by 1986, published pooled estimates on the relationship between vitamin C and beta-carotene intake and breast cancer. Heterogeneity was significant for some dietary factors and the authors used an approach, which they called conservative, that restricts the analysis to the studies showing lack of heterogeneity. Thus sources of inconsistencies were not subject to much investigation and some interesting information on between-study differences was probably lost. Their results support the hypothesis that increased consumption of fruit and

vegetables and a consequent higher intake of vitamin C and beta-carotene may reduce breast cancer, as I have found in my study.

Very good reviews were published[20-23] on melanoma but mostly had a narrative style. There are few studies[24-28] that tried to assess and quantify information from independent studies in a more systematic way employing meta-analytic techniques but they looked only at some risk factors and used different approaches to deal with heterogeneity.

Whiteman[24] calculated pooled estimates on sunburns on four studies that fulfilled some strict inclusion criteria. At the beginning all studies published between 1975 and 1993 were considered. However several papers at the end were excluded. First, all publications that did not present raw data on sunburns were not included. Second, severe inclusion criteria, defined as essential, were applied to choose a core group of studies. Even though only a small subgroup of studies was considered, between-study heterogeneity was significant. Thus another study was excluded, to obtain a homogeneous group of papers Sources of heterogeneity were not investigated and interpretations on inconsistencies were not proposed. This, in my opinion, neglected one of the more interesting aspects of meta-analysis. Furthermore the pooled estimates are calculated using a scores system but this method is very controversial. As a matter of fact, as I will discuss in Chapter 2, the subjective reviewer's point of view may heavily influence the results.

Bliss[25] conducted an individual data meta-analysis, on studies published before 1990, which look at pigmentation characteristics. The authors stated that they had included in the analysis melanoma case-control studies "which had included an independent physical examination of naevi by a trained individual, for which data collection was completed and where cases and controls were treated similarly". Thus studies with only self-reported nevus counts were excluded. Even though the subset of studies considered for the analysis was not large, between-study heterogeneity was found to be significant for some factors and in this

case the pooled estimate was not considered. Again inconsistencies and differences in patterns of estimates were not much investigated. The same group of authors that conducted this meta-analysis (International Melanoma Analysis Group, IMAGE) published a further meta-analysis on a subset of the studies included by Bliss[25], looking at influence of family history of the disease.[27] Similar methodology was used.

Nelemas[26], who looked at the influence of sunlight exposure to melanoma, in papers published before 1990, adopted an approach more similar to my own. The author started from the idea that non-experimental studies, such as case-control studies, do not allow for the assumption that the variation in study results is merely attributable to statistical sampling error. Thus he clearly showed that an important function of a meta-analysis is the exploration of sources of variation in study results. When studies with some degree of blinding were combined only a small and non-significant effect was evident. Conversely, in studies without blinding, the effect was considerably greater and significant because differential recall of past exposures may have introduced bias. Meta-regression, which could have been useful to investigate interaction between factors, was not applied because the authors considered the number of the studies available too low to produce reliable results. Only case-control studies published in English were included and this may have introduced a bias, as will be explained in Chapter 2.

A very recent meta-analysis[28] included case-control studies published in English between 1966 and 1999, which separately reported relative risks for melanoma associated with sun exposure during childhood and adulthood. Again heterogeneity was found significant for some subgroup of studies but was not deeply investigated.

1.5 Conclusions

When the magnitude of the underlying risks is small or when the results from individual studies disagree, meta-analysis is an attractive tool.

Occasionally, a meta-analyst has the opportunity to work with individual-level data. In most situations, relative to published data, individual-level data would provide great flexibility to address issues of control of confounding and exploration of effect modification or subgroup effects. More often, however, published reports of research are the only sources of data available.

A meta-analysis[19] that I carried out on published studies, looking at the association between vegetables and fruit consumption and breast cancer, is described in Chapter 3 to present an approved methodology for meta-analysis in epidemiology.

A systematic meta-analysis that assessed all risk factors for melanoma is illustrated in Chapter 4 and 5. As we have seen in the previous paragraph, few meta-analyses[24-28] were published on melanoma. Some of them used original individual records and this possibility enabled, for example, elimination of variations due to different coding and analytic procedures in the studies. However between-study heterogeneity was very often found significant, even when the authors considered subsets of studies identified with strict inclusion criteria. In fact many analysts identify heterogeneity and deal with it by excluding studies until a satisfactory degree of homogeneity is achieved. Authors sometimes exclude 25% of the data and still generalize to the total population. The problem is that they did not deeply investigate possible sources of variations and inconsistencies. Similarly, potential effect of publication bias was not explored in any meta-analysis.

This work differs from the previous analyses, which try to quantify and summarize previous studies, first of all because a deep exploration of between-study heterogeneity and possible sources of biases was carried out and it provided some clues in epidemiology of melanoma, looking extensively at inconsistencies and variability in the estimates. Thus

investigation of variation in the estimates by differences in study features, definitions, characteristics of the populations and of the analyses, is shown to be essential because interesting interpretations arise from these issues. Instead of using strict inclusion criteria or quality scores, to deal with differences among the studies, sensitivity analysis and heterogentiy analysis were carried out to examinate associations among factors influencing the estimates for all risk factors, looking at similarities and asymmetries. Thus much more studies were considered in this meta-analysis, compared to the previous ones. In fact the most recent papers were included and wider inclusion criteria were used, so that, for example, cohort studies were incorporated.

The best way to assess contrasts between risk factors is to concentrate on the studies that provide estimates of all measures of exposures together as this brings some consistency to the choice of measures used by the authors and the study methods. Thus comparisons, between risk factors and between different measures of exposures, are also carried out through a multivariate approach for multiple risk factors, published very recently[29] and never before used in the previous meta-analyses.

CHAPTER 2. HETEROGENEITY AND BIAS: STATISTICAL METHODS

2.1 Introduction

A good meta-analysis should help to understand differences in results from the mass of papers from which they are derived. Four are the main steps to carry out a meta-analysis: identify all relevant studies; assess study characteristics; deal with between-study heterogeneity and possible bias; and summarize the results.

The main goals of a meta-analysis in epidemiology are the estimation of an overall association and the identification of sources of bias and between-study heterogeneity. In particular investigation of biases and inconsistencies should become one of the key phase for a meta-analyst because it can lead to more insights than the mechanistic calculation of an overall measure of effect, which will be often be biased.

Many potential sources of heterogeneity occur within epidemiological research when using case-control and cohort designs. Colditz[30] in 1995 reviewed several meta-analyses in epidemiology and observed that the majority derived an overall quantitative estimates of the association between exposure and outcome but only a quarter of them tested for heterogeneity and even fewer explored for sources of variability.

In this Chapter I present some methodologies useful to deal with these typical issues of meta-analysis of observational studies: heterogeneity and bias.

Two main models to study variation in a meta-analysis are presented. One, the fixed effects model, considers the studies being analysed as universe of interest; the other, the random effects model, takes these studies as representing a sample from a larger population of possible studies.

Efficient methods that provide pooled trend estimates from dose-response data are illustrated. This technique takes into account the correlation among different exposures.

Multivariate analysis of multiple outcomes is briefly described in this Chapter. General linear MIXED models are applied in the next Chapters to compare few outcomes summarized in a multi-dimensional approach.

A brief description of the Bayesian method is introduced at the end of the Chapter. They were not applied to my data because Bayesian approaches are controversial: the definition of prior probability will often be based on subjective assessments and furthermore complex computational techniques are required for their application.

2.2 Bias in meta-analysis

One of the most crucial steps in a systematic meta-analysis is study identification. In order to control the biases the process of identifying and selecting studies is very important. Refined methods to search for studies are fundamental to include all potentially eligible studies. The effect on the estimates of the exclusion of each study not contributing to the analysis should be investigated. In fact, if the studies included are a biased sample of all the studies conducted, then the force of any possible inference is limited.

Publication bias is the most mentioned bias in meta-analysis because when it is present the significance of results may influence whether a study is submitted, positively reviewed and eventually accepted for publication or not. Commonly meta-analysts refer to publication bias but this is only one of the possible biases, included in the term "dissemination bias". In general when the analysis is influenced by the accessibility of research findings we should talk of dissemination bias. This depends not only on whether a study is published but also on when, where and in which format this occurs.[31] For example, language bias is related to the fact that studies without significant results are preferably published in languages other

than English and this implies that it will be more difficult to find such 'negative' studies. Authors try more likely to publish positive findings in an international, English language journal, whereas negative findings end in local journals. Therefore bias could be introduced in meta-analyses based exclusively on studies published in English. [32;33;34] Moreover if most of the major west European journals, published in languages other than English, are indexed in Embase or Medline, this is not the case for journals published in less developed countries. Obviously it will be very difficult to find studies that are published in journals not indexed in one of the major databases.

Searches in computerised databases are usually extended examining the reference lists of other studies and reviews. When reference lists are used, citation bias may have an important role. Citation bias leads to underreporting of 'negative' studies being referred to less often.[35;36] On the other hand, significant results are sometimes published in more papers, increasing the probability for them to be discovered (multiple publication bias).[37] Furthermore it is not always obvious that more publications come from a single study, and one dataset may thus be included in an analysis twice.[38]

Another source of bias comes from differences in methodological quality of studies. Methodological accuracy of smaller studies is not at the same level of larger studies and papers of lower quality also tend to show larger effect estimates.[39;40] In these cases there is often an interaction between sample size and statistical significance. To publish a non-significant result sample sizes must be very large. This is reasonable because the statistical power to detect a significant difference is low when samples are small.[41;42-44] The real problem arises when the true relationship is modest as in the majority of epidemiological analyses.[45] Actually for studies with small samples, the only results published will tend to be those that are significant and this can lead to a systematic overestimation of the true effect size.

2.2.1 Funnel plots and graphical tests to detect bias

In meta-analysis, funnel plot and related statistical analyses are the most commonly used methods for assessing the possible existence of publication bias. Funnel plots are simple scatter plots of the risk estimates, on the x axis, versus some measure of their precision, as standard error, variance, inverse of variance, sample size, on the y axis. The name 'funnel plot' arises from the fact that risk estimates from small studies will spread out more widely at the bottom of the graph, with the spread narrowing among larger studies that present more precise estimates. In the absence of bias the plot should look like a symmetrical inverted funnel. (See Figure 1.1) If there is a bias, because for example smaller studies without statistically significant effects remain unpublished, this will lead to an asymmetrical form of the funnel plot with a gap in a bottom corner of the graph. (See Figure 1.2) The more the asymmetry is pronounced, the more likely it is that the amount of bias will be substantial and the pooled effect calculated from meta-analysis will probably overestimate the true risk estimate.[46]

In absence of bias the shape of the plot depends on the choice of the axes. Standard error was shown to be the best choice for the vertical axis because the expected shape in the absence of bias corresponds to a symmetrical funnel, straight lines to indicate 95% confidence intervals can be included and emphasis of the plot is on smaller studies where bias is more likely. The only disadvantage, compared to other choices for the vertical axis, is that that axis has to be inverted to place the largest studies at the top of the graph.[47]

Visual evaluation of funnel plots may be subjective and more formal statistical methods, to examine associations between the study effects and size, were proposed.[46;48]

Figure 2.1. Asymmetrical funnel plot.

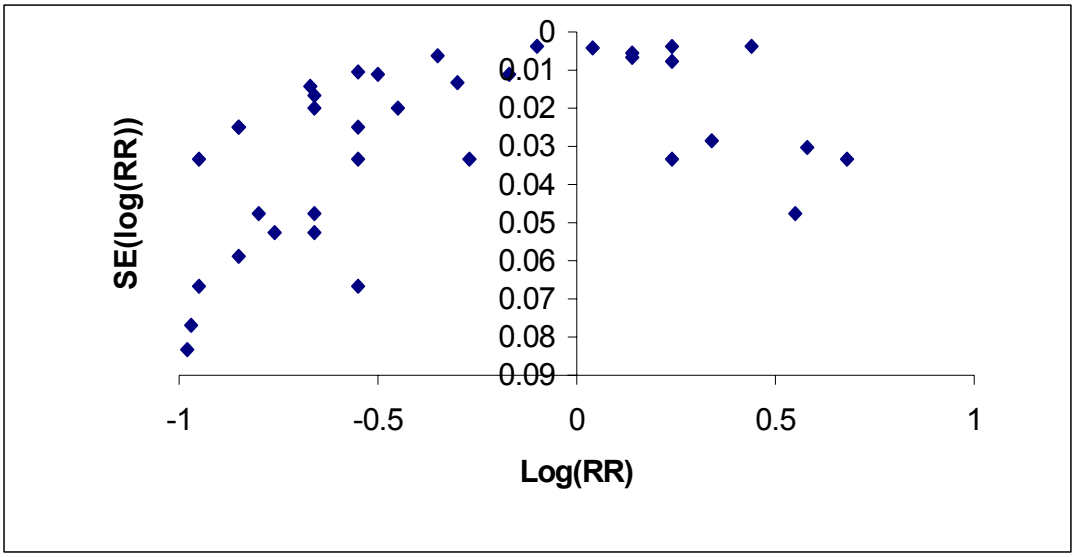
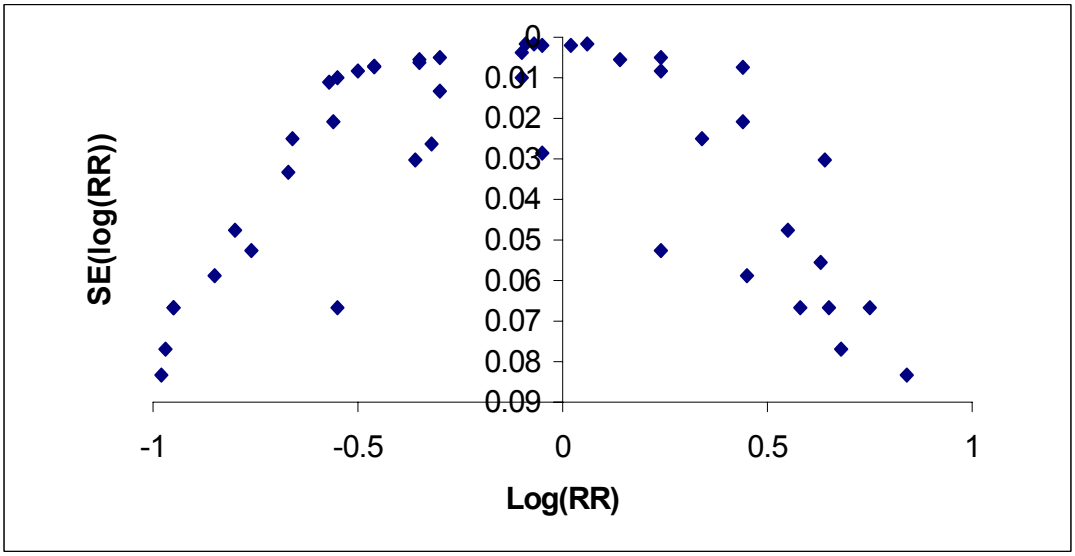


Figure 2.2. Symmetrical funnel plot.



Duval and Tweedie have proposed the so-called "Trim and fill" method. It is based on a rank data augmentation technique that adds studies to a funnel plot so that it becomes symmetrical.[49] Smaller studies at the bottom are omitted until the funnel plot is symmetrical (trimming). The trimmed funnel plot is used to calculate a pooled estimate by standard meta-analysis approach. The trimmed studies are then replaced and their missing counterparts

around the centre imputed or "filled". This provides an estimate of the number of missing studies and an adjusted pooled estimate that is obtained including the "filled" studies.

Begg and Mazumdar proposed a rank correlation method that uses Kendall's Tau to evaluate the association between the effect estimates and their variances.[48]

Egger introduced a linear regression approach, which is equivalent to a weighted regression of the estimate on its standard error:

$\log_e(RR)/SE(\log_e(RR)=a+b * (SE(\log_e(RR))^{-1}$

The intercept value (a) provides an estimate of asymmetry of funnel plot. Positive values of "a" indicate a trend towards higher levels of study estimate in publications with smaller samples sizes.[46]

Egger's method is more sensitive than Begg's rank correlation approach, but the sensitivity of both methods is generally low in meta-analyses based on less than 20 studies.[50] Both Egger's regression method and the "Trim and fill" method may be related to a great false-positive rate in detecting significant asymmetry of funnel plots. Furthermore Egger's method is known to be intrinsically biased.[51]

Copas proposed a model in which the probability that a study is included in a meta-analysis depends on its standard error. The model describes the process of study selection (publication bias) and evaluates the pooled RR for different parameter choices, which can be interpreted as the probabilities that a paper with a certain value of "standard error" is published (publication probability). For any given value of publication probability Copas and Shi proposed a method to estimate the number of studies that were undertaken but not published and the correspondent reduction in the estimated risk. As there are not enough data to choose a single "best" model, the authors proposed a sensitivity analyses in which the value of the estimated risk factor is computed under a range of assumptions on the severity of the selection bias.[52]

These statistical methods, which investigate the asymmetry of the funnel plot, try to estimate how big the impact of publication bias might be on the results. However none of them can be considered the ideal statistical method for assessing publication bias and any method should be considered indirect and exploratory. Modelling assumptions used may heavily influence the estimates adjusted for publication bias. Many factors may be involved in the publication process, and it is difficult, if not impossible, to adequately model them. These methods may detect "missing" studies even in the absence of bias, adding and adjusting for non-existent studies in response to funnel plot asymmetry arising from nothing more than random variation.[53] As a matter of fact their sensitivity is generally low in meta-analyses based on less than 20 studies, as I said previously.[50;51;54]

It was estimated that missing studies change the conclusions in less than 10% of meta-analyses, suggesting that publication bias, although widespread, may not be a major problem.[55] It is therefore wise to restrict the use of statistical methods that model selection mechanisms to the identification of bias rather than correcting it.[56;57]

2.3 Meta-analysis dilemma: heterogeneity

There may be different kinds of heterogeneity: population heterogeneity and methodological heterogeneity. The term 'population heterogeneity' covers factors such as study location, age, sex, types of diseases. Methodological heterogeneity has to do with differences between study designs and analyses.

When different studies give different results the aim of the meta-analysis should be to investigate the reasons why effects differ across studies, identifying methodological discrepancies among studies, assessing the possibility of confounding factors and evaluating differences among populations under study.[30;58;59]

As was seen in the previous Chapter, exploration of sources of heterogeneity can lead to insights over modification of apparent associations by various aspects of study design, exposure measure and population and may allow identification of features of study design that may have implications for future research.[59]

Subgroup analyses and "meta-regression" are techniques useful to work out when particular characteristics of studies are related to the sizes of the estimates. Subgroup analyses are meta-analyses on subgroups of the studies that partition the observed effect size variability into two components: the portion attributable to subject-level sampling error and the portion attributable to other between-study differences. This is obtained dividing results into different types of subjects, outcomes or study characteristics, but it requires cautious interpretation. When several outcomes are measured, but only a selected subset of them are reported and discussed, it is possible to have misleading results. Furthermore the more subgroup analyses are performed, the more likely it is that a statistically significant result will be found due simply to chance. Any subgroup analysis should be best considered as generating hypotheses for testing in the future and should have a scientific rationale.[60]

"Meta-regression" models represent a useful tool to investigate possible explanations for between-study heterogeneity because they allow also to test interactions between factors. The term meta-regression indicates the use of study-level covariates, as distinct from regression analyses that are possible when individual subjects data on outcomes and covariates are available. Subgroup analysis is equivalent to meta-regression with a categorical study-level covariate. Considering subgroup analysis formally as a meta-regression has advantages, since it properly focuses on the differences between subgroups, rather than the effects on each subgroup separately. Also random effects models allow for residual heterogeneity, not explained by sub-grouping.[58;61-63]

When there is a small number of studies and many differing characteristics, the risk of obtaining a spurious explanation from meta-regression is high. This is a particular problem in meta-analysis because there are many characteristics, which differ among the studies, and these can be highly correlated. Further summarizing of subject's characteristics at study level implies the risk of completely failing to detect genuine relationships between these characteristics and the size of risk factors. Meta-analysis carried out on individual subjects data can alleviate some of these problems. In particular within-study and between-study relationships can be more clearly distinguished, and confounding by individual level covariates can be investigated. However, as we have seen earlier, this kind of meta-analysis is much more expensive and time–consuming and very often not feasible.[61;62;64]

Since poor-quality studies sometimes produce systematically different results, a meta-analysis may yield misleading results if the quality of the studies is poor.[39;40;65] In an attempt to cope with these problems and to control heterogeneity, many researchers restrict analysis considering some inclusion criteria. However an extensive investigation of the effect of inclusion criteria on results is recommended to avoid introduction of reviewer's own bias.

Use of quality scoring in meta-analysis is controversial because it is not clear its validity and may not be associated with quality. It is very difficult to score and measure quality that is best evaluated qualitatively.[45;66] Greenland called quality scores "perhaps the most insidious form of subjectivity masquerading as objectivity" because they modify data information by using arbitrary judgments in their assignment.[67]

Sensitivity analysis is recommended rather than quality scores because it helps to establish the influence of individual study results to the overall pooled estimate. The main role of a sensitivity analysis is to discuss robustness of results and determine whether the assumptions or decisions to either exclude or include studies have a major effect on the final estimates.[68]

After exploration of the contributions of all known or suspected factors that may have introduced variation in the estimates, heterogeneity remains very often unexplained and statistical models that can take into account this variation are essential.[69]

2.3.1 Fixed effects models

Fixed effects models are based on the mathematical assumption that a single common (or 'fixed') effect underlies every study in the meta-analysis. In other words, if we were doing a meta-analysis of odds ratios, we would assume that every study is estimating the same odds ratio. Under this assumption, if all studies were infinitely large they would produce identical results. This means that between-study heterogeneity is not statistically significant.

The fixed effects model is used to obtain a pooled estimate "b" of the effect estimates, that is:

$$b=\frac{\sum_i w_i * b_i}{\sum_i w_i}$$

where b_i are the study results (as the $\log_e$(RR) estimates) and w_i are the weights of the studies. The study's weights is obtained calculating the inverse of the variance of the effect estimate: $w_i=1/[SE(\log(RR_i))]^2$.

In cancer studies the distinctions among the various measures of relative risk (e.g. odds ratios, rate ratios, and risk ratios) are ignored, because cancer is a rare disease in all populations under review. The relative risks and confidence intervals extracted from each study are converted to the natural log of the risk estimates and the associated variance.

If confidence intervals or standard errors of the relative risks are not available, crude data are used in Woolf's formula as follows: $se\left[\log(OR)\right] = \sqrt{1/a+1/b+1/c+1/d}$, where a, b c and d represent the values of Table 2.1.

Table 2.1. Table 2x2 with cases and controls by exposure categories.

	Cases	Controls
Exposed	a	b
Not exposed	c	d

When the standard error of the odds ratio is found in the publication then the approximate estimate of the standard error (SE) of the logarithm of the OR is obtained from:

SE(logOR)=SE(OR)/OR

When between-study heterogeneity is investigated the fixed effect approach may be applied to meta-regression models where the expected value of a study estimate is modelled as a fixed function of measures of study characteristics. Again this meta-analytic approach is based on weighted regression, where the log of the odds ratio from each study is the variable response and the weights depend on the precision of the estimates, i.e. the standard errors of the log(RR).

2.3.2 Random effects models

Random effects models make the assumption that individual studies are estimating different underlying risks. The idea of a random effects meta-analysis is to learn about the distribution of risks across different studies.

When large heterogeneity is found, diversities in the designs and analyses of the various studies should be taken into account in the final model and it can be assumed that the true effects estimated will vary among studies. There are two sources of variability that must be addressed, the usual sampling variation in the estimates and variation in the underlying parameter. To account for both sources of variation in the meta-analysis, the DerSimonian and Laird[70] method was used.

To evaluate between-study heterogeneity a sample test based on the statistic:

$Q_w = \sum_i w_i (b_i - \bar{b}_w)^2$

is used, where b_i is the *i*th effect estimate ($\log_e$(RR) estimates) and

$$\bar{b}_w = \sum_i w_i b_i / \sum_i w_i$$

is the weighted pooled estimator of effect estimate and w_i is the inverse of the *i*th sampling variance. Under the null hypothesis of no effect of the factor considered, Q follows approximately a Chi-squared with k-1 degrees of freedom, where k is the number of studies considered in the analysis.

The random effects model uses weighted least squares estimates of the effect estimates (as the logarithm of the RRs):

$$b_p^* = \sum_i w_i^* b_i / \sum_i w_i^*$$

where the weights are

$$w_i^* = (w_i^{-1} + \Delta_w^2)^{-1}$$

and Δ_w^2 gives an estimate of the degree to which studies have different assessments of the relative riss. Thus the calculations to obtain Δ_w^2 are based on the Chi-squared.[70]

Chi-squared test does not have a big power, therefore heterogeneity should not be considered statistically significant at the p-value=0.1 level of association.[71]

The consequence of performing a random rather than a fixed effects model is that the confidence intervals for the pooled estimate are wider. A random effect analysis therefore suggests more uncertainty in estimating the underlying parameter than a fixed effects model does. Moreover estimates from random effects models tend to be more sensitive to publication bias than fixed effect estimates, because smaller studies have larger relative weights. It follows that random effects models will be more strongly biased than fixed effects models by any tendency not to publish small statistically non-significant studies.

2.3.3 Dose response

An important criterion supporting causality of associations is a dose-response relation. In order to allow an estimation of the shape of a dose-response relation, fixed and random effects approaches for dose-response models may be used for summarising the results across the studies. An estimate of the change in the relative risk per unit of exposure within each study may be obtained, and they should be combined across studies.

The individual log relative risks are modelled as a function of exposure level in the following way:

$E(b_{ij})=\log (RR_{ij})=bx_{ij}$

where i=1,...,I is the index study, j=1,...,J-1 is the value of the exposure in the j-th non-reference exposure category within each study.

The dose-response of the logarithm of the relative risks for separate exposure levels depends on the same reference (Unexposed) group; therefore the estimates cannot be independent. The method of pooling proposed by Greenland and Longnecker[72] adjusts the estimates for within-study covariance and accounts for the correlation between estimates. This approach is based on constructing an approximate covariance estimate for the log odds ratios, from a fitted table that conforms to the log odds ratios. To fit the cell counts to the interior of the total data table an iterative algorithm, that is based on Newton Rapson method, is used and the asymptotic covariance is obtained.

It is necessary to assign numeric values to the categories before estimating coefficients. As a frequency distribution for exposure is very often not available, the method used is to assign category midpoints to categories. To fit the cell counts to the interior of the total data table, in order to obtain the asymptotic covariance, the number of cases and the number of observations for each exposure level is needed. When this information is not available, it is not possible to use the method of pooling proposed by Greenland and

Longnecker[72] to adjust the estimates for within-study covariance. In this case the inverse variance-weighted least squares estimate of the logarithm of the RR can be calculated from:

$$b=\frac{\sum w_j x_j y_j}{\sum w_j x_j^2}$$

where $w_j=1/\text{var}(\log(RR))$, $y_j=\log(RR)$ and x_j is the value of the exposure. When the j-1 values are independent, the standard error of log(RR) is:

$$SE(b)= 1/ \left(\sum w_j x_j^2\right)^{1/2}.$$

2.4 Multivariate analysis

Multivariate analysis is potentially much more informative and more correct than univariate analysis to evaluate multiple risk factors together. First because it permits to compare estimates extracted from the studies that provide all measures of exposures together and this allows having data with some consistency in study methods and in the populations considered. Second because it can take into account the correlation between risk factors. Actually estimated effects obtained from the same samples are correlated and an estimate of this correlation is needed to perform the analysis. However very often in meta-analysis of observational studies, as in my work, estimates of covariance are not available and independence has to be assumed.

Methods proposed in statistical literature are not easy to apply in practice, because self written programs have to be used. Arends showed that general linear MIXED model software is a very convenient framework for multivariate meta-analysis.[73]

Models were fitted using SAS (Proc Mixed[74]) that is based on an approximate likelihood approach. This method does not assume a normal distribution for the underlying parameters, as the mixture model introduced by DerSimonian and Laird[70] does, but the estimates are obtained with maximum likelihood algorithm.

Detailed description of statistical aspects of this approach can be found clearly described in the "Tutorial in Biostatistics" published in *Statistics in medicine,* where some SAS procedures, which I used for this work, are clearly described and interpreted.[29]

2.5 Bayesian meta-analysis

Bayesian approach to meta-analysis is based on the principle that each observation or set of observations should be viewed in conjunction with a prior probability describing existing knowledge about the phenomenon of interest. The new observation alters this probability to generate a posterior probability. In Bayesian terms, traditional statistical methods used in meta-analysis assume that the prior probability distribution is uniform, with all outcomes being equally probable. Bayesian method allows the incorporation of indirect evidence and opinions in the generation of the prior distributions.[75-77]

Specific advantages conferred by the Bayesian approach include: full allowance for all parameter uncertainty in the model, the ability to include other pertinent information that would otherwise be excluded, and the ability to extend the models to accommodate more complex, but frequently occurring, scenarios.

Bayesian models are available under both the fixed and random effect assumptions.[78] The confidence interval (or more correctly in Bayesian terminology, the 95% credible interval, which covers 95% of the posterior probability distribution) will often be wider than that derived from using the conventional models because another component of variability, the prior distribution, is introduced.

This approach is controversial because it depends heavily on opinions, and these will often vary considerably. To implement reliable Bayesian analyses, additional development in drawing prior probability distributions and conducting robust analyses is needed. Furthermore

Bayesian inference in meta-analysis requires complex computational techniques to be routinely applied.

2.6 Conclusions

Between-study heterogeneity is very frequent in meta-analyses of epidemiological data[59] and, if it is statistically significant, there are several choices open. One option is that of avoiding to calculate pooled estimates and to perform the meta-analysis. An unwise meta-analysis can lead to highly misleading conclusions. However a pooled risk estimate based on several small diverse populations and studies may provide a useful generalization of the results. Furthermore meta-analyses reporting effect estimates that may contain bias may provide relevant information, as long as potential underlying reasons for inconsistencies are addressed.

Finding systematic variation in results and identifying factors that may account for such variation aid in the interpretation of existing data and the planning and execution of future works. Subgroup analysis and meta-regression are described in this Chapter as statistical techniques of exploratory data analysis and they are applied in both the meta-analyses on breast cancer and melanoma. However for the systematic meta-analysis on melanoma there were more chances to explore deeply bias and heterogeneity. The reasons are more than one. First of all more studies were available. Secondly, the investigation of correspondences between all main risk factors allows more insights in epidemiology of melanoma. Thirdly, some analyses on different aspects of methodologies, as funnel plots, graphical tests and meta-regression, useful to detect significant variation in the estimates and bias are very recent.[47;50-52;54;61]

The danger of the testing approach for funnel plot is the temptation to assume that, if the test is not significant, there is no problem and hence the possibility of publication bias can

be ignored. Copas and Shy argued that publication bias is endemic to all empirical research even if it is not evident from the funnel plot. Approaches, which try to estimate exactly how many studies are missing, are very hazardous, and graphical tests make assumptions that cannot be tested. The sensitivity analysis proposed by Copas and Shi monitor how sensitively the results depend on the assumptions using a model to describe the process of study selection, estimate the effect of interest for different parameter choices within this model, and then check the fit of each estimate with the evidence in the funnel plot. Thus Copas and Shi's method was applied for all risk factors for melanoma. The application was quite straightforward because they published the S-plus routines, which were useful to carry out the calculations.

Meta-analysis needs only partially statistical skills but the statistical part constitutes a large and important fraction of what a meta-analysis consists of. The common technique for combining results across studies is the weighted average of study specific results. The choice of weights to be assigned to the studies and ways to deal with among-study variability differ from method to method.

A statistical issue is whether to incorporate between-study variation into the calculation of combined estimates. Thus fixed and random effects models are described. When existing studies are considered a random sample of a population of studies and heterogeneity is seen as an integral part of the data, then random effects model was used. Regression methods that take into account the correlation between exposures were applied to obtain summary relative risk for dose-response relationships.

Quality scores are adopted by some authors to deal with possible bias, imprecision and in general poor quality, which are generally more frequent in observational studies than in controlled trials. However in the absence of a rigorous and validated statistical method to

include quality assessment in the calculation of the summary estimates, weighting by quality is not considered in this meta-analysis.

Multivariate approach is applied to meta-analysis on melanoma in Chapter 4 and Chapter 5 to evaluate contrasts among multiple risk factors and to make comparisons with the classical pooled estimates, which are calculated with a different methodology. It was not used in breast cancer meta-analysis because practical applications with general linear MIXED model software have been explained only in a recent publication[73]. An hypothesis of independence among risk factors was assumed, even if it is unrealistic, because no estimates of the correlations between risk factors were available. This model has been applied to make comparisons with the pooled estimates obtained with the other models. An estimate of covariance can be obtained from the application of the model.

Bayesian approach is briefly presented but it is not applied in my meta-analyses because it leads to complicate calculations and so far only sophisticated software is available for this method. Moreover recent investigations demonstrated that, for practical purposes, the differences with the other methods are not substantial.[9]

In cancer studies distinctions among the various measures of relative risk are ignored in all meta-analyses that I carried out, because cancer is a rare disease in all populations under review.

CHAPTER 3. BREAST CANCER AND FRUIT AND VEGETABLES CONSUMPTION

3.1 Introduction

Breast cancer is a major public health problem in industrialised countries. It is estimated that one in eight American women will develop breast cancer in her lifetime.[79;80] The most convincing evidence that breast cancer is possibly preventable is that the rate of breast cancer increases after a woman migrates from a country with a low rate of cases to one with a higher rate. Dietary change was consistently suggested as a major contributor factor to this situation[81] even if a change[82;83] in health-care system, which may emphasize mammography and other methods of detecting breast cancer in its early stages, is also fundamental.

When breast cancer risk was examined in relation to intake of vegetables, fruits or both as a food group the results provided some evidence of a protective effect.[84] Fruits and vegetables are common sources of many candidate protective substances, including carotenoids, with and without vitamin A activity, and ascorbic acid. Carotenoids and Vitamin C may protect against breast cancer due to their role in antioxidant defence.

Among epidemiological studies, there is a convincing pattern showing that women with diet with high contents of vegetables and fruits, have a decreased risk of breast cancer. However, the results are not consistent. The aim of this work was to analyse published results that explore the relationship between breast cancer risk and the consumption of fruits and vegetables and two associated micronutrients (vitamin C and beta-carotene). A meta-analysis of all published studies from 1982 to 1997 was carried out starting from the published reviews[16;85-87] on vegetable and fruit consumption and breast cancer risk.

3.2 Materials and Methods

3.2.1 Selection of studies

Two bibliographic databases were screened from January 1982 to April, 1997:

- the online MEDLINE (National Library of Medicine, Bethesda, MD, USA (http://www.nlm.nih.gov/) using PUBMED (http://www.nlm.nih.gov/PubMed/) as system of querying from January 1966.
- Elsevier Science online EMBASE (http://www.ovid.com/) from January 1988.

No language restrictions were applied. The MEDLINE search was conducted using the following key-words: vegetable*, fruit*, Vitamin C, Betacarotene, Beta-carotene, Carotene in combination with breast, cancer*, tumour, tumor, neoplas*, malignan*, carcino*, metasta* and case control*, case-control*, cohort*, cross section*, cross-section*, follow up, follow-up, followup, prospective.

A possible search strategy using PUBMED on Text Word could be: (breast*[Text Word]) AND (cancer*[Text Word] OR tumour*[Text Word] OR tumor*[Text Word] OR neoplas*[Text Word] OR malignan*[Text Word] OR carcino*[Text Word]) AND (case control*[Text Word] OR case-control*[Text Word] OR cohort*[Text Word] OR cross section*[Text Word] OR cross-section*[Text Word] OR follow up*[Text Word] OR follow-up*[Text Word] OR followup*[Text Word] OR prospective[Text Word]) NOT (animal[Mesh]) AND ("1982"[PDat] : "1997"[PDat])

Successively the following mesh terms were used: vegetable, fruit, Vitamin C, Betacarotene associated with breast cancer, breast neoplasms and case-control studies, cohort studies, prospective, follow-up and cross-sectional studies. The final search strategy was carried out combining the search strategy for “text word” and “mesh terms”. Similar strategies were used to search EMBASE. The search was limited to human studies only.

The references of publications obtained from the MEDLINE search were also reviewed for relevant articles. A total of 45 articles[88;89-123;124;125-132] were identified, presenting results from

case-control and cohort studies, 27 dealing with vegetables, 20 with fruits, 30 with beta-carotene and 19 with vitamin C (Table 3.1).

At the first stage of the analysis, some inclusion criteria were identified to obtain a group of studies, each with a least a minimal information. This group of studies is known as the *main group* and the bulk of the meta-analysis is based upon these studies. In the sensitivity analysis, a comprehensive meta-analysis was carried out including all possible studies found to assess any bias induced by this selection.

The following inclusion criteria were used:

- The studies had to provide sufficient information to estimate the relative risk and 95% confidence intervals (i.e. they had to publish the Odds Ratios (OR), Relative Risks (RR), Standardize Incidence Ratio (SIR) or crude data and standard errors, variance, confidence intervals or p-value of the significance of the estimates). An estimate and its variance were required to calculate a weighted pooled-estimate of the RR. When the variances were not reported, they were calculated from the published confidence intervals, from the crude data or an estimate of them, or in an approximate way from the p-values.[133] Published results from three studies only[101;102;125] could not be included in the meta-analysis, because the authors did not present enough information to evaluate the variance.
- The studies had to be independent in order to avoid giving double weight to some studies. If results for micronutrients and food groups were derived from one study, but were published separately, both articles were considered. Simard[124] was not considered, because this study is based on a cohort which was also analysed in an article by Rohan[122]; the latter was chosen because the first presented only the percentages of people regularly eating fruit and vegetable. Cooper[89] and Ambrosone[88] were not included because they were not wholly independent from other studies

found.[94;123] They presented adjusted estimates that refer to very specific subgroups of the population. In Ambrosone's paper[88] the estimates are presented separately for women who had a family history of breast cancer. Cooper's paper[89] looked at oestrogen receptor status. The estimates for vegetable and fruit consumption from La Vecchia (1987)[108] paper were excluded from the analysis because updated figures were published subsequently in 1991.[113]

- As regards the consumption of food items and the intake of micronutrients the categories for which the results are reported had to be comparable. In the analysis of Vitamin C I calculated the relative risk of about "400 mg/day or more" compared to "70 mg/day or less", while for Beta-carotene I used "7000 μg/day or more" compared to "1000 μg/day or less". These categories are indicative and the rational for choosen them is explained in the following section. In five publications[93;110;121;127;130;131] the cut-off points for the highest frequencies were close to the lowest categories of all the other studies. This meant that these studies could not be used to give a relative risk estimate of the comparisons above. For this reason they were excluded from the main analysis. Some publications[90;100;101;105;112;117;119;127;128] at this stage were not considered, since it was not possible to assess comparability with the other studies because no value of the cut-off points was shown. Ingram[25] calculated RR estimates for quartiles and for median consumption, but without showing the values of the cutoff points. Only mean consumption was indicated and it appeared to be quite low. Consequently this study was excluded.
- The questionnaires to assess diet had to be equivalent. The vast majority (all except one) of the papers found presented estimates obtained from food frequency questionnaires (FFQ) that are simple methods, completed in only one session, that usually assesse intake over long time periods. Diet histories and other methods like

24-hour recalls or 7-day diaries are much more precise because they are often conducted with a nutritionist and provide comparable estimates to more intensive methods like food records. For this analysis we required that dietary information should be based on a standadised method of assessment as, at least, a food frequency questionnaire. Zemla[129] was not taken into account because it did not use a very simplified and not comparable questionnaire.

- The populations studied should be homogeneous, at least for the main risk factors for breast cancer. Hislop[97] was excluded in the first part of the analysis, because only women with benign breast diseases, having an increased risk of breast cancer, were considered. Kato presented two estimates calculated including as controls patients with benign breast disease and without including them. The estimates coming from the last group was chosen, even if they were not adjusted. I also excluded studies that presented the risk estimates for pre-menopausal women only[92;109], since a stronger influence of genetic predisposition for breast cancer at early ages is suggested.[134;135] In one study[96] risk estimates were given for pre- and post-menopausal women separately. Crude data (cases and controls for each categories) were also presented and pre and post-menopausal women were combined into one group in order to represent all women.

Twenty-seven studies were identified as fulfilling the entry criteria and this main group of studies, 18 for vegetables, 13 for fruits, 11 for beta-carotene, and 9 for vitamin C are listed in Table 3.2. The effects of excluding some estimates are critically investigated in the sensitivity analysis.

3.2.2 Extraction and unification of the data

A major concern in the discussion on the selection of controls for case-control studies has been the risk of introducing selection bias. Population controls, randomly sampled from the same population from which the cases originate, during a specified time interval in the same geographic area, are thought to be the best controls. The reason for this is that when cases and controls come from the same study base the risk for selection bias should be reduce. Patients, who were hospitalised even for other diseases, may be unrepresentative for the exposure distribution in the source population.[136] However population-based estimates are reliable if there is a full coverage of cases occurring in the population being studied and this is not always assured. In developing countries, for example, full ascertainment of cases of a specific disease is difficult to obtain. This is not only due to limited access to health care and lack of knowledge of how to access health care facilities, but also to financial barriers to health care for certain segments of the population. Furtheremore sometimes in population-based studies there is low rate of response among controls. In such circumstances hospital-based case-control estimates are the best choice.

In this meta-analysis one study[137] presented two different estimates, considering separately hospital and population controls. Results from population controls were chosen for the analysis but the choice it is not expected to influence the final pooled estimate because the two controls groups were consistent with each other, and in general their food consumption patterns resembled each other rather than the case pattern.

Whenever possible, a risk estimate for an overall item (food group "vegetable" and food group "fruit") was chosen. If such a risk estimate was not presented an indicator item (preferably a food item with a high density of the micronutrients of interest) was selected taking into account the availability of micronutrients as well. Between "cooked vegetables" and "raw vegetables" the cooked[91] ones w ere chosen because bioavailability of carotenoids in raw green leafy vegetables is low compared to cooked vegetables concentration of

betacarotene.[138] Thus, between "green vegetables" and "raw vegetables" the green ones were chosen[96]; between "yellow vegetables" and "other vegetables" the latter were chosen[16]; between "citrus fruits" and "fruits rich in beta-carotene" the latter were chosen[121]; between "citrus fruits" and "other fruits"[91] the latter were chosen. Pawlega[118] presented estimates only on boiled vegetables and these estimates were included.

When comparing categories with different units (e.g. frequencies per day, week, month, year or grams per day, week, month, year), it was observed that the upper limit of the cut-off points, that is the highest category, corresponds more or less to "one portion (around 250 g) per day or more". The reference category (low consumption) refers to "3-4 portions per week or less". These categories were chosen because they were the average of most common upper and lower limits of the cut-off points. This quantity is much lower than the 5-in-a-day portions suggested by the National Cancer Insitute in USA[139], but if I find a protective effect even with a lower consumption I have a stronger indication that high consumption of vegetables and fruits should be taken as an important suggestion even for hormone related cancers as breast cancer.

The distributions of lower and upper limits of the cut-off points for micronutrients were analysed and an average of the most common limits were chosen, as for vegetables and fruit consumptions. The categories were about "400 milligrams per day or more" of vitamin C compared with "70 milligrams per day or less" and intake of "7000 micrograms per day or more" of carotene compared with "1000 micrograms per day or less". Although this analysis is concerned with beta-carotene, Graham,[94] Hunter[99] and Kushi[106] focused on carotene intake. This difference was expected to be of minor importance and these studies were used. When estimates of vitamin C or beta-carotene were presented separately for participants who took supplements, and people who did not take any supplements, the former were chosen.

The studies show a large heterogeneity regarding adjustments that were made for age, energy intake, alcohol intake, age at menarche, place of birth, Quetelet index, education, socio-economic status, parity, menopausal status and social status. If the risk of breast cancer associated with dietary intake was expressed in more than one way, the estimate extracted from the study was the one that reflected the greatest degree of controlling for confounders. Some of the estimates were obtained from crude data and hence they were not adjusted for confounders.

Since a history of benign breast disease is a possible confounder, only risk estimates which were based on a comparison between controls without a history of benign breast disease and breast cancer cases were included in the meta-analysis, if both were presented.[101]

3.2.3 Analysis

Studies were classified as case-control or cohort and the meta-analysis was performed for each study design separately as well as for all studies combined. Analyses were also conducted on subgroups of studies based on other features (year of publication, geographical area, and characteristics in design).

The distinctions among the various measures of relative risk (e.g. odds ratio, rate ratio, risk ratio) were ignored in the overall combined analysis, assuming that breast cancer is a rare disease.[140]

Fixed and random effect dose-response models were used to estimate the dose-response relation across the studies. Estimate of the change in the relative risk per unit of exposure within each study was obtained from the publication.

A meta-analysis of epidemiological dose-response[141] data was carried out.

It was necessary to assign numeric values to the categories before estimating the coefficients and category midpoints were used here.[133] For the open-ended categories a value

for the maximum and minimum intake for each nutrient had to be specified in order to calculate the numeric assignments to the categories. For fruit and vegetable consumption the values assigned to the categories went from 0 to 8 times per week (there was only one study on vegetables for which a greater upper limit of consumption was accredited: 12 times per week). For vitamin C intake the values went from 20 mg to 480 mg per day, for beta-carotene from 1000 μg to 10000 μg per day. Estimates were obtained for different assumed values of the endpoints of the open-ended intervals, but no big changes were found between the estimates of the parameters of interest.

The values of the exposure levels were calculated using the cut-off points presented in the articles. When there was only a broad indication of the categories as "High", "Moderate" and "Low" an estimate of the values was taken from the average of the studies. For micronutrients the studies presented completely different distributions of intake. For this reason studies, which did not show the values of the cutoff points, were excluded from the analysis, as no commonality was present. Two studies, which were included in the analysis comparing "high" versus "low" consumption, were excluded from the dose-response analysis: Holmberg[98] because estimates for all cutoff points were not presented and Iscovich[101] because no information on variability (SE or p-value, or crude data) of the individual estimates was presented.

Estimates of the change in the relative risk per unit of exposure within each study obtained with the method of pooling proposed by Greenland and Longnecker[141] were combined across studies. Fixed effects model was used to pool the estimates of 11 studies on beta-carotene because no between-study heterogeneity was found. For vegetable and fruit consumption and vitamin C intake there was betweenstudy heterogeneity and so the random effects model was used to summarise 18, 13 and 9 studies, respectively.

3.2.3.1 Heterogeneity analysis

Studies included in this analysis differed in a number of aspects of their design and execution. Possible sources of heterogeneity are: publication year, country of origin, type of controls, type of study design, adjustment factors for the estimates, confounding factors, validation of the dietary questionnaire, type of questionnaire (number of items and definition of food groups), geographical region (Mediterranean vs. non-Mediterranean area), presentation of data in the papers, cut-off points and levels for categorisation of consumption, number of items in the food frequency questionnaire. Analyses were carried out in order to investigate reasons for heterogeneity. These analyses focused on the association between vegetable intake and breast cancer risk, because the greater number of studies permitted investigation of further sub-groups. The main effects and interactions between the factors characterizing the studies were explored by analysis of a variance model. PROC GLM in SAS was used to fit the random effects models on the log(RR).[74] The weight for each study was equal to the inverse of the sum of the within-study variance and the residual between-study variance, in order to correspond to a random effect analysis.

3.2.3.2 Sensitivity Analysis

Sensitivity analyses were carried out to evaluate if variations or violations of the inclusion criteria, or decisions about the category cutoff values influenced the results. In order to check if the exclusion criteria may have influenced the results, meta-analysis was carried out including all possible studies found. Four studies[88;89;108;124] were not included because they were not independent. A further two studies[102;125] were not considered because it was not possible to obtain any variability of the relative risk estimate. Finally a number of "distortion hypotheses"[142] were also considered, to try to account for the effects of selection publication bias.

3.3 Results

3.3.1 Characteristics of studies

An overview of the 27 studies included in the first selected groups is given in Table 3.2. Fifteen studies were carried out in European countries, eight in North America, three in Asian countries and one in Australia. Five studies were cohort studies by design and 22 studies were case-control studies. Seven case-control studies used population controls. These 27 studies included a total of 23143 cases.

Twenty-six studies used a food-frequency questionnaire to obtain information on diet; one used a diet history. Large differences within the studies were observed regarding the number of food items (8 to 236). In all studies the relationship between breast cancer and food/nutrient intake was based on partitioning the intake distribution into tertiles, quartiles or quintiles.

3.3.2 Pooled relative risk estimates

The extracted estimates and confidence intervals of the risk for breast cancer associated with the consumption of vegetable and fruit are presented in Figure 3.1 and 3.2, respectively, comparing highest versus lowest consumption. The relative risk for nutrient intakes and breast cancer risk, are presented in Figure 3.3 and 3.4.

The fixed effects model is quite unrealistic insofar as it implies that only sampling error is associated with differences among estimates from studies with identical measured characteristics. Actually the Chi-squared estimates that measure the between-study heterogeneity in the analysis referring to vegetables (Chi-squared=42.4, with d.f.=17, $p<0.001$) and fruit consumption (Chi-squared=37.5, with d.f.=12, $p<0.001$) and vitamin C intake (Chi-squared=18.4, with d.f.=8, $p<0.02$), are all significant. This is an indication that

the homogeneity assumption at the base of the fixed effects model may be not correct. A random effects model was performed for vegetable, fruit and Vitamin C to take account of the variation among studies in the model. Thus, it is not assumed that the studies represent the same effect. Rather, the effects estimated from the different studies come from some underlying distribution of effects. Since no heterogeneity problem was detected for publications on breast cancer risk and beta-carotene (Chi-squared=9.3, with d.f.=10, p=0.5) a fixed effects model was used.

Relative Risk and Confidence Intervals for the food groups and micronutrients, comparing "High intake" versus "Low intake", are presented in Table 3.3. These estimates suggest a significant protective effect for breast cancer due to higher vegetable consumption, high intake of Vitamin C and beta-carotene. The pooled estimate for fruit intake indicates that this food group may have a possible protective effect, but without statistical significance.

As can be seen from Table 3.4 there are some indications that an increase in the consumption of vegetables and fruit is associated with an increase in the protective effect.

3.3.3 Results from heterogeneity analysis

Only one of the features considered seems to explain some of the between-study heterogeneity: validation of the questionnaire (Table 3.5). Use of validated questionnaire is associated with a weaker protective effect of vegetable consumption. We can also observe that the kind of controls used, in case-control studies, may be an important factor, even if it is not statistically significant. When population controls are considered, pooled-estimates are below 1 but the upper limit of the confidence interval is close to 1 (OR=0.73 with 95% C.I. 0.51, 1.04). At the opposite, in case-control studies with hospital controls we have a significant protective effect (OR=0.68 with 95% C.I. 0.56, 0.82). This may suggest that studies less susceptible to selection bias indicate a less strong protective effect.

None of the two factor interactions between the 7 characteristics of the studies listed in Table 3.5 was found to be statistically significant, although testing for interactions here has a low power.

The number of categories used for the consumption classification was also considered to see if this factor influences the results. Studies were separated into two groups: those that consider 3 or less levels and the ones that consider 4 or more levels. This factor did not seem to be relevant to explain variability between studies.

3.3.4 Results from sensitivity analysis

The largest case control study is Negri's[113] and no differences were observed in the estimates in Table 3.5 when this study was omitted, except that the estimates for case-control versus cohort studies became more similar.

The decisions on the indicator item selected, when an overall item (food group "vegetable" and food group "fruit") was not presented, were checked. Estimates for "cooked vegetables" in Franceschi's paper[91], are not significantly below 1, whereas for "raw vegetables" the Odds Ratio is 0.73 (with 95% C.I. 0.60-0.88): therefore the decision was conservative. The estimate chosen from Hirose's paper[96] was the one from "green vegetables", but when the estimate from "raw vegetables" (OR=1.05 with 95% CI 0.92-1.19) was included in its place the pooled estimate did not change (OR=0.74 with 95% CI 0.64-0.85). In Hislop's paper[97] estimates for "yellow vegetables" and "other vegetables" where very similar (OR=0.8 with 95% CI 0.56-1.12 and RR=0.82 with 95% CI 0.55-1.22, respectively). The estimates in Richardson[121] for "citrus fruits" and for "fruits rich in beta-carotene" were exactly the same. The decision to choose "citrus fruits" instead of "other fruits" in Franceschi's paper[91] did not significantly modify the pooled estimate that became 0.95 (with 95% CI 0.82-1.11).

The nine estimates excluded because the cut-off points were not indicated in the papers did not show any significant effect for the food groups and the micronutrient of interest. For vegetables, Van't Veer[127] and Ingram[100] presented non-significant estimates greater than 1, whereas in Iscovich's paper[101] the OR was almost significant below 1. For fruits, all these three papers[100;101;127] indicate a non-significant protective effect. The same results are indicated for Vitamin C in all the papers[100;104;128] excluded and for beta-carotene in 5[105;117;119;127;128] out of 8 papers[90;100;105;112;119;127;128] excluded.

The results, shown in Table 3.6, obtained after inclusion of all possible studies (23 for vegetables, 17 for fruits, 25 for beta-carotene and 16 for vitamin C) are very similar to those obtained previously with the first group of selected studies.

3.3.5 Publication bias

The existence of hypothetical unpublished data was considered and the influence that such data would have on my results was studied. It was assumed that some more studies had data on fruit/vegetable consumption or micronutrients and breast cancer risk but that their results were not published because they were non-significant. To be conservative, it was postulated that the association with breast cancer for those studies was not protective. An average relative risk of 1.2 (with 95% CI 0.8; 1.8) was assumed, in order to have confidence intervals crossing one, because otherwise I would expect to have seen reports on significant associations. The distortion analysis showed that even if I found another seven studies with information on breast cancer and vegetable consumption, ten studies on breast cancer and beta-carotene, and another one on Vitamin C, the hypothetical pooled estimates of the relative risk ratio would still be significantly below one.

3.4 Discussion

The quantitative analysis of the published studies on the relationship between the risk of breast cancer and dietary habits suggests a moderate protective effect due to high consumption of vegetables and related micronutrients. For fruit intake, study results were less clear. Only two studies show a significant protective effect of high fruit intake for breast cancer.[107;126] If the consumption pattern for fruits is more homogenous than for vegetables, or vice versa, in any given population, and both groups are equally protective per amount consumed, then the food group with the wider range of exposures is more likely to yield a larger effect. From this point of view, vegetables might better discriminate between cases and controls. For many cancers the evidence for a protective effect of vegetables is rather stronger than that for fruits, perhaps reflecting the fact that vegetables are generally consumed in greater quantities than fruits, and thus in more variable quantities within populations.[85] Furthermore many yellow-orange vegetables such as carrots, squash and sweet potatoes, and dark green vegetables, such as broccoli and spinach are very good sources of nutrients such as vitamin C and carotenoids.[144]

Forest plots are not used in this thesis because I agree with Charles Poole who commented a paper on Forest plots[143]. This Professor of the Department of Epidemiology, University of North Carolina, explained in the Education and Debate section of BMJ his aversion to the sizing of symbols for point estimates in proportion to their meta-analytic weight, writing that: "The expressed motivation for this special effect is to counteract a presumably irresistible urge for the proverbial clinician's eye to be drawn to the widest confidence intervals and thus to the least precise estimates. To my admittedly jaded meta-analytic eye, however, the distended blobs representing the low-variance estimates in these displays connote not precision but its opposite. […]" Furthermore forest plots came from specially produced computer programs. Most standard statistical packages cannot easily

produce such a plot. Important information on the papers is available on the tables presenting all the main study features.

The distributions of consumption of fruit/vegetable and intake of micronutrients extracted from the papers are partitioned into different percentiles: median, tertiles, quartiles or quintiles. Unlike pooled meta-analyses, I was not able to repartition vegetables and fruit consumption and micronutrients intake, but I have based the analysis on the partitions selected by the authors of the original reports. The pooled estimate that compared the highest with the lowest category of intake found in the papers was considered in order to reduce misclassification. This method of examining the associations addresses only the question whether a difference in risk exists between extreme categories of exposure. An important consideration is that a woman is not likely to change her diet from that of the highest to that of the lowest quintile, and the present estimates are intended primarily to reflect the strength of an observed association – an important criterion of causality. One limitation of this approach it that it may attenuate the summary relative risks because I have to pooled together estimates related to different percentiles. In fact if the intake is related to breast cancer risk the relative risk generated by a study that partitions fat intake into quintiles will generate a larger relative risk between the highest and the lowest categories of intake than does a study that partitions according to tertiles.

Only few prospective studies offered information on breast cancer and vegetable/fruit consumption.[99;122;130] Cohort studies offer the advantage of collecting data before the onset of the disease and are less likely to be biased. A protective effect for high fruit and vegetable intake was detected in each of the mentioned studies, but this effect was not statistically significant.

A large heterogeneity due to mode and quality of dietary assessment has to be considered as well. The studies mentioned in this review assessed dietary intakes at a range of

different time periods (current diet, 1, 2, 5 years prior to the interview). A high correlation, however, exists between recalled past diet and current diet[145], partly because recalled diet is heavily influenced by current diet and partly because diet tends to remain relatively constant over time. Heterogeneity due to the various time frames it is not likely to account for the overall heterogeneity.

Food Frequency Questionnaires (F.F.Q.) were used by all the studies included in this meta-analysis. This kind of questionnaire is characterised by several inaccuracies.[142] In order to improve data quality, two criteria are important to be evaluated: Validity and Reliability. Most of the included studies presented information on Reproducibility and/or Validity. Only two studies[96 103] provided very limited information on the dietary assessment instrument. Furthermore they used reduced questionnaires, which might not be able to detect variability in food intake within the study population. However, when these two studies were eliminated from the analysis, the conclusions remained the same.

A comparison between Mediterranean countries and other European countries, with respect to cancer mortality rates and food availability patterns, suggests that low consumption of animal fat and high consumption of fruits and vegetables may contribute to a low incidence of coronary heart disease and several forms of cancer. Several studies[105-107] in the Mediterranean area indicated that vegetable intake or related dietary factors may protect against breast cancer.

I would wexpect that the beneficial effects of vegetables and fruit consumption were higher in populations who had lower baseline vegetables intakes, but in the heterogeneity analysis, high vegetables consumption seems to have a comparable protective effect in geographical areas where a “Mediterranean diet“ is prevalent (Italy, Greece and Spain) compared to “non-Mediterranean areas“ (USA, Argentina, Japan, Sweden, Denmark, Poland, Switzerland and Canada). Looking at the few papers that presented the values of the cut-off

points considered for the frequencies of vegetables consumption, it was not found a difference in the two groups: highest and lowest cut off points are similar (7 times per week vs 0-2 per week), but obviously the quantities could be different.

In mediterranean countries it was observed that breast cancer rates are usually relatively low compared with most other Western countries. The reason for this pattern has not been clearly understood. A "Mediterranean diet" is characterised by a high intake of vegetables and fruits in parallel with a high consumption of total fat,[146] a low intake of animal fats and a higher consumption of olive oil compared with non-Mediterranean countries. Olive oil is high in monounsaturated fats and relatively low in saturated fats, as well as linoleic acid, and contains a relatively high amount of antioxidants, including alpha-tocopherol. Furthermore, a diet rich in monounsaturated fats presumably yields tissue structures that are less susceptible to oxidative damage than would be the case in high polyunsaturated diets.[147] For this reason a protective effect of vegetables and fruit consumption on breast cancer could be confounded by factors biologically independent of their content in beta-carotene or vitamin C.

Many studies on diet and breast cancer risk investigated the relevance of a high fat intake as a risk factor for breast cancer, but the results are not consistent.[145] The food pattern may be of special interest as high fat or energy intake is often associated with a low consumption of fruits and vegetables. Therefore, it is possible that a high fruit and vegetable consumption simply implies a lower intake of fat. An adjustment for energy-intake to address the issue of diet composition rather than absolute intake can be conceived.[91;92;122;125-127;130] However, there seems to be no particular influence on the estimated relative risks due to energy adjustment.

The confounding issue is particularly a problem in the epidemiology of weak associations; the majority of breast cancer risk factors being rather weak with estimates not

exceeding 2. At this level, confounding by some yet unrecognised factors cannot be ruled out. Specific dietary habits might reflect education and consequently socio-economic status, which in turn, may be closely related to reproductive history. Adjustment for parity did not significantly explained between-study heterogeneity (p=0.33), however, when I looked at the pooled estimates of vegetables consumption in the two subgroups (adjusted vs not adjusted), I could verify that RRs not adjusted for parity indicate a significant protective effect (RR=0.68; 95% C.I.: 0.61; 0.76) wereas the pooled estimate from RRs adjusted for parity was not significant (RR=0.79; 95% C.I.: 0.55; 1.14). These findings are only indicative because the number of studies was too small to have enough power to test the relevance of all possible confounding factors.

Meta-analysis has to date been applied mainly to the results of randomised trials therapy. Although examples of meta-analysis directed at risk factors for disease exist there is no general agreement on whether studies with heterogeneous results should be combined, how differences in study quality should be taken into account, and whether studies with heterogeneous design carried out in different countries should be combined. However, in a sensitivity-analysis, none of these considerations weakened the conclusion drawn from this meta-analysis. There might be a moderate protective effect for breast cancer due to a higher consumption of fruit and vegetable. A meta-analysis cannot replace a well-conducted study, but in combination with narrative and quantitative methods a meta-analysis can be a useful tool for preliminary investigation.

Finally, greater attention needs to be given to the problem of colinearity between antioxidants and the confounding effect caused by antioxidants. Confounding by antioxidant micronutrients is of particular concern, as intakes of these nutrients tend to be positively correlated. In addition, measurement of dietary intake does not in any way eliminate the possibility that some other factor in fruits and vegetables is responsible or that it simply

reflects evidence of a protective effect of vegetarianism for breast cancer. Either measure may be a marker for other substances. Dietary fibres are found in vegetables and fruits, but they also derive from legumes and unrefined grains. Among the studies considered for this review only two presented risk estimates that differentiate between fibres derived from fruits and vegetables and fibres from other sources.[92;131] Both investigators showed a significant protective effect for fibres derived from fruits and vegetable sources, but not for grain fibres. These results emphasise once more the possible role of fruits and vegetables as a food group in breast cancer prevention.

The exclusion of the pubblications[92;96;109] which presented analyses separately for menopausal status does not change the results because they obtained results consistent with this meta-analysis: significantly protective values for high vegetable consumption and beta-carotene intake, and not significantly protective effect for fruit consumption.

3.5 Tables

Table 3.1. List of studies with indication of which estimates were excluded from meta-analysis and reasons for exclusion.

Veg.	Fr.	Betac.	Vit. C	First Author	Year of pub.	N. of cases	N. of controls	Main reasons for exclusion
N.I.	N.I.	Exc.	Exc.	Ambrosone[88]	1995	336	413	No indep.
N.I.	N.I.	Exc.	N.I.	Cooper[89]	1989	451	451	No indep.
Exc.	Exc.	Exc.	Exc.	Freudenheim[92]	1996	297	311	Only pre-menop.
Exc.	Exc.	Exc.	Exc.	Hislop[97]	1990	398	398	Benign breast disease
Exc.	Exc.	Exc.	Exc.	Ingram[100]	1991	99	209	Not com. and no inf.
Exc.	Exc.	Exc.	Exc.	Iscovich[101]	1989	150	150	No inf., no var.
N.I.	N.I.	Exc.	Exc.	Jarvinen[102]	1997	88	4697*	No var.
N.I.	N.I.	Exc.	Exc.	Katsouyanni[104]	1988	120	120	No inf.
N.I.	N.I.	Exc.	N.I.	Lee[109]	1991	200	120	Only pre-menop.
N.I.	N.I.	Exc.	N.I.	Marubini[112]	1988	214	215	No inf.
N.I.	N.I.	Exc.	N.I.	Paganini-Hill[117]	1987	123	10473*	No inf.
N.I.	N.I.	Exc.	N.I.	Potischman[119]	1990	83	113	No inf.
Exc.	N.I.	N.I.	N.I.	Simard[124]	1990	68	343	No indep.
Exc.	Exc.	N.I.	N.I.	Toniolo[125]	1989	250	499	No var.
Exc.	Exc.	Exc.	N.I.	Van't Veer[127]	1990	133	238	No inf. and No comp.
N.I.	N.I.	Exc.	Exc.	Yuan[131]	1995	834	834	Continuous estimates and No comp.
N.I.	N.I.	Exc.	Exc.	Zaridze[128]	1991	139	139	No inf.
Exc.	N.I.	N.I.	N.I.	Zemla[129]	1984	328	585	No FFQ

N.I., No information to obtain the estimate; Exc., excluded from the meta-analysis;. No indep, Not independent from other studies; No inf, No information on cut-off points, No comp, Not comparable categories, No var, No estimate of variance; No FFQ, No Food Frequency Questionnaire; (*) Number refers to the cohort size

Table 3.2. List of studies included with indication of which estimates were included and excluded from meta-analysis and reasons for exclusion.

Veg	Fr.	Betac	Vit. C	First Author	Year of pub	Type of study	Country	N. of cases	N. of control	Main reasons for excl
Inc.	N.I	Exc.	N.I.	Ewertz [90]	1990	CC	Denmark	1474	1336	No inf.
Inc.	Inc	N.I.	N.I.	Franceschi[91]	1995	CC	Italy	2569	2588	
Inc.	N.I	N.I.	Exc.	Graham[93]	1982	CC	USA	2024	1463	No com.
N.I.	N.I	Inc.	Inc.	Graham[94]	1991	CC	USA	439	1463	
N.I.	N.I	Inc.	Inc.	Graham[95]	1992	Co	USA	344	18586*	
Inc.	Inc	N.I.	N.I.	Hirose[96]	1995	CC	Japan	1186	23163	
Inc.	N.I	N.I.	N.I.	Hislop[132]	1986	CC	Canada	846	862	
Inc.	Inc.	Inc.	N.I.	Holmberg[132]	1994	CC	Sweden	380	432	
Inc.	N.I	Inc.	Inc.	Hunter[99]	1993	Co	USA	1439	89494*	

Table 3.2b. List of studies with indication of which estimates were included and excluded from meta-analysis and reasons for exclusion.

Veg	Fr.	Betac	Vit. C	First Author	Year of pub	Type of study	Country	N. of cases	N. of control	Main reasons for excl.
Inc.	N.I.	N.I.	N.I.	Kato[103]	1992	CC	Japan	908	908	
Inc.	Inc.	N.I.	N.I.	Katsouyanni[105]	1986	CC	Greece	120	120	
N.I.	N.I.	Inc.	Inc.	Kushi[106]	1996	Co	USA	879	34387*	
Inc.	Inc.	N.I.	N.I.	Landa[107]	1994	CC	Spain	100	100	
Exc	Exc	Inc.	N.I.	La Vecchia[108]	1987	CC	Italy	1108	1281	No indep.
Inc.	Inc.	Exc.	N.I.	Levi[110]	1993	CC	Switzerland	107	318	No comp.
N.I.	N.I	Inc.	N.I.	London[111]	1992	CC	USA	402	403	
Inc.	Inc.	N.I.	N.I.	Negri[113]	1991	CC	Italy	2860	6147	
N.I.	N.I	Inc.	Inc.	Negri[114]	1996	CC	Italy	2569	2588	
Inc.	Inc.	Inc.	Inc.	Nunez[115]	1996	CC	Spain	139	136	
N.I.	Inc.	N.I.	N.I.	Nunez-Martin [116]	1995	CC	Spain	30	30	
Inc.	N.I.	N.I.	N.I.	Pawlega[118]	1992	CC	Poland	127	250	
Inc.	N.I	N.I.	Inc.	Qi[120]	1994	CC	China	244	244	
N.I.	Inc.	Exc.	N.I.	Richardson[121]	1991	CC	France	409	515	No comp.
N.I.	N.I.	Inc.	N.I.	Rohan[123]	1988	CC	Australia	451	451	
Inc.	Inc.	Inc.	Inc.	Rohan[122]	1993	Co	Canada	519	56837*	
Inc.	Inc.	N.I.	N.I.	Trichopoulou[126]	1995	CC	Greece	820	1548	
Inc.	Inc.	Exc.	Inc.	Verhoeven[130]	1997	Co	Netherlands	650	62573*	No comp.

Veg., Vegetables; Fr., Fruits; Vit. C., Vitamin C; Betac., Beta-carotene;
Year of pub., Year of publication; Exc., Estimates excluded from the meta-analysis;
Inc., Estimate included in the meta-analysis; N.I., No Information to obtain the estimate.
CC, Case-control study; Co, Cohort study; No indep., Not independent from other studies; No inf., No information on cut-off points; No comp., Not comparable categories; No var., No estimate of variance
(*) indicates that the number refers to the cohort size.

Table 3.3. Estimates from meta-analysis of the RR of getting breast cancer.

Food groups, micronut.	N. of studies	RR	Low 95%CI	Up 95%CI	Heterog.
Random effects model					
Vegetable	17	0.75	0.66	0.85	p<0.001
Fruit	12	0.94	0.79	1.11	p<0.001
Vitamin C	9	0.80	0.68	0.95	p=0.02
Fixed effects model					
Beta-carotene	11	0.82	0.76	0.91	p=0.51

Table 3.4. Dose-response estimates from meta-analysis.

Vegetables: Random effects model. Heterogeneity p<0.001				
Consumption/Intake	**N of studies**	**RR**	**Low 95% CI**	**Up 95% CI**
3 vs 1 portions/ week	16	0.91	0.89	0.93
4 vs 1 portions/ week	16	0.87	0.85	0.88
5 vs 1 portions/ week	16	0.83	0.81	0.84
6 vs 1 portions/ week	16	0.79	0.77	0.80
Fruits: Random effects model. Heterogeneity p<0.001				
3 vs 1 portions/ week	11	0.93	0.88	0.97
4 vs 1 portions/ week	11	0.89	0.85	0.93
5 vs 1 portions/ week	11	0.86	0.82	0.90
6 vs 1 portions/ week	11	0.83	0.79	0.87
Vitamin C: Random effects model. Heterogeneity p=0.013				
100 vs 50 mg/ day	9	0.96	0.90	1.03
200 vs 50 mg/ day	9	0.89	0.84	0.96
300 vs 50 mg/ day	9	0.83	0.78	0.89
400 vs 50 mg/ day	9	0.77	0.72	0.83
Beta-carotene: Fixed effects model. Heterogeneity p=0.77				
2000 vs 1000 μg/ day	11	0.98	0.97	0.99
3000 vs 1000 μg/ day	11	0.96	0.94	0.97
4000 vs 1000 μg/ day	11	0.93	0.92	0.95
5000 vs 1000 μg/ day	11	0.91	0.90	0.93

Table 3.5. Estimates from meta-analysis, for breast cancer risk and vegetable consumption for sub-groups of studies.

Possible heterogeneity factors	**N of studies**	**RR**	**Low CI**	**Up CI**	**Significance of factors**
<= 3 categories of consumpt.	7	0.76	0.63	0.91	p=0.98
> 3 categories of consumption	10	0.73	0.60	0.89	
Case-control studies	14	0.71	0.60	0.81	p=0.30
***Cohort studies**	3	0.86	0.73	1.01	
Validated questionnaire	6	0.85	0.71	1.01	p=0.09
Non-validated questionnaire	11	0.66	0.55	0.81	
Energy adjustment	5	0.73	0.64	0.83	p=0.56
No energy adjustment	12	0.72	0.61	0.84	
Adjustment for confounders	10	0.68	0.56	0.83	p=0.22
***No adjustm. for confounders**	7	0.86	0.77	0.97	
Mediterranean countries	6	0.67	0.54	0.87	p=0.48
Non Mediterranean countries	11	0.77	0.66	0.92	
<=50 items in FFQ	7	0.79	0.67	0.93	p=0.71
>50 items in FFQ	10	0.71	0.57	0.87	
Popul. controls (CC studies)	4	0.73	0.51	1.04	p=0.76
Hospital controls (CC studies)	10	0.69	0.57	0.82	

***Except for this estimate, Random effects models were used.

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Table 3.6. Estimates from meta-analysis, from random effects models, when all possible studies are included.

Food groups, and micronutrients	N of studies	RR	Low 95% CI	Up 95% CI
Vegetable	23	0.74	0.65	0.84
Fruit	18	0.91	0.79	1.05
Vitamin C	16	0.78	0.66	0.93
Beta-carotene	26	0.79	0.71	0.89

3.6 Figures

Figure 3.1. RR estimates and CI of breast cancer and vegetables

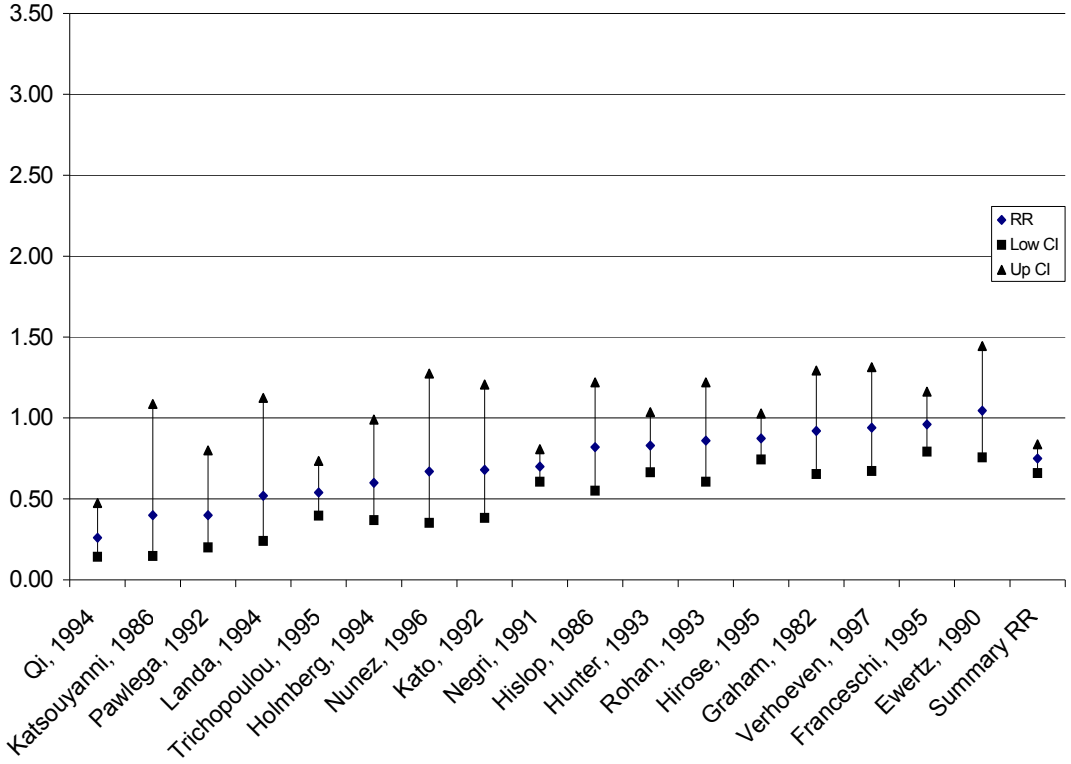


Figure 3.2. RR estimates and CI of breast cancer and fruit.

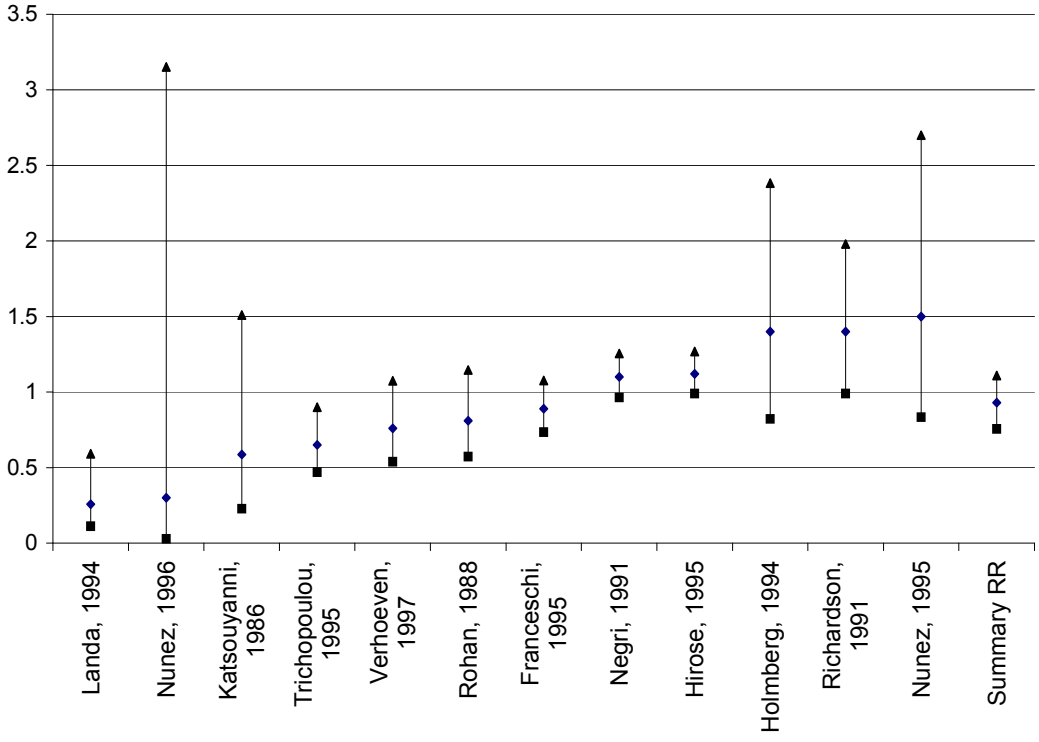


Figure 3.3. RR estimates and CI of breast cancer and Beta-carotene.

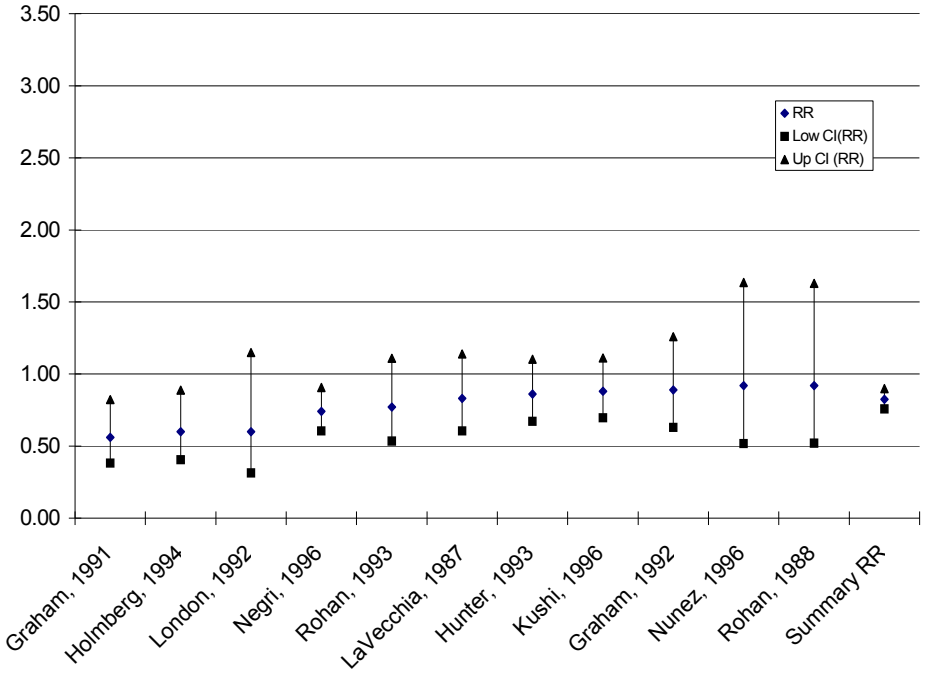
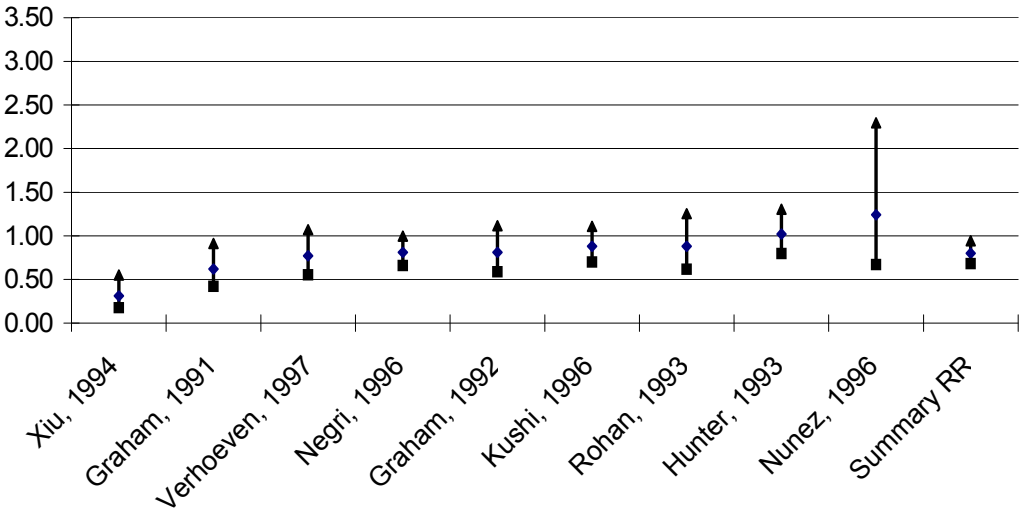


Figure 3.4. RR estimates and CI of breast cancer and Vitamin C.



Legend of Figures
RR, Relative Risk of "high consumption" compared to "low consumption" (for food groups representing "greater than 1 portion per day" versus less than 3-4 portions per week", about "400 milligrams per day or more" of vitamin C compared with "70 milligrams per day or less" and intake of "7000 micrograms per day or more" of carotene compared with "1100 micrograms per day or less"); CI, Confidence Intervals.
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CHAPTER 4. MELANOMA AND PIGMENTED LESIONS

4.1 Introduction

Skin cancer is the most common form of cancer, affecting nearly one million people each year in USA. Non-melanoma skin cancers, including basal cell (BCC) and squamous cell carcinomas (SCC), account for the vast majority of all new skin cancers. Cutaneous malignant melanoma is the least common form of skin cancer but the most serious and account for about three-quarters of all skin cancer deaths.[148]

The outcome of this systematic meta-analysis was histological confirmed cutaneous malignant melanoma (CMM), which is commonly divided into four histological types. These are superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma and acral lentiginous melanoma. The majority of melanomas (around 90%) are cutaneous lesions (superficial spreading and nodular melanomas). Melanomas also occur in the pigmented cells of the retina and on the mucous membranes of the nasopharyngeal sinuses, vulva, and anal canal. Mucosal melanoma and melanomas located on the palms, digits, soles, and nail beds (where acral lentiginous melanoma is found) are unique because they cannot be directly attributable to sun exposure and a different aetiology has to be invoked.[149;150] Acral lentiginous melanoma was not studied from an epidemiological point of view because it is very rare in white-skinned populations. Lentigo maligna melanoma, that is the invasive form of lentigo maligna, clinically resembles other pigmented lesions such as solar lentigo or a superficial malignant melanoma. The data related to lentigo are consistent with very heavy repeated exposures over many years.

Although cutaneous malignant melanoma is still a relatively rare neoplasm in many populations, incidence of cutaneous malignant melanoma was increasing world-wide in

Caucasian populations for several decades; between the early 1960s and the late 1980s annual increments of 3 to 7% were observed in 24 populations of mainly European origin,[151] making melanoma the most rapidly increasing cancer in white populations, except for lung cancer in women.[152] However, recent trends showing a deceleration or levelling off of the rate of increase in melanoma risk in cohort born after 1950 in some of those populations.[153-157] As a result of the increasing incidence, CMM is now one of the more common cancers in white populations. It ranks 4th in man and 3rd in women in high incidence areas such as Australia and New Zealand (non-Maoris) and about 6th in medium incidence areas like the U.S. (whites), Scandinavia and parts of Canada.[156] Another important point concerning disease burden is that in the U.S. it is the most common cancer in the 25-29 age group in females, and the second most common cancer (after breast cancer) in the 30-34 age group.[158]

Earlier detection, resulting mainly from increased awareness by health providers and the general public, had an impact, as seen in the higher rate of increase in localized tumours. However there is supporting evidence that the trends reflect real changes more than increased diagnosis: in the U.S. from 1974-1997, all stages increased by comparable amounts and a decrease in the incidence rates of thick lesions shortly after the increase in thin melanoma in U.S., Australia and Scotland was not observed.[153;156;159] Furthermore models taking into account a birth cohort effect proved to explain the observed trends more than those with a period effect.[160] The steadily increasing incidence in all countries over the past 40 years cannot be associated with the increased awareness resulting in attention in melanomas that may have started to develop 10-20 years previously. Most important, mortality increased for decades in most fair-skinned populations,[159] although there is evidence that CMM mortality rates are levelling or beginning to fall in recent generations in the U.S., Australia, Canada, Scandinavian countries and U.K.[161]

Melanoma is very rare in black people and we know very little about risk factors for cutaneous melanoma in more pigmented ethnic groups and in Asians. Epidemiological studies summarized by this meta-analysis concern Caucasian populations. Among people who are not black, melanocytic naevi (both common acquired and dysplastic or atypical) were identified as the most important phenotypic risk factor and there was recent activity directed at a better epidemiological understanding of this topic.[150;152;162-165]

Benign naevi (moles) are non-malignant aggregations of melanocytes. Melanocyte density varies with different anatomical sites of the skin, increasing in density from trunk to extremities, head, and foreskin. In white populations, benign naevi are uncommon in the newborn, and increase during childhood and particularly after adolescence, reaching a peak in early adulthood; the prevalence falls thereafter.

Because two-thirds to three-quarters of patients with melanomas report previous lesions and 25-50% have histological confirmation of an associated nevus, at least some naevi are probably precursors of melanoma.[166] Hence, the study of acquired melanocytic naevi may tell us a great deal about the origins of malignant melanoma. A fairly large body of evidence suggests that the number of melanocytic naevi represents the best predictor for cutaneous malignant melanoma and that large atypical naevi may play an independent role.[167-173] Risk of melanoma rises with increasing number and clinical atypia of naevi, from small risk in those with few small non dysplastic naevi, to higher risks with larger non dysplastic, to very high risk in those with multiple clinically dysplastic naevi.[174;175]

Atypical naevi, present in 2 to 5% of Caucasian adults, are usually larger with a more variegated appearance, in comparison to ordinary naevi. The term “atypical nevus” is frequently used clinically raising the suspicion of naevi likely to be hiding underlying dysplasia from benign congenital or acquired naevi, whereas dysplasia is a pathologic diagnosis.

Sun exposure is the principal environmental risk factor implicated in naevi development and in melanoma occurrence.[172;176] Number of naevi seems to be higher in those with high propensity to burn and light skin colour. Solar ultraviolet radiation may be important at two stages, in the production of benign acquired naevi, and in the transformation or promotion of such naevi into malignant melanoma.[177] The biological correlates of such associations are still poorly understood, but it is plausible that factors that affect the density of melanocytic naevi may also influence the development of malignant melanoma later in life.

4.2 Materials and methods

4.2.1 Data sources and search strategy

Two bibliographic databases were screened until September 30, 2002:

- the online MEDLINE (National Library of Medicine, Bethesda, MD, USA (http://www.nlm.nih.gov/) using PUBMED (http://www.nlm.nih.gov/PubMed/) as system of querying from January 1966.
- Elsevier Science online EMBASE (http://www.ovid.com/) from January 1988.

No language restrictions were applied. The MEDLINE search was conducted using the following KEY-WORDS for titles and abstracts: nevi*, naevi*, nevo*, naevo*, nevu*, naevu*, mole*, pigmented lesion*, skin lesion*, cutaneous lesion*, melanocytic lesion*, in combination with melanoma and case control*, case-control*, cohort*, cross section*, cross-section*, follow up*, follow-up*, followup*, prospective.

A possible search strategy on Text Word in PUBMED cound be: (nevi*[Text Word] OR naevi*[Text Word] OR nevo*[Text Word] OR naevo*[Text Word] OR nevu*[Text Word] OR naevu*[Text Word] OR mole*[Text Word] OR moles*[Text Word] OR pigmented lesion*[Text Word] OR skin lesion*[Text Word] OR cutaneous lesion*[Text Word] OR melanoc* lesion*[Text Word]) AND (melanoma*[Text Word]) AND (case control*[Text

Word] OR case-control*[Text Word] OR cohort*[Text Word] OR cross section*[Text Word] OR cross-section*[Text Word] OR follow up*[Text Word] OR follow-up*[Text Word] OR followup*[Text Word] OR prospective*[Text Word]) NOT (uveal*[Text Word] OR ocular*[Text Word] OR animal*[Mesh]) AND ("1966"[PDat] : "2002"[PDat])

Similar search strategies were used with the following MESH TERMS: nevus, moles associated with melanoma and case-control studies, cohort studies, cross-sectional studies, and prospective studies. The final search strategy was carried out combining the search strategy for "text word" and "mesh terms". Similar search strategies were used to search EMBASE. The search was limited to human studies only.

Other sources were the references of the retrieved articles and preceding reviews on the topic. All the retrieved references were entered into the bibliography management software Reference Manager 9,[178] to facilitate the search for duplicate references.

4.2.2 Selection of studies

Primary inclusion criteria were developed for the selection of all relevant articles: case-control, cohort or cross-sectional studies published as an original article. Ecological studies, case reports, reviews, editorials, were not considered eligible. On the basis of primary inclusion criteria the initial relevance of all retrieved articles was evaluated on the basis of title and abstract.

As a second step, some inclusion criteria were identified, to obtain a subset of studies, with at least minimal information and comparable results. The following inclusion criteria were used:

1. The studies have to provide sufficient information to estimate the relative risk and 95% confidence intervals (i.e. they have to publish the Odds Ratios or Relative Risks or crude data and standard errors, variance, confidence intervals or p-value of the

significance of the estimates). An estimate and its variance are required to calculate a weighted pooled estimate of the RR.

2. The studies have to be independent in order to avoid giving double weight to some studies.
3. For the naevi counts the results reported have to be comparable. For this reason naevi counts must not be restricted only to the presence of large naevi, which have probably a special aetiology. Congenital naevi have not been considered in this meta-analysis because presence of large congenital naevi is associated to a very high risk of melanoma and those patients already need to be watched over with particular care, whereas there are many anamnestic difficulties to find small congenital naevi.[20;179]
4. The populations studied should be homogeneous, at least for the main risk factor for melanoma. Studies could not include only cases of plantar foot, soles and vulva, because a distinct aetiology for non sun-exposed sites was suggested.[180] Studies conducted exclusively on infant melanoma were excluded because they are not many and melanoma on infants is very rare.[181] Furthermore very often infant melanoma may be indicators of a different pathology, as for example children with pigmentous Xeroderma,[182] or completely different risk factors, mainly genetic.[181]

4.2.3 Extraction and unification of the data

A questionnaire was developed to collect some information about each study:

- General information: year of publication, study design, study location, latitude of the region, mean age of study population;
- Exposure information: definition of common naevi used, body district where the naevi were counted, number and profession of observers, categorization;

- Case information: inclusion or exclusion of specific histological types of melanoma, inclusion of cases with family history of melanoma, number and source of cases, participation rates of cases, percentages of fair skinned people in cases and in controls;
- Case-control study information: source controls, matching design, blinding of case status, blinding of interviewers, response rates of controls;
- Follow-up information**:** source study population, years of follow-up, blinding on exposure status and completeness of follow-up;
- Statistical information: statistical methods used, adjusting for confounding variables (demographic factors, such as age and sex, baseline host characteristics such as hair, eye and skin colour and inherent tendency to burn or tan easily, atypical moles, sun exposure…), type of effect estimates (odds ratio, relative risk, standardized incidence ratio) with corresponding measures of precision, according the specific exposure category.

Preparation of the data of the original studies for the meta-analysis was carried out according to a two-step procedure.

First, since the number of common naevi was given by a range, to each class was assigned the number of naevi corresponding to the midpoint of the range. Highest categories of naevi count are often open, therefore, a value for the maximum number of naevi had to be specified in order to calculate numeric assignments to the categories. When no information about distribution of common naevi was available a fix value of 120 was set as the maximum value for the open-end categories. This choice was done investigating the distributions of naevi, looking at lower and upper limits for naevi density categories, in all included studies, and corresponding rough variation of number of controls and ORs. The analysis is not straightforward because the number of categories published varies from 2 to 6. It was seen

that the percentages of controls in classes with more than 100 naevi are very low (from 2% to 7%). The studies with three categories, where the mean lowest limit for the highest category is 53, present a mean percentage of controls in the upper categories of 18. The studies which consider four categories, and mean lowest limit for the highest category is 87, show that the mean percentage of controls in the upper category decreased to 8; the two studies that published six categories, and the mean lowest limit for the highest category is 110, the percentages of controls in the upper categories is only 4.5. In total eight studies consider 100 as lowest limit for the upper category. Thus an upper limit for the highest categories of 120 was considered as a reasonable value because it includes all possible situations. Effect of this assignment on this estimate was evaluated in the sensitivity analysis.

For the upper categories of atypical naevi it was assigned the same amplitude as the preceding category, because the risk estimate is more sensitive to changes of small numbers of atypical naevi. A dichotomous categorization has also been evaluated.

Secondly, the distinction among various measures of relative risk (e.g. odds ratio, rate ratio, risk ratio) was ignored assuming that melanoma is a rare disease. Consequently, every measure of association, adjusted for maximum number of confounding variables concerning each level of naevi count, and the corresponding confidence interval, was translated into log relative risk and corresponding variance with the formula proposed by Greenland.[133] When estimates are not published, they will be calculated from crude data. To obtain the standard error of the log odd ratio from crude data, Woolfe's formula was implemented. For Standardized Incidence Rates (SIR) the number of cases can be used to estimate the standard error of the log(SIR). If only the p-value is published then a "test-based" estimate is considered.[133]

MacKie[183] reported adjusted odds ratio separated for males and females and they were considered as two independent estimates.

Tucker[172], Rodenas[184] and Grob[170] published odds ratios for common naevi with diameter smaller and greater of 5 mm separately; the first estimate was included in the meta-analysis.

Bain[185] showed two estimates of risk for palpable and total self-reported nevus count; first one was considered for the meta-analysis.

Marrett[186] used a self administered whole-body diagram to assess nevus density with qualitative indications and the four categories "none", "few", "moderate" and "many" were transposed into the following numerical categories: "0", "1-24", "25-49" and "50+", respectively.

As for the meta-analysis on vegetables and fruit, it was decided to choose results from population controls if data from case-control studies were presented separately for hospital and population controls. As was said previously, patients who are hospitalised even for other diseases may be unrepresentative for the exposure distribution in the source population.[136] However we have to take into account that population-based study requires full coverage of cases occurring in the population being studied and this is not always assured. Furtheremore sometimes in population-based studies there is low rate of response among controls. In these cases hospital-based estimates have to be preferred.

In this meta-analysis two papers presented a single RR calculated considering hospital together with population controls, thus there was not need to make a choice.

4.2.4 Data analysis strategy

The data thus obtained were used for the statistical analysis performed with a six-step procedure. Details of statistical methods are reported in Chapter 2.

In the first-step, a linear model was fitted, within each study, to estimate the relative risk, per one nevus of increase. The model was fitted according to the method proposed by

Greenland and Longnecker, which provides an estimator of the natural logarithm of RR, and of its standard error, requiring the estimates and the number of subjects at each category of naevi count. This dose-response model takes into account that the estimates for separate naevi categories depend on the same reference group. When number of subjects at each category of naevi count was not available from the papers, coefficients were calculated ignoring the correlation between the estimates of risk in the separate exposure levels.

In the second step, the summarized RR was estimated pooling the study-specific estimates by the classical fixed effects and random effects models.[70;133] The homogeneity of the effect across studies is assessed using the large sample test based on the Chi-squared statistic.[70] In the third step a further analysis was carried out estimating pooled RR for common and atypical together, through the bivariate approach proposed by Houweling.[29] Covariance between common and atypical naevi risk estimates was not available, and independence was assumed in the model. An estimate of the covariance was obtained from the model. Log(RR) was fitted with PROC MIXED.[74]

In the fourth step, possible sources of heterogeneity were investigated; sub-groups analyses and analysis of variance models were carried out to investigate between-study heterogeneity. Factors that were investigated are: publication year, country of origin, latitude, type of study design, matching design, sources of cases and controls, family history of cases, adjustment for confounding, type of naevi categorization, self count of moles. Main effects and interactions between the factors were explored by analysis of variance model. SAS Proc GLM was used to fit the random effect models on the log(RR).[74]

In the fifth step, sensitivity analysis was carried out to evaluate if violations of the inclusions criteria, variation in assignments for mid points and upper limits, or changes on subgroups heterogeneity analysis may affect the results. The influence of single papers was also assessed.

Finally, in the sixth step, hypothesis that publication bias might affect the validity of the estimates was tested by a funnel-plot-based approach using the adjusted rank correlation method (Begg's method)[48] and linear regression analysis (Egger's method)[46]. "Trim and fill" and Copas and Shi[52] methods were applied. Estimates of the likely number of missing studies and of the adjusted RRs, calculated by imputing suspected missing studies, are provided. However, the adjusted results are not intended to give "better" estimates, but they can be used as a form of sensitivity analysis for estimating the likely impact of publication bias in the meta-analysis.

4.3 Results

4.3.1 Literature search and selection

Five hundred and twenty-six articles were identified from MEDLINE, one hundred and forty from EMBASE (with seventy-six references found in both databases). After elimination of duplicates, I obtained five hundred and ninety studies. On the basis of title and keywords, references were evaluated using primary inclusion criteria. Of those, fifty-five articles were identified as potential for meta-analysis; other thirteen articles were identified checking the reference list for a total of sixty-eight articles. Of those forty-six were identified as fulfilling the secondary inclusion criteria, for a total of forty-seven datasets.

4.3.2.Study characteristics

An overview of the forty-seven datasets included in the selected groups is given in Tables 4.1. These forty-seven datasets included a total of 10499 cases and 14256 controls. Among the thirty-eight datasets dealing with common naevi, twenty-six presented the risk estimates for the whole body nevus count and seventeen for naevi count on arms. Twenty-seven datasets published the risk estimates for atypical naevi. Twenty-four studies were carried out in

European countries, fourteen in North America, seven in Australia and one in Argentina. I finally arrived at eight cohort studies, all dealing with atypical naevi, thirty-seven case-control studies and two nested case-control studies.

Seventeen case-control studies used controls were population based, seven of them had cases from hospitals and controls drawn from the population. Eleven studies had both cases and controls from hospitals, five wrote cases from population (registries) and controls from hospitals. Among the studies with cases drawn from hospitals, two used controls drawn from visitors to the hospitals and one controls from neighbourhood. Two case-control studies used both, population and hospital-based controls. For one study information on source of cases and controls was not available. (Table 4.1)

Of the thirty-eight datasets dealing with common naevi, nine presented estimates of risk based on self-assessment of the naevi count, while for all the twenty-seven datasets on atypical naevi assessment of naevi count was performed by physicians.

4.3.3 Pooled RR estimates

Relative risk estimates, and corresponding 95% confidence intervals, for the increase of one common nevus on whole body and arms, are presented in Figures 4.1 and 4.2, respectively. Relative risks for one atypical nevus are presented separately for case-control studies and cohort studies in Figures 4.3a and 4.3b, respectively.

Fixed-effect model is quite unreasonable as it implies that only sampling error is associated with differences among estimates from studies with identical measured characteristics. Actually Chi-squared estimates, which evaluate between-study heterogeneity, are all significant (Chi=181.970, d.f.=25, $p<0.001$, for common naevi on whole body; Chi=111.738, d.f.=16, $p<0.001$, for common naevi on arms; Chi=390.148, d.f.=27, $p<0.001$, for atypical naevi). This is an indication that homogeneity assumption, at the base of the fixed

effects model, is probably not correct. Random effects models were performed for common naevi on whole body, common naevi on arms and atypical naevi, to take into account the variation among studies.

Pooled Relative Risks (RR) and Confidence Intervals (C.I.), calculated from dose–response models, for common naevi (whole body and arms) and atypical naevi are presented in Table 4.2 and Table 4.3. Statistically significant associations were found between naevi (common and atypical) count and melanoma. Summary estimates for common naevi, counted on whole body, indicate a significant risk for melanoma even for a medium-low density of naevi, indicated by the category "16 to 40" naevi compared to "0 to 15" naevi (pooled RR=1.47; 95% C.I.: 1.36, 1.59). The increase in the number of naevi is clearly associated with an increase in risk. People with very high naevi density ("100+" naevi) present a highly significant risk, almost 7 times greater (pooled RR=6.89; 95% C.I.: 4.63, 10.25) than people with very few naevi ("0-15" naevi).

The count on a district (arms) confirms the association between common naevi and melanoma. Risk for people with more than 10 common naevi on arms is almost five times greater than risk for people with no naevi on arm (pooled RR=6.82; 95% C.I.: 3.05, 7.62).

Atypical naevi count confirms to be a highly significant risk factor for melanoma. Presence of any atypical nevus increased the risk of 10 times compared to absence of atypical naevi (RR=10.12; 95% C.I.: 5.04, 20.32). Summary relative risks for having only one atypical nevus are already considerable (RR=1.60; 95% C.I.: 1.38; 1.85), rising up to 10.49 (95% C.I.: 5.05; 21.76) for 5 atypical naevi (Table 4.3).

To make a more reliable comparison between the two types of naevi, a further analysis was conducted on the thirty-eight studies that published both estimates only, on both common and atypical naevi. This analysis gives us an indication of a substantial correlation (R=0.36), suggesting that common and atypical naevi are not independent. I obtained similar

results to the ones obtained in the univariate analysis: pooled estimate for the increase of one atypical nevus (RR=1.515 and 95% C.I.: 1.372, 1.674) is much higher ($p<0.001$) than that for the increase of one common nevus (RR=1.016 and 95% C.I.: 1.012, 1.021).

4.3.4 Heterogeneity analysis

Studies included in this work vary in a number of aspects of their design and analysis. As previously stated, several factors that may have induced differences in outcomes were investigated with sub-group analyses and analysis of variance models. RR estimates, for one common (on whole body) and atypical nevus, by sub-group factors are shown in Table 4.4 and Table 4.5. One study[187] did not publish much information on the study design and was not included in several sub-groups examined for heterogeneity analysis.

Within-subgroup heterogeneity remains highly significant for all subgroups identified by all possible factors (data not shown).

Heterogeneity may be investigated in several ways. If we look at the Chi-squared that evaluates any differences among groups (it compares pooled estimates of each subgroup with the overall pooled estimate)[133] it can be observed that all, but few factors, explain between-subgroup heterogeneity. Among studies considering common naevi in all body, only "dichotomization of exposure" and "adjustment for chronic sun" do not explain any between-study variability (Chi-squared $p=0.502$ and $p=0.918$, respectively). In publications analysing atypical naevi, "adjustment for acute sun exposure" and "adjustment for chronic sun exposure" do not seem to play a significant role (Chi-squared $p=0.19$ and $p=0.715$, respectively). (Data not shown).

If we investigate between-study heterogeneity by meta-regression, we can notice that only source of cases is an important factor that significantly affects the estimates for common naevi on arms. Studies with cases drawn from hospitals presented estimates lower than the

ones from studies with cases drawn from population (See Figure 4.4). Pooled estimate, for the increase of one nevus in arms, for the former (RR=1.08, 95% C.I.: 1.036; 1.125) is significantly lower (p=0.05, Table 4.4) than the estimate for the latter (RR=1.172, 95% C.I.: 1.117; 1.229). If I consider studies with cases drawn from hospitals and controls drawn from population the pooled estimate is even lower (RR=1.07, 95% C.I.: 1.02; 1.122).

Similar results are obtained analysing studies on atypical naevi (Table 4.5 and Figure 4.5): when controls are drawn from hospitals the pooled estimate, for one nevus of increase, is significantly (p=0.02) lower (RR=1.424, 95% C.I.: 1.306; 1.553) than the pooled estimate of studies with controls drawn from population (RR=1.64, 95% C.I.: 1.227; 2.192) or other sources (RR=1.625, 95% C.I.: 1.166; 2.264). If I consider the six studies with both, cases and controls drawn from hospitals, the pooled RR is even lower (RR=1.312, 95% C.I.: 1.253; 1.375).

Type of study is an important factor ($p<0.001$) explaining a lot of between-study variability on atypical naevi (Table 4.5). Actually Figure 4.6 shows that RRs, for one atypical nevus, in case-control studies are much lower and more precise than in cohort studies.

Very likely type of study is related to the type of categorization used for the estimates, because cohort studies used dichotomous categories to evaluate whether atypical naevi are present. Actually type of categorization is a significant factor for atypical naevi.

Thirteen out of twenty-eight studies, which investigate the association between atypical naevi and cutaneous melanoma, published the results for a dichotomous exposure, in terms of presence or absence of atypical nevus (Figure 4.7). The pooled estimate (RR=2.86; 95% C.I.: 2.05, 3.99) that evaluates the risk for the presence of any atypical nevus (dichotomous categorisation) is significantly (p=0.010) higher than in studies that consider more categories for naevi count (RR=1.60; 95% C.I.: 1.38, 1.85) (See Table 4.5). A great difference was also found when I considered only studies that used dichotomization

(presence/absence) of atypical naevi (Table 4.6). Cohort studies showed a huge relative risk for the presence of any number of atypical naevi (RR=39.20; 95% C.I.: 23.61; 65.08) whereas, if we consider only case-control studies, the pooled RR for the presence of atypical naevi is much lower, even if it is still quite high and statistically significant (RR=4.54; 95% C.I.: 2.65, 7.80) (Table 4.6).

When only case-control studies are considered, we can observe a considerable reduction also in the risk estimates from dose-response models (Table 4.7). Actually the RR for the increase of five atypical naevi (RR=6.36 95%; C.I.: 3.80, 10.33) is twice lower than the RR calculated considering all types of studies together (RR=10.48, 95% C.I.: 5.05; 21.76).

Cohort studies did not publish estimates for common naevi and it was not possible to investigate this aspect as with atypical naevi. Some study features were investigated only on case-control studies, because it was not possible to extract much information from the papers on the cohort studies.

Likelihood ratio test indicates that few two-factor interactions are statistically significant, in the subgroup of case-control studies analysing atypical naevi, but I was dealing with very sparse and zeros in the tables. Testing for interactions here has low power.

4.3.5 Sensitivity analysis

Number of naevi increases until the age of 30-50 and then it decreases, thus age was considered the most important confounding variable for the aetiology of melanoma. The estimates included in the analyses were adjusted for age or come from study with matching for age, except for Dabkowski.[188] Excluding the latter, the pooled estimate for the increase of one common nevus (RR=1.02; 95% C.I.: 1.01; 1.02) and one atypical nevus (RR=1.99; 95% C.I.: 1.72, 2.29) do not significantly change.

The choice of an upper limit for the highest category is necessary to obtain a mean value for the highest category in the dose-response analysis. The decision to assign a value of 120 common naevi to the upper category with open end, for count on whole body, was investigated. Pooled random effect estimates, obtained assigning alternative upper limits for the open-end categories, are shown in Table 7. As can be seen, pooled estimates are sensitive to changes in assignments. There is a clear decreasing trend in RR estimates with increasing numbers for the upper category. Furthermore if we estimate the RR for the upper category, "100+" naevi, considering only the studies that present as lower limit a value greater than 100 we obtain a lower estimate (RR=5.73; 95% C.I.: 3.61; 9.10). Thus we should be careful in the interpretation of the estimates for the highest categories because, at it was seen previously, very few subjects belong to the upper categories and in this case the RRs are not very stable.[189]

Impact of inclusion criteria was analysed. Three studies were excluded for different reasons not related to dependence from other studies: Cockburn[190] was non considered because only the risk for large nevus (larger than a pencil eraser) was estimated, while Green[191] and Rolon[192] were not included because only acral melanomas were considered in their studies. The pooled random effects estimate for the increase of one common nevus does not change appreciably (the RR without Green was RR=1.02; 95% C.I.: 1.01, 1.02) when Green[191] was included in the analysis (RR=1.02; 95% C.I.: 1.01, 1.02). Only a slight difference was observed in the RR, for the increase of one common nevus on arms, when Rolon[192] was included in the analysis (RR=1.13 with 95% C.I.: 1.09, 1.17; and RR=1.12 with 95% C.I.: 1.08; 1.16; with and without Rolon[192], respectively). If we consider large naevi (larger than a pencil eraser), defined in the Cockburn paper[190] as atypical naevi and we included in the analysis the estimate published for dyzygous twins, a very slight decrease was

observed (RR=1.95; 95% C.I.: 1.70; 2.23; whereas the overall estimate is RR=1.96 with 95% C.I.: 1.71; 2.26 for each atypical nevus).

Following considerations by some authors,[193;194] the method of assessment of naevi would be an important aspect in study design, to be considered for the inclusion criteria. Actually self-assessment of the number of melanocytic naevi is difficult to perform accurately, and people severely underestimate the actual number. Although, the pooled RR, for common naevi on whole body (RR=1.020; 95% C.I.: 1.015, 1.025), from the studies (n=5) with self assessment of naevi count, was found very similar to the estimate obtained from studies (n=20) with assessment of naevi count by physician (RR=1.018; 95% C.I.: 1.013, 1.023). For naevi count on arms, similar results were found. The pooled estimate from the studies (n=4) with self-assessment (RR=1.081; 95% C.I.: 1.023; 1.143) is not significantly different from the pooled RR from the studies (n=13) with assessment by physician (RR=1.144; 95% C.I.: 1.098, 1.193)

4.3.6 Publication bias

Investigation of publication bias, for common naevi counted in whole body, gives us some indications that probably some studies with no significant results were not published. Rank correlation analysis (Begg's method[48]) of the funnel plot (Figure 4.8), indicates that smaller studies tend to report greater relative risk than greater studies (p=0.008). Similarly linear regression analysis (Egger's method[46]) also indicates a general trend towards asymmetry of the funnel plot (p=0.004). The "Trim and fill" analysis suggested that the number of missing studies may be five and their inclusion would lead to a slightly lower pooled estimate (RR=1.016; 95% C.I.: 1.012, 020).

Exploration among studies on atypical naevi (Figure 4.9) also shows that smaller studies tend to report greater relative risk than in general (p=0.019). Similarly linear

regression analysis (Egger's method) indicates a trend towards asymmetry of the funnel plot ($p<0.001$). Using the "Trim and fill" analysis, four studies were identified in order to achieve symmetry of funnel plot. When the analysis was restricted to case-control studies no missing studies were identified.

Lastly, no asymmetry on the funnel plot (Figure 4.10) were observed for common naevi counted on arms with Begg's method ($p=0.387$) and linear regression analysis on the funnel plot (Egger's method) ($p=0.241$).

Using sensitivity analysis for publication bias proposed by Copas and Shi[52] a strong positive trend in the funnel plot was found only for common naevi in whole body. Figure 4.11 shows the relative risks from the included studies plotted against a measure of the uncertainty (standard error of the relative risk) in that relative risk. This uncertainty decreases as the size of the study increases, so that large studies are on the left of the plot and small studies on the right. The plot shows a trend for smaller studies to give more positive results than the larger studies. The basic idea of this method is that there should be no relation between study outcome and study size; the relation that we observe is simply an artefact of the process of selecting these studies. As the number of unpublished studies increases the estimates of the relative risk fall sharply.

As was seen with Egger and Begg methods, for common naevi counted in arms there is no big evidence for the presence of publication bias. For atypical naevi Copas and Shi method give an indication for a continuous estimate less than 2 as reasonably consistent with the data. For example, with a RR=1.54 (95% C.I.: 1.29; 1.84) I get a p-value for the asymmetry of the funnel plot equal to 0.09.

4.4 Discussion

One of the main problems with studies on naevi is to ensure valid counts. Naevi counts, published in even the most detailed studies, cannot be confirmed using biopsies. In 1990, the International Agency for Research on Cancer proposed a detailed protocol to standardize methodologies in nevus studies. However, even with a greater degree of standardization, problems arise in the inter-observer variation: up to about 10% of the variation in full body counts by different counters may be due to this.[177] In our analysis, self-assessment of the number of common melanocytic naevi does seem to have significantly affected the estimates. The pooled estimate from the studies with self-assessment of naevi count was found very similar to the estimate obtained from studies with assessment of naevi count by physicians. Moreover as long as the error rates in counting are similar in different phenotype or sun exposure groups, this will not be a source of error in determining the aetiology of naevi. For atypical naevi none of the studies presented estimates obtained with self-assessment because trained personnel is needed to define clinically atypical naevi.

In heterogeneity analysis it was seen that studies with controls are drawn from hospitals presented lower estimates than studies with controls drawn from population. These works probably published the most reliable estimates because usually assessment of naevi was much more precise in hospital-based studies. Population-based studies used weak and over-simplified measures of nevus count, such as self-assessment by the subjects or a very limited examination, and overall the data may be deficient of the details given through skilled examination. Actually in cohort studies, broad naevi classifications, as “presence/absence”, are used largely, whereas cases-control studies presented estimates for more complex categorizations of naevi counts. This may be one of the reasons for the differences found in the estimates, classified by type of study. RRs extracted from cohort studies are much higher than ORs published in case-control studies. However there may be another important factor that could have influenced the results. The populations of the two types of studies are

probably particularly different. Several characteristics were analysed and it was noted that mean age in case-control studies and in cohort studies is significantly ($p<0.001$) different: 50.9 and 34.9, respectively (fifteen case-control studies and seven cohort studies published information on age of subjects). Cohort studies include much younger subjects and this disparity may play a special role for large naevi. Actually, for the aetiology of melanoma and naevi, age was considered the most important confounding variable and the number of large naevi at younger ages is the main predictor of melanoma occurrence.

Usually studies carried out in clinical situations, using short interviews by busy medical doctors, are usually of less value also because they include patients who are hospitalised for some diseases and, as I said previously, they may be unrepresentative for the exposure distribution in the source population.[136] However in regard to the assessment of naevi, in studies with controls drawn hospitals information is likely to be much more consistent and unbiased. Thus the lower RR found, including only case-control studies with controls drawn from hospitals, may be more reliable than the RR calculated considering population-based studies. Actually even if hospital-based case-control studies use control groups drawn from several disease categories, the large majority exclude other skin diseases and other cancers.

Some of the host factors, such as pigmentation, are clearly genetic. Others, such as the number of naevi, density of freckles and skin type, are considered a combination of genetic factors and the influences of sun exposure. In many epidemiological studies nevus density was consistently correlated with pigmentary traits, similar to those associated with malignant melanoma, and with intense sun exposure and history of sunburns.[195-198]

Persons with many benign naevi have more cutaneous “naevomelanocytes” and therefore have more total melanocytes at risk of undergoing malignant transformation than people with few nevi. Numerous moles might also indicates a greater genetic tendency to

form melanoma. Recent progress in the genetics of melanoma has led to the identification of two melanoma susceptibility genes: the tumour suppressor gene CDKN2A and the CDK4 gene. CDKN2A mutations have been detected in at most 50% of melanoma-prone families that have been examined in Europe, North America, and Australia and CDK4 mutations have been described in only three families. Therefore, other genes remain to be identified. These highly penetrant genes can explain the high incidence of melanoma in rare families while the familial clustering of few melanoma cases might result from low penetrant susceptibility genes and/or shared environmental exposures. To assess the patterns of familial aggregation of three melanoma risk factors (great number of naevi, light phototype and high degree of sun exposure) a recent study was carried out on 66 French families with at least two melanoma cases.[199] It was found that melanoma may not only result from specific genetic and environmental determinants but also from those underlying melanoma-associated phenotypes with complex gene-gene and gene-environment interactions. Furthermore the majority of data suggest that dysplastic naevi are independent risk factors for melanoma. Presence of dysplastic naevi does not appear to co-segregate with mutation in CDKN2A or CDK4.[200]

In the heterogeneity analysis of this work, adjustment for sunlight indicators and other phenotypic factors do not seem to play an important role in explaining variability in the estimates. However relationship among naevi, sun exposure and phenotypic factors is certainly quite complex. Actually individuals who are prone to burning (red hair, dense freckling, very sensitive skin) may avoid sun exposure and develop fewer naevi than might be expected.[177] Moreover it was suggested that the relation between sun exposure and melanocytic naevi might have parabolic dose-response curve.[201]

Autier and co-authors in a very recent publication on naevi[202] suggest that UVB doses would represent the principal environmental determinant of the progression from small to larger naevi. However they found a total absence of influence of sunburns and of difference

in latitude on small naevi, suggesting that biological events giving rise to small naevi could be linked to a component of sunlight other than UVB doses.

A very recent study suggests an interesting new hypothesis on sun exposure and naevi, based on a "divergent pathway" model for melanoma occurring in different body sites.[203] They found that melanomas on the head and neck were more likely to arise in people with few naevi, many solar keratoses, and who presented high levels of occupational sun exposure. In contrast, melanomas of the same histological type arising on the trunk tended to occur among people with many naevi, few solar keratoses, and lower levels of occupational sun exposure. They suggest that after initiation by sunlight, melanocytes of nevus-prone individuals are induced to proliferate and become neoplastic with little (if any) further requirement for sun exposure. In contrast, people with a low tendency to develop naevi require ongoing exposure to sunlight to drive the development of melanoma, beyond that required for initiation. Among these people, melanomas will tend to be on sun exposed body sites and will be associated with chronic sun exposure. This work stimulates further analyses in this field to look more deeply into risk factors for different body sites.

4.5 Appendix: Reasons for exclusion

One study[190] was not considered because the authors estimated the risk only for large nevus. Two papers[191;192] were not included because considered only acral melanomas. Nineteen articles were excluded because they were not independent from other studies.

The paper by Nordlung[204] was not included and the one by Roush[205] was preferred because the latter used unconditional logistic regression to compute the odds ratio and it eliminated cases and controls with family history of melanoma.

Augustsson[206] and Stierner[207] were not included and the one by Augustsson[208] was preferred because the latter used total body naevi counts, whereas the former presented

estimates for naevi counts calculated separately for body areas. For the same reason between Garbe (1994)[165] and Rieger[209] and between Garbe (1989)[210] and Kruger[211] the ones by Garbe were preferred.

The 204 cases and 200 controls analysed by Weiss[212] in 1990 were included in the multicenter case-controls study with 1079 cases and 778 controls presented by Weiss[213] in 1991.

Between Dubin's paper, published in 1986,[164] and Dubin's paper, published in 1990,[214] the last was analysed because it excluded patients with non-melanoma skin cancer from controls.

A paper by Bataille[173] was not included because it presented a comparison study of two case-control studies of melanoma in Australia and England using the same mole-counting protocol that examiners had previously reported in 1996.[215]

Rodenas published two papers in 1996[184] and in the 1997[216] on the same collective of subjects. Out of the two, the 1996 one was chosen, where common naevi are better described, by categorizing separately common naevi with diameter 2-5 mm, common naevi with diameter greater than 5 mm and clinically atypical naevi.

The 106 cases analysed by Carli in 1995[217] and in 1996[218] were included in a following case-controls study published in 1999[219] with 131 cases and 176 controls.

Weinstock (1989)[220] and Bain (1988)[185] reported the same nested case-control study with self-reported nevus count. The second one was chosen because it published the results also for raised self-reported nevus count.

Between Westerdahl's[221] and Masback's[222] papers the former was analysed because it presented crude and adjusted odds ratios, while the latter presented a stratified analysis by several other factors.

Zaridze published in Vopr.Onkol[223]and the Int.J.Cancer[224] results concerning the same case-control study. The first was chosen because it is in an English version.

Osterlind (1990)[225] and Green (1985)[167] published the same estimates of odds ratio presented in Osterlind (1988)[226] and Green (1985)[167] respectively, with similar description of the same study. The choice between them was considered indifferent.

Landi's study published in 2002[227] was not included because it analyses a subset of patients reported in 2001.[228]

Schneider (1994)[229]and Moore (1997)[230] analysed the same population of employees of the Lawrence Livermore National laboratory. The second was chosen because it reported the results for a greater number of cases, 69 versus 9.

Rigel published in 1988[231] was not included and the one published in 1989[232] was preferred because in the estimates of risk it used only invasive melanomas, while the first paper reported two-thirds of *in situ* lesions of the prospectively diagnosed melanomas.

Rationales behind the selection of a study, between two dependent papers, are summarised in Table 4.9.

4.6 Tables

Table 4.1a. Characteristics of the studies on pigmented lesions.

First author	Year pub	Country	Study design	N° cases	N° control	Case source	Control source	Common naevi		Atypical naevi
								Body	Arms	
Augustsson [208]	1991	Sweden	CC	121	378	Pop	Pop	Yes	-	Yes
Autier[233]	1994	Belgium, France, Germany	CC	420	447	Hosp	Neigh.	-	Yes	-
Bain [185]	1988	USA	N CC	98	190	Pop	Pop	-	Yes	-
Bataille[215]	1996	England	CC	426	416	Pop	Hosp	Yes	-	Yes
Beral [162]	1983	Australia	CC	287	574	Hosp	Pop	Yes	-	-
Carli [219]	1999	Italy	CC	131	176	Hosp	Pop	Yes	-	Yes
Chen [234]	1996	USA)	CC	548	494	Pop	Pop	-	Yes	-
Cristofolini[235]	1987	Italy	CC	103	205	Hosp	Hosp	Yes	-	Yes
Dabkowski[188]	1997	Poland	CC	74	300	Hosp	Pop	Yes	-	Yes
Dubin[214]	1990	USA	CC	289	527	Hosp	Hosp	Yes	-	-
Elwood[236]	1986	England	CC	83	83	Pop	Hosp	Yes	Yes	-
Elwood[237]	1990	England	CC	195	195	Pop	Hosp	-	Yes	-
Garbe [165]	1994	Germany; Austria; Switzer.	CC	513	498	Hosp	Hosp	Yes	-	Yes
Garbe [210]	1989	Germany	CC	200	200	Hosp	Hosp	Yes	-	Yes
Green[167]	1985	Australia	CC	183	183	Pop	Pop	-	Yes(1)	-
Grob [170]	1990	France	CC	207	295	Hosp	Pop	Yes	Yes(1)	Yes
Grulich[171]	1996	Australia	CC	242	276	Hosp	Pop	Yes	Yes	Yes
Halpern[238]	1991	USA	CC	105	181	Hosp	Pop	Yes	-	Yes
Halpern[239]	1993	USA	Co	2	89	-	-	-	-	Yes
Holly[169]	1987	USA	CC	121	139	Hosp	Hosp	Yes	-	Yes
Holman [150]	1984	Australia	CC	511	511	Pop	Pop	-	Yes	-
Kang[240]	1994	USA	Co	2	155	-	-	-	-	Yes
Kelly[241]	1997	Australia	Co	20	278	-	-	-	-	Yes
Landi [228]	2001	Italy	CC	183	179	Hosp	Pop+ Hosp	Yes(2)	-	Yes
Loria [242]	2001	Argentina	CC	101	249	Hosp	Hosp	Yes	Yes	-
MacKie [183] **(wom.)**	1989	Scotland	CC	181	181	Pop	Hosp	Yes	-	Yes
MacKie [183] **(men)**	1989	Scotland	CC	99	99	Pop	Hosp	Yes	-	Yes
MacKie [175]	1993	Scotland	Co	5	116	-	-	-	-	Yes
Marghoob[243]	1994	USA	Co	-	124	-	-	-	-	Yes
Marrett [186]	1992	Canada	CC	583	608	Pop	Pop	Yes	-	-
Mastrangelo [244]	2000	Italy	CC	99	104	Hosp	Pop	-	Yes	-
Moore [230]	1997	USA	N CC	69	69	Pop	Pop	Yes	-	Yes
Naldi[245]	2000	Italy	CC	542	538	Hosp	Hosp	Yes	-	-
Osterlind[226]	1988	Denmark	CC	474	926	Pop	Pop	-	Yes	-
Rhodes	1980	USA	CC	138	217	N.A	N.A	-	-	Yes
Rigel [232]	1989	USA	Co	1	281	-	-	-	-	Yes

Table 4.1b. Characteristics of the studies on pigmented lesions.

First author	Year pub	Country	Study design	N° cases	N° control	Case source	Control source	Common naevi		Atypical naevi
								Body	Arms	
Rodenas [184]	1996	Spain	CC	105	138	Hosp	Visitors to the hosp	Yes	-	Yes
Roush [205]	1988	Australia	CC	246	134	Hosp	Pop+Hosp	Yes	-	Yes
Snels [246]	1999	Holland	Co	3	166	-	-	-	-	Yes
Sorahan[247]	1985	England	CC	58	182	Hosp	Hosp	-	Yes(1)	-
Swerdlow[168]	1986	Scotland	CC	180	197	Hosp	Hosp	Yes	Yes	-
Tiersten [248]	1991	USA	Co	4	157	-	-	-	-	Yes
Tucker[172]	1997	USA	CC	716	1014	Hosp	Hosp	Yes	-	Yes
Weiss [213]	1991	Germany	CC	1079	778	Hosp	Hosp	Yes	-	Yes
Westerdahl[221]	1995	Sweden	CC	400	640	Pop	Pop	-	Yes(1)	-
White [249]	1994	USA	CC	256	273	Pop	Pop	-	Yes	-
Zaridze [224]	1992	Russia	CC	96	96	Hosp	Visitors to the hosp	-	Yes	-

N.A.=not available; Pop=population; Hosp=Hospital; Neig=Neighbourhood; CC=Case Control study; Co=Cohort study; N CC=Nested Case-control; (1) Only one arm; (2) Only back

Table 4.2. Estimates from meta-analysis for common naevi.

Whole body			
N° nevi	**RR**	**Low 95% CI**	**Up 95% CI**
0-15	1.00		
16-40	1.47	1.36	1.59
41-60	2.24	1.90	2.64
61-80	3.26	2.55	4.15
81-100	4.74	3.44	6.53
101+	6.89	4.63	10.25
Arms			
N° nevi	**RR**	**Low 95% CI**	**Up 95% CI**
0	1.00		
1-5	1.44	1.29	1.60
5-10	2.48	1.90	3.23
11+	4.82	3.05	7.62

For whole body N° of studies = 26 Heterogeneity Chi = 181.970 $p < 0.001$
For arms N° of studies = 17 Heterogeneity Chi = 111.738 $p < 0.001$

Table 4.3. Estimates from meta-analysis for atypical naevi.

Nevi	N.	RR	Low 95% CI	Up 95% CI	Heterog Chi	p-value
Absent	13	1.00			85.340	<0.001
Present		10.12	5.04	20.32		
0	15	1.00			221.876	<0.001
1		1.60	1.38	1.85		
2		2.56	1.91	3.43		
3		4.10	2.64	6.35		
4		6.55	3.65	11.75		
5		10.49	5.05	21.76		

Table 4.4. Sub-groups analysis for common naevi.

	Common naevi (whole body)					Common naevi (arms)				
Variables	**N° of studies**	**RR**	**Low 95% CI**	**Up 95% CI**	**P-value**	**N° of studies**	**RR**	**Low 95% CI**	**Up 95% CI**	**P-value**
Country										
Australia	3	1.013	1.005	1.022		3	1.147	1.065	1.235	
North America	6	1.016	1.010	1.022		3	1.117	1.041	1.198	
North Europe	6	1.027	1.013	1.041		6	1.178	1.084	1.281	
Mediterr. Europe	7	1.017	1.008	1.027		3	1.045	0.993	1.101	
Central Europe	4	1.022	1.017	1.028	0.594	2	1.146	0.999	1.315	0.485
Public. year										
83-89	9	1.023	1.012	1.034		7	1.168	1.107	1.232	
90-01	17	1.018	1.013	1.022	0.383	2	1.100	1.051	1.152	0.163
Matching										
Individ. matching	9	1.026	1.018	1.035		9	1.153	1.096	1.214	
Freq. Matching	8	1.017	1.013	1.021		7	1.109	1.051	1.17	
No matching	8	1.012	1.005	1.019	0.103	1	1.078	1.006	1.154	0.602
Source of Cases										
Hospital	19	1.019	1.015	1.024		8	1.08	1.036	1.125	
Population	7	1.018	1.010	1.025	0.738	9	1.172	1.117	1.229	0.052
Source of Controls										
Hospital	14	1.022	1.016	1.028		5	1.125	1.049	1.207	
Population	8	1.018	1.011	1.026		10	1.143	1.086	1.202	
Other	4	1.011	1.004	1.018	0.259	2	1.08	1.055	1.106	0.726
Dichotomous exp.										
No	16	1.018	1.013	1.023		14	1.146	1.095	1.199	
Yes	10	1.021	1.014	1.028	0.485	3	1.039	1.011	1.068	0.095
Self count of moles										
No	20	1.018	1.013	1.023		13	1.144	1.098	1.193	
Yes	5	1.020	1.015	1.025	0.434	4	1.081	1.023	1.143	0.277
Adjusted for phenotype charact.										
No	12	1.016	1.011	1.022		6	1.082	1.021	1.147	
Yes	14	1.021	1.015	1.027	0.355	11	1.155	1.105	1.207	0.145
Adjusted for chronic sun exposure										
No	22	0.015	0.013	0.016		14	0.278	0.222	0.306	
Yes	4	0.015	0.012	0.018	0.918	5	0.265	0.244	0.344	0.716
Adjusted for acute sun exposure										
No	17	1.017	1.012	1.022		8	1.157	1.100	1.218	
Yes	9	1.023	1.015	1.031	0.254	9	1.103	1.049	1.158	0.258
Adjusted for atypical naevi										
No	15	1.022	1.016	1.028						
Yes	11	1.015	1.010	1.020	0.229					

p-values: for Significance of factor; RR, Relative risk estimates for one common nevus

Table 4.5. Sub-groups analysis for atypical naevi.

Variables	N° of studies	RR	Low 95% CI	Up 95% CI	P-value
Type of study					
Case-control	20	1.56	1.41	1.72	
Cohort	8	4.35	2.82	6.69	<0.001
Dichotomous exp.					
No	15	1.60	1.38	1.85	
Yes	13	2.86	2.05	3.99	0.01
Country					
Australia	3	1.77	1.14	2.76	
North America	10	2.52	1.94	3.26	
North Europe	6	2.09	1.58	2.76	
Mediterr. Europe	5	1.72	1.37	2.15	
Central Europe	4	1.44	1.24	1.69	0.45
Publication year					
80-89	8	1.76	1.42	2.18	
90-94	10	2.63	1.85	3.76	
96-01	10	1.69	1.38	2.07	0.22
Matching	Case-control studies only				
Individ. matching	5	1.40	1.18	1.65	
Freq. matching	6	1.47	1.28	1.70	
No matching	7	1.74	1.45	2.08	0.302
Source of Cases					
Hospital	14	1.52	1.37	1.69	
Population	5	1.51	1.18	1.92	0.179
Source of Controls					
Hospital	9	1.42	1.31	1.55	
Population	6	1.64	1.23	2.19	
Other	3	1.63	1.17	2.26	0.023
Family history of melanoma					
No	6	1.75	1.39	2.20	
Yes	13	1.46	1.31	1.62	0.265
Adjusted for phenotype charact.					
No	8	1.59	1.36	1.86	
Yes	11	1.46	1.31	1.62	0.517
Adjusted for chronic sun exposure					
No	14	1.55	1.37	1.76	
Yes	5	1.43	1.21	1.67	0.716
Adjusted for acute sun exposure					
No	11	1.59	1.36	1.85	
Yes	8	1.42	1.29	1.56	0.494
Adjusted for common nevi					
No	8	1.509	1.216	1.872	
Yes	11	1.513	1.362	1.681	0.830

P-values: for Significance of factor; RR, Relative risk estimates for one atypical nevus

Table 4.6. Estimates from studies that used dichotomization of atypical naevi number, by type of study.

	N of studies	Naevi	RR	Low 95% CI	Up 95% CI	Heterogeneity Chi- squared	p-value for Chi
Only case-control	7	Absent	1.00				
		Present	4.54	2.65	7.80	19.766	0.003
Only cohort studies	6	Absent	1.00				
		Present	39.20	23.61	65.08	4.81	0.440

Table 4.7. Estimates from meta-analysis for atypical naevi from case-control studies.

N° naevi	RR	Low 95% CI	Up 95% CI
0	1.00		
1	1.45	1.31	1.60
2	2.10	1.71	2.54
3	3.03	2.23	4.06
4	4.39	2.91	6.47
5	6.36	3.80	10.33

N° of studies = 13 Heterogeneity Chi = 64.694 $p < 0.001$

Table 4.8. Estimates from meta-analysis for common naevi on whole body. Sensitivity analysis.

Last category was calculated assigning 100 to the upper value			
N° naevi	**RR**	**Low 95% CI**	**Up 95% CI**
0-15	1.00		
16-40	1.56	1.42	1.72
41-60	2.55	2.09	3.11
61-80	3.95	2.95	5.27
81-100	6.10	4.16	8.94
101+	13.08	7.59	22.53
Last category was calculated assigning 150 to the upper value			
N° naevi	**RR**	**Low 95% CI**	**Up 95% CI**
0-15	1.00		
16-40	1.40	1.31	1.50
41-60	2.02	1.76	2.33
61-80	2.81	2.28	3.46
81-100	3.90	2.96	5.13
101+	6.92	4.69	10.21

Table 4.9. List of studies excluded with reasons for exclusion.

Articles	Main reasons for exclusion
Augustsson, 1991 [206]	Not independent from Augustsson ,1991 [208]
Bataille, 1998 [173]	Not independent from Bataille, 1996 [215]
Carli, 1995 [217]	Not independent from Carli, 1999[219]
Carli, 1996 [218]	Not independent from Carli,1999[219]
Dubin, 1990[214]	Not independent from [164]
Green, 1986 [250]	Not independent from Green, 1985[167]
Green, 1999 [191]	Only acral melanoma
Kruger, 1992[211]	Not independent from Garbe, 1989[210]
Masback, 1999 [222]	Not independent from Westerdahl, 1995[221]
Osterlind, 1990[225]	Not independent from Osterlind, 1988[226]
Rieger, 1995[209]	Not independent from Garbe, 1994[165]
Rodenas, 1997[216]	Not independent from Rodenas, 1996[184]
Rolon, 1997[192]	Only acral melanoma
Nordlung, 1985[204]	Not independent from Roush, 1988[205]
Stierner, 1992[207]	Not independent from Augustsson, 1991[208]
Weinstock, 1989[220]	Not independent from Bain, 1988[185]
Weiss, 1991[213]	Not independent from Weiss, 1990[212]
Zaridze, 1992[223]	Not independent from Zaridze, 1992[224]
Landi, 2002[227]	Not independent from Landi, 2001[228]
Schneider, 1994[229]	Not independent from Moore, 1997[230]
Cockburn, 2001[190]	Estimates of risk only for large naevi
Rigel, 1988[231]	Not independent from Rigel, 1989[232]

4.7 Figures

Figure 4.1. RR and CI for the increase of one common nevus on whole body.

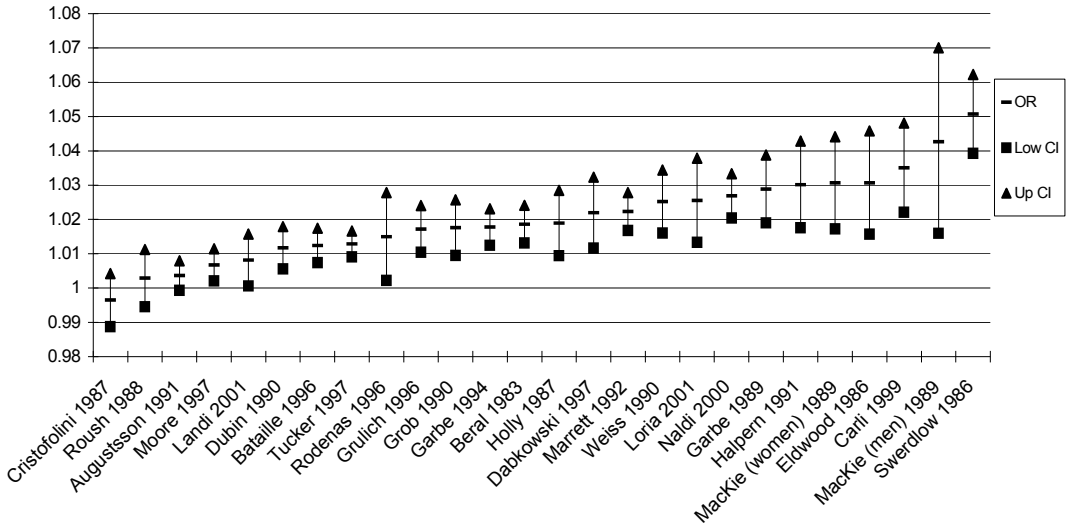


Figure 4.2. RR and CI for the increase of one common nevus on arms.

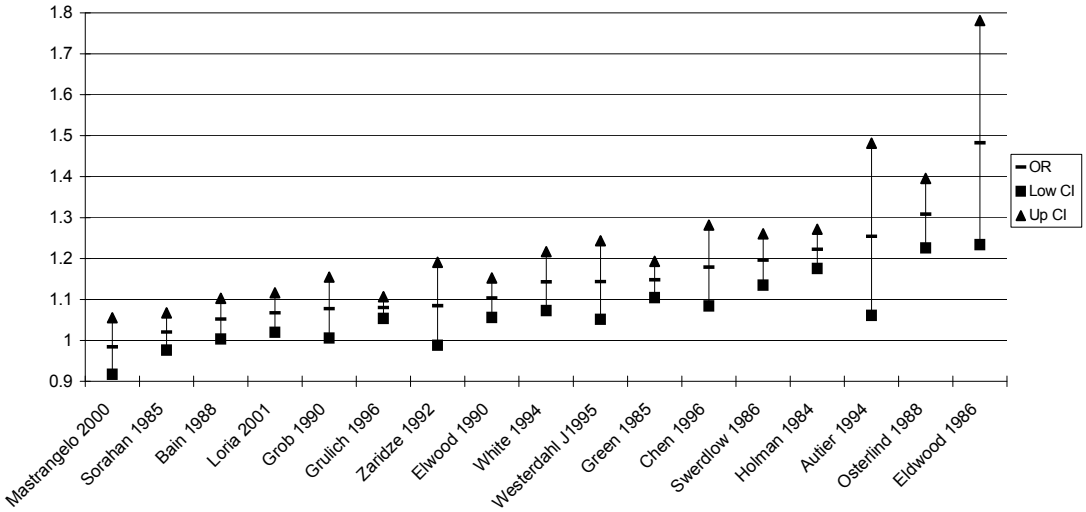


Figure 4.3a. RR and CI for the increase of one atypical nevus in case-control studies.

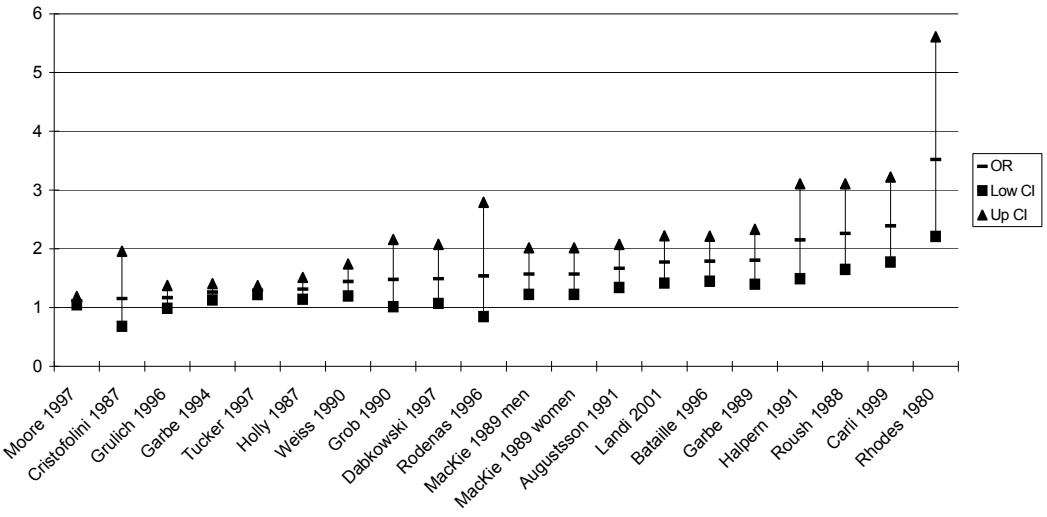


Figure 4.3b. RR and CI for the increase of one atypical nevus in cohort studies.

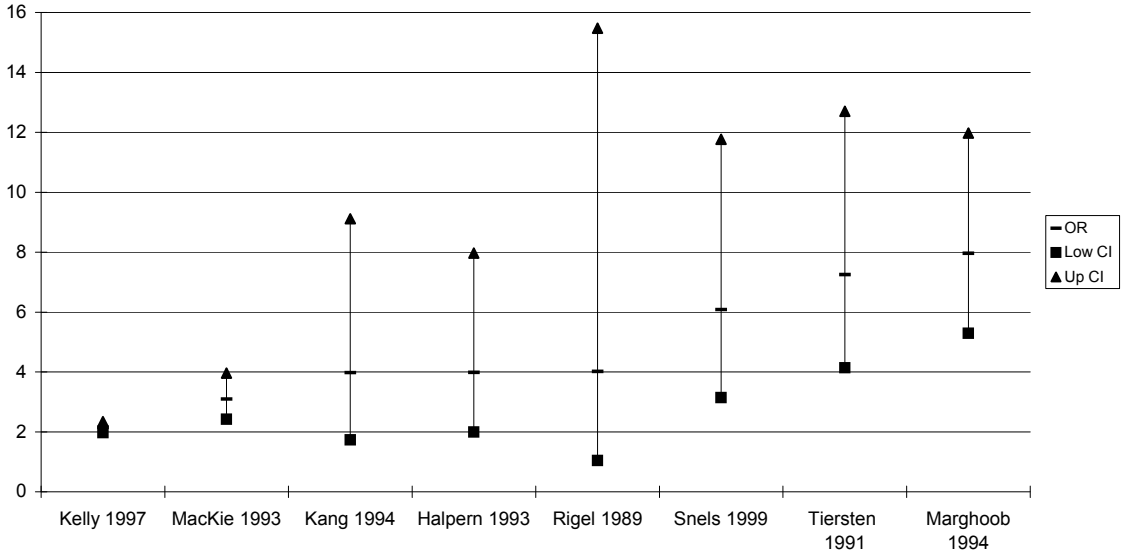
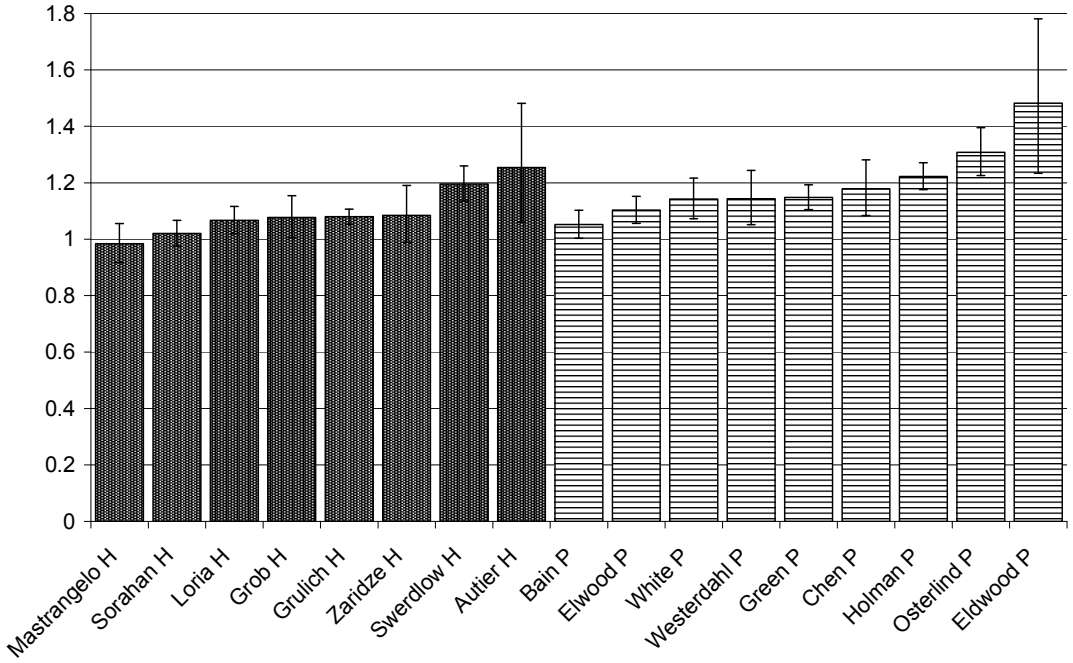
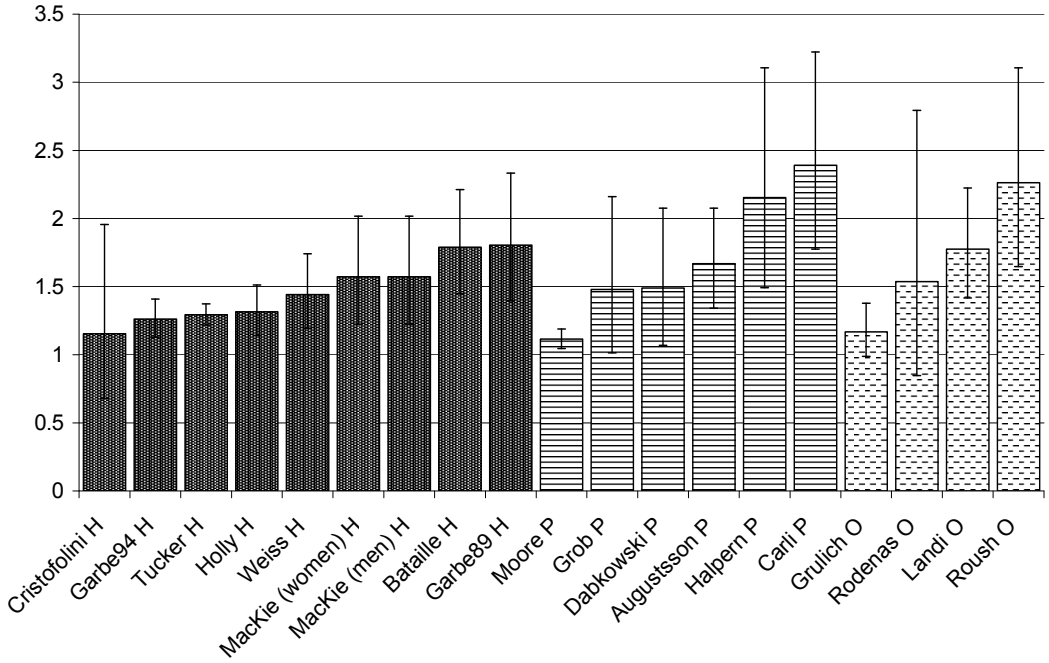


Figure 4.4. RR and CI for one common nevus on arms by source of cases.



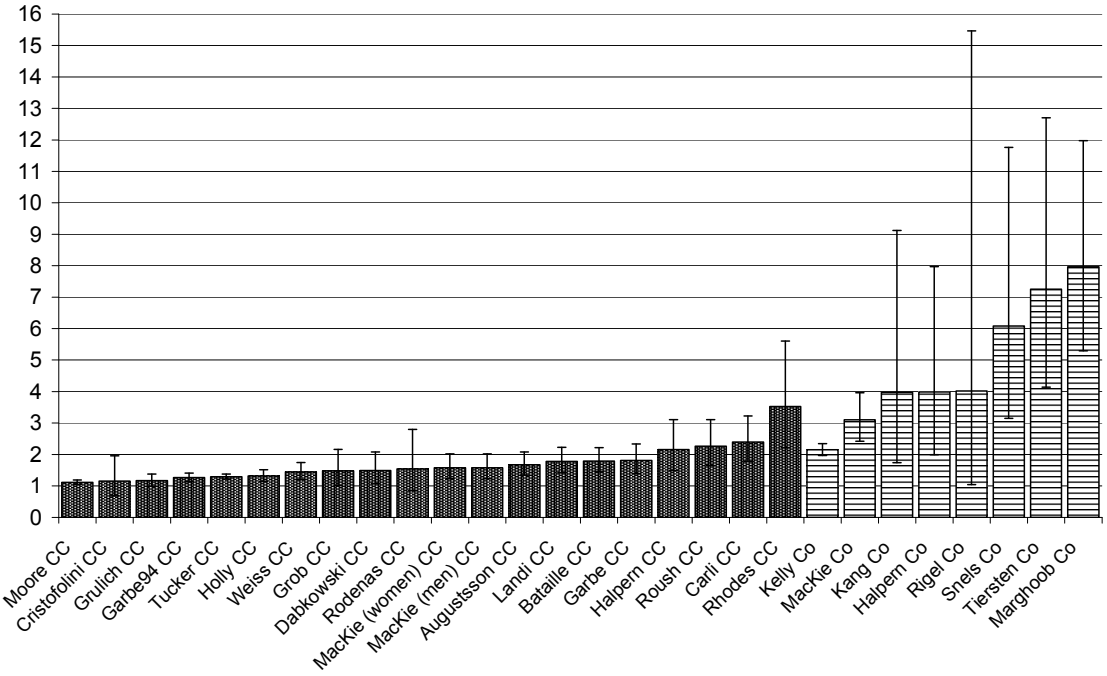
Studies with cases drawn from hospitals are indicated with dark bars, drawn from population with grey bars.

Figure 4.5. RR and CI for one atypical nevus by source of controls.



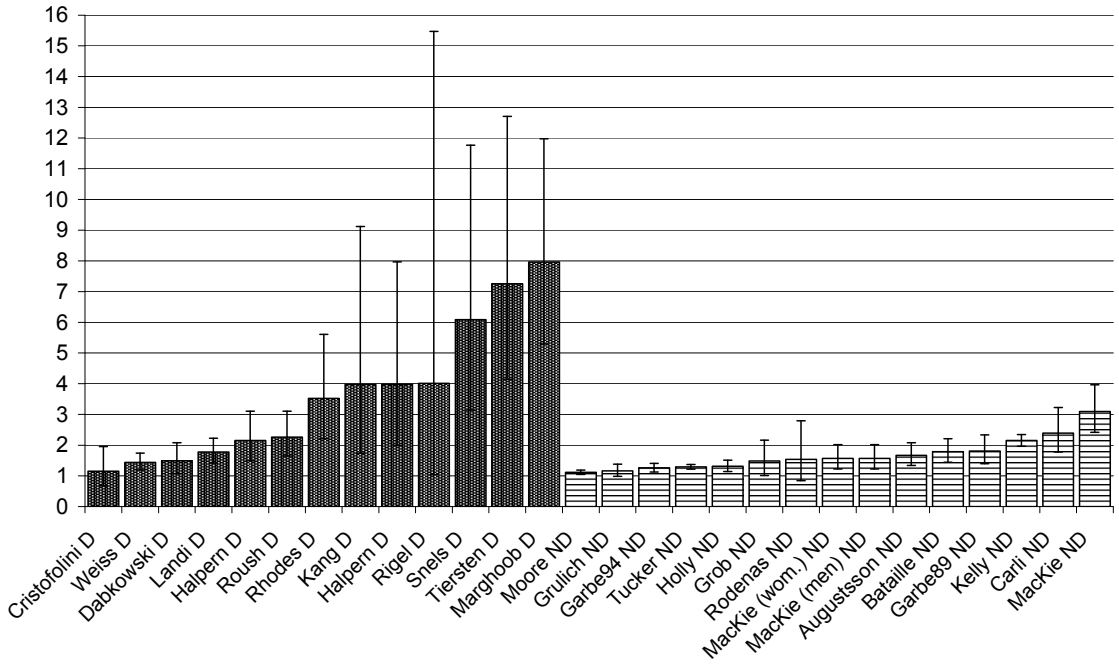
Hospital base studies are indicated with dark bars, Population–based studies with grey bars and Other sources for controls with dashed lines.

Figure 4.6. RR and CI for one atypical nevus by type of study.



Case-Control studies are indicated with dark bars and Cohorts with grey bars.

Figure 4.7. RR and CI for one atypical nevus by type of categorization.



Estimates on naevi categorized in a Dichotomous variable are indicated with dark bars and in a variable Not Dichotomous with grey bars.

Figure 4.8. Funnel plot for common naevi on whole body.

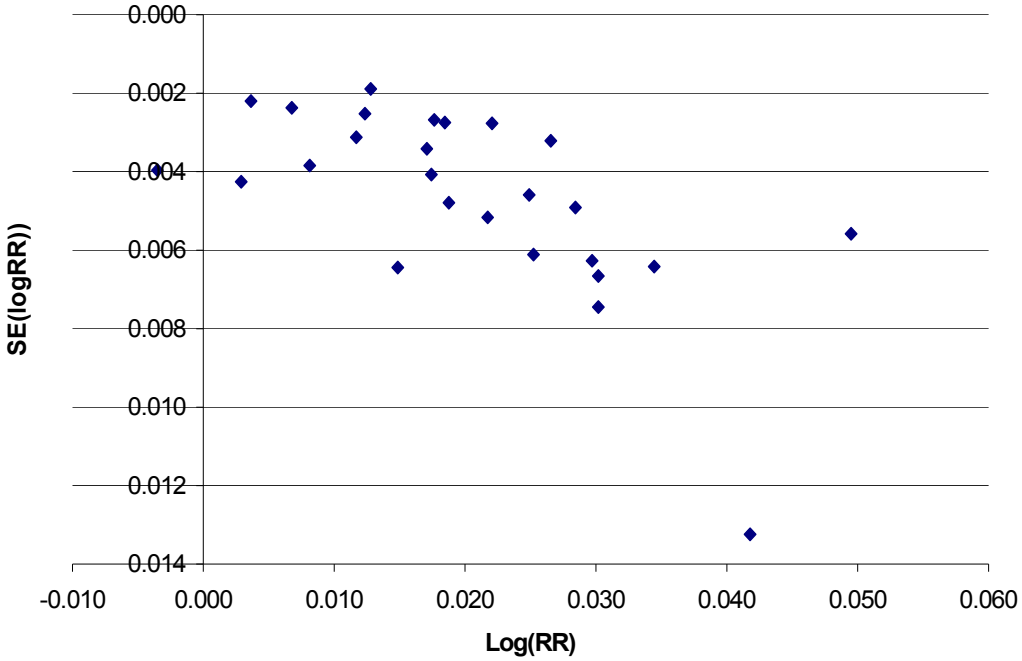


Figure 4.9. Funnel plot for atypical naevi.

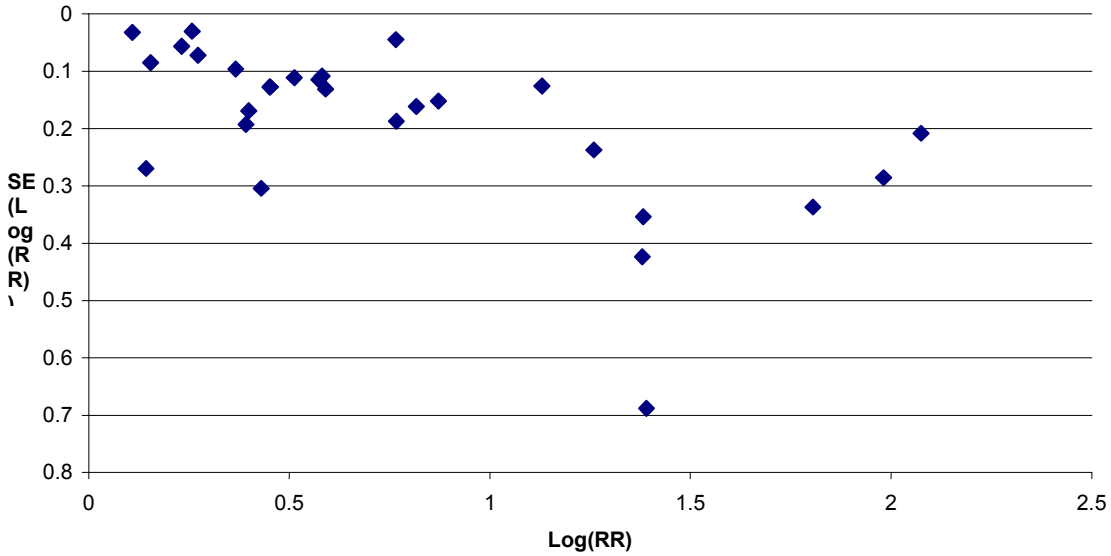


Figure 4.10. Funnel plot for common naevi on arms.

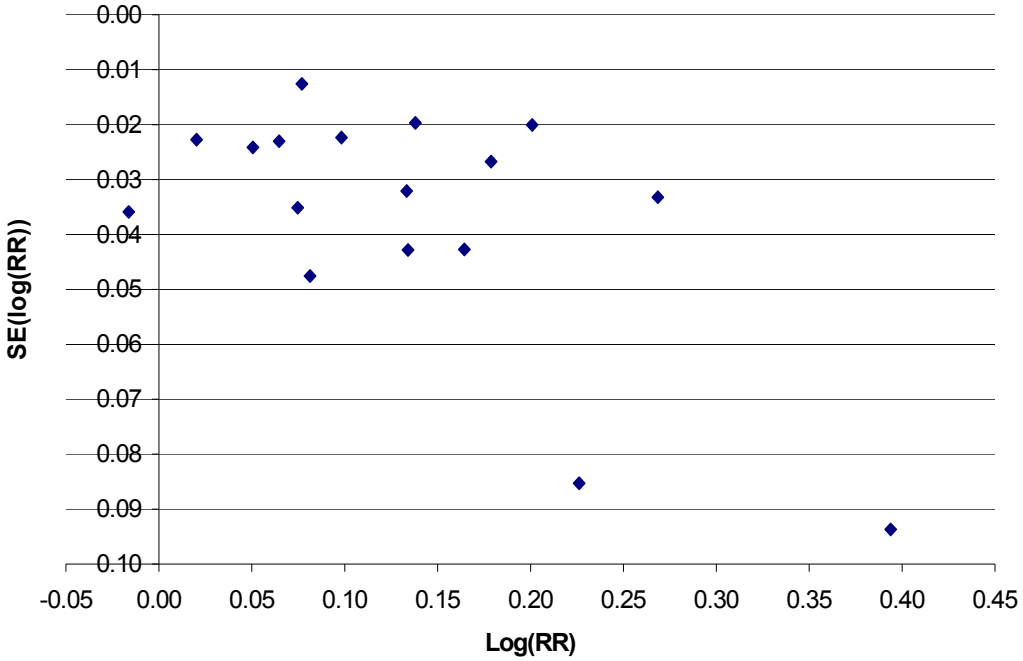
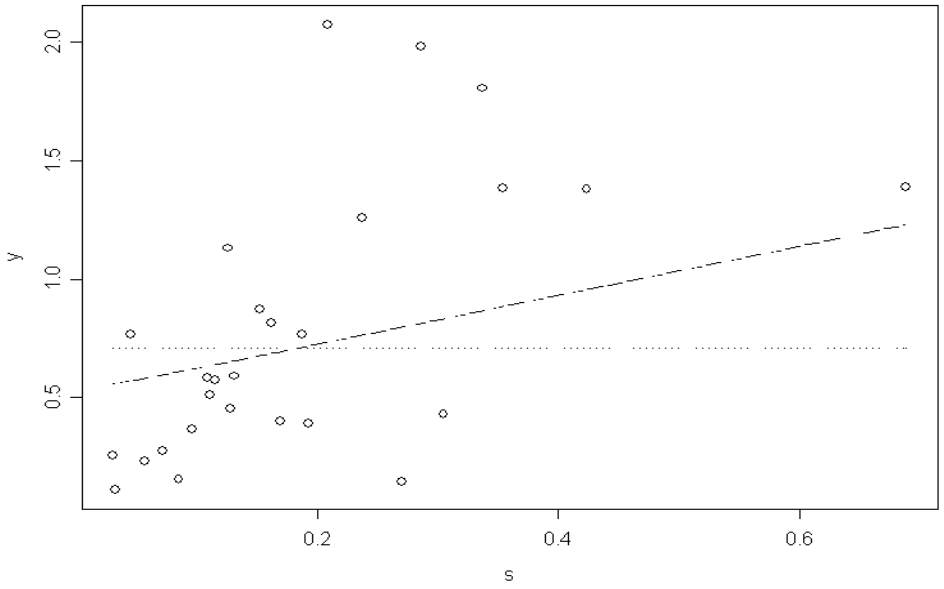


Figure 4.11. Log Relative risk (y) vs its Standard error (s) for common naevi.



CHAPTER 5. MELANOMA AND THE OTHER RISK FACTORS

5.1 Introduction

A systematic meta-analysis on all published studies from January 1984 to December 1999 is performed in this Chapter. All main risk factors for melanoma are investigated, except for the naevi count that was evaluated in the previous Chapter.

The published literature shows strong associations of melanoma with several factors. Highly renowned groups such as the International Agency for Research on Cancer have accepted sun exposure as the main cause of cutaneous melanoma in humans.[176] However complete or more convincing answers are still needed to many questions on sun exposure. They include whether the pattern of sun exposure is really important and acts independently of amount of sun exposure and whether sunburn makes a specific contribution to the risk of skin cancer. It is often difficult to separate the interrelations between sunburn history, sun exposure habits, ability to tan and other phenotypic factors. UV radiation may act as both an initiator through sunburn, for example, and a promoter, producing naevi and promoting action on them.[251]

Assessment of sun exposure has been investigated in this chapter looking also at differences between intermittent and chronic patterns of exposure and a possible association with sunburns. Several phenotypic characteristics are also analysed (hair colour, eye colour, skin colour, presence of freckles and phototype), trying to investigate interrelationships and associations with the influence of adjustment.

Most of the evidence relevant to the effects of different patterns of sun exposure comes from epidemiological studies and it is not easy to separate the effects of different patterns of exposure with epidemiological methods. The evidence for the association between sun and melanoma is often conflicting and the mechanism remains unclear. Several

methodological problems may bias the association between sunlight exposure and melanoma risk.[26] A deep exploration of between-study heterogeneity and possible sources of bias has been carried out searching for significant differences by study features, definitions adopted, characteristics of the populations and of the types of analyses.

In 1992, IARC concluded that there is limited evidence for the carcinogenicity of exposure to ultraviolet radiation from sunlamps and sun-beds and that there is inadequate evidence in humans for the carcinogenicity of fluorescent lighting.[176] Most of the studies on sources of ultraviolet radiation, other than the sun, have several methodological limitations.[252;253] Furthermore the power of these studies cannot be very high because of the presumed lag time between the relevant exposure and diagnosis of melanoma generated by this exposure. At the moment there is not enough evidence of any effect of other sources of ultraviolet radiation. Thus these factors are not included in this meta-analysis.

It was decided not to evaluate risk factors mentioned in certain publications where highly contradictory evidence of an effect was reported, such as sunscreen[254] and oral contraceptives[22;255] These two risk factors were investigated in two recently published meta-analyses[256;257] where no association with melanoma was found. Diseases, therapies or situations that may become big risk factors for melanoma and were studied only on very small populations such as immunosuppressors and PUVA therapy[258] or ionising radiations[259] are not considered for the meta-analysis.

Cutaneous lesions, which may be considered indicators of acute and chronic exposure to UV radiations, are included as photodamage indicators. In particular, in this chapter I analysed solar lentigo, actinic keratosis, solar elastosis and presence of epithelioma.

Risk associated with family history of melanoma is evaluated by looking at this factor alone, in the absence of other contemporaneous risk factors. Those with a family history of melanoma and dysplastic naevi and/or who have a large number of naevi

themselves, are at a very high risk of developing a melanoma over their lifetime.[27] The risk associated with this situation, called atypical mole syndrome,[260;261] can be estimated as the product of the risk associated with the number of naevi and with family history of melanoma.

Personal history of melanoma has not been included in the meta-analysis because some studies estimated a very high risk of melanoma associated with a previous melanoma.[20;262] Such individuals are well known to be at high risk.

Socio-economic level is regarded by some authors[237;263;264] as a risk factor of melanoma. There is a strong social class gradient in risk: melanoma is much more common in higher socio-economic groups, and some studies showed that this is a social rather than a specific occupational factor. However it was decided not to include socio-economic level in the meta-analysis because there is evidence that at least a large proportion of this socio-economic gradient can be explained by variations in sun exposure. [265] The increased risk for higher social classes may be due to greater opportunities for leisure and vacation activities involving sun exposure. Actually in several case-control studies the association is weakened adjusting for sun exposure.[252]

Diet was not considered, although various associations with dietary factors were suggested for melanoma, because the available information is very limited.[266] More work on dietary factors is needed and it would be particularly interesting to conduct this in a population where solar radiation is a less important feature.

Age and sex are considered as confounding factors. They are interrelated and associated with different behaviours with sun exposure and they have a cohort effect on chronic sun exposure.[267] In most societies, melanoma incidence rates are somewhat higher in women than in men during the reproductive age groups, being similar after the age of 60 years.[268]

Identification of intermediate variables, that are dependent variables in causal chain of melanoma, is very important for the definition of disease model and consequent analysis of variables involved. High number of naevi is associated with cutaneous sensitivity to sun therefore we could consider pigmented lesions as an effect modifier. If naevi are considered precursors of melanoma, and their number is defined by sun exposure, the variable number of naevi is in the causal chain of sun exposure with melanoma, and adjusting for number of naevi would introduce a bias toward null effect. In Figure 5.1 a simplified disease model, which summarises some hypotheses on the main associations and interactions between risk factors and melanoma, is presented. Interpretation of factors as effects modifiers or intermediate variables, and their possible interactions, are discussed in the heterogeneity and sensitivity analyses of next paragraphs.

5.2 Methods

A literature search was performed to identify publications that contain suitable information on the association between all risk factors (except for naevi) and melanoma, over the period January 1984 to December 1999. Four hundred ninety-eight papers in total were found, but only one hundred and ten were independent.

A detailed description of data sources, search strategy and data collection can be found in Chapter 4, where the meta-analysis on naevi is presented. A detailed description of the statistical methods adopted can be found in Chapter 2. As previously, statistical significance for the heterogeneity Chi-squared is assumed at the p-value=0.1, given the low power of this test.[71] A bivariate approach was used to compare intermittent versus chronic sun exposure and sunburns in childhood versus sunburns in adulthood. Conditional independence was assumed at the beginning of the model because estimates on the covariance, between the

two variables to be compared, were not available. However an estimate of the correlation was obtained from the bivariate model.

In general the estimates extracted are the published Odds Ratios (OR), Relative Risk (RR) or Standardized Incidence Ratio (SIR), for the highest category compared to the lowest category of exposure, adjusted for the maximum number of confounders.

Inclusion criteria adopted for all following analyses are the following:

- The studies have to provide sufficient information to estimate the relative risk and 95% confidence intervals (i.e. they have to publish OR, RR, SIR or crude data and standard errors, variance, confidence intervals or p-value of the significance of the estimates).
- The studies have to be independent in order to avoid giving double weight to some studies. Rationales for the choice to include a particular study, among a set of dependent studies, can be seen in the previous Chapter on naevi. In general the publication with the widest sample size was chosen.
- The populations studied had to be homogeneous, at least for the main risk factor for melanoma. Thus studies could not include only cases of lentigo maligna melanoma (LMM) and acral lentiginous melanoma (ALM), as there is evidence of a different aetiology for these lesions.

Most results were for all subjects, combining sexes; some of them presented results separately for women and men with no combined data. They were used in that form, producing a number of independent datasets higher than the number of studies included in the meta-analysis.

I finally obtained a total of seventy-four independent studies that presented the minimum information necessary for the analysis and satisfied all inclusion criteria.

Several methodological issues, that may have biased the results, and differences in study designs and in population features were explored as potential sources of variation between estimates: type of study, blinding of subjects and/or interviewers, sources of cases and controls, mean age of cases and controls, percentages of fair skin in cases and controls, inclusion among controls of subjects with any tumours or with dermatological problems, inclusion of subjects with family history, exclusion of maligna lentigo and acral melanoma, latency period considered, country, latitude, publication year and adjustment for some possible confounding factors (age, sex, naevi, phenotype, phototype, sunburns, chronic, acute and total sun exposure, family history…**).**

Heterogeneity analysis looking at the influence of adjustment for several confounding factors was always evaluated on the estimates coming from saturated models (including naevi for sun exposure analysis).

For each risk factor, a table describing some general features of each study included in the analysis, graphs of ORs extracted from the papers, with corresponding confidence intervals, and funnel plots are presented. Sometimes further graphs are used to present relative risks included in the analysis, to obtain more readable estimates. When variation among definitions and methods of assessment of exposures are a matter of concern a further table with some brief descriptions is included. When some features significantly explain between-study heterogeneity, more figures are presented to show differences among studies in terms of these characteristics. When publication bias is found to be significant, a plot, proposed by Copas and Shi[52] investigating a trend between the estimates and their standard error, has been added.

5.3 Ultraviolet radiations exposure**:** Introduction

The "Consensus Development Conference on Sunlight, Ultraviolet Radiation, and the Skin" in 1991 stated that the only established exogenous causal factor for cutaneous melanoma in white populations is sun exposure.[269] Similar conclusions were reached by the International Association for Research on Cancer,[176] which has reviewed in great detail the relation between melanoma and sun exposure. Several pieces of epidemiological evidence were found.

Firstly, the closer to the equator one is, the higher the mortality and incidence rates of melanomas are. This has been reported in many areas such as North America and Australia. Melanoma is more common in the white population of Australia than any where in the world. Actually most Australians experience an average of seven hours or more of bright sunlight per day and the majority of the population lives closer to the equator. The relationship with latitude is less clear in Europe where melanoma incidence rates are higher in Scandinavia than they are in southern Europe, probably reflecting different skin pigmentation and the importance of recreational sunlight exposure and vacation habits.[250] That trend did not apply in Europe as a whole but something similar was still seen within individual countries**.** Furthermore there is a relationship between the length of time that people have lived at lower latitudes and the risk of melanoma. Migrant studies have shown that people who move from less sunny places to countries such as Australia, New Zealand, Israel and California present lower rates than the native born population.[252]

Secondly, the highest rates are seen in body sites that received the greater amount of sun exposure such as the face, ears, and shoulders. Per unit area of epidermis, the male ear has the highest incidence of melanoma of any part of the body.[166] However the distribution of melanoma is unlike non melanoma skin cancers that occur almost exclusively on exposed skin areas and that are clearly more common in individuals with a great deal of outdoor exposure. Furthermore there is a difference in the distribution of melanoma between sexes that is consistent with different exposure habits. It was seen a considerable change, with time, in the

sites distribution, with marked increase for the trunk in males and in lower limb for females.[160]

Thirdly, cutaneous malignant melanoma is a disease of Caucasian people (fair skin being more susceptible to the ill effects of sunlight) and is more frequent in sun-sensitive people. The risk was found higher in individuals with both phenotypic susceptibility and a history of sun exposure.[152]

Fourthly, the incidence is higher in people with benign sun-related skin conditions. Patients with melanoma have a significant excess of solar elastosis, actinic keratoses, and non-melanoma skin cancers, which are considered measurements of cumulative sun damage.[270]

Fifthly, even if the main evidence is based on analytical epidemiological studies, mutagenic and oncogenic activities of sun exposure are well documented in experimental animals and their role in skin ageing and the association with squamous cell carcinomas of the skin is well established.[176]

Melanocytes are in the skin and their normal function is to respond to UVR by producing and distributing pigments. However melanoma does not simply increase with an increasing amount of accumulated exposure to ultraviolet radiation. This is illustrated by the fact that the incidence of melanoma is higher among indoor than among outdoor workers[271;272]

Sun exposure is particularly difficult to assess as methods of recording and coding vary a lot between studies. The measurement of sun exposure is extremely complex, and no objective methods exist for assessment of different types of exposure, for definition of different categorizations of level of exposure or even consistency on the use of particular reference groups. Inadequate definitions result in non-differential misclassification, that is,

errors in the exposure classification that are independent of the case-control status and this may modify the results toward no effect.

Biases may also be induced by inclusion of some histological types of melanoma. Actually superficial spreading melanoma and nodular melanoma are the only histological types relevant to test the intermittent sun exposure hypothesis. As lentigo maligna melanoma is strongly associated with the chronic sun exposure, and acral lentiginous melanoma occurs on the non-sun exposed site, their inclusion could modify the risk estimates for intermittent sun exposure.[273]

The period in which sunlight exposure is measured is also important and the risk estimates are expected to vary according to induction period. However conflicting results have been reported, some[274;275] indicating that events 20 years prior to diagnosis may contribute significantly, others[276-278] relating melanoma risk to early childhood ultraviolet exposure and extending the, so called, latent period to 40-50 years prior to melanoma appearance. In this analysis definitions that considered widest induction periods were included.

The physical dose of solar radiation, received from a given pattern of exposure, will vary with geographical location and latitude that define the background level of exposure. An hour of exposure at the latitude of, for example, Queensland gives about three times the UV exposure of an hour of exposure in mid latitude Europe. Elwood and Diffey[279] showed that, for identical outdoor exposure patterns, an individual at higher latitude will receive a relatively lower amount of total ultraviolet dosage. The absorbed dose will vary with personal skin characteristics as well as clothing. The presence of constitutional factors (hair colour, eye colours...) may increase the sensitivity of the skin to sunlight and become important risk factors for melanoma.

For melanoma, the pattern of sun exposure several decades before diagnosis is the important factor, but this will be difficult to ascertain by a retrospective study. However the evidence implicating sun exposure in the aetiology of melanoma derives largely from case-control studies. These types of studies have the major disadvantage that the information collected concerns events occurred in the past. Prospective studies, collecting exposure data before disease onset, would be better. In fact retrospective assessment of sun exposure implies potential for significant recall bias: if patients with melanoma or the interviewers are aware that sun light might be associated with the disease it is more likely that sun exposure will be reported.[280] Many studies were conducted before the 1990s, and at that time much professional opinion was against the concept that melanoma could be related to sun exposure. [21;281] Therefore (with the possible exception of Australia) there was little public knowledge about the dangers of sun exposure.

Another crucial aspect of case-control studies is selection of representative controls. If they were recruited from inpatients of various hospital departments, it is unlikely that such controls would be representative of the case base, and thus results may be potentially biased.[26] In fact some of them use patients with other skin conditions, and even other skin cancers, as controls. At the opposite the response rates in studies with population based controls have in general been adequate. Comparisons with the source population give good evidence of comparability between the selected controls and their sources populations in general demographic features.[252] Furthermore it is difficult to realize cohort studies because melanoma, even in high-risk countries, is still a rare disease and sun exposure is not recorded in any existing database.

5.3.1 Extraction and homogenisation of the data on sun exposure

Results were classified as relating to intermittent, chronic (occupational) or total sun exposure. Intermittent sun exposure was generally assessed by posing questions about specific activities that would be likely to represent relatively severe intermittent exposure such as recreational activities: sunbathing, water sports, and vacations in sunny places. While occupational exposure to the sun is considered to be a more regular and chronic exposure. Total accumulation exposure is a sum of both types of exposures.

Several studies presented more measures for the same pattern of exposure and it was decided to include the exposures concerning the periods of life that were widest and most recent. Regarding childhood exposure and adulthood exposure the second option was chosen because there is evidence that self-reported childhood sun exposure is less reproducible than sun exposure at older ages.[282] This choice was checked in the heterogeneity analysis looking at the relevance of the latent period considered. When the decision about the most appropriate definition is not straightforward, the one that presented the highest prevalence among controls was chosen.

The estimates adjusted for demographic factors, such as age and sex, and baseline characteristics, such as ethnic origin, skin pigmentation and inherent tendency to burn or tan easily, were favoured. Those who do not suffer when lying in the sun are likely to spend more time doing so, therefore an analysis looking at sun exposure without adequate adjustment for the sun sensitivity factors, skin pigmentation and tendency to burn will underestimate the true relationship. Such adjusted measures were used instead of measures adjusted for factors which themselves could be related to sun exposure, such as number of naevi. As was seen previously, melanoma risk is strongly related to the number of naevi and naevi are increased in individuals with high levels of sun exposure. Thus, naevi may lie in the causal pathway between sun exposure and melanoma and in this case the adjustment for naevi would be not appropriate because it would decrease the true association.[283] The mechanism for the

association between sun exposure and melanoma may be related to the induction and/or transformation of naevi or the number of naevi could be considered as a potential confounder. There is no consensus on this issue, but I decided to treat the number of naevi not as confounders but as intermediates, investigating this choice in the sensitivity analysis. Relative risk estimates adjusted for the greater number of confounders, excluding naevi, were extracted from the papers. If all the published estimates were adjusted for naevi a crude estimate from published raw data was calculated.

5.3.2 Total sun exposure

5.3.2.1 Materials and methods

Among the fifteen papers that investigated association between melanoma and total sun exposure, five[164;236;251;284;285] were not independent, reporting results already described in other papers. I finally arrived at ten eligible independent case-control studies and eleven datasets because Graham[286] only presented estimates separately for gender. All of them presented estimates referring to melanoma on all body.

Details of all these studies, including number and sources of cases and controls, country of origin and assessment of exposure are given in Tables 5.1 and Table 5.2. In bold are indicated the definitions corresponding to the definitions for the estimates included in the analysis. As can be seen in Table 5.2 there are several measures of total sun exposure, which included a variety of definitions, measured with questionnaires, concerning all life or shorter periods. Definitions of total sun exposure range from a general indication of history of intensive sun exposure, to overall sun exposure, cumulative sun exposure in hours during the whole or mean hours during summer. Some of them used very sophisticated classifications with sun exposure indexes, or accurate calculations of the total number of hours of exposure or the number of hours per day, whereas some others used only broad categories. When it was

necessary to choose among different measures, the definitions that seem to indicate the highest levels of exposure were preferred, in order to decrease misclassification.

5.3.2 2 Results

Odd ratios extracted from the included papers were plotted, with their confidence intervals, in two figures (Figures 5.2a and 5.2b), grouping highest estimates together to be more readable.

A random effects model was adopted because there is significant heterogeneity between published estimates (Chi squared=63.49 with 10 d.f.).

Final pooled RR is not significantly greater than one (RR=1.29 with 95% C.I.: 0.82; 2.02), suggesting no statistically significant association between total UV radiation and risk of melanoma.

5.3.2.3 Heterogeneity analysis

The lack of standardized measurements for sunlight exposure and the use of different baseline and exposure categories represent the first serious problem in assessing the effect of UV exposure. It is difficult to investigate heterogeneity looking at sun exposure definitions because they are described in a qualitative subjective way. Only induction period, considered in the descriptions of the total sun exposure, may be quantitatively evaluated: the majority of the studies considered "all life long" whereas two studies asked questions on last 20 year (Cristofolini 1987[235]) and last 10 years (White 1994[249]). The estimates are not significantly different from each other.

Looking at all possible factors that may have induced differences in outcomes, not due to sampling variation, subgroup analysis shows that heterogeneity within subgroups of studies remains significant. However from meta-regression it was seen that three factors

explain some of the between-study heterogeneity: “sources of controls”, “adjustment of the estimates for phenotype” and “publication year”.

Estimates with the indication of the source of controls are presented in Figure 5.3. As can be seen, the seven studies with controls not drawn from hospitals showed higher and more precise values compared to studies with controls drawn from hospitals. The pooled RR for studies with controls not drawn from hospitals indicates a slightly significant risk for total sun exposure (RR=1.78; 95% C.I.: 1.00; 3.17; Chi=35.83, d.f.=6, p<0.001). This estimate is different, at 8%, from the pooled relative risk of the subgroup of case-control studies with controls drawn from hospitals, which suggests a not significant effect of total sun exposure (RR=0.75 with 95% C.I.: 0.40, 1.40; Chi=14.97, d.f.=3, p=0.002). This finding may be explained by considering that recall bias can influence results because controls with diseases may be more aware of the effect of UV radiation and may more easily remember episodes of sun exposure. In fact three out of four estimates obtained from case-controls with controls based on hospitals, come from studies that include subjects with dermatological problems or any tumours (Graham 1985[286], Dubin 1990[214]). All four studies with controls drawn from hospitals declared to have included subjects with family history. Thus recall bias was hypothesized as an important factor because it may modify the results towards a null effect. This hypothesis is also supported by the trend found by looking at publication years. As we have said, recall bias may be a problems for many studies performed after the 1990s. From Figure 5.4 it can be seen that studies published after 1990, when dangers of sun exposure became a public issue, showed an increased significant relative risk (RR=2.24 and 95% C.I.: 1.27; 3.96; Chi=18.00, d.f.=4, p=0.001). Whereas studies published before 1990, when much professional and common opinion did not yet agree with the perception that melanoma could be related to sun exposure, indicated a significantly (p=0.05) lower risk (RR=0.77 and 95% C.I.: 0.54; 1.116; Chi=11.28, d.f.=5, p=0.05) than the studies published afterwards.

Investigating influence of adjustment for confounders on the fully adjusted estimates (see Figure 5.5), it can be observed that the four estimates adjusted for phenotype are higher than not adjusted. The pooled RR of the subgroups of studies for which we have estimates adjusted for phenotype indicates a significant risk for total sun exposure (RR=2.51, 95% C.I.: 1.18, 5.27), with a reduction in the Chi-squared (Chi=10.6 with 3 d.f., p=0.013). The pooled RR for subgroups of estimates not adjusted for phenotype is lower and not significant (RR=1.00, 95% C.I.: 0.59, 1.71; Chi=32.71 with 7 d.f., p<0.001). Meta-regression gives a difference between the two groups significant at 9%. In fact adjustment for phenotype may influence estimate of exposure to the sun because fair people probably receive less sun exposure. If we do not take this into account we will obtain estimates that are lower not because of the sun effect but because of phenotype: fair people do not go in order to get suntanned. The fact that adjusted estimates are higher than unadjusted suggests that sun exposure may have a significant effect.

Exclusion of lentigo maligna and acral melanoma do not have any significant effect on the results. Similarly, country and average latitude do not seem to have a significant effect in explaining between-study heterogeneity.

It is important to notice that caution should be taken in the interpretation of these results because the number of studies included in the meta-analysis is not large and there may be a problem of multiple testing.

5.3.2.4 Sensitivity analysis

The choice to exclude from meta-analysis estimates adjusted for naevi was investigated and a new analysis was carried out including all relative risks adjusted for the maximum number of confounders. The results (RR=1.36 with 95% C.I.: 0.86, 2.16; Chi=55.92, d.f.=10, p<0.001) are very similar to the final RR calculated on estimates not adjusted for naevi.

Green published two papers on the same collective of subjects, in 1985 and 1986. Out of the two, I considered, as usual, the paper[284] in which estimates of the relative risk are adjusted for the major number of confounder. This estimate is much greater than the other and it has very wide confidence intervals: RR=5.3 (95% C.I.: 0.9; 30.8). The RR adjusted for less confounders is lower (RR=1.7 and 95% C.I.: 0.4; 7.8) and it differs from the previous one because it does not take into account eye colour and inherent tendency to burn.[250] A further pooled RR was calculated with the Green's lowest estimate and a very similar value of the pooled RR was found: RR=1.31 (95% C.I.: 0.83, 2.03).

Rodenas (1996)[184] presented a very high estimate, but its weight is quite low (w=5.96) and it does not influence the pooled RR.

It is interesting to note that there is a significant ($p<0.001$) difference between mean percentages of fair skin between cases (mean=41.65, s.e.=17.75) and controls (mean=23.72, s.e.=13.28), among the 9 studies that published this information. Green and Rodenas are the two studies with the lowest percentages of fair skin in the controls (6% and 5.8%, respectively) and with a big difference between cases and controls (percentages in cases are: 13% and 36%, respectively). However they adjusted the estimates for phenotype. Meta-regression shows that percentages of fair skin, in cases and in controls, do not seem to significantly explain the variability among the estimates, probably because the majority of the estimates are adjusted for phenotype or phototype.

Investigation of the funnel plot (Figure 5.6) with Copas and Shi methods gives no indication of publication bias.

5.3.3 Intermitted sun exposure

5.3.3.1 Materials and methods

Forty-three papers provided information on association between melanoma and intermittent exposure to UV radiations, in terms of a specific recreational or vacation exposure. One of them (Sorahan 1985[247]) did not publish any information to calculate the standard errors of the estimates and seventeen[164;212;219;222;224;225;233;251;284;285;287-294] of them were not independent. The twenty-five independent studies, included in the analysis, are all case-control studies (Moore 1997[230] is a nested case-control). Herzfeld (1993)[295] presented data on melanoma only on the trunk of males. Holly (1995)[296] published data regarding a female population. Chen (1996)[234] was not included for the calculation of the main pooled estimate because it was not possible to extract an estimate not adjusted for naevi. This decision was tested in the sensitivity analysis where further pooled estimate that includes fully adjusted estimates was calculated.

Details of the studies are presented in Tables 5.3.

The measures used to define intermittent sun exposure were recreational or vacation exposures, either in general or related to specific activities. Definitions adopted by different authors are summarized in Table 5.4. As can be seen there is a variety of different measures of intermittent exposure. Some of them include an indication of the amount of time spent outdoors during leisure and of the frequency of participation in sun-based activities such as sunbathing, swimming, boating skiing, gardening. Very sophisticated definitions are also used, with sun exposure indexes or accurate number of hours of exposure. When it was necessary to choose among different measures, the definitions that seem to indicate the highest levels of exposure were preferred, in order to decrease misclassification. “Sunbathing” was one of the activities favoured to represent the highest level of intermittent sun exposure, among the different situations evaluated by the studies (boating, skiing, gardening…). As mentioned at the beginning, when the decision about the most appropriate definition is not straightforward the variable with the highest prevalence in controls was chosen. However the

estimates of intermittent sun exposure, coming from the same study, were quite similar and the choice among them was considered not influential for the final pooled result.

5.3.3.2 Results

Estimates included for the calculation of the final pooled RR are plotted in two Figures 5.7, grouping highest estimates in the same plot to be more readable. As can be seen there is reasonably consistent evidence for a positive association between intermittent sun exposure and melanoma. The random effects model gives an indication of a significant risk: RR=1.58 (95% C.I.: 1.26; 1.99).

5.3.3.3 Heterogeneity analysis

A considerable between-study heterogeneity was found (Chi-squared=133.36 with 23 d.f.; $p<0.001$). The variations in ORs and RRs is likely to be related to many factors and probably the considerable diversity among the definitions of intermittent sun exposure plays an important role. Latency period was evaluated quantitatively and it does not justify differences in the estimates.

Meta-regression indicates that none of the considered factors seem to be statistically significant in explaining between-study heterogeneity. Heterogeneity within subgroups of studies remains highly significant. Nevertheless there are some similarities in the type variability among estimates found in total sun exposure. Pooled RR of the subgroups of studies with controls not coming from hospital indicates a higher significant risk for intermittent sun exposure (RR=1.75; 95% C.I.: 1.34; 2.29), compared to the subgroup of studies with controls based on hospitals that suggests a lower and not significant effect of sun exposure (RR=1.12; 95% C.I.: 0.76, 1.65). Again this difference may be explained by the problem of recall bias (towards a null effect) because controls with diseases may be more

aware of the effect of UV radiation and may easily remember episodes of sun exposure. Actually four (Bell 1987[297], Dubin 1990[214], Weiss 1991[213] and Wolf 1998[298]) out of five case-control studies with controls drawn from hospitals included subjects with dermatological problems.

Investigation of influence of adjustment for phenotype and/or phototype, which was carried out on fully adjusted estimates, indicates trends similar to the one found for total sun exposure. The pooled RRs of the subgroups of estimates, adjusted for these features, indicate a higher significant risk for intermittent sun exposure (RR=2.24; 95% C.I.: 1.70, 2.95 for phenotype and RR=2.53; 95% C.I.: 1.64, 3.91 for phototype). The pooled RRs of not adjusted estimates are lower and not significant (RR=1.08 with 95% C.I.: 0.82, 1.42 for phenotype and RR=1.21 with 95% C.I.: 0.96, 1.53 for phototype). These results are consistent with the previous considerations that if we do not take into account phenotype and phototype we will obtain estimates that are lower not because of the sun effect but because people with sensitive skin do not go for tanning.

Few studies published percentages of fair phototype (12 out of 24) in cases and in controls, but where it was possible to investigate differences in prevalence of fair phototypes there was not seen to be a significant difference.

As for total sun exposure, exclusion of lentigo maligna and acral melanoma do not have any significant effect on the results.

The measurements which give the strongest associations for intermittent sun exposures were the ones obtained in the sixteen studies conducted in European countries (RR=1.92 and 95% C.I.: 1.41, 2.653; Chi=94.61, d.f.=15, $p<0.001$), where it is possible to clearly see the effect of sunny vacations. Positive results were also reported in sunnier countries as Italy and Spain (Rosso 1998[299], Carli 1995[217] and Rodenas 1996[184]). A less consistent pattern was seen in Australia and USA where the pooled estimate considering only

these two countries (9 studies) is not any more significant (RR=1.19 and 95% C.I.: 0.90, 1.56; Chi=26.86, d.f.=8, p<0.001). If we investigate the influence of intermittent sun exposure during vacations we do not always obtain very reliable estimates. In Australia, for example, people go to places which enjoy a lower rather than a higher level of sunshine exposure. The major study performed in Queensland (Green 1986[250]) does not show a significant association with intermittent sun exposure. Similar results were found in the other very detailed study carried out in Australia (Holman 1986[300]), which showed positive association with some but not all measures of intermittent sun exposure. In this analysis we have to take into account that in areas with very high levels of sunshine the effect of intermittent sun exposure may be seen more for some specific recreational activities. In a sunny environment, recreational activities should involve high frequency or intensity of sun exposure to result in an intermittent rather than a constant pattern. It is probably easier to study intermittent exposure in countries as Europe where many people have a little regular exposure and increase sun exposure during holidays and recreational activities.

5.3.3.4 Sensitivity analysis

The choice to exclude from meta-analysis estimates adjusted for naevi was investigated and a new analysis was conducted including relative risks adjusted for the maximum number of confounders, including naevi. The pooled estimate is very similar to the previous pooled relative risk (RR=1.58 and 95% C.I.: 1.27, 1.97; Chi=133.27, d.f.=25, p<0.001).

Chen (1996)[234] only presented estimates separately for the four body sites and adjusted for naevi and did not publish information to calculate the crude estimates. For this reason it was excluded from the main analysis and it was included in this phase of the work. The four estimates being very similar (Chi-squared for heterogeneity is 0.08 with 3 d.f., p-

value=0.99) in order to not give to much importance to this study a weighted average of the four estimates was considered for the analysis on the fully adjusted estimates.

The highest estimates were presented by Grob (1990)[170] and Autier (1998)[301] and it is interesting to note that they are the only two studies, included for the final pooled RR, that presented an estimate based on a sun exposure index and not looking only to a single activity.

Looking at the funnel plot (Figure 5.8), Copas and Shi method gives no indication of publication bias for intermittent sun exposure

5.3.4 Chronic sun exposure

5.3.4.1 Materials and methods

Thirty-nine papers were identified concerning association between melanoma and chronic sun exposure. Among those articles, eight[164;212;225;236;251;290-292] presented results already described in other papers. I analysed twenty-eight independent case-control studies, plus one nested case-control study and two cohort studies. Two of them (Osterlind 1988[302] and Pion 1994[303]) presented estimates separately for sex therefore I finally arrived at thirty-three eligible independent datasets.

Herzfeld (1993)[295] presented results on melanoma found only on trunk of male subjects. The estimate extracted from Graham (1985)[286] concerned data on a male population and the estimate from Holly (1995)[296] on a female population.

Chen (1996)[234] was not included for the calculation of the main pooled estimate because it was not possible to extract estimate not adjusted for naevi, as for the other two measures of sun exposure. This decision was tested in the sensitivity analysis where a further pooled estimate, which includes fully adjusted estimates, was calculated.

Details of the studies included in the analysis and definitions of exposure, used to identify chronic or occupational sun exposure, are described in Table 5.5 and Table 5.6.

Regular outdoor activities through occupation are likely to give the best measure of chronic or regular, as opposed to intermittent, sun exposure. As can be seen, occupational exposures were assessed with differing degrees of detail. Several of the studies used only very general definitions, for example occupation being predominantly outdoors or predominantly indoor, which is likely to be inadequate. Some others were more accurate and gave detailed evaluation involving assessment of habits and a quantification of time that subjects spend outdoors. In general, chronic exposure should be easier to document than intermittent sun exposure.

5.3.4.2 Results

ORs and RRs, with their confidence intervals, are plotted in two Figures 5.9, grouping highest estimates in the same plot, to be more readable. As can be seen there are several studies that present estimates lower than 1, indicating a protective effect of chronic sun exposure, but the confidence intervals very often includes 1,indicating a not significant effect of chronic sun exposure on melanoma. Even if there is a problem of heterogeneity (Chi-squared=85.3, with 31 d.f., $p<0.001$), a general suggestion of a protective effect is emerges from the analysis, even if not significant. In fact the majority (18 out of 31) of the estimates are below 1.

The pooled RR, obtained from the random effects model, is: RR=0.93 (95% C.I.: 0.84; 1.04).

5.3.4.3 Heterogeneity analysis

Results from meta-regression indicate that three study features are statistically significant in explaining variability between studies, even if heterogeneity within subgroups remains significant: “source of controls”, “inclusion of subjects with dermatological problems” and “country of the study”. Two-factors interactions were not significant.

For chronic sun exposure, the relationship between source of controls and sun exposure, in the evaluation of the risk of melanoma, is different from that obtained for intermittent and total sun exposure. From Figure 5.10 it can be seen that the majority of the studies (thirteen out of eighteen), with controls not drawn from hospitals, showed a protective effect (RR lower than one) and six of them were statistically significant. The pooled RR of this subgroup of studies (RR=0.84, 95% C.I.: 0.71, 0.99; Chi-squared=50.03, 17 d.f., $p<0.001$) is significantly different ($p=0.06$) from the one obtained considering the hospital-based studies (RR=1.07, 95% C.I.: 0.92, 1.24; Chi-squared=33.51, 13 d.f., $p=0.001$).

In Figure 5.11 a histogram presents studies with the indication of inclusion of subjects with dermatological problems. As can be seen, the fives studies, in which it was stated that these subjects had been included, showed all rates greater than one. The pooled RR of the twenty studies that did not include subjects with dermatological problems, or did not say anything about it, (RR=0.82; 95% C.I.: 0.69, 0.97; Chi-squared=42.99, d.f=20., $p=0.001$) is significantly ($p=0.01$) lower than the RR of the studies that declared to have included them (RR=1.39; 95% C.I.: 1.09, 1.76). For this latter sub-group of studies the fixed effects model was used because the Chi-squared was not any more significant (Chi=5.27, d.f.=4, $p=0.26$).

Similar results are obtained considering the subgroup of twenty-two studies coming from Australia, USA or UK. The majority of them (fifteen) showed rates lower than one (See Figure 5.12). The Pooled (RR=0.87, 95% C.I.: 0.79, 0.96, with Chi-squared=43.2, d.f.=21 $p=0.003$) is significantly lower ($p=0.02$) than the one obtained for the other countries (RR=1.32, 95% C.I.: 0.91, 1.92, with Chi-squared=30.63, d.f.=8, $p<0.001$).

Studies conducted in these countries in general presented a better design: many had controls community based (13 out of 22 studies); all of them excluded subjects with dermatological problems; the majority presented quite detailed information on sun exposure. One of the reasons may be related to the fact that the USA and Canada present quite high

incidence of melanoma and for many years melanoma was a matter of concern. Much professional opinion maintained that melanoma could be related to sun exposure and many studies were planned to investigate this association.

Thus the indication of a protective effect for chronic sun exposure comes from studies that presented a better design: population-based controls, which stated that subjects with dermatological problems had been excluded and which were carried out in "high incidence" countries. In fact, the pooled RR calculated on the four studies that have all these three features suggests a significantly protective effect for chronic sun exposure and a not significant heterogeneity (pooled RR=0.64, 95% C.I.: 0.51, 0.81, with Chi-squared=3.35, d.f.=3 p=0.34).

Elwood arrived at similar conclusions, in a review published in 1996[21], where he found an agreement between western Australia and northern hemisphere studies in terms of low risk of melanoma seen with heavy occupational sun exposure. In the very detailed study published in 1985[304] Elwood suggests that the association with occupational exposure may be non-linear, with an increase in risk related to small amounts of occupational exposure and a decrease in risk with long continued heavy exposure. This mixed overall pattern may explain the inconsistent results arising from many other studies that do not assess the chronic sun exposure in enough detail. Elwood[21] looked at the ratio of the risk ratios for intermittent sun exposure compared to occupational sun exposure and he found that the ratios of intermittent to chronic exposure tend to be positive for the studies with control group drawn from the community, or hospital control group which excludes patients with skin diseases or other cancers. This finding suggests that in well-conducted studies it is easier to find a clear distinction between the two estimates and a lower protective effect for long continued chronic sun exposure.

Among the studies classified as non hospital-based there are four that have singular designs: Cooke (1984)[305], Vagero (1986)[306], Goodman (1995)[307] and Freedman (1997)[308]. Three of them also have big weights with a vast quantity of cases and controls. Goodman's paper compares incidence cases of melanoma, recorded by population-based registry, to incidence cases of all other forms of registered cancers, with respect to declared occupation. Cooke's paper compares observed and expected number of incidence cases for several occupational unit groups, in the New Zealand cancer registry. Freedman's paper compares deaths from melanoma with non-cancer deaths, drawn from a database supported by two American national health institutes. Potential sunlight exposure was assessed by usual occupation recorded on the death certificate. Vagero's paper presented an analysis based on incidence cases obtained from an extended Swedish cancer registry, created from a linkage of the Swedish Cancer Registry to the population census. For each case, census information such as occupation was known.

Their design being different from the others and obviously information about sun exposure not being very detailed, a further analysis was carried out looking at their influence on the results. After their exclusion, the subgroup of eighteen remaining studies in high incidence countries, provides an estimate indicating a clear significant protective effect of chronic sun exposure: RR=0.81 (95% C.I.: 0.72, 0.88) and heterogeneity was not any more significant (Chi-squared=23.485 with 17 d.f.; p=0.134). Similar results are found when these four studies are excluded from the subgroups of studies obtained looking at sources of cases and controls. The subgroup formed by population-based studies presents a heterogeneity Chi-squared not any more significant (12.49 with 10 d.f.; p=0.23) and the pooled estimate obtained with the fixed effects model suggests a higher significant protective effect of chronic sun exposure (RR=0.76; 95% C.I.: 0.67; 0.85). These results provide good evidence against

there being a clear increase in risk at maximum levels of chronic exposure, and a protective effect is suggested.

Results on adjustment for phenotype and naevi, on estimates coming from saturated models, are similar to those obtained for total and intermittent sun exposure. The pooled RRs of the twenty-three estimates not adjusted for phenotype (RR= 0.92; 95% C.I.: 0.85, 1.01) and of the twenty-eight estimates not adjusted for naevi (RR= 0.89; 95% C.I.: 0.80, 0.99) are lower than the pooled RRs of the estimates adjusted for phenotype (RR=1.23; 95% C.I.: 0.75, 1.99) and for naevi (RR=2.33; 95% C.I.: 1.51, 3.62).

Just few studies adjust for total sun exposure (Freedman (1997)[308]), intermittent sun exposure (Cristofolini (1987)[235], Weiss (1991)[213]) or sunburns (Cristofolini (1987)[235]).

Not many studies published percentages of fair phototype (14 out of 28), but where it was possible to investigate differences in percentages of fair phototypes between cases and controls a not significant influence of this difference on the estimates was observed.

Twenty-two studies indicated that they had excluded lentigo maligna and acral melanoma but their estimates are not significantly different from the others. “Blinding of subjects” and “matching” are also not significant in explaining variability among the estimates.

5.3.4.4 Sensitivity analysis

The choice to exclude from meta-analysis estimates adjusted for naevi was evaluated and a new analysis was conducted including relative risks adjusted for the maximum number of confounders, including naevi. The pooled RR is very similar to the previous one calculated on all not fully adjusted estimates: RR=0.94 (95% C.I.: 0.84, 1.05), with a highly significant between study heterogeneity (Chi=98.65, d.f.=32, $p<0.001$).

As in the previous analyses on sun exposure, Chen (1996)[234] presented only estimates separately for the four body sites and adjusted for naevi and did not publish information to calculate the crude estimates. For this reason it was excluded from the main analysis and it was included in the previous pooled RR, calculated on estimates adjusted for naevi.

Analysis was repeated with exclusion of the four peculiar big studies (Freedman 1997[308], Goodman 1995[307], Vagero 1986[306] and Cooke 1984[305]). Results are very similar to the previous ones and no consistent significant protective effect of chronic/occupational UV exposure is suggested RR=0.93 (95% C.I.: 0.81, 1.09); heterogeneity Chi-squared test remains still highly significant (Chi=71.07 d.f.= 27, $p<0.001$).

Two of them (Osterlind 1988[302] and Pion 1994[303]) presented estimates separately for sex but their estimates are quite similar to the others and the difference by sex is not significant.

No indication of publication bias was found (Figure 5.13).

5.3.5 Discussion on intermittent and chronic sun exposure

Interesting considerations come from the heterogeneity analysis looking at study design differences. Data from detailed studies with controls drawn from community are consistent with the intermittent sun exposure hypothesis: particularly intense exposure to sunlight increases the risk of melanoma, while more regular, chronic exposure has a neutral or even protective effect. Very similar results were found in Nelemans's[26] (RR=1.57 95%C.I.: 1.29-1.91 for intermittent; RR=0.73 95%C.I.: 0.60-0.89 for chronic sun exposure) and Elwood's[280] (RR=1.71 95%C.I.: 1.54-1.90 for intermittent; RR=0.86 95%C.I.: 0.77-0.96 for chronic sun exposure) meta-analyses that include holder studies.

Elwood did not investigate between-study heterogeneity looking at possible study features and he presented only a pooled estimates excluding different RRs. Whereas Nelemans clearly use a methodology quite similar to mine showing that an important function of a meta-analysis is the exploration of sources of variation in study results. The author found that when studies with some degree of blinding were combined only a small and non-significant effect was evident for exposure to sunlight. Conversely, in studies without blinding, the effect was considerably greater and significant because differential recall of past exposures may have introduced bias. Meta-regression, which could have been useful to investigate interaction between factors, was not applied because the author considered the number of the studies available too low to produce reliable results. In fact the number of studies was lower than mine because Nelemans included only case-control studies and papers published in English.

To make a comparison between intermittent and chronic sun exposure on more comparable estimates a further analysis was performed on the nineteen studies that published both estimates. In Figures 5.14 are presented, for each study, intermittent and chronic sun exposure RRs estimated. As can be seen, in the majority of the studies the RR for intermittent sun exposure is higher than the RR for chronic sun exposure, even if the differences are not always statistically significant. In fact meta-regression indicates a significant difference between the two estimates ($p=0.015$).

Analysing intermittent and chronic sun exposure in a bivariate approach, I obtained an indication of a considerable correlation ($R=0.46$) between the two variables but the pooled estimates for intermittent and chronic are quite similar to the ones obtained previously: 1.66 with 95% C.I. (1.23, 2.24) and 0.965 with 95% C.I. (0.76, 1.22), for intermittent and chronic sun exposure respectively. Looking at factors explaining heterogeneity, the inclusion in the model of the variable indicating presence of subjects with dermatological problems makes the

variables “sources of cases”, “source of controls” and “countries” not any more significant, suggesting an association between them. All these features represent a sort of indicator of a well-done study design, as I discussed in the heterogeneity analysis of the univariate approach.

More than in other situations these analyses should be considered with caution because of two main problems concerning quantification of exposure and its association with melanoma: recall bias and a considerable variability among definitions and measures of exposure. However, differences in results relating to different types of sun exposure argue against significant recall bias.

One study[309] had the chance to evaluate publication bias because it found that the degree of tanning reported was lower in the second survey following the diagnosis of the melanoma. However the questions and the contexts in which they were administered were not identical.

A case-control study performed in twins, from 1980 to 1991, showed contrasting results on the effect of a possible recall bias, which was suggested for sunbathing, mole and freckling frequency and not for burning and tanning.[310] However, as we said before, in the 1980s when some of the big studies were carried out, there was little public perception of the risks of sun exposure in regard to melanoma. The acceptance of sun exposure as a danger, something regularly commented on in the press, came later.

Assessment of exposure by questionnaire in a way which will allow different types of exposure to be separated, is difficult and requires that an interview be conducted lasting between 30 and 90 minutes.[252] Studies carried out in clinical situations often using short interviews by busy medical doctors are not very reliable.

Obviously none of the studies had the opportunity to compare responses directly with any pre-recorded data on sun exposure, as such data do not form part of any medical or

employment record. The lack of cohort studies is actually related to the fact that melanoma is in absolute terms a rare disease, and that sun exposure is not systematically recorded in any existing database, in the way, as for example, drug use may be recorded by medical doctors. It has therefore been difficult, so far, to design retrospective cohort studies. Actually in the few nested case-control studies, data on sun exposure are very limited. On the other hand, major case-control studies are characterized by good study designs by counting all newly incidence melanoma cases in a defined population, completing interview data on a large proportion of cases and controls and using detailed interview techniques.

The complexity of the relationship between solar exposure and melanoma should not be surprising, as sun exposure has a wide range of effects on the skin.[176] In fact the effects of UV exposure are modified by skin responses that attempt to protect the organism. Thus the increased risk associated with intermittent exposure may be because such exposures occur on relatively unprotected skin, giving high transmission to the level of the melanocytes. Regular exposure on tanned and thickening skin may be more effectively blocked at the epidermal level.[252]

In some studies the researcher tried to look at the effect of sun exposure at different times in life but they were not able to make any firm conclusions primarily because recorded sun exposure for an individual tended to be somewhat similar throughout life. Actually subjects tended to behave in the same way at each period of life and it is difficult to separate the effects of differences in sun exposure at different ages.[252] However this may represent an interesting aspect worthy of further investigation because migrant studies indicate that the risk of melanoma is much lower in subjects who arrive in a country such as Australia after the age of 15, whereas the risk in those who arrive at around age 5 is similar to the risk of the native country. This suggests that exposure in childhood may be particularly important.

Some authors suggest that the various opportunities for sun exposure may combine their effect throughout life.[301] Impact of adult sun exposure on melanoma risk may be influenced by sun exposure experiences during childhood. An adult with moderate sun exposure, but who was heavily exposed to the sun during childhood, could perhaps be at greater risk of developing a melanoma than an adult with high sun exposure but who was protected against solar radiation during childhood.

Thus impact of sun exposure on melanoma risk in different periods of life was investigated in this work, analysing effects of sunburn experiences in childhood and as adult.

5.3.6 Sunburn history: Introduction

Sunburn is an inflammatory reaction that arises following acute exposure of the skin to intense solar radiation. It is noticeable by erythema, pain and dermal edema, and if severe, by blistering and desquamation of epidermis.

Many studies show positive associations between melanoma risk and a history of sunburn but a straightforward interpretation of this association is complicated. In fact such experience, unlike everyday sun exposure, is unlikely to be forgotten and, for this reason, many studies consider sunburn as a marker of acute sun exposure.[24]

Furthermore this inflammatory reaction may represent the increased risk of those with high susceptibility rather than a direct effect of the presence of sunburn. Sunburns and reported skin types are obviously highly correlated but the reasons for this may not be simple. It would seem reasonable to assume that subjects would report their skin type based upon their past sunburn experience. Actually skin type is usually described in terms of tanning ability or as susceptibility to burning and it may becomes a crude surrogate measure for sunburn history. It is arguable whether this factor should be treated as a confounder for the association between sunburn and melanoma. While skin type certainly confounds the

association between sun exposure and sunburn, it cannot confound any association between sunburn and melanoma. Thus sunburn is considered by many authors[24;250;220] a biological marker of high dose of ultraviolet radiation penetrating to the melanocytes at the base of the epidermis, regardless of the degree of pigmentation in the epidermis. Nevertheless a history of sunburn should be associated with unusually intense sun exposure and skin sensitivity and therefore both questions must be addressed to make the data meaningful.

Investigating whether the risk related to sunburn is constant throughout life or whether a critical period exists during which exposure is more harmful, Weinstock (1989) [220] found significant positive effects with sunburns at ages "15-20" but not at age "30+" and these results are adjusted for skin sun sensitivity. The biological hypothesis is based on the idea that, among older subjects, the accumulation of UV may have induced skin damage over time and this may leave them more susceptible to the harmful effects of following sun exposure. Therefore one may speculate that young individuals, who experience severe, blistering sunburn, may show a short-term high risk of melanoma during those years. Those among them who do not develop melanoma at young age may subsequently tend to avoid sun exposure, thus we find a decreasing risk of melanoma at older ages, similar to those who never experienced sever sunburn whit blistering.[251]

It would have been useful to distinguish whether the influence of age may be explained looking at a specific and critical age related sensitivity to sunlight in childhood or to a difference in dose. In this meta-analysis it was not possible to go into much depth because few studies actually include this information. In order to take into account age effect, separate meta-analyses on sunburns in childhood and in adulthood were carried out.

As for sun exposure, measures adjusted for demographic factors, such as age and sex, and baseline characteristics, such as ethnic origin, skin pigmentation, were favoured, instead of measures adjusted for factors which themselves could be related to sun exposure,

such as number of naevi. The early influence of sun exposure may be related to the natural history of acquired naevi, whose frequency rises from childhood to peak in late adolescent and early adult life, and then diminishes in the late twenties and subsequently. If the number of naevi in young adults is related to previous sun exposure, UV may increase the frequency of naevi and also stimulate their dysplastic development. The adjustment for naevi may therefore not be appropriate because, if these relationships are true, it will diminish the true association, as naevi would lie in the causal pathway between sunburns and melanoma. Thus crude estimates are preferred to estimates adjusted for naevi. This choice has been evaluated in the sensitivity analyses.

5.3.7 Sunburn in childhood

5.3.7.1 Materials and methods

Seventeen papers regarding the association between melanoma and sunburn in childhood were found. Among these, four[225;290;291;299] were not independent. I finally arrived at thirteen eligible independent papers: twelve case-control studies and one nested case-control (Moore 1997[230]), which evaluated risk of all-body melanoma. Holly 1995[296] presented data concerning a population of only women.

Details of all these studies, including number of cases and controls, country of origin and definition of exposure, are given in Tables 5.7. The definition of an episode of "sunburn" varied considerably among studies (Table 5.8). When in a study more variables indicating sunburn experiences were published, the one that expressed the greater level of severity was chosen to minimize the chance of misclassification. For example, from "Sunburn with blistering" and "Painful sunburn" the first one was chosen. Zanetti[292] presented three estimates for sunburns in childhood: "often", "severe" and "yes". In order to be conservative last one was chosen because it presented the lowest value.

In this analysis "childhood" was defined as considering subjects of no more than fifteen years of age. However classification of periods of life varied a lot among publications, from use of specific age categories, to use of undefined periods such as "childhood", "adolescent" and "adulthood". Weinstock (1989)[220] was not included because the age period considered is "15-20" and it was not coherent with the other definitions of childhood sunburns.

5.3.7.2 Results

Estimates included for the calculation of the final pooled RR are plotted in the Figures 5.15, grouping in the same plot estimates with very wide confidence intervals to have more readable values. As can be seen, even if the Chi-squared test shows significant between study heterogeneity (Chi-squared=63.33, d.f.=12, $p<0.001$), there is convincing consistent evidence for a positive association between sunburns in childhood and melanoma. Random effects model gives an indication of a significant risk: RR=2.23 (95% C.I.: 1.54; 3.23).

5.3.7.3 Heterogeneity analysis

Meta-regression indicates that none of the study features seem to be statistically significant in explaining between study heterogeneity. However we have to take into account that the number of studies included in this meta-analysis is not large and it is difficult to find significant reliable results.

From meta-regression, adjustment fon any confounders does not seem to significantly explain variability among the estimates. However a history of sunburn indicates both an unusually intense exposure and skin sensitivity, and therefore studies which assessed sunburn while controlling for sensitivity, through a separate question on tendency to burn, are important. Looking at the influence of adjustment by sub-group analysis, on the fully adjusted

estimates, it was observed that heterogeneity is not any more significant (Chi-square=14.58, d.f.=9, p=0.10) for the subgroup of 10 studies that adjusted for phenotype. Furthermore, the pooled estimate from the fixed effects model is lower than the one obtained in the main analysis, but it still statistically significant RR=1.67 (95% C.I.: 1.39, 2.01). Only three studies did not adjust for phenotype and the pooled estimate from these three studies is not very reliable because it presents very wide confidence intervals (RR=1.96; 95% C.I.: 0.51, 7.61). If we look at the six studies that published two estimates, adjusted and not adjusted, we have results similar to the ones obtained for sun exposure. The pooled adjusted estimate, obtained with the fixed effects models (heterogeneity is not any more significant: Chi=7.61, d.f.=6, p=0.18 and Chi=6.48, d.f.=6, p=0.26, for adjusted and not adjusted respectively), is lower (RR=2.03, 95% C.I.: 1.43; 2.90) than the not adjusted one (RR=2.82, 95% C.I.: 2.02; 3.95). However the pooled RR remains highly significant.

When I considered the six studies that adjusted for phototype or other measures of skin sensitivity, again the pooled adjusted estimate, obtained with the fixed effects model (heterogeneity not significant: Chi=6.75, d.f.=5, p=0.24) is lower (RR=1.61, 95% C.I.: 1.24; 2.11) than the not adjusted one (RR=2.02, 95% C.I.: 1.01; 5.04). However the pooled RRs remains highly significant. Therefore this meta-analysis demonstrated a strong association between melanoma risk and sunburns history, which persisted after controlling for tendency to burn and other measures of skin sensitivity.

Only six studies published information on percentages of fair skin subjects in the sample under study. Looking at these percentages in cases and controls it was seen a highly significant difference (p<0001) in the percentages of fair skin subjects between cases and controls in the different studies (mean percentage=45.7 in cases and 27.2 in controls). However this difference is not significantly associated with the estimates, probably because the majority of them are adjusted for phenotype or phototype.

None of the studies adjusted for total sun exposure or chronic sun exposure and only one (Autier 1998[301]) adjusted for intermittent sun exposure.

5.3.7.4 Sensitivity analysis

The pooled RR calculated on fully adjusted estimates shows a decreased risk, even if still significant, for sunburns in childhood RR=1.87, (95% C.I.: 1.32, 2.67). Heterogeneity also decreases but remains highly significant: Chi-squared=48.65 with 12 d.f., p<0.001.

Holly 1995[296] presented data concerning a female population but the results from this study are not significantly different from the others.

A slight asymmetry can be observed in the funnel plot. However the sensitivity analysis on publication bias proposed by Copas and Shi evaluates a not significant p-value (p=0.36) for the fit to the funnel plot (Figure 5.16) suggesting a not significant effect of publication bias.

5.3.8 Sunburns in adulthood

5.3.8.1 Materials and methods

Thirty-five papers were identified on the association between melanoma and sunburn in adulthood. Ten studies[164;217;219;225;250;251;265;290;291;293;299], out of the thirty-five papers found, presented results on data already used in other papers. One study (Sorahan 1985[247]) was excluded because it did not publish information to calculate the variance of the estimates. I finally used twenty-five independent case-control studies (two were nested case-control studies: Weinstock 1989[220] and Moore 1997[230]) and twnty-six datasets because Mackie (1989)[183] presented estimates separately for sex. Holly (1995)[296] and Weinstock (1989)[220] analysed female populations. As for sun exposure, Chen (1996)[234] and Green (1986)[250] were not included for the calculation of the main pooled estimate because it was not possible to

extract an estimate not adjusted for naevi. A further pooled estimate with their inclusion is presented in the sensitivity analysis.

General features of studies included in the analysis are shown in Tables 5.9.

The variety of definitions for sunburns, considered by the authors, are presented in Tables 5.10. Those chosen for the meta-analysis are indicated in bold. As can be seen the definitions of an episode of sunburn vary considerably among studies. Sunburn was defined considering "blistering", "erythema", asking about type of pain, using a "vacation sunburns score", or evaluating simply "ever sunburns".

To assess the event in adulthood it was decided to include studies with clear indication that experiences occurred at major age (>19 years of age) or more general, when the exact age was not expressed. The choice to include both types of definitions was investigated in the heterogeneity analysis looking at latency period.

When papers presented more definitions of sunburn I included the ones with the widest period of exposure (for example, between "sunburns 5 years before diagnosis" and "sunburns 18-20 years before diagnosis" I have chosen the later) and the most severe sunburn description (for example, between "painful erythema" and "blistering sunburns" the latter was chosen). Grob (1990)[170] presented estimates for two quite comparable definitions of sunburn: "Frequency of sunburns per year in recent years: >2" and "Severity of sunburn in recent years: at least one severe burn". The first one was chosen because apparently the second one presented some inconsistencies with levels of sunburn severity assigned to people that never had sunburns.

Carli published estimates obtained by two different datasets, acquired from the same study, in two papers: the one published in 1999[219] included the data investigated in 1995[217]. In the 1999 Carli presented two estimates with huge confidence intervals, regarding sunburns in adulthood and in childhood: OR=2.7 (95% C.I.: 0.4; 29.4) and OR=4.9 (95% C.I.: 0.4; 265.0)

respectively. In 1995 Carli presented a more precise estimate concerning sunburns lifetime: OR=1.8 (95% C.I.: 0.6, 5.5). The reason for the considerable difference in the estimates was not worked out by looking at the difference in the sets of confounders (sex, country of birth and residence, for the study in 1995, and age, sex and level of education, for the study in 1999) and by looking at the categories that are compared ("Never, occasionally, easily, usually" in 1995 and "0, 1-2, 3-5, >5" in 1999). To be consistent with the previous choices the most recent study (Carli 1999[219]), which analyses the biggest number of cases and controls, was chosen for the main pooled RR, but this choice was investigated in the sensitivity analysis.

5.3.8.2 Results

Estimates, included for the calculation of the final pooled RR, are plotted in two Figures 5.17a and 5.17b. Highest estimates are grouped in the same plot, to be more readable. It can be noticed that all the estimates are greater or equal to 1 indicating a risk factor for adulthood sunburns and, even if the lowest limits of the confidence intervals are not all above 1, there is reasonable consistent evidence for a positive association between adulthood sunburns and melanoma.

The random effects model gives an indication of a significant effect (RR=1.84; 95% C.I.: 1.54, 2.20) and between-study heterogeneity is highly significant (Chi-squared=47.6 with d.f=22., p=0.001).

5.3.8.3 Heterogeneity analysis

Meta-regression indicates that the only characteristic explaining variability among the estimates is latitude: at higher latitudes we have a greater association between sunburns and melanoma (p=0.022). Latitude was calculated by looking at the city where the study is

conducted, whereas for studies performed on whole regions the average latitude was considered. When I categorized the variable "latitude", comparing the studies done in countries at latitude greater than 50 with the ones conducted in countries at latitude lower than 50 (see Figure 5.18), the p-value was even lower than considering latitude as continuous variable (p=0.005). The studies are quite well distributed in the two subgroups and the average latitude among all the studies is 48. Chi-squared tests, assessing heterogeneity within the two subgroups of studies, are not any more significant (Chi=14.82, d.f.=10, p=0.139 for latitude<50, and Chi=15.59, d.f.=10, p=0.148 for latitude>50). The pooled estimates, from the fixed effects model, is much higher for the studies at higher latitudes (RR=2.34; 95% C.I.: 1.93, 2.82) suggesting an higher risk for melanoma due to sunburns at higher latitudes. However for the studies carried out at lower latitudes the pooled estimate still indicates a significant association between melanoma and sunburns (RR=1.42; 95% C.I.: 1.23, 1.65).

One study (Weinstock 1989[311]) was not included in the heterogeneity analysis that evaluates latitude because it was carried out in different parts of the USA.

This finding may be explained taking into account that at higher latitudes the frequency of fair skinned people is greater and also intermittent sun exposure probably plays a special role inducing more easily sunburn episodes. Actually several authors[169;311;312] consider sunburn history to be an important indicator of intermittent sunlight exposure. Furthermore some suggest that the effects of intermittent sunlight exposure can be best studied in populations living at higher latitudes.[300,302] Elwood show that, for identical outdoor exposure patterns, an individual at higher latitude will receive a relatively higher amount of total ultraviolet dosage from the intermittent component of their outdoor exposure.[279]

Subgroup analysis shows that pooled estimates do not change significantly in terms of the different factors considered. However the Chi-squared, testing heterogeneity, becomes not significant for many subgroups of studies. As we have seen previously, lentigo maligna is

very probably associated with sun exposure and the inclusion of this melanoma type could introduce an overestimation of the relative risk. If we observe the Chi-squared for the sub-group of studies, which do not include acral and lentigo melanoma, we note that heterogeneity is not any more significant (Chi-squared=7.13 with d.f.=5, p=0.211; pooled RR=2.019 95% C.I.: 1.47; 2.76). Looking at other features, typical of well conducted studies, we can see that in the sub-group of the studies that used blinding for interviewers again the Chi-squared is not any more significant (Chi-squared=11.61 with d.f.=7, p=0.312; pooled RR=1.81 95% C.I.: 1.51; 2.16). Similar results are obtained for the subgroup of studies that use detailed definitions of exposure with a clear indication of latency period considered (Chi-squared=14.33 with d.f.=8, p=0.07; pooled RR=1.78 95% C.I.: 1.35; 2.35). In all these situations the estimates are significantly greater than one, suggesting a significant positive association with melanoma, and there is not indication of heterogeneity.

In only two papers (Dubin 1990[214]and Wolf 1998[298]) the authors stated that controls with dermatological problems were included and in one paper (Nelemans 1993[287]) the author wrote that other cancers were included among controls, but their estimates are very similar to the others.

As for sunburn in childhood there is a highly significant difference (p<0001) in the percentages of fair skinned subjects between cases and controls, in the thirteen studies that published this information (mean percentage=54.6 in cases and 39.4 in controls). However this difference is not significantly associated with the estimates and again the adjustment for phenotype or phototype, of the majority of them, probably has a significant effect on the estimates.

Looking at the influence of adjustment, on the estimates obtained from saturated models, similar results to the ones observed for sunburns in childhood were found. Meta-regression shows that the adjustment for phenotype and phototype does not seem to

significantly explain variability among the estimates. For the subgroup of ten studies that adjust for phototype or other measures of skin sensitivity, that are adjusted also for phenotype, the pooled estimate (RR=1.53 95% C.I.: 1.31, 1.78) is slightly lower than the one obtained on the sixteen not-adjusted estimates (RR=1.59 95% C.I.: 1.37, 1.85) but it still statistically significant. Both the pooled estimates are obtained with the fixed effects models because heterogeneity was not any more significant (Chi=14.30, d.f.=9, p=0.11 and Chi=20.06, d.f.=15, p=0.17, for adjusted and not adjusted respectively). Again we can see that the association between melanoma risk and sunburn history persists after controlling for tendency to burn and other measures of skin sensitivity.

Only one study adjusted for total sun exposure and intermittent sun exposure (Chen 1996[234]) and only one (Cristofolini 1987[235]) adjusted for intermittent and chronic sun exposure.

5.3.8.4 Sensitivity analysis

Two studies are notable: MacKie (1989)[183] presented a very high risk estimate (for men RR=9.3 95%; C.I.: 2.39; 24.95) and Autier (1998)[301] showed a very precise estimate, and then a considerable weight (Standard Error=0.150). In Mackie, the odd result is due to the fact that for the highest category of exposure (severe sunburn = "3+") the number of controls was zero. In Autier, on the other hand, analyses were based on a considerable number of subjects and on a high prevalence of exposure results. A further meta-analysis was carried out taking out MacKie (males estimates, 1989) and Autier (1998). We can observe a reduction of between-study heterogeneity, 34.9 with 20 d.f., p=0.02, whereas final risk estimate does not change significantly: pooled RR=1.79 95% C.I. (1.51; 2.12).

When the fully adjusted estimates are considered and Chen (1996)[234] and Green (1986)[250] are included in the analysis, a considerable reduction in between-study

heterogeneity can be noticed (Chi-squared=34.2 with 24 d.f., p=0.08). The pooled RR is very similar to the one obtained in the main analysis (RR=1.59; 95% C.I.: 1.38; 2.82).

Looking at the funnel plot (Figure 5.19) we can notice a clear asymmetry that suggests a possible problem of publication bias. Investigating publication bias with the sensitivity analysis proposed by Copas and Shi[52], a quite strong positive trend in the funnel plot was found, well fitted by the dashed line in Figure 5.20. In this figure, where the log-RRs are plotted against a measure of the uncertainty of those log-RRs (e.g. the standard deviation="s"), a trend can be seen with smaller studies (the ones on the right, with greater standard deviation) that give more positive results than larger studies. The dotted line is the usual random effects model. As the number of unpublished studies increases, and the probability of publication bias decreases, the estimate of the pooled relative risk becomes smaller. Thus the main pooled estimate calculated at the beginning is probably too high. However the precision of the studies is sufficiently strong for the overall evidence of a positive increased in risk and even if we assume 45 unpublished studies the pooled RR remains significant: 1.29 (with 95% C.I.: 1.07; 1.55). In this case the p-value for publication bias, calculated with the method proposed by Copas and Shi, is not any more significant (p=0.10).

When I evaluated the funnel plot looking at the studies grouped by latitudes, the indication of publication bias evidently decreases: for studies carried out at lower latitudes it is not any more significant. At higher latitudes sensitivity analysis proposed by Copas and Shi shows that, with only three unpublished studies added, the indication of publication bias disappeared but the pooled estimate does not present a considerable change: RR=2.18 (with 95% C.I.: 1.59; 2.98).

5.3.9 Discussion on sunburn

The greater danger to the validity of the results of meta-analysis on sunburns is misclassification of sunburn exposure. There is a strong possibility that the interpretation of sunburn has a cultural element, which is different among populations. Systematic misclassification also may occur among the studies as a result of different definitions of sunburns or according to constitutional factors, as skin colour, for example. Furthermore, as for the measures of sun exposure, systematic misclassification of sunburn exposure could occur in those studies where cases reported higher sunburn exposures than controls due to recall bias. However a population-based case-control study on melanoma, published in 1995, looked at the reliability of reported sunburn history. One hundred subjects were interviewed in 1987 and in 1989 and re-interviewed after more than 10 years and the results excluded a significant effect of recall bias.[313]

The greater consistency of a positive association for sunburn, compared to that with intermittent exposures, may indicate a specific relationship of melanoma with sunburn per se, or it may be that sunburn is simply a more easily remembered measure of intermittent exposure to the sun.[169;311] A relationship between sunburns and intermittent sun exposure is suggested also by the association, that we found, between sunburn and latitude, but it is not easy to understand if sunburn is an indicator of intermittent sun exposure and it lies in the causal chain with melanoma.

Tanning ability is one of the pigmentary characteristics that very likely has a modification effect on the relationship between sun exposure measures and the risk of melanoma. Subjects with little or no ability to tan usually exhibit higher risk relative risk, for sun exposure and for history of severe sunburn than good tanners. For good tanners, moderate exposure very likely is protective against melanoma, and excessive sun exposure increases the risk but not as high as for poor tanners. One can in fact speculate that tanning may confer a protecting effect on the skin and that moderate sun exposure may actually protect against

melanoma by promoting a tan in individuals who do so easily. However heterogeneity analysis suggests that the association between melanoma risk and sunburn history may persist after controlling for tendency to burn and other measures of skin sensitivity. Probably excessive sun exposure overwhelms the protective mechanism provided by tanning.

Even if pooled estimates for sunburns in childhood are higher than pooled estimates for sunburns in adulthood, the difference among them was not considered valuable. To make an evaluation on more comparable estimates a further analysis was carried out on the eleven studies that published both estimates, in childhood and in adulthood. As can be seen in Figure 5.21, in nine, out of eleven studies, the estimates are higher in childhood than in adulthood. The estimates are higher in adulthood in two studies: Osterlind (1988)[226], but the values are very similar, and Westerdhal (1994)[293] who presented an estimate for sunburns in childhood equal to 1. Meta-regression shows not significant difference among the estimates of the two groups (p=0.338).

The bivariate approach, on these pairs of estimates, indicates a slightly higher risk for sunburn in childhood (RR=1.87 and 95% C.I. 1.27; 2.75) than sunburn in adulthood (RR=1.45 and 95% C.I. 1.16; 1.82) but both RR suggest a significant association between melanoma and sunburns. Measures of the correlation between the estimates of sunburn in childhood and in adulthood were not available. Therefore the bivariate model was built assuming independence. From the model an estimate of the correlation equal to 0.17 was found suggesting that independence is not a trusty hypothesis. As for the univariate analysis, meta-regression with the bivariate approach shows that latitude and adjustment for phenotype (for sunburn in adulthood) are factors that significantly explain between-study heterogeneity. No significant differences between sunburns in adulthood and in childhood were found also in two meta-analyses[24,28] and one overview.[280] However in the recent work published by

Whiteman[28] conclusions are somewhat different because the author, looking at ecological studies, suggests a special role of sunlight in childhood.

In these comparisons it should be taken into account that there is some evidence that self reported childhood sun exposure is less reproducible than sun exposure at older age and this may question the veracity of comparisons of period-specific exposure for sunburns.[282]

5.3.10 Conclusions on sunburns and sun exposure

Given these results showing the link between sun exposure, sunburns and melanoma, the first step in photoprotection should be to reduce exposure to sunlight sufficient to cause sunburn. However avoidance of sunburn may be a confusing target for education campaigns, because we cannot exclude the idea that sunburns may be merely an indicator of the combination of susceptibility to sun and intermittent exposure.

This is an important concept as sunscreens filter out only a proportion of the solar spectrum and could allow prolonged sun exposure, a situation that could lead to increased melanoma.[301] It is in fact suggested that the use of sunscreens may fail to achieve sun protection if the behavioural change, which they produce, is increased suntan. People should be advised that the use of sunscreen may prolong recreational sun exposure, because the marketing of these products persuades that excessive sun exposure is safe with their use[314]. In reality it is not clear what exactly could be the benefit of sunscreen against the risk of melanoma: whereas it may be used to block certain types of ultra-violet light from reaching the skin, it may allow an increased exposure to other types which may be harmful.[152] A broader message to reduce total sun exposure, by changing patterns of outdoor exposure, using clothing and shade, may be more effective. However, we should think that reduction of exposure to sunlight, particularly only an internediate reduction, may actually increase the risk of melanoma by changing someone from a regular frequent exposure pattern to an

episodic exposure pattern. Thus the finding that regular frequent exposure to sunglight might be associated with a lower relative risk of melanoma should be taken into account in public health programs. Furthermore, it is important to remember that sun exposure shows a protective effect against many other types of cancer.[315-319] The lack of sufficient UV-B radiation is considered the cause for a large proportion of twelve types of cancers.[320;321] The ideal case would, therefore, be to find a way of obtaining the beneficial effects of UVB exposure without suffering the increased incidence of skin cancer. Thus, further studies on the relationship between vitamin D, regional UV doses and the rates of different types of cancers should be worthwhile.

5.4 Family history of melanoma

5.4.1 Introduction

The first report in malignant melanoma was that of familial melanoma, documented in 1820.[322] Subsequently several other investigations on familial melanoma were published. At present a family history is considered an extremely important risk factor for cutaneous malignant melanoma and researches into potential melanoma susceptibility genes are ongoing. Approximately 8-12% of melanoma patients has a family history.[323]

It was reported worldwide that persons with the atypical mole (dysplastic nevus) syndrome are at much higher increased risk. Families with multiple cases of melanoma often exhibit the dysplastic nevus syndrome, a syndrome characterized by multiple atypical moles that continue to appear in adulthood. Greene (1985)[324] estimates that a person who has dysplastic naevi and at least two family members with melanoma has a 500-fold increase in melanoma risk. However so few people have this syndrome that in unselected series they account for less than 5% of total melanoma incidence. Furthermore one must take into

account the fact that in many of these families dysplastic naevi as well as environmental factors are involved.

As I said previously, in this work I considered family history alone, without the contemporaneous presence of dysplastic naevi.

5.4.2 Materials and methods

Thirteen publications were identified on the association between family history and melanoma. Among them two[225;250] were not independent. I determined eleven eligible independent case-control studies that evaluated risk of melanoma in all body. Holly 1995 [296] analysed a female population.

The results of each study were transformed in order to obtain a dichotomous exposure: subjects were classified as having a positive family history of melanoma if they reported one or more affected first-degree relatives. Some studies also collected information on more distant relatives but this information is unlikely to be complete and has not been analysed in this work.

Estimates and some information for Holly (1987)[169], Swerdlow (1986)[168] and Walter (1999)[325] were obtained from the meta-analysis on individual data published by Ford (1995). In Swerdlow, controls did not have any subject with family history, therefore 0.5, instead of 0, was assigned into the cell of the 2x2 table used for calculation of the estimates.

Some general features of the studies included in the meta-analysis are presented in Tables 5.11.

5.4.3 Results

Estimates, adjusted for the maximum number of confounders, are presented with their confidence intervals in two Figures 5.22a and 5.22b, to be more readable.

The fixed effects model was used to pool the estimates because between-study heterogeneity was not significant (Chi=9.567, d.f.=10, p=0.48). The pooled estimate (RR=1.79, 95% C.I.: 1.46; 2.19) indicates a highly significant association between family history and melanoma and this is consistent with the pooled analysis on individual data published by Ford (1995) (RR=2.24, 95%CI: 1.76-2.86). For Ford's meta-analysis, which include studies published before 1990, strict inclusion criteria were considered. The authors stated that they had included in the analysis melanoma case-control studies "which had included an independent physical examination of naevi by a trained individual, for which data collection was completed and where cases and controls were treated similarly". Even though the subset of studies considered for the analysis was not large (only 8 studies) between-study heterogeneity was found to be significant for some factors and in these cases the pooled estimate was not calculated. Inconsistencies and differences in patterns of estimates were not investigated.

5.4.4 Sensitivity analysis

The analysis was repeated on not adjusted estimates in order to evaluate effect of confounders but the results are very similar. Fixed effects models are used to obtain the pooled estimate (RR=1.87, 95% C.I.: 1.55; 2.27) because heterogeneity was not significant (Chi=11.61, d.f.=10, p=0.31).

The funnel plot (Figure 5.23) seems clearly asymmetrical suggesting a significant effect of publication bias. However both methods proposed by Copas and Shi and Egger tests give indication of no significant effect of a possible publication bias.

5.4.5 Discussion on family history

The familial relative risk was quite similar in all studies, like in Ford's meta-analysis[27], suggesting similar risks for family history even in completely different countries, which present a variability of 10 fold of incidence rates. This provides an indication for the hypothesis of independence between sun exposure and genetic susceptibility. However Siskind, in a recent publication (2002)[326], found a significant interaction between the effect of sun exposure on melanoma risk and familial susceptibility to melanoma. He suggested that melanoma may develop in a susceptible subset of the population who receives a threshold UV dose and that high genetically susceptible subjects have a lower threshold and reach this earlier than others in the same environment. In area of high solar radiation, within high-risk families, genetic factors rather than differences in sun exposure determine who will get the disease. Within families at relatively low genetic risk cumulative sun exposure is likely to be a more important determinant of melanoma risk.

5.5 Indicators of Photodamage

5.5.1 Introduction

Solar keratoses are benign tumours that are at least ten times commoner than skin cancers and premature skin ageing (photoageing of the skin) is even more common.[327] Melanoma-susceptible individuals may have two distinct cutaneous phenotypes: one associated with multiple naevi, without signs of marked photoageing, the other characterized by solar damage with actinic keratoses but no excess of naevi.[328]

Actinic keratoses are also called solar keratoses because they indicate that sun damage has occurred. They are precursors of cancers, which means they can be the first step in the development of skin cancer. It is estimated that up to 10 percent of active lesions will take the next step and progress to squamous cell carcinoma.[328]

As indicators of actinic damage I considered the presence of lesions as solar lentigo, elastosis, actinic keratoses, and others objective measurements of actinic damage as cutaneous microphotography. Cutaneous tumours, different from melanoma, are also included because European cancer registries demonstrated an increased incidence of melanoma following an initial diagnosis of squamous cell skin cancer (SSC) and basal cell skin cancer (BSC).

5.5.2 Materials and methods

Among the nineteen papers that were found on the association between indicators of actinic damage and melanoma, five[164;225;251;284;300] reported results concerning data already published in other articles. I finally arrived at fourteen independent papers on eleven case-control studies (Moore 1997[230] is a nested case-control study) and three cohort studies. One of the cohort studies (Lindelof 1991[329]) presented results separately for women and men. All papers analysed the risk associated with melanoma on all the body.

Some general features of the studies included in the analysis are listed in Table 5.12.

Definitions adopted by the various authors, presented in Table 5.13, are classified into two groups:

-Pre-Malignant and Cancer Lesions (PMCL): actinic keratoses and other cutaneous tumours were included in this group.

-Other Indicators of Actinic Damage (OIAD): solar lentigo, elastosis and indicators found by cutaneous microphotograph were included in this group.

As can be seen in Tables 5.13, definitions used by the authors of the papers are very mixed. Some of them are very precise, where the extent of actinic damage is measured by a technique (CMT) that takes an impression of the skin texture and it is graded using a microscope. Other works use much more rough definitions quantifying lentigines with very broad categories, as “any” versus “none” or “many” versus “few”, or considering only the

presence of “splotchy freckles”. Furthermore it must be remembered that the distinction between solar lentigines and freckles is not always straightforward in case-control studies on melanoma and this may be a further cause of bias.

From the fourteen datasets found, I obtained 18 estimates, adjusted for the greater number of confounders, on “pre-malignant and cancer lesions” (n=10) and on “other indicators of actinic damage” (n=8).

5.5.3 Results

ORs and RRs for the presence of pre-malignant cancer lesions and other indicators of photodamage, are plotted in three Figures 5.24 and 5.25, with their confidence intervals. From these extracted estimates a strong positive association with melanoma is suggested. The calculation for the pooled RR confirms this indication: 2.98 (95% C.I.: 2.04; 4.36), for the presence of indicators of actinic damage considered all together. However there is a very high value for the Chi-squared (Chi=141.62, d.f.=17, $p<0.001$) revealing highly significant heterogeneity.

Looking separately at the two main sub-groups of studies, for “pre-malignant and cancer lesions (PMCL)” I obtained a very high estimate (RR=4.33 with 95% C.I.: 2.74; 6.82) which is more than twice that calculated for “other indicators of actinic damage (OIAD)” (RR=1.96 with 95% C.I.: 1.06; 3.61). The difference between the two is significant at 8%. However heterogeneity remains highly significant with $p<0.001$ for both groups (Chi=44.58 with d.f.=9 and Chi=65.65 with d.f.=7, for PMCL and OIAD respectively).

5.5.4 Heterogeneity analysis

“Matching” can be considered a marker of fine design. In fact when confounding cannot be controlled by randomization, individual cases may be matched with individual controls that

have similar confounding factors, such as age, to reduce the effect of the confounding factors on the association being investigated in analytic studies. However matching can be counterproductive if one matches in a case-control study on strong correlates of exposure in the base population that are not risk factors (or proxy risk factors) for the disease. This type of overmatching results in a decrease in statistical efficiency (i.e., less precision for a given number of cases and controls) The problem is that the net effect of matching in case-control studies (but not in cohort studies) is to introduce selection bias that must be controlled in the analysis.

A considerable reduction in heterogeneity, among OIAD studies, can be observed when I classified studies by "matching". Heterogeneity analysis shows that Chi-squared indicates not significant heterogeneity in both sub-groups of studies, matched (Chi=3.21; d.f.=4; p=0.52) and unmatched (Chi=0.59; d.f.=1; p=0.79). The latter was calculated excluding one odd estimate (Halpern, 1991[238]), which increases heterogeneity very much. Pooled estimates of the two subgroups, calculated with the fixed effects model, are quite different: for matched studies I obtained a pooled estimate significantly (p=0.01) greater (RR=3.47 with 95% C.I.: 2.57; 4.69) than for unmatched studies (RR=1.35 with 95% C.I.: 1.06; 1.71). In all studies matching was carried out by age, sex and place of residence and estimates were obtained by a conditional logistic model adjusted for some important confounding variables. Thus there not seem to be a problem of overmatching and the analysis looks well conducted.

These findings, obtained exploring heterogeneity, should be interpreted with concern because the number of studies is very low and there may be a problem of multiple testing. However the decrease in heterogeneity in the subgroup of matched studies is an indication suggesting that the pooled estimate is reliable and that the association between melanoma and "pre-malignant and cancer lesions" and "other indicators of actinic damage" is significant.

5.5.5 Sensitivity analysis

Halpern (1991)[238] presented a very odd estimate. It is the only estimate indicating a protective effect of the indicators of photodamage and the RR is also significant. If I exclude this study from the subgroup of OIAD I can observe a considerable reduction in heterogeneity, which, however, remains highly significant (Chi=27, with d.f.=6, $p<0.001$) and the increase in the pooled estimates leaves a considerable relative risk (RR=2.60 with 95% C.I.: 1.66; 4.09).

Lindelof (1991)[329]) presented results separately for sex but the confidence intervals of the RRs overlaps indicating not statistically significant difference between men and women.

There is some indication for publication bias when I considered PMCL together with OIAD (Figure 5.26). However with only three added publications the p-value for the funnel plot is no longer significant and the pooled estimate does not change very much: (RR=2.18 with 95% C.I.: 1.59; 2.98). If the analysis is carried out separately, for the two main sub-groups of indicators of photomage, the suggestion for publication bias disappears.

5.5.6 Discussion on indicators of photodamage

It is important to take into account that the apparent association of melanoma with some indicators of photodamage may be due to intensified medical surveillance in patients with a history of other cancers, a shared internal pathway of cancer induction and adverse effects of agents used in the treatment of non melanoma skin cancers.[330] A shared internal pathway of induction may be important in the association of actinic keratoses and photoageing with melanoma. Actinic keratoses, for example, are more common in fair-skinned, fair-haired, light-eyed individuals subjected to high levels of sun exposure. Because their skin has less protective pigment, they are the most susceptible to sunburn that is strongly associated with

solar keratoses.[331] Strong association between degree of photoageing and lifetime sun exposure has also been found in extensive longitudinal studies conducted in Australia.[327]

5.6 Host factors

5.6.1 Freckles

5.6.1.1 Materials and methods

Thirty-two studies recorded an assessment of individual propensity to freckles and melanoma. Eight of them (seven original works[164;217;224;225;250;251;332] and one revision of literature[25]) showed results already analysed in other publications. I finally arrived at twenty-four independent case-control studies (Moore 1997 is a nested case-control study). Mackie (1989)[183] presented estimates separately for sex, Chen (1996)[234] separately for four body sites (head and neck, upper limbs, lower limbs and truck). Hezerfeld (1993)[295] analysed a male population and Holly (1995)[333] a female population.

Some general features, definitions and categorizations used by the authors are described in Tables 5.14 and 5.15. As can be seen measurements and classifications of freckles density varied substantially between studies. Classification of freckling varied from 2 to 5 categories; some authors were interested in a classification between people with freckles and those without freckles and others evaluated their intensity with more precise categorization. Some authors asked about freckles after sun or on summertime (Chen 1996[234], Dubin 1986[164], Elwood 1986[236], Marret 1992[186] and Moore 1997[230]). Some of them used very detailed definitions (Holly, 1987[169]) or diagrams for comparison (Grulich, 1996[171]), some others used a general indication of "freckles". Some authors asked about freckles as adult (Holly 1995[333] and Osterlind 1988[226]) some other as child or teenager (White 1994[249], Grulich 1996[171], Gallagher 1986[332] and Chen 1996[234]).

Elwood in 1986[236] and in 1990[237] published two different estimates, evaluating freckles as adult and in childhood. Freckles in adulthood is the definition chosen for the main analysis, as I did in the rest of the analysis, thinking that more recent information is probably more reliable. However these estimates present huge confidence intervals whereas RRs for freckles in childhood are much more precise. Therefore the choice to include Elwood's estimate on freckles as adult was evaluated in the sensitivity analysis.

In order to reduce the problem of misclassification bias the estimates extracted are those comparing highest categories versus lowest categories, as I did for the other risk factors. However this choice was checked in the sensitivity analysis because many authors consider only two rough categories. A further pooled estimate was calculated reducing to a dichotomy the larger number of groupings. Cases and controls from the categories defined as "none, few, sparse, absent, no" were included into the category "none/few", and the subjects belonging to categories defined as "some, many, moderate, yes, present" into the category "many/some". Thus we can make a comparison with results of the meta-analysis on individual data published by Bliss[25].

Many of the papers presented both estimates: for dichotomous exposure and for a higher number of categories. When the former was not published it was calculated from the raw data.

To be able to evaluate influence of adjustment for various confounders, a further pooled estimate was obtained considering only the raw data in the calculation of the pooled RR for a dichotomous exposure.

5.6.1.2 Results

ORs adjusted for the maximum number of confounders are presented in Figures 5.27, with their confidence intervals. As it ca be seen, for several studies, the risk for high density of

freckles is 2 to 3 times greater than the risk of having no or sparse freckling. In fact the pooled estimate, obtained from the random effects model, indicates high density of freckles as a highly significant risk factor: (RR=2.11; 95% C.I.: 1.76; 2.53). However the between-study heterogeneity is considerable (Chi=71.03; d.f.=24; p<0.001)

Chen (1996)[234] presented only estimates separately for the four body sites, and not raw data. However, the four estimates being very similar (Chi-squared is 0.38 with 3 d.f., p-value for heterogeneity is 0.94), a weighted average of the four estimates was included. This decision was made in order not to give too much weight to this study, mainly in the subgroups analysis that investigates heterogeneity. The choice of including only a weighted average of the four estimates was evaluated in the sensitivity analysis.

5.6.1.3 Heterogeneity analysis

Interesting results, similar to sunburns and chronic sun exposure, were found for freckles density from meta-regression analysis. Two factors, "Latitude" and "country", significantly explain variability among the estimates. In Figure 5.28 and in Figure 5.29 histograms present the studies by country (classified in two broad categories: "at higher" and "at lower incidence") and latitude (higher and lower than 50). As can be seen, at high latitude and in "low incidence" countries the estimates are higher albeit less precise.

Looking at study location, I obtained similar results to the ones found for chronic sun exposure (see Figure 5.28). Considering the subgroup of fourteen studies coming from Australia and USA (classified as "high incidence" countries), the pooled estimates for high density of freckles (RR=1.72; 95% C.I.: 1.42, 2.09) is significantly lower (p=0.006) than the one obtained for the other countries (RR=3.21; 95% C.I.: 2.29, 4.46). The Chi-squared indicates still significant heterogeneity within the 14 "high incidence" countries (Chi=33.56 and d.f.=13; p=0.001) and within the others (Chi=24.53 and d.f.=10; p=0.006).

The distribution of freckles in cases and in controls, in the studies that published this information, was investigated to see if it might be a different distribution by country. The percentages of subjects in the highest category of freckles and in the lowest category, calculated on the total number of subjects included in the study, were evalauted for the various countries. Percentages in highest categories of papers published "high incidence" countries were found not significantly different from the percentages in the highest categories of other countries. This could suggest that the indication for a different estimate in "high incidence" countries may be mainly due to different study features. However we should be carefull in the interpretation of these results, because the same highest categories (usually it is "many freckles") may have a different meaning in "high incidence" countries, where fair-skinned people are characterized by high frequence of freckles, compared to other countries.

As for chronic sun exposure, an indication for a lower RR should be considered carefully because RRs obtained from "high incidence" countries are probably the most reliable. The vast majority of the studies conducted in these countries does not in fact include subjects with dermatological problems (only 1 in 14); in most of them (10 out of 20 studies) controls are drawn from community; the vast majority of studies that adopts blinding for interviewers is in high incidence countries; almost 50% of them adjust for phototype and phenotype and the few studies that adjust for family history or sunburns are among them.

As can be seen in plot 5.29, at latitudes greater than 50 the association between freckles and melanoma is significantly ($p=0.04$) greater (RR=2.81; 95% C.I.: 2.02; 3.91) than at lower latitude (RR=1.83; 95% C.I.: 1.45; 2.31). In both sub-groups, heterogeneity decreases but it is still highly significant (for "latitude>50": Chi=23.59, d.f.=9; $p=0.005$; for "latitude<50": Chi=40.75, d.f.=13; $p<0.001$). Tucker (1997)[172] was not included, when the factor "latitude" was explored in the heterogeneity analysis, because it was carried out in two

very distant cities (Philadelphia and S. Francisco). This finding may be related to a higher frequency of people with fair phenotype at higher latitude, as I suggested for sunburns.

Bliss (1995)[25] found that freckle density at young ages defines a more extreme phenotypic risk group than at older ages. In this analysis I was unable to analyse the interaction with age. The only observation that I could make regards the influence of adjustment for some confounders and on the latency period considered. Few studies (n=8) evaluate the risk for high density of freckles in childhood, but for this subgroup of studies the pooled RR (RR=1.82; 95% C.I.: 1.56; 2.11; Chi=14.25; d.f.=7; p=0.16), is not significantly (p=0.75) different from the RR calculated for high density freckles in adulthood (RR=2.15; 95% C.I.: 1.69; 2.74; Chi=57.70; d.f.=17; p<0.001). As in the meta-analysis published by Bliss, phenotype and phototype do not seem to play an important role because adjustment for these confounders does not influence significantly the estimates for freckles density.

5.6.1.4 Sensitivity analysis

When the dichotomous exposure is considered ("none/few" versus "many/some" freckles), I obtained results very similar to the ones obtained in the meta-analysis on individual data published by Bliss[25]. The subjects with medium-high density of freckles are at very high risk, compared to subjects with medium-low density of freckles (RR=2.16; 95% C.I.: 1.86; 2.51). Between-study heterogeneity is still very elevated (Chi=86.89; d.f.=24; p<0.001).

In order to evaluate effect of adjustment, the pooled RR on the dichotomous exposure is calculated considering raw data. I observed a higher RR, but the change is not considerable (RR=2.33; 95% C.I.: 1.98; 2.75; Chi=111.46, with d.f.=23; p<0.001). For this last estimate Chen has not been included because raw data were not available from this paper.

As mentioned previously, Elwood in 1990[237] and in 1986[236] presented two estimates concerning freckles in adulthood and in childhood and the first ones were included in the

main analysis. If estimates evaluating freckles in childhood are considered for the pooled RR, results do not change notably (RR=2.13; 95% C.I.: 1.77; 2.57). Chi-squared decreases but the between-study heterogeneity remains highly significant (Chi=77.36; d.f.=24; p<0.001)

When the estimates published by Chen (1996)[234], on the four body sites considered separately, are included as they are, without any weighting average, a considerable change was not found (RR=2.06; 95% C.I.: 1.74; 2.45). Chi-squared decreases a bit but the between-study heterogeneity remains highly significant (Chi=71.41; d.f.=27; p<0.001)

Looking at the funnel plot a clear asymmetry can be observed (Figure 5.30). From the sensitivity analysis, investigating publication bias, proposed by Copas and Shi, we have a suggestion of a significant effect of publication bias. The publication probability becomes 1 (no bias) if we add 7 unpublished studies and, in this case, the pooled RR decreases. However the pooled RR remains highly significant (RR=1.99; 95% C.I.: 1.48; 2.68)

It is interesting to note that from the analysis on publication bias, as from heterogeneity analysis on "countries", we have an indication to take into consideration an estimate of risk lower than the main pooled Relative Risk found in "Results" section (RR=2.11; 95% C.I.: 1.76; 2.53).

Looking at the weights of the study estimates it can be seen that there is a very influential study (Holly, 1995[333]). The weight of the estimate extracted from this study is greater than 80. However the pooled RR, calculated with the exclusion of it, does not change very much (RR=2.16; 95% C.I.: 1.78; 2.63; Chi=67.25; d.f.=24; p<0.001)

5.6.2 Eye colour

5.6.2.1 Materials and methods

Thirty-four papers published information on eye colour and melanoma. Five[164;217;225;251;332] among them analysed data already used for other publications. I finally arrived at twenty-nine

independent case-control studies (Moore 1997[230] is a nested case-control study) and thirty datasets because Graham (1985)[286] published estimates separately for men and women. In all studies, except that of Chen (1996)[234], risk associated with melanoma in all body was evaluated. Hezerfeld (1993)[295] presented estimates only for men and Holly (1995)[333] only for women.

General features of all studies included in the analysis are reported in Tables 5.16 and categories and descriptions of assessment for eye colours are presented in Tables 5.17.

Classification of eye colour was very similar across studies. The majority of the authors considered three qualitative categories: "black/brown", "blue or blue/grey" and "green or green/grey/hezel". It was difficult to define a single category of colour at consistent higher risk. However the main analysis and the investigation for heterogeneity were performed on "blue eye" because more studies were available (30 versus 21 for "green eye"). Results on "green eye" are presented in the sensitivity analysis.

As mentioned previously, Chen (1996)[234] presented only estimates separately for the four body sites. However, these estimates are very similar (Chi-squared is 1.23 with 3 d.f., p-value for heterogeneity is 0.74), therefore, in order to not give to much weight to this study a weighted average of the four estimates was included. This choice was checked in the sensitivity analysis.

5.6.2.2 Results

Extracted OR and corresponding confidence intervals are presented in two plots (Figures 5.31). As can be seen, all of them indicate a positive association between blue eye colour and melanoma and the majority is statistically significant.

The pooled estimate indicates that subjects with blue eyes are at higher risk than subjects with dark eyes (RR=1.51; 95% C.I.: 1.30; 1.76). Even if the methods of assessment

and the categories used to classify eye colour are quite consistent, a considerable between study heterogeneity is indicated by the high significant value of the Chi-squared (Chi=94.53; d.f.=29; p<0.001).

5.6.2.3 Heterogeneity analysis

Adjustment for phenotype explains some variability between the estimates. In Figure 5.32 indication of adjustment is presented for each estimate comparing "blue versus black" eye colour. Similarly to the results obtained in the meta-analysis published by Bliss[25], we can notice that not adjusted estimates are higher than adjusted estimates. In fact seven, out of nineteen, not adjusted estimates are around two or above. Meta-regression indicates an almost significant difference between adjusted and not adjusted estimates (p=0.059). Furthermore heterogeneity, in the subgroup of estimates adjusted for phenotype, is not any more significant, whereas for not adjusted is still highly significant (Chi=8.6, d.f.=10, p=0.57 for adjusted and Chi=79.43, d.f.=18, p<0.001 for not adjusted). The pooled RR from the fixed effects model on estimates adjusted for phenotype (hair colour, freckles, skin colour…) are lower (RR=1.25, 95% C.I.: 1.11; 1.42) than the not adjusted estimates (RR=1.68, 95% C.I.: 1.34; 2.11). This suggests a considerable correlation between these host factors.

Only five studies adjust for a measure of sun exposure, as intermittent, chronic sun exposure or sunburns, and five studies adjust for naevi. However these adjustments do not seem to influence significantly the estimates.

Heterogeneity Chi-squared test is highly influenced by one study (Osterlind, 1988[226]), which presented an estimate below one (suggesting a negative association) and a very high weight (w=113.6), and by two studies (Dabkowski, 1997[188] and Beitner, 1990[334]), which showed estimates above one and not too big weights (w=41.2 and 11.2). Excluding these three estimates the heterogeneity is not any more significant for both the analyses.

5.6.2.4 Sensitivity analysis

The analysis conducted on “green” versus “dark” eye colour leads to considerations similar to the previous results on “blue” eyes. The pooled RR suggests “green” eye colour as a significant risk factor (RR=1.41, 95% C.I.: 1.14; 1.75 with Chi=57.17; d.f.=20; $p<0.001$).

Very similar results are obtained in the heterogeneity analysis performed on “green” versus “black”. As we have seen for “blue”, the pooled relative risk (RR=1.15, 95% C.I.: 0.95, 1.41) on estimates adjusted for phenotype is lower than that on not adjusted estimates (RR=1.54, 95% C.I.: 1.15; 2.07), and it is not significant. However the difference between the two estimates is not statistically significant. The pooled RR, calculated on adjusted estimates, is obtained with the fixed effects model because heterogeneity was not significant (Chi=8.06, d.f.=6, p=0.233).

As was the case for freckles, a pooled estimate was calculated reducing to a dichotomy the larger number of groupings. Data from the categories defined as “black” and “brown” were included into the category “dark”, and data belonging to the categories described as “hazel”, “green”, “grey”, “blue” into “light”. The calculations on crude data, when they were available, show that the pooled RR does not change significantly (RR=1.56, 95% C.I.: 1.36; 1.76; Chi=67.05; d.f.=25; $p<0.001$). Chen (1996)[234], Holly (1995)[333], Holman (1984)[150] and Beitner (1990)[334] could not be included in this pooled RR.

Graham (1985)[286] published estimates separately for sex, with slightly higher estimates for men, but the difference was not statistically significant.

When the four estimates published by Chen (1996)[234], for the four different body sites, are included in the analysis, as they are, the pooled relative risk does not change significantly (RR=1.53, 95% C.I.: 1.34, 1.76; Chi-squared=95.59, d.f.=32; $p<0.001$).

No indication for publication bias was found (Figure 5.33).

5.6.3 Hair colour

5.6.3.1 Materials and methods

There are forty identified publications, which analysed association between melanoma and hair colour. Five[164;212;217;225;251;332] of them were not independent from other studies. I finally arrived at thirty-five case-control studies (Weinstock 1991[335] and Moore 1997[230] are nested case-control studies) and thirty-six datasets, because Graham presented estimates separately for sex. In all but Chen (1996)[234], risk associated with melanoma in all body was evaluated.

General features of studies included in the analysis are shown in Tables 5.18. Definitions, categorization and methods of assessment of hair colour are presented in Tables 5.19. As can be seen definitions and assessments vary considerably between studies. In some studies the interviewer was a dermatologist or trained physician, in others a general questionnaire was used; some authors referred to independent colour or density charts, for classification of pigmentary characteristics, whereas in others broad categorical rating was used. Some studies asked about a natural hair colour, other simply about hair colour; some questions were about adult hair colour (at time of interview), others about hair colour at 20 years or in childhood. Lock-Anderson (1998) presented two estimates, as adult and before age of 7; hair colour as child was chosen because the majority of the studies considered hair colour before age of 20.

As for eye colour, classification of hair colour was quite similar across studies. The majority of the authors considered three main categories for hair colour: “black or brown”, “blond, fair or auburn” and “red or blond-red”. Generally people with “red or blond red” hair were considered at higher risk than “blond, fair or auburn”. Actually the risk for “red” versus “black” hair colour was evaluated in all thirty-five studies, whereas “blond” versus “black” was calculated in only twenty-seven studies. For this reason the main analysis was conducted

for “red” versus “black” estimates. In the sensitivity analysis further investigations on “blond” hair were carried out.

Chen (1996)[234] only presented estimates separately for the four body sites. However, these estimates are very similar (Chi-squared is 2.79 with 3 d.f., p-value for heterogeneity is 0.42), therefore, in order to not give to much weight to this study a weighted average of the four estimates was included. This choice was checked in the sensitivity analysis.

5.6.3.2 Results

OR are presented with their confidence intervals in Figures 5.34. The pattern of risk of melanoma by hair colour is reasonably consistent across studies and red hair is shown to be significantly associated to melanoma. In the vast majority of the studies statistical significance is reached.

The pooled estimate indicates that subjects with “red” hair colour are at significantly higher risk than subjects with “dark” hair colour (RR=2.79, 95% C.I.: 2.15; 3.61). This association is much stronger than that observed for eye colour. However, a very high between study heterogeneity was found (Chi=135.82; d.f.=35; $p<0.001$).

Hair colour was also considered on an ordinal categorical scale and the possibility of a trend across hair colour categories was investigated. In order to apply this model the data were categorised into four distinct groups, corresponding to the levels of an ordinal categorical variable: black = 0; brown = 1; blond = 2; red = 3. A meta-analysis, on the fourteen publications that reported estimates for all four categories, was carried out. The approach of pooling, proposed by Greenland and Longnecker[72], described in Chapter 2, was used to take into consideration correlation between cateogories. As can be seen from Table 5.20, the pooled estimates showed a marked positive trend and subjects with red hair are clearly at greater risk compared to subjects with brown and blond hair. The random effects

model was used for the calculation of the pooled RR because a significant heterogeneity was found (Chi=63.11; d.f.=13, p<0.001).

5.6.3.3 Heterogeneity analysis

Heterogeneity become any more significant when two studies (Beitner, 1990[334] and Grob, 1990[170]), which presented very large estimates, were excluded from the main analysis.

From meta-regression it was found that estimates adjusted for phenotype or for phototype are almost significantly (p=0.059) lower than the ones not adjusted. In fact we can see in Figure 5.35 that the not adjusted estimates are very high and nine (out of twenty-one) are around or above 4. The pooled RR, calculated with the fixed effects model on the estimates adjusted for phenotype or photoype (hair colour, freckles, skin colour, skin reaction, skin type…), is lower (RR=2.08, 95% C.I.: 1.61; 2.69 and Chi=19.97, d.f.=13, p=0.10) than the one, from random effects model, on not adjusted estimates (RR=3.51, 95% C.I.: 2.38; 5.18 and Chi=112.21; d.f.=21; p<0.001). The pooled RR on not adjusted estimates is still highly significant but the decrease, compared to the pooled RR on adjusted estimates, suggests a considerable correlation between these host factors. As we saw previously for eye colour.

Only three studies adjusted for intermittent or chronic sun exposure, some more for sunburns (6) and for naevi (11). However these adjustments do not significantly influence the estimates.

Heterogeneity decreases substantially (Chi=35.92; d.f.=29; p=0.18) when the pooled RR was calculated on the raw data, recoded to reduce to a dichotomy the larger number of groupings. For hair colour “black” and “dark brown” were combined into “dark” category and “light brown”, “blond” and “red” into “light” category. The pooled RR becomes lower but it still significant (RR=1.72, 95% C.I.: 1.59; 1.85). The relative risk was calculated with the fixed effects model because there was not any more heterogeneity. Autier (1995)[288], Chen

(1996)[234], Holly (1995)[333], Holman (1984)[150], Beitner (1990)[334] and Rosso (1998)[299] did not present crude data and they were not included in this part of the analysis.

Bliss (1995)[25] showed higher relative risk for hair colour assessed in younger individuals. In fact it is possible that melanoma risk is related to hair colour at a younger age, and that hair colour in older individuals is a less accurate measure. However when I looked at differences between hair colour in childhood and hair colour with the other more general definitions, I did not find any significant difference among the estimates.

5.6.3.4 Sensitivity analysis

Pooled estimate of "blond" versus "black" indicates that subjects with "blond" hair colour are also at significantly higher risk than subjects with "dark" hair colour (RR=1.94, 95% C.I.: 2.44; 2.69), even if the RR is lower than that for "red" colour. A very high between study heterogeneity (Chi=315.54; d.f.=26; $p<0.001$) was found also for this comparison and, as for "red" versus "black", is largely due to Beitner (1990)[334].

When the four estimates published by Chen (1996)[234], for the four different body sites, are included in the analysis, as they are, the pooled relative risk does not change significantly (RR=2.73, 95% C.I.: 2.18; 3.41 and Chi-squared=137.96, d.f.=38; $p<0.001$).

The funnel plot is clearly asymmetrical (Figure 5.36) however the method proposed by Copas and Shy does not suggest a statistically significant effect of publication bias. As a check, I verified the results from the other methods that investigate asymmetry of the funnel plot. Begg's method, which considers the Kendall's score, gives a slight indication of publication bias ($p=0.047$). However Egger's linear regression approach does not ($p=0.106$) and "Trim and fill" random effects method does not suggest to consider "missing" studies to adjust the pooled estimate for a possible publication bias.

5.6.4 Skin colour

5.6.4.1 Materials and methods

In twenty-eight papers it was possible to find an estimate of the association between melanoma and skin colour. Four[217;224;225;332] sets of analysed data were already investigated in other publications. The twenty-four independent papers presented results all acquired from case-control studies (one is a nested case-control study: Weinstock, 1991[335]). I finally arrived at twenty-five datasets because Graham (1985)[286] published estimates separately for sex. Hezerfeld (1993)[295] published estimates only on men and Holly (1995)[333] and Weinstock (1991)[335] only on women. In all but Chen (1996)[234] study, it was evaluated risk for all body.

General features of studies included in the analysis are shown in Table 5.21 and methods of assessment for skin colour are presented in Table 5.22. Some studies used previously validated skin colour charts; some used prosthesis and wigmakers' samples whereas others made a simple broad visual assessment. Some authors judged skin colour with scores on a quartile or quintile scale, others considered a rough coding classification as "dark", "medium", "fair". Some papers investigated skin colour presenting estimates for sun-exposed and unexposed body sites (e.g. upper inner arm) and the latter was chosen for the meta-analysis.

It is hard to measure skin colour because it exhibits a much narrower colour range than hair or eye colour. Therefore, in order to reduce the problem of misclassification bias, it was decided to include the estimates comparing highest versus lowest categories. However in the sensitivity analysis a pooled RR was calculated reducing to a dichotomy the larger number of groupings, because many authors considered only two broad categories. Data from the categories defined as "dark" and "medium" were collapsed into "dark/medium", and data belonging to the categories described as "light" or "fair" and "very fair" into the category "light". Calculations are on the studies that published raw data. Three studies (Holman

1984[150], Holly 1995[333] and Chen 1996[234]) were not included because they presented only adjusted estimates.

5.6.4.2 Results

ORs, adjusted for the maximum number of confounders, are presented in Figures 5.37, with their confidence intervals. As can be seen, the majority indicates that people with light skin are at considerable higher risk compared to people with dark skin.

Coefficients of the studies were summarized with random effects models, heterogeneity being highly significant (Chi=160.77, d.f.=24, p<0.001), and the pooled relative risk for "light" skin colour, compared to "dark", is 2.02 (95% C.I.: 1.60; 2.55).

5.6.4.3 Heterogeneity analysis

Even if adjustment for phenotype and phototype are not significant in explaining variability among the estimates, it was observed a reduction in the pooled RR calculated on the estimates adjusted for phenotype or phototype. Pooled estimate on unadjusted is RR=2.48 (95% C.I.: 1.61, 3.82; Chi=123.22, d.f.=12, p<0.001) whereas the pooled RR on adjusted estimates is RR=1.71 (95% C.I.: 1.44, 2.03; Chi=17.41, d.f.=11, p=0.096). Similar trends were observed previously for hair and eye colour suggesting a correlation between these factors.

Seven studies adjusted for a measure of sun exposure (intermittent, chronic sun exposure or sunburns) and eight studies adjusted for naevi. However these adjustments do not significantly influence the estimates.

5.6.4.4 Sensitivity analysis

Rodenas (1996)[184] presented an adjusted estimate much lower than the crude one (OR=4.2 and 95% C.I. 1.67, 10.57; OR=0.5 and 95% C.I. 0.11, 2.18, for crude and adjusted estimates

respectively), and there is a suspicion of a type error. However these two ORs are not outliers and their weights are not large (w=1.77 and 4.51, for adjusted and not-adjusted estimates respectively) therefore they have no considerable influence on the pooled estimates.

In Nelemans (1993)[336] it is not completely clear which are the reference categories. In the first table that was published in their paper, only the highest category was indicated ("light") and it is not obvious which is the reference category; in the second table they presented cases and controls were from North Europeans and Middle Europeans and probably North Europeans were considered as "light skin". If this hypothesis is correct this study should not be included in the meta-analysis because classification of all North European people as light skin subjects is too much rough. However the weight of this study is not very big (w=10.42).

Male estimates extracted from Graham (1985)[286] are quite unstable because very few subjects are in the reference category; however its weight is quite low (w=2.69).

Freedman (1997)[308] compared deaths from melanoma with non-cancer deaths, drawn from a database supported by two American national health institutes. Subjects were classified in "fair" or "other white" and were defined fair skinned if they were Caucasians and coming from Britain, Ireland, Germany, Scandinavia, Poland or other Northern European countries. Weight of the relative risk estimate published in this paper is really huge (w=435) but the estimate is very low and not significant (RR=1.01 and 95% C.I. 0.92, 1.11) therefore the decision to include this estimate is conservative.

A further analysis was carried out excluding the four previously cited odd studies (Rodenas, 1996[184], Nelemans, 1993[336], Graham, 1985[286] and Freedman, 1997[308]). Heterogeneity decreases but remains highly significant (Chi=67,22, d.f.=19, $p<0.001$) and the pooled RR is very similar to the one obtained in the main analysis (RR=2.11 and 95% C.I. 1.62, 2.47). However when I exclude another study (Dabkowski 1997 [188]), which presented a

very high estimate (OR=15.41 and 95% C.I. 7.82, 30.38) and influenced heterogeneity very much, Chi-squared decreases considerably (Chi=28.51; d.f.=18, p=0.056) and the pooled estimate remains highly significant (RR=1.77 and 95% C.I. 1.53, 2.04).

Pooled RR on the dichotomous exposure, "dark/medium" versus "light", is very similar to the one comparing adjusted estimates of the highest versus the lowest categories (RR=1.84 and 95% C.I. 1.48, 2.29). Chi-squared remains highly significant (Chi=153.87, d.f.=21, p<0.001).

Gender does not seem to have any influence in results variation. In fact estimates from Hezerfeld (1993)[295], which considered only men, are very similar to the results published by Holly (1995)[333] and Weinstock (1991)[335] that regarded only women.

A clear asymmetry can be seen in the funnel plot shown in Figure 5.39. Sensitivity analysis on publication bias, proposed by Copas and Shi, gives an indication of an overestimation of the risk for light skin colour. However with only four more papers p-value for publication bias is not any more significant and the new pooled RR still indicates light skin colour as a significant risk factor (RR= 1.75 and 95% C.I. 1.35, 2.27).

5.6.5 Skin phototype

5.6.5.1 Materials and methods

Twenty-two articles on the association between melanoma and phototype (also known as skin type) were identified: three[212;217;332] of them analysed data published in other papers. I finally narrowed this down to nineteen independent case-control studies and twenty datasets (Mackie 1989[183] presented estimates separately for sex). Holly (1995)[333] published estimates only on women.

Marret (1992)[186] published two estimates looking at "skin's reaction to strong sunlight after the first summer exposure" and "after repeated exposures". The second one was

chosen because it was considered more appropriate to have a reliable estimate for skin phototype.

Studies included in the meta-analysis are presented in Table 5.23 with some general features. Definitions of "skin type" used by the authors are described in Tables 5.24. As can be seen some papers evaluated "skin type" in general and some others presented more detailed definitions, considering reaction of the skin after few days of sun exposure, investigating differences between childhood and adulthood, or using Fitzpatrick classification.

Fitzpatrick created a standard method for classifying individual skin types, according to their skin colour and burning and tanning responses to sun light exposure. This classification of skin type into four categories, from type I (always burn, never tan) to type IV (never burn, tan easily), is widely used clinically, although the precise questions asked and the responses coded vary between studies.

The main analysis was conducted considering the estimates calculated for the highest category at risk (type I), as I did for the other risk factors, in order to reduce misclassification. Actually skin type is a subjective, self-reported assessment and, as such, is prone to substantial misclassification.

Five authors presented only dichotomous estimates, whereas ten papers considered skin type as an ordinal categorical variable and published three estimates comparing each skin type level with the fourth level (never burn, tan easily). A further investigation was carried out on these studies that published three estimates, in order to evaluate a trend in melanoma risk among skin type categories.

5.6.5.2 Results

ORs, with their confidence intervals, are shown in Figures 5.40. As can be seen, all but two studies (Lock-Andersen, 1998[337] and Ammannatti, 1987[338]) presented positive relative risk

estimates and the majority are significant. The pooled relative risk (RR=2.29 and 95% C.I. 1.73, 3.05) showed that people who always burn and never tan are at significantly higher risk than people who never burn and tan easily.

Dose-response model was used considering skin type as an ordinal categorical variable, categorized as follows: IV type (no burn, tan easily) is coded with 0; III type is coded with 1; II type is coded with 2; I type (burn, no tan) is coded with 3. Statistical methods, proposed by Greenland and Lognecker[72], was applied on the ten articles that published all three ORs, comparing I, II and III versus IV skin type. Random effects model was used even if the between study heterogeneity was not highly significant (p=0.072), given the low power of Chi-squared test. A clear increasing trend in melanoma risk, with decreasing skin type levels, was found (see Table 5.25).

5.6.5.3 Heterogeneity analysis

Random effects model was used to calculate pooled RR because heterogeneity is significant (Chi=63.98; d.f.=19; p<0.001). However from meta-regression it was found that none of the study characteristics, analysed to investigate heterogeneity, is significantly associated with the variability between the estimates.

Even if adjustment for phenotype is not significant in explaining variability among the estimates, it was observed a reduction in the pooled RR calculated on the adjusted estimates. Pooled RR of not adjusted estimates is RR=2.66 (95% C.I.: 1.68, 4.22; Chi=20.58, d.f.=8, p=0.008) whereas the pooled RR of adjusted estimates is RR=2.06 (95% C.I.: 1.44, 2.95; Chi=38.28, d.f.=10, p<0.001). Similar trends were observed for hair, eye and skin colour, suggesting a correlation between these host factors.

Being described usually in terms of tanning ability or as susceptibility to burning, skin type may become a crude surrogate measure for sunburn history. Actually the pooled RR

of estimates adjusted for sunburns is a much lower (RR=1.60 and 95% C.I.: 1.17, 2.19), even if not statistically different (p=0.378), than pooled RR of unadjusted estimates (RR=2.55 and 95% C.I.: 1.78, 3.63). Between-study Chi-squared, calculated on adjusted estimates, is not significant (Chi=3.07, d.f.=4, p=0.801), whereas it remains highly significant (Chi=59.28, d.f.=14, p<0.001) for unadjusted.

5.6.5.4 Sensitivity analysis

Heterogeneity is highly influenced by the study published by Rodenas (1996)[184], which presented a very large estimate (OR=29.8 and 95% C.I. 8.90, 99.89). However this OR does not have a considerable weight (w=2.69), the confidence intervals being quite wide, and exclusion of this study does not make a big change in the pooled estimate (RR=2.06 and 95% C.I. 1.61, 2.63)

Mackie (1989)[183] presented estimates separately for sex and Holly (1995)[333] published estimates only on women. Again the confidence intervals of the ORs for men and women published by Mackie overlaps and I cannot suggest a significant influence of gender. However it is interesting to note a similar trend for almost all studies that presented separately for sex. In Mackie (1989) for freckles, Graham (1985) for eye colour, Lindelof (1991) for photodamage, Graham (1985) for skin colour and Mackie (1989) for sunburns in adulthood estimates for men are higher and with wider confidence intervals. It is not clear if this finding is due merely to chance or if it is due to some other unknown factor.

Sensitivity analysis, proposed by Copas and Shi, suggests that there may be some indications for publication bias but, with only two papers more, p-value for the funnel plot (Figure 5.41) is not any more significant and the pooled relative risk does not show a considerable decrease (RR=2.01 and 95% C.I. 1.44, 2.82)

5.6.6 Discussion on host factors

If in ascertaining ability to tan problems of recall bias and reliability may arise, in the findings related to differences in eyes or hair colour definitions, it is more difficult to raise the question of misclassification bias. Therefore relative risk estimates for the established host factors should be quite reliable.[309]

In heterogeneity analysis I found suggestions for a considerable correlation between skin, hair and eyes colours, as is suggested in the literature. At the opposite in Bliss (1995)[25] individual subjects meta-analysis, hair, eye and skin colours appear largely independent and the authors discuss the possibility of combining them to demarcate high risk groups who can be targeted for prevention.

Clearly hair and eye colours cannot be considered directly in a causal relationship with melanoma and are likely to be risk factors by virtue of their correlation with skin phenotype. Their association with melanoma may be because it is easier to have an indicative measure of hair and eye colour. They have a wide colour range in many populations whereas skin colour is difficult to measure and has a much narrower colour range. This makes more difficult to obtain a reliable estimate of risk for skin colour.

5.7 Conclusions

A systematic meta-analysis looking at all published studies for all risk factors for melanoma was carried out to obtain summary estimates and investigate between-study heterogeneity.

Published literature showed strong associations of melanoma with several factors. Sun exposure is one of the main factors analysed and discussed and it is the most important modifiable factor in the aetiology of melanoma. Influence of sun exposure is investigated in this chapter considering several variables, indicating direct or indirect measure of sun exposure: cumulative exposure, acute intermittent exposure, chronic exposure and history of

sunburns in childhood and adulthood. The overall conclusion of a positive association with intermittent exposure and a protective or uncertain effect for chronic sun exposure is consistent with the previous meta-analyses. Another related characteristic, shown to be strongly positively associated with melanoma, is the frequency of previous sunburns. However it is important to take into account that for all these variables there is a relevant problem of measurement of exposure due to absence of standardized instruments of measurement. Recall bias in this situation is a very important problem.

Some photodamage indicators, which may also be considered indicators of acute and chronic exposure to sun radiations, are analysed and the pooled estimates show that they are highly associated to melanoma.

Some genetically controlled features influence individual susceptibility to the sun. These include skin pigmentation, which, in turn, is related to hair and eye pigmentation; and tendency to burn or ability to tan, which is clinically characterized as skin type. The historically classic risk factors (fair or red hair, blue eyes, and fair skin) are confirmed to be highly associated with melanoma and pooled estimates are calculated. These are not modifiable risk factors but higher risk individuals may be identified and targeted for increased frequency of screening and intensified efforts at primary intervention. The other set of factors, which are very strong and useful clinically as risk indicators, includes the number of acquired naevi and presence of atypical naevi, as we have seen in the previous chapter.

The remaining major factor is family history, which confers an extremely high risk estimated by a pooled estimate not affected by heterogeneity.

Fixed effects and random effects models have been applied to compared usually highest versus lowest category of exposure. Dose-response models are used to estimate the dose-response relationship across categories of exposure. Multivariate approach was considered to compare multiple risk factors.

Between-study heterogeneity was found to be significant for the majority of the risk factors considered. Therefore results from subgroup analysis and meta-regression should be regarded with much attention. The studies were classified according to several features that may have been responsible for the variability in the estimates. I had expected in advance that specific methodological aspects, such as type of study, source of cases and controls, matching, blinding, exclusion of lentigo maligna, acral melanoma or subjects with family history, adjustment for certain confounders, or features of the population under study as age, latitude or country of the study, would be associated with considerable changes in the estimates. Thus several important conclusions were made looking at the factors found to be significantly associated with variation in the estimates.

Sensitivity analyses are also carried out to investigate choices regarding inclusion or exclusion of studies, methodologies applied and influence of singles studies. Publication bias was expplored to verify relibility of pooled estimates.

5.8 Tables

5.8.1 Total sun exposure Tables

Table 5.1. Characteristics of the studies on total sun exposure.

First author, Year	Country	N° Cases	N° Controls	Source Cases	Source Controls
Cristofolini, 1987 [235]	Italy	103	205	Hospital	Hospital
Dabkowski, 1997[188]	Poland	74	300	Hospital	Population
Dubin., 1990[214]	USA	289	527	Hospital	Hospital
Elwood, 1985[304]	Canada	595	595	Population	Population
Graham, 1985[286] **(women)**	USA	186	319	Hospital	Hospital
Graham, 1985 [286] **(men)**	USA	218	202	Hospital	Hospital
Green, 1986[250]**-1985**[167]	Australia	183	183	Population	Population
Grob, 1990[170]	France	207	295	Hospital	Population
Holman, 1986[300]	Australia	507	507	Population	Population
Rodenas, 1996[184]	Spain	105	138	Hospital	Visitors to hospital
White, 1994[249]	USA	256	273	Population	Population

Table 5.2a. Definitions of total sun exposure.

First author, year		Definition of exposure
Cristofolini, 1987	**1**	**Heavy or frequent exposure to sunlight in the last 20 years: yes**
Dabkowski, 1997	**1**	**History of intensive UV exposure: yes**
Dubin, 1990	1	Hours of sun exposure 0-5 years previously, h/day: 5+
	2	Hours of sun exposure 6-10 years previously, h/day: 5+
	3	Hours of sun exposure 11-20 years previously, h/day: 5+
	4	**Overall sun exposure: Much**
Elwood, 1985	**1**	**Whole-body equivalent hours of sun exposure from all sources per year. One whole body equivalent hour of exposure represents 1 hr of sun exposure of the whole body surface, and recorded exposures were converted to equivalent hours using estimates of the proportion of surface area exposed.: 500+**
Graham, 1985 (men)	**1**	**Cumulative sun exposure, hours (thousands): >160**
	2	Average annual sun exposure, h/year: >3200
	3	Exposure level: 5
Graham, 1985 (women)	**1**	**Cumulative sun exposure, hours (thousands): >100**
	2	Average annual sun exposure, h/year: >2000
	3	Exposure level: 5
Green, 1986-1985	**1**	**Cumulative hours of sun exposure during whole of life: 50000**
	2	Cumulative hours of sun exposure during ages 10-19 years: 5000
	3	Cumulative hours of sun exposure during the 5 years prior to case diagnosis: 5000

Definitions included are in bold.

Table 5.2b. Definitions of total sun exposure.

First author, year	Definition of exposure
Grob, 1990	**1 Cumulative lifetime outdoor sun exposure index: SU=sun exposure unit (a day with at least 2 h of direct sun exposure): >2000SU**
Holman, 1986	**1 Mean total outdoor exposure in summer, hr/week: >23**
Rodenas, 1996	**1 Total sun exposure (hrs): > 8501**
White, 1994	**1 Average yearly sun exposure (hours) over the 10 years before reference date, h/year. Reference date was 2 years prior to diagnosis for the cases and a comparable date for controls: 500-2880** 2 Sun exposure index at ages 2-10 years. Sun exposure index was calculated as number of days per year in the sun divided clothing category: 91-365 3 Sun exposure index at ages 11-20 years. Sun exposure index was calculated as number of days per year in the sun divided clothing category: 91-365

Definitions included are in bold.

5.8.2 Intermittent sun exposure Tables

Table 5.3a. Characteristics of the studies on intermittent sun exposure.

First author, Year	Country	N° Cases	N° controls	Source of Cases	Source of controls
Autier, 1998[301]	Germany, France and Belgium	420	447	Hosp	Pop
Beitner, 1990[334]	Sweden	523	505	Hosp	Pop
Bell, 1987[297]	UK	268	1577	Hosp	Hosp
Carli, 1995[217]	Italy	106	109	Hosp	Pop
Chen, 1996[234] **(head/neck)**	USA	85	494	Pop	Pop
Chen, 1996[234] **(lower limb)**	USA	97	494	Pop	Pop
Chen, 1996[234] **(trunk)**	USA	265	494	Pop	Pop
Chen, 1996[234] **(upper limb)**	USA	101	494	Pop	Pop
Dubin, 1990[214]	USA	289	527	Hosp	Hosp
Dunn-Lane, 1993[339]	Ireland	100	100	Hosp	Hosp
Elwood, 1985[304]	Canada	595	595	Pop	Pop
Fritschi, 1996[340]	Canada	103	533	Pop	Pop
Green, 1986[250]	Australia	183	183	Pop	Pop
Grob, 1990[170]	France	207	295	Hosp	Pop
Herzefeld, 1993[295] **(men, trunk)**	USA	324	415	Pop	Pop
Holly, 1995[296] **(women)**	USA	452	930	Pop	Pop
Holman, 1986[300]	Australia	507	507	Pop	Pop

Table 5.3b. Characteristics of the studies on intermittent sun exposure.

First author, Year	Country	N° Cases	N° controls	Source of Cases	Source of controls
Lock-Andersen, 1998[337]	Denmark	168	176	Hosp	Pop
Moore, 1997[230]	USA	69	69	Pop	Pop
Nelemans, 1993[336]	Netherlands	141	183	Pop	Pop
Osterlind, 1988[302]	Denmark	474	926	Pop	Pop
Weiss, 1991[213]	Germany	204	200	Hosp	Hosp
Westerdahl, 1995[221]	Sweden	400	640	Pop	Pop
Wolf, 1998[298]	Austria	193	319	Hosp	Hosp
Zaridze, 1992[224]	Russia	96	96	Hosp	Visitor to hosp

Table 5.4a. Definitions of intermittent sun exposure.

First author, Year		Definizione dell'esposizione
Autier, 1998	1	Index of sun exposure during childhood (residency>=1 year in Mediterranean, tropical or subtropical area starting before 10 years old; ever sunburn between 5 and 10 years, and between 10 and 14 years old; never been protected against sunlight during holidays in sunny resorts: high (2 risk factors)
	2	**Index of sun exposure during adulthood (on average >2 holidays weeks spent each year in sunny resorts; during holidays, sun exposure during the hot hours of the day; search for suntan during residence of >=1 year in Mediterranean, tropical or subtropical area; ever sunburn after 14 years old; sunscreen use; never suffered from non-malignant skin disease that lasted for >=1 year): extreme (5-7 risk factors)**
Beitner, 1990	**1**	**Number of sunbathes April-Sept. Per year: >30**
	2	Sunbathing vacations abroad: >= once a year
Bell, 1987	**1**	**Frequent sunbathing: yes**
	2	Keen gardener: yes
	3	Outdoor sportsman: yes
Carli, 1995	1	Cumulative sunbathing until age 20 yr (hr): >800
	2	**Sunbathing (hr/yr) after age 20 yr: >80**
Chen, 1996	**1**	**Total recreational sun exposure index. Sun exposure index was created by assigning scores to 2 recreational sun exposure history variable, number of vacations to places sunnier than the usual residence and number of days spent in outdoor recreational activities per year, and summing the scores for 2 periods of time (before 16 years and during last 10 years): level 4**
Dubin, 1990	**1**	**Recreation type: mostly outdoors**
Dunn-Lane, 1993	**1**	**Holidays in the sun: yes**
	2	Sunbathing at every opportunity: yes

Definitions included are in bold.

Table 5.4b. Definitions of intermittent sun exposure.

First author, Year		Definizione dell'esposizione
Elwood, 1985	**1**	**Recreational, summer, group A equivalent hours of exposure/season. One whole body equivalent hour of exposure represents 1 hr of sun exposure of the whole body surface, and recorded exposures were converted to equivalent hours using estimates of the proportion of surface area exposed. group A referred to activities where to activities where a bathing suit or very light clothing to be worn (e. g. sunbathing, swimming).: 160+**
	2	Vacation, summer, groups A&B equivalent hours of exposure/season. One whole body equivalent hour of exposure represents 1 hr of sun exposure of the whole body surface, and recorded exposures were converted to equivalent hours using estimates or the proportion of surface area exposed. Group A referred to activities where a bathing suit or very light clothing to be worn (e. g. sunbathing, swimming). Group B referred to activities where light clothing would to be worn (e. g. team games, camping, gardening).: 40+
	3	Sunny vacations per decade, number (equivalent hours of exposure). One whole body equivalent hour of exposure represents 1 hr of sun exposure of the whole body surface, and recorded exposures were converted to equivalent hours using estimates of the proportion of surface area exposed.: 4+
Fritschi, 1996	1	Hobbies, gardening: yes
	2	**Hobbies, outdoor sports: yes**
Green, 1986	**1**	**Recreation hours spent in sun on beach over whole life: 5000**
	2	Recreation hours spent on the beach in sun during ages 10-19 years: 500
	3	Recreational hours spent on the beach in the sun during the 5 years prior to case diagnosis: 500
Grob, 1990	**1**	**Outdoor leisure sun exposure per year in the last 2 years: SU=sun exposure unit (a day with at least 2 h of direct sun exposure): >60 SU**
Herzfeld, 1993	**1**	**Participate in water sports: yes**
	2	Bathe more than once per day: yes
Holly, 1995	1	Time spent outdoors on weekends with arms and legs exposed to the sun (past 10 years): >=3/4 of time
	2	**Time sunbathing in a typical year (past 10 years): >= once/week**
Holman, 1986	**1**	**Recreational outdoor exposure proportion in summer at ages 10-24 yr, %: >60**
	2	Boating-frequency of participation in summer: once or more/wk
	3	Fishing-frequency of participation in summer: once or more/wk
	4	Swimming-frequency of participation in summer: once or more/wk
	5	Sunbathing, ages 15-24 yr-frequency of participation in summer: once or more/wk
	6	Sunbathing, 0-9 yr pre-diagnosis, frequency of participation in summer: once or more/wk
	7	Mean time spent outdoors in summer aged 10-20 (h/week): >=16

Definitions included are in bold.

Table 5.4c. Definitions of intermittent sun exposure.

First author, Year		Definizione dell'esposizione
Lock-Andersen, 1998	1	Childhood: sun exposure in leisure time: always or very often
	2	**Adulthood: sun exposure in leisure time: always or very often**
	3	VAS, visual analogue scale with end points: 0=never sun exposed; 10=always sun exposed.
Moore, 1997	1	Did you ever sunbathe?: yes
	2	**Sunbathing during 10 years prior to diagnosis (hr/wk): >=0,45**
	3	Sunbathing ages 15-25 (days/year): >=7,5
	4	Hiking: yes
	5	Swimming: yes
	6	Boating-sailing: yes
	7	Water skiing: yes
Nelemans, 1993	**1**	**Sunbathing: yes**
	2	Water sports: yes
	3	Vacations in sunny countries: yes
	4	<15 years of age, swimming pools only: yes
	5	<15 years, open waters only: yes
	6	<15 years, any type of polluted water: yes
	7	15-25 years of age, swimming pools only: yes
	8	15-25 years of age, open waters only: yes
	9	15-25 years of age, any type of polluted waters: yes
	10	>25 years of age, swimming pools only: yes
	11	>25 years of age, open waters only: yes
	12	>25 years of age, any type of polluted water: yes
	13	Age at which swimming was learned (years): <9
Osterlind, 1988	**1**	**Sunbathing: at some time**
	2	Boating: at some time
	3	Skiing: at some time
	4	Swimming (outdoor): at some time
	5	Vacations in sunny resorts: very sunny resorts
Rodenas, 1996	**1**	**Outdoor leisure exposure (hrs): >=951**
	2	Childhood sunbaths: >=211
Rosso, 1998	**1**	**Holidays at beach (no. of hours in a lifetime): >4000**
	2	Holidays at beach during childhood (n. of hours in a lifetime): >1600
	3	Holidays t beach during adulthood (n. of hours in a lifetime): >2200
	4	Outdoor sports (n. of hours in a lifetime): >2800
Walter, 1999	**1**	**Beach vacation in the past 5 years: yes**
	2	Beach vacations at age 12 years: yes
	3	Beach vacations at age 18 years: yes
Weiss, 1991	**1**	**Recreational sun exposure: high/medium**

Definitions included are in bold.

Table 5.4d. Definitions of intermittent sun exposure.

First author, Year		Definizione dell'esposizione
Westerdahl, 1995	**1**	**Sunbathing frequently during the summer (April-September): yes**
	2	Sunbathing vacations abroad (per year): one or more
Wolf, 1998	**1**	**Leisure sun exposure: >50%**
	2	Vacation in sunny areas: every other year
	3	Sunbath per year: >30
Zaridze, 1992	**1**	**Sunbathing at age 18-20: often**

Definitions included are in bold.

5.8.3 Chronic sun exposure Tables

Table 5.5a. Characteristics of the studies on chronic sun exposure.

First author, Year	Type of study	Country	N° Cases	N° controls	Source of Cases	Source of controls
Autier, 1994[233]	CC	Germany, France and Belgium	420	447	Hospital	Population
Beitner, 1990[334]	CC	Sweden	523	505	Hospital	Population
Bell, 1987[297]	CC	UK	268	1577	Hospital	Hospital
Carli, 1995[217]	CC	Italy	103	109	Hospital	Population
Chen, 1995 [217] **(head/neck)**	CC	USA	85	494	Population	Population
Chen, 1996[234] **(lower limb)**	CC	USA	97	494	Hospital	Hospital
Chen, 1996 [234] **(trunk)**	CC	USA	265	494	Hospital	Hospital
Chen, 1996 [234] **(upper limb)**	CC	USA	101	494	Hospital	Hospital
Cooke, 1984[305]	Cohort	New Zealand	501	n.a.	Population	Population
Cristofolini, 1987[235]	CC	Italy	103	205	Population	Population
Dubin, 1990[214]	CC	USA	289	527	Hospital	Hospital
Dunn-Lane, 1993[339]	CC	Ireland	100	100	Other	Other
Elwood, 1985[304]	CC	Canada	595	595	Hospital	Hospital
Elwood, 1986[236]	CC	UK	83	83	Hospital	Hospital
Freedman, 1997[308]	CC	USA	12156	23845	Population	Population
Garbe, 1989[210]	CC	Germany	200	200	Hospital	Population
Goodman, 1995[307]	CC	USA	3527	53129	Population	Population
Graham, 1985[307] **(men)**	CC	USA	218	202	Population	Population
Grob, 1990[170]	CC	France	207	295	Hospital	Population
Herzfeld, 1993[295] **(men, trunk)**	CC	USA	324	415	Hospital	Population
Holly, 1995[296] **(women)**	CC	USA	452	930	Hospital	Hospital
Holman, 1986[300]	CC	Australia	507	507	Population	Population

Table 5.5b. Characteristics of the studies on chronic sun exposure.

First author, Year	Type of study	Country	N° Cases	N° controls	Source of Cases	Source of controls
Lock-Andersen, 1998[337]	CC	Denmark	168	176	Hospital	Population
Nelemans, 1993[336]	CC	Netherlands	141	183	Population	Population
Osterlind, 1988[302] **men**	CC	Denmark	194	390	Population	Population
Osterlind, 1988[302] **(women)**	CC	Denmark	280	536	Population	Population
Pion, 1994[303] **(women)**	Nested CC	USA	1494	4467	Population	Population
Pion, 1994[303] **(men)**	Nested CC	USA	1305	3910	Population	Population
Rodenas, 1996[184]	CC	Spain	105	138	Hospital	Visitors to hospital
Rosso, 1998[299]	CC	Italy	260	416	Population	Population
Vagero, 1986[306]	Cohort	Sweden	4706	2630458*	Population	Population
Walter, 1999[325]	CC	Canada	583	608	Population	Population
Weiss, 1991[213]	CC	Germany	204	200	Hospital	Hospital
Westerdahl, 1994[293]	CC	Sweden	400	640	Population	Population
White, 1994[249]	CC	USA	256	273	Population	Population
Wolf, 1998[298]	CC	Austria	193	319	Hospital	Hospital

(*) number refers to the cohort size; n.a.: not available

Table 5.6a. Definitions of chronic sun exposure.

First authorm, year	Exposure definitions
Autier, 1994	**1 Past or present occupation in farming or building construction: yes** 2 Duration of occupation in farming or building construction: >30 years
Beitner, 1990	**1 Outdoor workers: yes**
Bell, 1987	**1 Occupation: outdoor**
Carli, 1995	**1 Outdoor job: yes**
Chen, 1996	**1 Total years in outdoor jobs: 5+**
Cooke, 1984	**1 Occupational units groups: who have worked outside of any building for 10 h or more per week (outdoor)**
Cristofolini, 1987	**1 Main occupation: outdoor**
Dubin, 1990	**1 Occupation type: mostly outdoors**
Dunn-Lane, 1993	**1 Work outside for >30 hrs/week: yes**
Elwood, 1986	**1 Outdoor exposure (h): 25000+**
Elwood, 1985	**1 Occupational, summer, equivalent hours of exposure/season: One whole body equivalent hour of exposure represents 1 hr of sun exposure of the whole body surface, and recorded exposures were converted to equivalent hours using estimates of the proportion of surface area exposed.: 400+**
Freedman, 1997	**1 Occupation: outdoor (non-farmer)**
Garbe, 1989	**1 Occupational sun exposure: sometimes/nearly every time**
Goodman, 1995	**1 Sun exposure during work: mainly outdoor**

Definitions included are in bold.

Table 5.6b. Definitions of chronic sun exposure.

First author, year	Exposure definitions
Graham, 1985	**1 Hours of cumulative occupational sun exposure: >45000**
Grob, 1990	**1 Indoor or outdoor occupation: outdoor only**
Herzfeld, 1993	**1 Occupation: mostly outdoor**
Holly, 1995	**1 Time spent outdoors on weekdays with arms and legs exposed to the sun: >1/2 of time**
Holman, 1986	**1 Outdoor work in summer: >2 hr/day**
Lock-Andersen, 1998	**1 Sun exposure in working hours: always or very often**
Nelemans, 1993	**1 Occupational exposure: ever**
Osterlind, 1988	**1 Working outside in the summer (held for at least 6 months): yes** 2 Working in outdoor occupations: yes
Pion, 1994	**1 Occupational location: outdoor**
Rodenas, 1996	**1 Occupational exposure (hrs): >11501**
Rosso, 1998	**1 Outdoor work (n. of hours in a lifetime): >22000**
Vagero, 1986	**1 Type of work: outdoor**
Walter, 1999	1 Outdoor activity days between ages 10 and 20: >100 **2 Outdoor job hours 5 years ago: >0**
Weiss, 1991	**1 Occupational sun exposure: continuously/part-time**
Westerdahl, 1994	**1 Outdoor employment during the summer: yes**
White, 1994	**1 Lifetime occupational sun exposure (%): >50**
Wolf, 1998	**1 Occupational sun exposure: yes**

Definitions included are in bold.

5.8.4 Sunburns in childhood Tables

Table 5.7. Characteristics of the studies included on sunburns in childhood.

First author, Year	Country	N° Cases	N° controls	Source of Cases	Source of controls
Autier, 1998[301]	Germany, France and Belgium	420	447	Hospital	Population
Carli, 1995[217]	Italy	103	109	Hospital	Population
Cristofolini, 1987[235]	Italy	103	205	Hospital	Hospital
Elwood, 1984[294]	Canada	595	595	Population	Population
Elwood, 1990[237]	UK	195	195	Population	Hospital
Holly, 1987[169]	USA	121	139	Hospital	Hospital
Holly, 1995[296] **(women)**	USA	452	930	Population	Population
Holman, 1986[300]	Australia	507	507	Population	Population
Moore, 1997[230]	USA	69	69	Population	Population
Osterlind, 1988[302]	Denmark	194	390	Population	Population
Rodenas, 1996[184]	Spain	105	138	Hospital	Visitors to hospital
Westerdahl, 1994[293]	Sweden	400	640	Population	Population
Zanetti, 1992[292]	Italy	260	416	Population	Population

Table 5.8. Definitions for sunburns in childhood.

First author, year	Definizione dell'esposizione
Autier, 1998	**1 Ever sunburn between 5 and 10 years and between 10 and 14 years old: yes**
Carli, 1995	**1 Childhood sunburn (pain for 2 or more days or blistering): usually**
Cristofolini, 1987	**1 Painful and blistering sunburn in childhood and adolescence**
Elwood, 1984	**1 Childhood sunburn: severe or frequent**
Elwood, 1990	**1 Severe sunburn (pain for 2 or more days or blistering) age 8-12**
Holly, 1987	**1 Sunburns with blisters, score value: the score combined number of sunburns that caused blisters reported during elementary school, high school and young adult years: 3+**
Holly, 1995	**1 Sunburn before age 12 years: severe/frequent burns** 2 Number of painful sunburns during elementary school>=7
Holman, 1986	**1 Sunburn causing pain for >=2 days, <10 years of age: 5+**
Moore, 1997	**1 Sunburn with blistering: one or more episodes** 2 Painful sunburn: one or more episodes
Osterlind, 1988	**1 Number of sunburns before age 15: 5+**
Rodenas, 1996	1 Childhood painful erythema: often **2 Childhood blistering sunburns: yes**
Westerdahl, 1994	**1 Number of sunburns before age 15 years: >5 times**
Zanetti, 1992	1 Sunburns in childhood: often 2 Sunburns in childhood: severe **3 Sunburns in childhood: yes**

Definitions included are in bold.

5.8.5 Sunburns in adulthood Tables

Table 5.9a. Characteristics of the studies on sunburns in adulthood.

First author, Year	Country	N° Cases	N° controls	Source of cases	Source of controls
Autier, 1998[301]	Germany, France and Belgium	420	447	Hospital	Population
Beitner, 1990[334]	Sweden	523	505	Hospital	Population
Carli, 1995[217]	Italy	131	174	Hospital	Population
Carli, 1999[219]	Italy	103	109	Hospital	Population
Chen, 1996[234] **(head neck)**	USA	85	494	Population	Population
Chen, 1996 [234] **(lower limb)**	USA	97	494	Population	Population
Chen, 1996 [234] **(trunk)**	USA	265	494	Population	Population
Chen, 1996 [234] **(upper limb)**	USA	101	494	Population	Population
Cristofolini, 1987[235]	Italy	103	205	Hospital	Hospital
Dabkowski, 1997[188]	Poland	74	300	Hospital	Population

Table 5.9b. Characteristics of the studies on sunburns in adulthood.

First author, Year	Country	N° Cases	N° controls	Source of cases	Source of controls
Dubin, 1990[214]	USA	289	527	Hospital	Hospital
Dunn-Lane, 1993[339]	Ireland	100	100	Hospital	Hospital
Elwood, 1985[304]	Canada	595	595	Population	Population
Elwood, 1986[236]	UK	83	83	Hospital	Hospital
Elwood, 1990[237]	UK	195	195	Population	Hospital
Green, 1985[312]	Australia	183	183	Population	Population
Grob, 1990[170]	France	207	295	Hospital	Population
Holly, 1995[296] (men)	USA	452	930	Population	Population
Holman, 1986[300]	Australia	507	507	Population	Population
MacKie, 1989[183] (women)	UK	181	181	Population	Hospital
MacKie, 1989[183] (men)	UK	99	99	Population	Hospital
Moore, 1997[230]	USA	69	69	Population	Population
Nelemans, 1993[336]	Netherlands	141	183	Population	Population
Osterlind, 1988[302]	Denmark	194	390	Population	Population
Rodenas, 1996[184]	Spain	105	138	Hospital	Visitors to hospital
Weinstock, 1989[311] (women)	USA	130	300	Population	Population
Westerdahl, 1994[293]	Sweden	400	640	Population	Population
Wolf, 1998[298]	Austria	193	319	Hospital	Hospital
Zanetti, 1992[292]	Italy	260	416	Population	Population

Table 5.10a. Definitions of sunburns in adulthood.

First author (year)	Definizione dell'esposizione
Autier, 1998	**1 Ever sunburn after 14 years old: yes**
Beitner, 1990	**1 Erythema after sunbathing: often, very often**
Carli, 1995	**1 Adulthood sunburn (pain for 2 or more days or blistering): usually**
Carli, 1999	**1 Number of sunburns (pain for 2 or more days or blistering): 6+**
Chen, 1996	**1 Number of sunburns (pain for 2 or more days or blistering): 3+**
Cristofolini, 1987	**1 Sunburn: frequent/constant**
Dabkowski, 1997	**1 History of sunburn: yes**
Dubin, 1990	**1 Severe sunburn with blistering: ever**
Dunn-Lane, 1993	**1 History of severe sunburn with blistering: ever**
Elwood, 1985	**1 Vacation sunburn score: was taken as the sum of the maximum grading score for each of the four body areas.: very severe, 10-12**
Elwood, 1986	**1 History of sunburn causing pain for 2 days or more: yes**
Elwood, 1990	1 Severe sunburn (causing blistering or pain for 2 days or more) 5 years before diagnosis: moderate/max **2 Severe sunburn (causing blistering or pain for 2 days or more) 18-20 years before diagnosis: moderate/max**

Definitions included are in bold.

Table 5.10b. Definitions of sunburns in adulthood.

First author (year)	Definizione dell'esposizione
Green, 1986	**1 Pain longer than 48 hours, with or without blistering: 6+**
Grob, 1990	**1 Frequency of sunburns per year in recent years: >2**
	2 Severity of sunburn in recent years: at least one severe burn
Holly, 1995	**1 Ever sunburned with pain that lasted for>=2 days: yes**
	2 Ever sunburned with pain that lasted for >=2 days in past 10 years: >=4 episodes
	3 Number of painful sunburns over age 30 years: >=7
	4 Number of painful sunburns during ages 23-30 years: >=7
Holman, 1986	**1 Highest severity of past sunburn: blistering sunburn**
	2 Sunburn causing pain for >= 2 days, during last 10 years: yes
MacKie, 1989	**1 Number of severe sunburns in life: 3+**
Moore, 1997	1 Sunburn with blistering (18-29): one or more episodes
	2 Painful sunburn (18-29): one or more episodes
	3 Sunburn with blistering (30+): one or more episodes
	4 Painful sunburn (30+): one or more episodes
Nelemans, 1993	**1 History of sunburns: yes**
Osterlind, 1988	**1 Number of sunburns in last 10 years: 5+**
Rodenas, 1996	1 Adulthood painful erythema: often, very often
	2 Adulthood blistering sunburns: yes
	3 Total episodes of blistering sunburns: >=2
Weinstock, 1989	**1 Number of blistering sunburns reported after age 30 y: 5+**
	2 Number of blistering sunburns reported per year after age 30 y: at least 1/5 y
Westerdahl, 1994	1 Episodes of sunblisters: yes
	2 Formation of ulcer: yes
	3 Average number of episodes of sunburn per year: >=3
	4 Number of sunburns (causing severe erythema and pain for a few days) after age 19 years: >5 times
	5 History of sunburns: yes
Wolf, 1998	**1 History of sunburn: yes**
	2 Erythema due to sunlight: often
	3 Skin rash due to sunlight: yes
Zanetti, 1992	**Severe sunburns lifelong: ever**

Definitions included are in bold.

5.8.6 Family history Tables

Table 5.11a. Characteristics of the studies on family history.

First author, Year	Country	N° Cases	N° controls	Sources of cases	Sources of controls
Cristofolini, 1987[235]	Italy	103	205	Hospital	Hospital
Dubin, 1986[164]	USA	289	527	Hospital	Hospital
Green, 1985[167]	Australia	183	183	Population	Population
Holly, 1987[169]	USA	121	139	Hospital	Hospital

Table 5.11b. Characteristics of the studies on family history.

First author, Year	Country	N° Cases	N° controls	Sources of cases	Sources of controls
Holly, 1995[333] (women)	USA	452	930	Population	Population
Holman, 1984[150]	Australia	507	507	Population	Population
MacKie, 1989[183] (women)	Scotland	280	280	Population	Hospital
Osterlind, 1988 [226]	Denmark	474	926	Population	Population
Swerdlow, 1986[168]	Scotland	180	197	Hospital	Hospital
Walter, 1999[325]	Canada	583	608	Population	Population
Westerdahl, 1994[293]	Sweden	400	640	Population	Population

5.8.7 Indicators of photodamage Tables

Table 5.12. Characteristics of the studies on indicators of photodamage.

First author, Year	Type of study	State	N cases	N controls	Source cases	Source control
Bataille, 1998[328]	CC	Australia	259	281	Hosp	Hosp
Dubin, 1990[214]	CC	USA	289	527	Hosp	Hosp
Frish, 1996 [341]	Cohort	Denmark	134	37674*	Pop	Pop
Garbe, 1989[210]	CC	Germany	200	200	Hosp	Hosp
Garbe, 1994[165]	CC	Germany, Austria, Switzer.	513	498	Hosp	Hosp
Green, 1986[250]	CC	Australia	183	183	Pop	Pop
Halpern, 1991[238]	CC	USA	105	181	Hosp	Pop
Holly, 1987[169]	CC	USA	121	139	Hosp	Hosp
Holman, 1984[273]	CC	Australia	507	507	Pop	Pop
Lindelof, 1991[329] (women)	Cohort	Sweden	9	934*	Pop	Pop
Lindelof, 1991[329] (men)	Cohort	Sweden	6	1039*	Pop	Pop
Marghoob, 1995[342]	Cohort	USA	10	290*	Pop	Pop
Marrett, 1992[186]	CC	Canada	583	608	Pop	Pop
Moore, 1997[230]	Nested CC	USA	69	69	Pop	Pop
Osterlind, 1988[343]	CC	Denmark	474	926	Pop	Pop

(*) number refers to the cohort size.

Table 5.13a. Definitions and classification for indicators of photodamage.

First author (year)	Definitions	Classification
Bataille, 1998	Number of solar keratoses on left forearm: 10+	Pre-mal. and cancer les.
Bataille, 1998	Degree of solar elastosis: severe	Other indic. of act. damage
Dubin, 1990	Prior non-melanoma skin cancer or solar keratosis: yes	Pre-mal. and cancer les.
Frisch, 1996	Previous basal cell carcinoma: yes	Pre-mal. and cancer les.

Table 5.13b. Definitions and classification for indicators of photodamage.

First author, year	Definitions	Classification
Garbe, 1989	Actinic lentigines: moderate to large numbers	Other indic. of act. damage
Garbe, 1994	Actinic lentigines: many	Other indic. of act. damage
Green, 1986	Lentigines on arms: any	Other indic. of act. damage
Green, 1986	Actinic tumor on face:yes	Pre-mal. and cancer les.
Halpern, 1991	Actinic damage: severe	Other indic. of act. Damage
Holly, 1987	Previous skin cancer: yes	Pre-mal. and cancer les.
Holman, 1984	Cutaneous microphotograph (grade): 6+	Other indic. of act. Damage
Holman, 1984	History of non-melanocytic skin cancer: yes	Pre-mal. and cancer les.
Lindelof, 1991 (women)	Previous basal cell carcinoma (BCC): yes	Pre-mal. and cancer les.
Lindelof, 1991 (men)	Previous basal cell carcinoma (BCC):yes	Pre-mal. and cancer les.
Marghoob, 1995	Patients who had a BCC and/or squamous cell carcinoma (SCC): yes	Pre-mal. and cancer les.
Marrett, 1992	Splotchy freckles: yes	Other indic. of act. damage
Moore, 1997	Previous skin cancer: yes	Pre-mal. and cancer les.
Osterlind, 1988	Cutaneous microphotograph (grade): 4+	Other indic. of act. damage

5.8.8 Freckles Tables

Table 5.14a. Characteristics of the studies on freckles.

First author, Year	Country	N° Cases	N° Controls	Sources of cases	Source of controls
Carli, 1999[219]	Italy	131	174	Hosp	Pop
Chen, 1996[234] (head/neck)	USA	85	494	Pop	Pop
Chen, 1996[234] (lower limb)	USA	97	494	Pop	Pop
Chen, 1996[234] (trunk)	USA	265	494	Pop	Pop
Chen, 1996[234] (upper limb)	USA	101	494	Pop	Pop
Dubin, 1990[214]	USA	1103	585	Hosp	Hosp
Elwood, 1984[294]	England	595	595	Pop	Pop
Elwood, 1986[236]	England	83	83	Hosp	Hosp
Elwood, 1990[237]	England	195	195	Pop	Hosp
Green, 1986[250]	Australia	183	183	Pop	Pop
Grulich, 1996[171]	Australia	242	276	Hosp	Hosp
Halpern, 1991[238]	USA	105	181	Hosp	Pop
Herzfeld, 1993[295]	USA	324	415	Pop	Pop
Holly, 1987[169]	USA	121	139	Hosp	Hosp
Holly, 1995[333]	USA	452	930	Pop	Pop
MacKie, 1989[183] (women)	Scotland	181	181	Pop	Hosp

Table 5.14b. Characteristics of the studies on freckles.

First author, Year	Country	N° Cases	N° Controls	Sources of cases	Source of controls
MacKie, 1989[183] (men)	Scotland	99	99	Pop	Hosp
Marrett, 1992[186]	Canada	583	608	Pop	Pop
Moore, 1997[230]	USA	69	69	Pop	Pop
Nelemans, 1993[336]	Netherl.	141	183	Pop	Pop
Osterlind, 1988[226]	Denmark	474	926	Pop	Pop
Rodenas, 1996[184]	Spain	105	138	Hosp	Visitors to hosp
Tucker, 1997[172]	USA	716	1014	Hosp	Hosp
Walter, 1990[344]	Canada	583	608	Pop	Pop
Westerdahl, 1995[221]	Sweden	400	640	Pop	Pop
White, 1994[249]	USA	256	273	Pop	Pop
Wolf, 1998[298]	Austria	193	319	Hosp	Hosp
Zaridze, 1992[224]	Russia	96	96	Hosp	Other

Table 5.15a. Definitions and categories for freckles.

First author, year	Definition of exposure	Categories
Carli, 1999	Tendency to freckles	Present vs Absent
Chen, 1996	Number of freckles on face after sun exposure before age 25 years	"50+" vs "0"
Dubin, 1990	Freckles are brown spots which come and go with sun exposure: degree of freckling on summertime. History of freckles.	Yes vs No
Eldwood, 1986	Freckles as an adult, summer only	Many vs None
Elwood, 1990	Current freckling on the face and arms.	Many vs None
Green, 1986	Propensity to freckles.	Present vs Absent
Grulich, 1996	Freckling as a 15 year old from a groups of diagrams of the face.	Many vs None
Halpern, 1991	Freckles.	Present vs Absent
Herzfeld, 1993	Freckles	Yes vs No
Holly, 1987	Freckles < 3mm; Profile: Flat, macular; Colour: Light to medium brown; Border: Even and sometimes poorly defined.	Many vs None
Holly, 1995	Presence of freckles as a young adult	Yes vs No
MacKie, 1989 (women)	Freckling tendency	Some vs None
MacKie, 1989 (men)	Freckling tendency	Some vs None
Marrett, 1992	Freckles after sun exposure	Many vs None or few
Moore, 1997	Freckles that change with sun	Yes vs No
Nelemans, 1993	Degree of freckling	Many vs None

Table 5.15b Definitions and categories for freckles.

First author, year	Definition of exposure	Categories
Osterlind, 1988	Degree of freckling on the arms as an adult reported by the subject.	Many vs None
Rodenas, 1996	Freckles on face, forearms, shoulders and upper back were assessed by comparison with an analog scale from 0 to 100 (maximum intensity).	"40+" vs "0"
Tucker, 1997	Freckling pattern	Many vs None or few
Walter, 1990	Density of normal freckles	Density categories: 4 vs None
Westerdahl, 1995	Freckles.	Yes vs No
White, 1994	Number of freckles on face before age 20 years	"50+" vs "0"
Wolf, 1998	Freckles	Many/several vs NOne
Zaridze, 1992	Freckles on arms	Yes vs No

5.8.9 Eye colour Tables

Table 5.16a. Characteristics of the studies on eye colour.

First author, year	Country	N° Cases	N° controls	Sources of cases	Sources of controls
Ammannatti, 1987[338]	Italy	104	104	Hospital	Hospital
Augustsson, 1991[208]	Sweden	121	378	Population	Population
Beitner, 1990[334]	Sweden	523	505	Hospital	Population
Bell, 1987[297]	UK	268	1577	Hospital	Hospital
Carli, 1995[217]	Italy	106	109	Hospital	Population
Chen, 1996[234] **(head/neck)**	USA	85	494	Population	Population
Chen, 1996[234] **(lower limb)**	USA	101	494	Population	Population
Chen, 1996[234] **(trunk)**	USA	97	494	Population	Population
Chen, 1996[234] **(upper limb)**	USA	265	494	Population	Population
Cristofolini, 1987[235]	Italy	103	205	Hospital	Hospital
Dabkowski, 1997[188]	Poland	74	300	Hospital	Population
Dubin, 1990[214]	USA	289	527	Hospital	Hospital
Dunn-Lane, 1993[339]	Ireland	100	100	Hospital	Hospital
Elwood, 1984[294]	Canada	595	595	Population	Population
Elwood, 1986[236]	UK	83	83	Hospital	Hospital
Graham, 1985[286] **(men)**	USA	218	202	Hospital	Hospital
Graham, 1985[286] **(women)**	USA	186	317	Hospital	Hospital
Green, 1986[250]	Australia	183	183	Population	Population
Grulich, 1996[171]	Australia	244	276	Hospital	Hospital
Halpern, 1991[238]	USA	105	181	Hospital	Population
Herzfeld, 1993[295] **men**	USA	324	415	Population	Population
Holly, 1987[169]	USA	121	139	Hospital	Hospital

Table 5.16b. Characteristics of the studies on eye colour.

First author, year	Country	N° Cases	N° controls	Sources of cases	Sources of controls
Holly, 1995[333] (women)	USA	452	930	Population	Population
Holman, 1984[150]	Australia	507	507	Population	Population
Lock-Andersen, 1998[337]	Denmark	168	176	Hospital	Population
Marrett, 1992[186]	Canada	583	608	Population	Population
Moore, 1997[230]	USA	69	69	Population	Population
Nelemans, 1993[336]	Netherlands	141	183	Population	Population
Osterlind, 1988[226]	Denmark	474	926	Population	Population
Rodenas, 1996[184]	Spain	105	138	Hospital	Visitors to hospital
Rosso, 1998[299]	Italy	260	416	Population	Population
Westerdahl, 1994[293]	Sweden	400	640	Population	Population
Wolf, 1998[298]	Austria	193	319	Hospital	Hospital

Table 5.17a. Categories and methods of assessment for eye colour.

First author, year	Categories	Methods of assessment
Ammannati, 1987	1)black/brown; 2)blue; 3)grey/green	Questions to the interviewees
Augustsson, 1991	1)brown/mixed; 2)blue/grey; 3)green	Assessed by interviewer
Beitner, 1990	1) brown, mixture (mainly brown); 2)blue, grey-blue; 3)green	Questions to the interviewees
Bell, 1987	1)brown; 2)blue	Questions to the interviewees
Carli, 1995	1)black/brown; 2)green; 3)grey/blue	Questions to the interviewees
Chen, 1996	1)med/dark brown; 2)grey/hazel; 3)blue	Assessed by direct inspection against a group of standardized photos of eye colour (by interviewer)
Cristofolini, 1987	1)black; 2)hazel, green, grey; 3)blue	Standard questionnaire and assessment by the interviewing dermatologist
Dabkowski, 1997	1)other; 2)blue/green	Questionnaire filled in together with the interviewer
Dubin, 1990	1)brown; 2)grey; green; hazel; 3)blue	Assessed by interviewer
Dunn-Lane, 1993	1)brown/hazel; 2)blue/grey/green	Questions to the interviewees
Elwood, 1984	1)brown; 2)grey; green; hazel; 3)blue	Assessed by interviewer
Elwood, 1986	1)brown; 2)green, hazel; 3)blue, grey	
Graham, 1985 (women)	1)brown; 2)hazel; 3)blue-green; grey; 4)blue	Questions to the interviewees

Table 5.17b. Categories and methods of assessment for eye colour.

First author (year)	Categories	Methods of assessment
Graham, 1985 (men)	1)brown; 2)hazel; 3)blue-green; grey; 4)blue	Questions to the interviewees
Green, 1986	1)brown; 2)blue, grey; 3)green, hazel	
Grulich, 1996	1)brown; 2)hazel; 3)green; 4)blue/grey	
Halpern, 1991	1)brown; 2)blue; 3)hazel/other	Questions to the interviewees
Herzfeld, 1993	1)other; 2)blue	Interview by trained
Holly, 1987	1)dark; 2)light	Questions to the interviewees
Holly, 1995	1)brown; 2) green, gay, hazel; 3) blue	Questions to the interviewees
Holman, 1984	1)brown; 2)hazel; 3)green; 4)grey; 5)blue	Assessed by interviewer
Lock-Andersen, 1998	1)brown; 2)grey/green; 3)blue	Assessed by interviewer
Marrett, 1992	1)brown; 2)blue; 3)hazel, green, grey	Questions to the interviewees
Moore, 1997	1)other; 2)blue	Questionnaire filled in together with the interviewer
Nelemans, 1993	1)brown; 2)grey/green; 3)blue	Examined by a physician trained in dermatology
Osterlind, 1988	1)brown; 2)grey/green; 3)blue	Inspection of the left eye
Rodenas, 1996	1)black/brown; 2)hazel; 3)blue/green	
Rosso, 1998	1)dark; 2)blu/green/hazel	
Westerdahl, 1994	1)other; 2)blue	Questions to the interviewees
Wolf, 1998	1)brown; 2)green; 3)grey; 4)blue	Questions to the interviewees

5.8.10 Hair colour Tables

Table 5.18a. Characteristics of the studies on hair colour.

First author, Year	Country	N° Cases	N° controls	Sources of cases	Sources of controls
Ammannatti, 1987[338]	Italy	104	104	Hosp	Hosp
Augustsson, 1991[208]	Sweden	121	378	Pop	Pop
Autier, 1995[288]	Germany, France and Belgium	420	447	Hosp	Pop
Beitner, 1990[334]	Sweden	523	505	Hosp	Pop
Bell, 1987[297]	UK	268	1577	Hosp	Hosp
Carli, 1999[219]	Italy	131	174	Hosp	Pop
Chen, 1996[234] (head/neck)	USA	85	494	Pop	Pop
Chen, 1996[234] (lower limb)	USA	97	494	Pop	Pop
Chen, 1996[234] (trunk)	USA	265	494	Pop	Pop
Chen, 1996[234] (upper limb)	USA	101	494	Pop	Pop
Cristofolini, 1987[235]	Italy	103	205	Hosp	Hosp

Table 5.18b. Characteristics of the studies on hair colour.

First author, Year	Country	N° Cases	N° controls	Sources of cases	Sources of controls
Dubin, 1990[214]	USA	289	527	Hosp	Hosp
Dunn-Lane, 1993[339]	Ireland	100	100	Hosp	Hosp
Elwood, 1984[294]	Canada	595	595	Pop	Pop
Elwood, 1986[236]	UK	83	83	Hosp	Hosp
Elwood, 1990[237]	UK	195	195	Pop	Hosp
Garbe, 1994[165]	Germany; Austria; Switzer.	513	498	Hosp	Hosp
Graham, 1985[286] **(women)**	USA	186	317	Hosp	Hosp
Graham, 1985[286] **(men)**	USA	218	202	Hosp	Hosp
Green, 1986[250]	Australia	183	183	Pop	Pop
Grob, 1990[170]	France	207	295	Hosp	Pop
Grulich, 1996[171]	Australia	244	276	Hosp	Hosp
Halpern, 1991[238]	USA	105	181	Hosp	Pop
Herzfeld, 1993[295] **(men)**	USA	324	415	Pop	Pop
Holly, 1987[169]	USA	121	139	Hosp	Hosp
Holly, 1995[333] **(women)**	USA	452	930	Pop	Pop
Holman, 1984[150]	Australia	507	507	Pop	Pop
Lock-Andersen, 1998[337]	Denmark	168	176	Hosp	Pop
Marrett, 1992[186]	Canada	583	608	Pop	Pop
Moore, 1997[230]	USA	69	69	Pop	Pop
Nelemans, 1993[336]	Netherlands	141	183	Pop	Pop
Osterlind, 1988[226]	Denmark	474	926	Pop	Pop
Rodenas, 1996[184]	Spain	105	138	Hosp	Visitors to hosp
Rosso, 1998[299]	Italy	260	416	Pop	Pop
Weinstock, 1991[335] **(women)**	USA	130	300	Pop	Pop
Weiss, 1991[213]	Germany	204	200	Hosp	Hosp
Westerdahl, 1994[293]	Sweden	400	640	Pop	Pop
White, 1994[249]	USA	256	273	Pop	Pop
Wolf, 1998[298]	Austria	193	319	Hosp	Hosp
Zanetti, 1988[290]	Italy	260	416	Hosp	Hosp

Table 5.19a. Definitions and methods of assessment of hair colour.

First author, year	Category	Definitions	Assessment
Ammannati, 1987	1) black/brown; 2)blond/red	Hair colour	Questions to the interviewees
Augustsson, 1991	1)dark brown; 2)light brown; 3)red/blond	Hair colour at the age of 20	Estimated and rated according to a three-point scale
Autier, 1995	1)black; 2)brown; 3)blond; 4)red	Hair colour	By interview and by observation with guidance charts

Table 5.19b. Definitions and methods of assessment of hair colour.

First author, year	Category	Definitions	Assessment
Beitner, 1990	1)black; 2)ash-blonde, brown; 3)red; 4)blonde	Original hair colour	Questions to the interviewees
Bell, 1987	1)other; 2)dark; 3)auburn + red	Hair colour	Questions to the interviewees
Carli, 1999	1)black; 2)dark brown; 3)light brown; 4)blond/red	Hair colour	Questions to the interviewees
Chen, 1996	1)dark brown; 2)light/med brown; 3)blonde; 4)red/auburn	Natural hair colour at age 20 years	Graded visually by the subject against a color sample chart
Cristofolini, 1987	1)black; 2)brown; 3)light brown, blond or red	Hair colour (adult)	Standard questionnaire and assessment by the interviewing dermatologist
Dubin, 1990	1)dark brown; 2)black; 3)light brown; 4)blond; 5)red	Natural colour during the winter at age of 20 years	Self-administered reliability questionnaire
Dunn-Lane, 1993	1)brown/black; 2)blond; 3)red	Hair colour at age 20	Questions to the interviewees
Elwood, 1984	1)black; 2)dark brown; 3)brown; 4)red and light brown; 5)blonde	Adult hair colour	Direct comparison with prosthesis and wigmakers samples
Elwood, 1986	1)black, dark brown; 2)light brown; 3)red, blonde	Natural hair colour in childhood and as a young adult	Structured questionnaire and comparison chart developed by the Western Canada Melanoma Study
Elwood, 1990	1)black/dark brown; 2)mid brown; 3)fair; 4)blonde; 5)light red; 6)dark red	Hair colour	By interviewer using standardized hair samples
Garbe, 1994	1)black or brown; 2)blond; 3)red	Hair colour at the age of 20	Dermatologic examinations
Graham, 1985	1)brown or black; 2)blond; 3)red	Hair colour as a child	Questions to the interviewees
Green, 1986	1)black/dark brown; 2)light brown/blonde; 3)red	Hair colour at age 21 as graded against a standard colour chart	Questions to the interviewees
Grob, 1990	1)black; 2)brown; 3)blond; 4)red/reddish blond	Natural colour of hair	Questions to the interviewees
Grulich, 1996	1)black/dark brown; 2)medium brown; 3)light brown; 4)blonde; 5)red-brown/red	Natural hair colour at the age of 15 years	Questions to the interviewees

Table 5.19c. Definitions and methods of assessment of hair colour.

First author, year	Category	Definitions	Assessment
Halpern, 1991	1)black; 2)brown; 3)blond/red	Natural hair colour at age 20	Questions to the interviewees
Herzfeld, 1993	1)other; 2)blond or red	Hair colour	Interview, administered by trained
Holly, 1987	1)brown or black; 2)red or blond	Natural hair colour at age 20	Questions to the interviewees
Holly, 1995	1)black or brown; 2)blond or ligth brown; 3)red	Hair colour	Questions to the interviewees
Holman, 1984	1)black or dark brown; 2)light brown; 3)fair or blond; 4)red	Natural hair colour	Graded visually against 23 samples of human hair selected from the JL International Colour Range
Lock-Andersen, 1998	1)black/brown; 2)blond; 3)red	Hair colour before age of 7	Assessed by a pre-tested questionnaire
Marrett, 1992	1)black; 2)brown; 3)light brown; 4)blond or fair; 5)red	Natural hair colour at age 20	Questions to the interviewees
Moore, 1997	1)other; 2)blond or red	Hair colour	Questionnaire, done face-to-face
Nelemans, 1993	1)brown/black; 2)blond; 3)red/fair	Hair colour	Examined by a physician trained in dermatology
Osterlind, 1988	1)dark brown/ black; 2)light brown; 3)blond/fair; 4)red	Natural hair colour	Graded visually against 20 samples of human hair
Rodenas, 1996	1)black; 2)brown; 3)blond/red	Hair colour at age 20 to 30	
Rosso, 1998	1)black/brown; 2)light brown; 3)blond/red	Hair colour	Using a visual scale on 11 levels
Weinstock, 1991	1)black; 2)dark brown; 3)light brown; 4)blonde or auburn; 5)red	Natural hair colour at age 20	Questions to the interviewees
Weiss, 1991	1)black/dark; 2)blond; 3)red	Natural hair colour at age 20	
Westerdahl, 1994	1)dark brown/ black; 2)light brown; 3)blond/fair; 4)red	Hair colour	Questions to the interviewees
White, 1994	1)brown/black; 2)red/blond	Hair colour before any greying	Questions to the interviewees
Wolf, 1998	1)black; 2)brown; 3)blond; 4)red	Hair colour before any greying	Questions to the interviewees
Zanetti, 1988			Using a visual scale on 11 levels

Table 5.20. RR estimates from meta-analysis.

	RR	Low CI	Up CI
"brown" versus "black"	1.34	1.18	1.53
"blond" versus "black"	1.80	1.58	2.06
"red" versus "black"	2.42	2.12	2.76

5.8.11 Skin colour Tables

Table 5.21. Characteristics of studies on skin colour.

First author, Year	Country	N° Cases	N° Controls	Source of cases	Source controls
Ammannatti, 1987[338]	Italy	104	104	Hosp	Hosp
Carli, 1999[219]	Italy	131	174	Hosp	Pop
Chen, 1996[234] **(head/neck)**	USA	85	494	Pop	Pop
Chen, 1996 [234] **(lower limb)**	USA	97	494	Pop	Pop
Chen, 1996[234] **(trunk)**	USA	265	494	Pop	Pop
Chen, 1996[234] **(upper limb)**	USA	101	494	Pop	Pop
Cristofolini, 1987[235]	Italy	103	205	Hosp	Hosp
Dabkowski, 1997[188]	Poland	74	300	Hosp	Pop
Dubin, 1986[164]	USA	1103	585	Hosp	Hosp
Elwood, 1984[294]	Canada	595	595	Pop	Pop
Elwood, 1990[237]	England	215	215	Pop	Hosp
Freedman, 1997[308]	USA	12156	23845	Other	Other
Graham, 1985[286] **(women)**	USA	186	319	Hosp	Hosp
Graham, 1985[286] **(men)**	USA	218	202	Hosp	Hosp
Green, 1986[250]	Australia	183	183	Pop	Pop
Grob, 1990[170]	France	207	295	Hosp	Pop
Herzfeld, 1993[295] **(men)**	USA	324	415	Pop	Pop
Holly, 1995[333] **(women)**	USA	452	930	Pop	Pop
Holman, 1984[150]	Australia	507	507	Pop	Pop
Lock-Andersen, 1998	Denmark	168	176	Hosp	Pop
Marrett, 1992[186]	Canada	583	608	Pop	Pop
Nelemans, 1993[336]	Netherlands	141	183	Pop	Pop
Osterlind, 1988[226]	Denmark	474	926	Pop	Pop
Rodenas, 1996[184]	Spain	105	138	Hosp	Visitors to hosp
Walter, 1990[344]	Canada	583	608	Pop	Pop
Weinstock, 1991[335] **(women)**	USA	130	300	Pop	Pop
Wolf, 1998 [298]	Austria	193	319	Hosp	Hosp
Zaridze, 1992[224]	Russia	96	96	Hosp	Other

Table 5.22. Methods of assessment for skin colour.

First author, year	Methods of assessment
Ammannati, 1987	Questionnaire
Carli, 1999	Interview
Chen, 1996	Assessed by the nurse-interviewer at the inner aspect of the upper arm
Cristofolini, 1987	Standard questionnaire and assessment by the interviewing dermatologist
Dabkowski, 1997	Questionnaire filled in together with the interviewer
Dubin, 1986	Skin colour chart used during an interview (of area exposed to sun and not exposed)
Elwood, 1984	Prosthesis and wigmakers samples used during an interview (of area exposed to sun and not exposed)
Elwood, 1990	Visual-skin colour chart
Freedman, 1997	
Graham, 1985	Ascertained by direct questions to the interviewees, self-assessed complexion colour
Green, 1986	Determined by interviewer (left forearm)
Grob, 1990	Determined by interviewed: Complexion colour
Herzfeld, 1993 (men)	Interview, administered by trained
Holly, 1995 (women)	Interview, self-assessed complexion colour
Holman, 1984	Interviewers use goggles fitted with monochromatic filters and complexion chart (left dorsum of hand, left shoulder tip and upper inner arm)
Lock-Andersen, 1998	Measurements were taken by metropolitan interviewers using goggles fitted with monochromatic filters; in rural areas skin colour was graded visually against a 10-step complexion chart
Marrett, 1992	Assessed by the interviewer, who compared the skin on the subject's upper inner arm to a 15 colour prosthetic skin-tone panel
Nelemans, 1993	Examined by a physician trained in dermatology
Osterlind, 1988	Complexion chart at 3 sites-the dorsum of the left hand, the tip of the left shoulder and the inner side of the left upper arm
Rodenas, 1996	Determined by interviewed on sun-exposed skin (dorsum of hand and upper inner arm)
Walter, 1990	Assessed using prosthetic skin sample on the inner surface of the upper left arm
Weinstock, 1991 (women)	Questionnaire
Wolf, 1998	Questionnaire
Zaridze, 1992	

5.8.12 Phototype Tables

Table 5.23. Characteristics of the studies on skin phototype.

First author, year	Country	N° Cases	N° Controls	Source of cases	Source of controls
Ammannati, 1987[338]	Italy	104	104	Hosp	Hosp
Augustsson, 1991[208]	Sweden	121	378	Pop	Pop
Autier, 1995[288]	Germany, France and Belgium	420	447	Hosp	Pop
Bataille, 1996[215]	England	426	416	Pop	Hosp
Beitner, 1990[334]	Sweden	523	505	Hosp	Pop
Carli, 1999[219]	Italy	131	174	Hosp	Pop
Chen, 1996[234] **(head/neck)**	USA	85	494	Pop	Pop
Chen, 1996[234] **(lower limb)**	USA	97	494	Pop	Pop
Chen, 1996[234] **(trunk)**	USA	265	494	Pop	Pop
Chen, 1996[234] **(upper limb)**	USA	101	494	Pop	Pop
Elwood, 1984[294]	Canada	595	595	Pop	Pop
Elwood, 1986[236]	UK	83	83	Hosp	Hosp
Garbe, 1989[210]	Germany	200	200	Hosp	Hosp
Garbe, 1994[165]	Germany; Austria; Switzer.	513	498	Hosp	Hosp
Holly, 1995[333] **(women)**	USA	452	930	Pop	Pop
Lock-Andersen, 1998[337]	Denmark	168	176	Hosp	Pop
MacKie, 1989[183] **(women)**	Scotland	181	181	Pop	Hosp
MacKie, 1989[183] **(men)**	Scotland	99	99	Pop	Hosp
Marrett, 1992[186]	Canada	583	608	Pop	Pop
Rodenas, 1996[184]	Spain	105	138	Hosp	Visitors to Hosp
Rosso, 1998[299]	Italy	260	416	Pop	Pop
Weiss, 1991[213]	Germany	204	200	Hosp	Hosp
Wolf, 1998[298]	Austria	193	319	Hosp	Hosp
Zanetti, 1988[290]	Italy	260	416	Hosp	Hosp

Table 5.24a. Definitions and methods of assessment for phototype.

First author, year	Definition of exposure
Ammannati, 1987	Phototype
Augustsson, 1991	Melski classification
Autier, 1995	Skin phototype
Bataille, 1996	Fitzpatrick classification
Beitner, 1990	Fitzpatrick classification
Carli, 1999	Fitzpatrick classification

Table 5.24b. Definitions and methods of assessment for skin phototype.

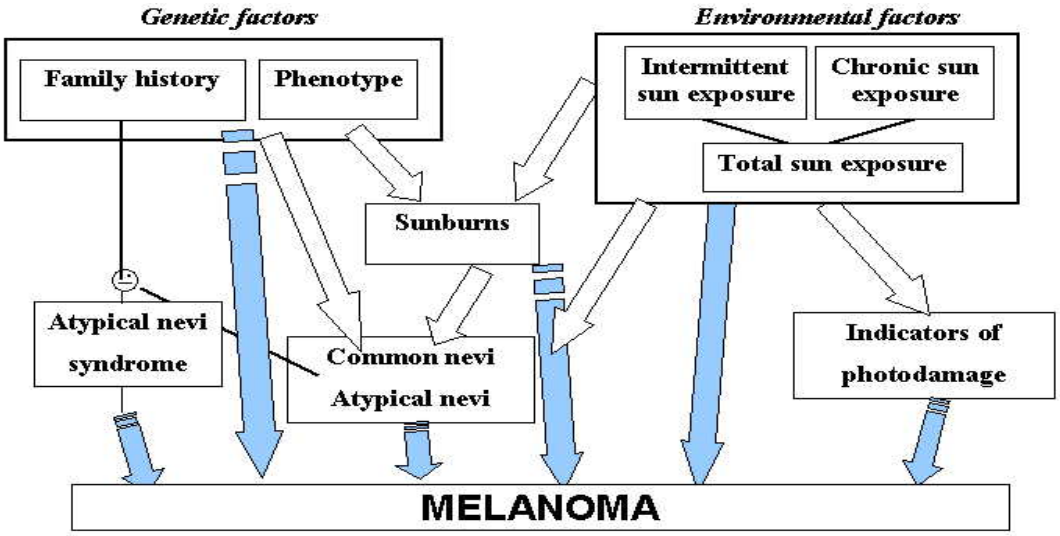
First author, year	Definition of exposure
Chen, 1996	After repeated and prolonged exposure to sunlight, skin become i) only freckled or no suntan at all (skin type I), ii) only mildly tanned due to a tendency to peel (skin type II), iii) moderately tanned (skin type III), or iv) very brown
Elwood, 1984	Sensitivity to sun burning and tanning as an adult and in childhood
Elwood, 1986	Usual skin reaction to exposure to sunlight, over a few days of sun
Garbe, 1989	Pigmentation behaviour after sun exposure
Garbe, 1994	Skin type
Holly, 1995	Skin's reaction after a few days of exposure to the sun
Lock-Andersen, 1998	Skin type
MacKie, 1989	Skin type graded: 1= always burns never tans; 2= usually burns, tans rarely and with difficulty; 3= rarely burns, tans easily; 4= tans very readily, never burns
Marrett, 1992	Skin's reaction to strong sunlight after the first summer exposure (repeated exposure)
Rodenas, 1996	Skin reaction to an initial sun exposure (45 to 60 minutes of noontime exposure in the early summer) taking into account the reaction after 24 hr (propensity to burn) and after seven days (ability to tan). Fitzpatrick Classification
Rosso, 1998	Skin reaction to sun exposure when 20 years old
Weiss, 1991	Skin type
Wolf, 1998	Skin phototype

Table 5.25. RR estimates from Meta-analysis for skin phototype.

	RR	Low CI	Up CI
III vs IV: "tan with protection" vs "tan, no burn"	1.27	1.16	1.40
II vs IV: "burn then tan" vs "tan, no burn"	1.62	1.47	1.78
I vs IV: "burn, never tan" vs "tan, no burn"	2.06	1.87	2.26

5.9 Figures

Figure 5.1. Scheme summarising some hypotheses on the disease model.



5.9.1 Total sun exposure Figures

Figure 5.2a. RR and CI for total sun exposure (first group of studies).

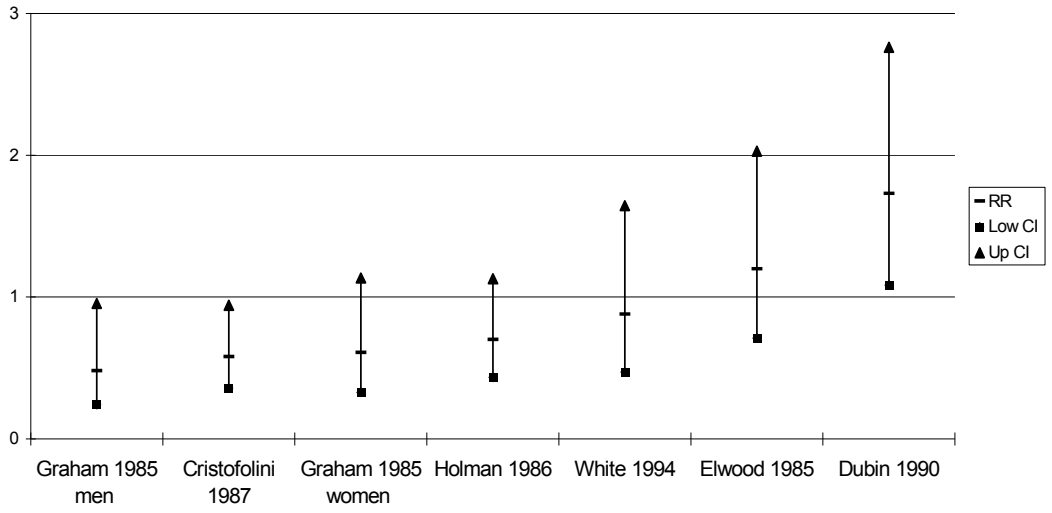


Figure 5.2b. RR and CI for total sun exposure (second group of studies).

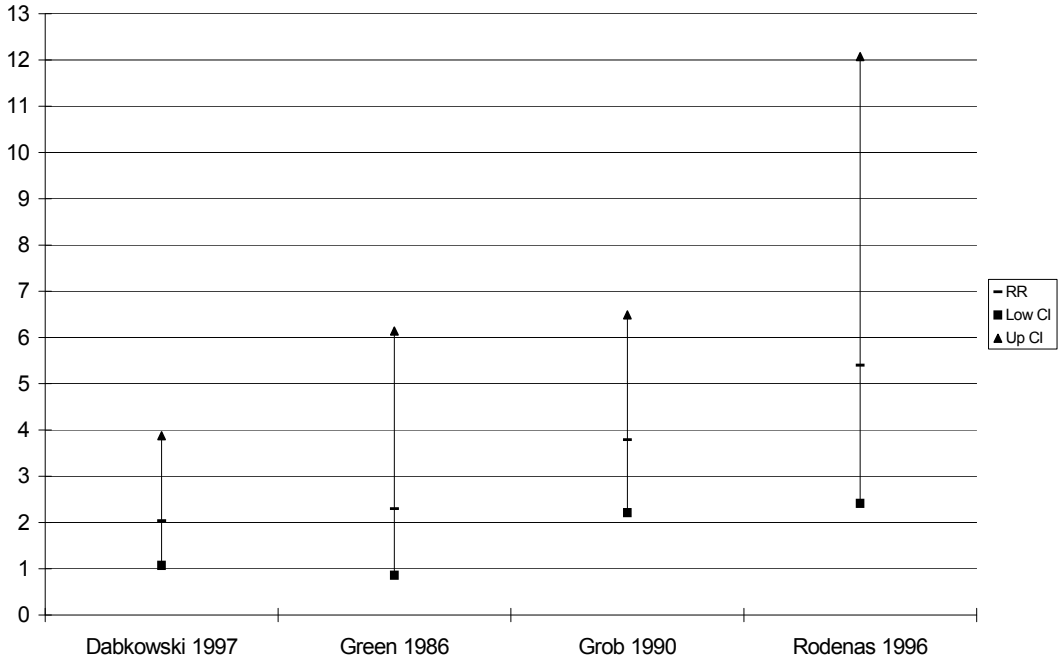
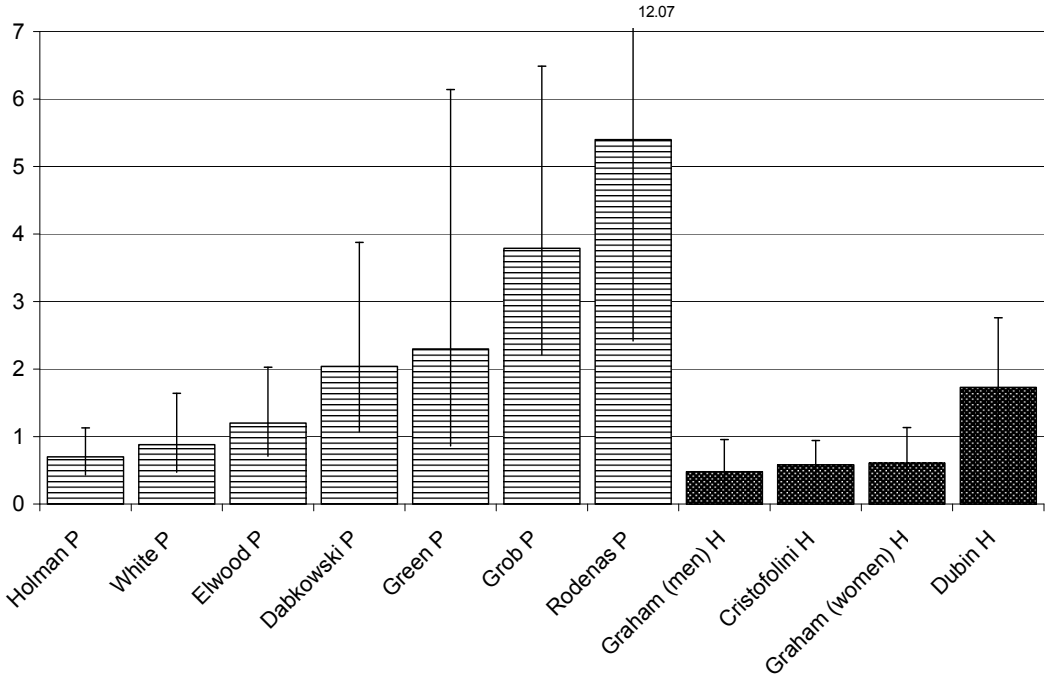
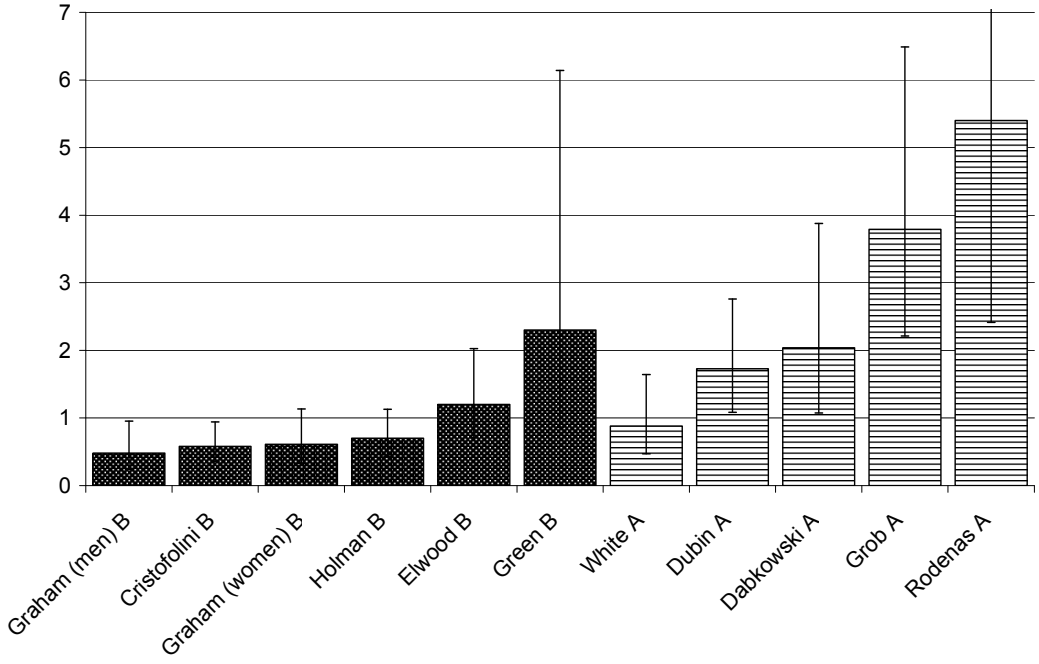


Figure 5.3. RR and CI for total sun exposure by type of controls.



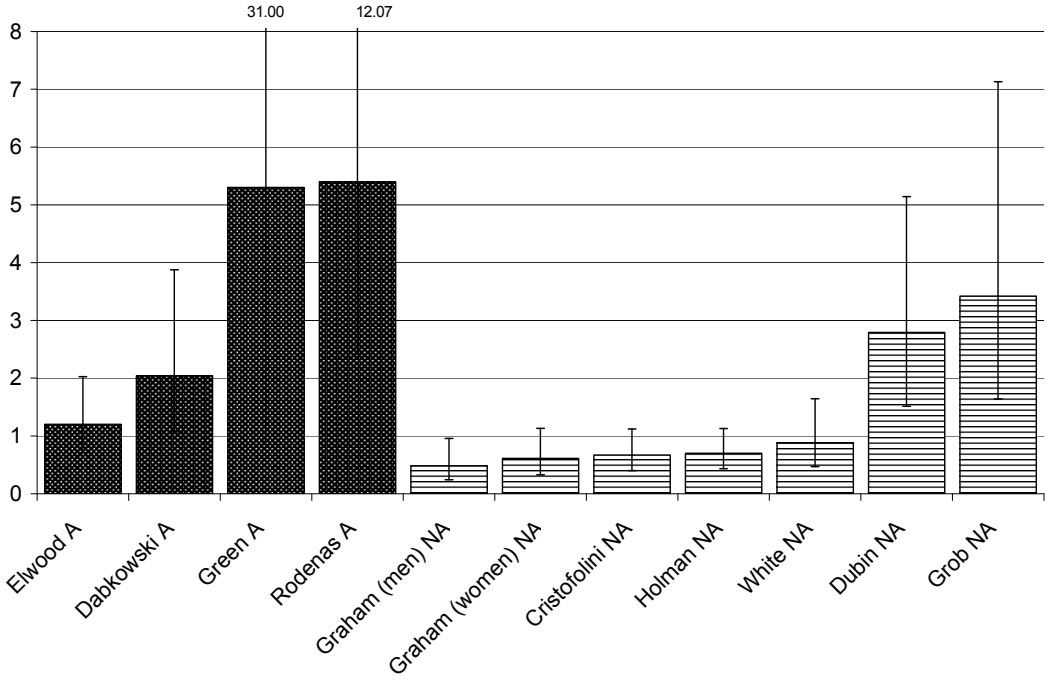
Hospital-based studies are indicated with dark bars and Population-based and other designs with grey bars.

Figure 5.4. RR and CI for total sun exposure by publication year.



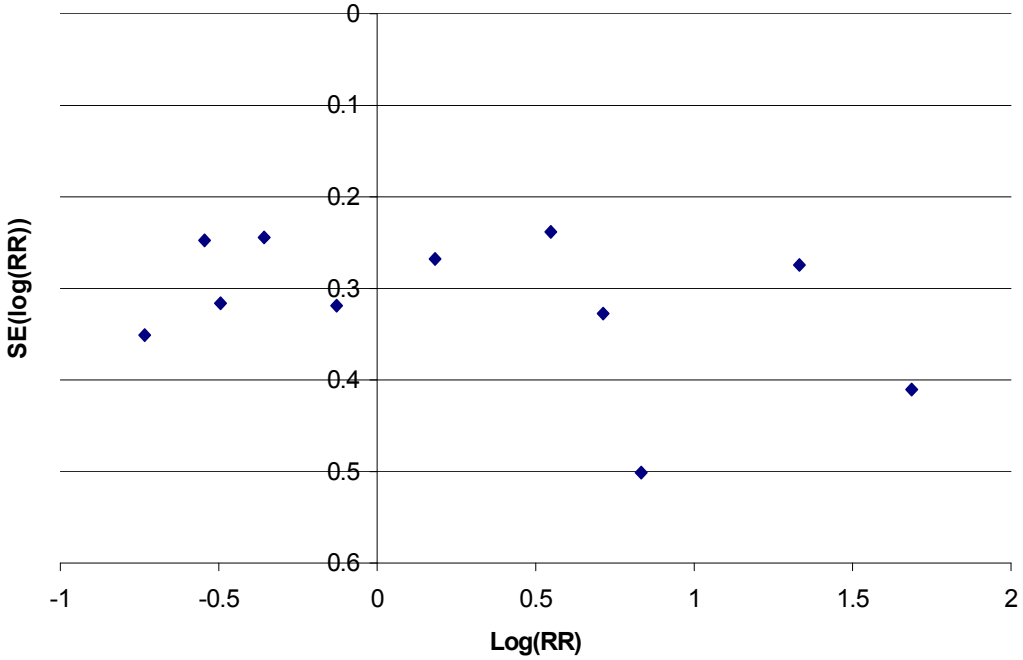
Studies conducted Before 1990 are indicated with dark bars and studies conducted in 1990 or After with grey bars.

Figure 5.5. RR and CI for total sun exposure by adjustment for phenotype.



Estimates Adjusted for phenotype are indicated with dark bars and
Not Adjusted estimates with grey bars.

Figure 5.6. Funnel plot on total sun exposure.



5.9.2 Intermittent sun exposure Figures

Figure 5.7a. RR estimates and CI on intermittent sun exposure (first group of studies).

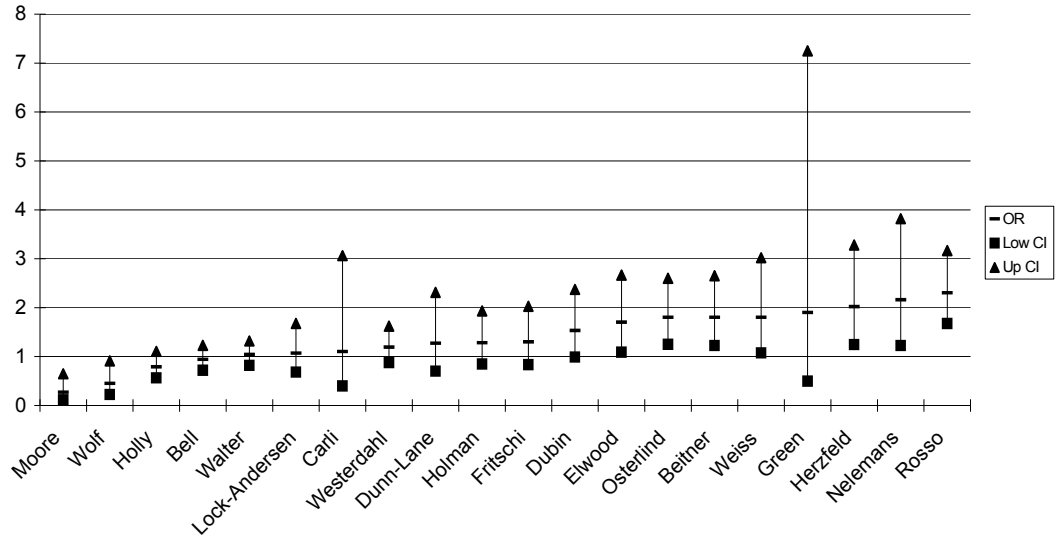


Figure 5.7b. RR estimates and CI on intermittent sun exposure (second group of studies).

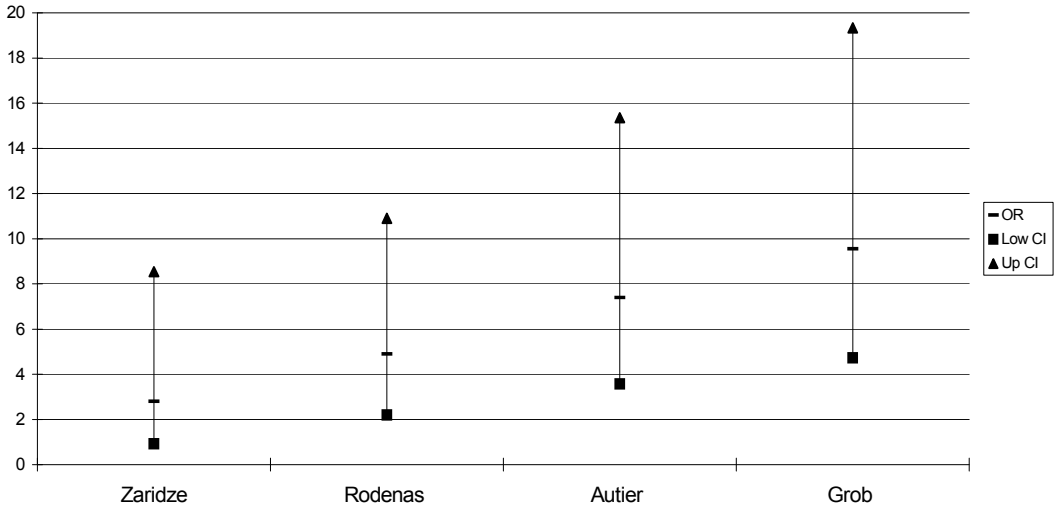
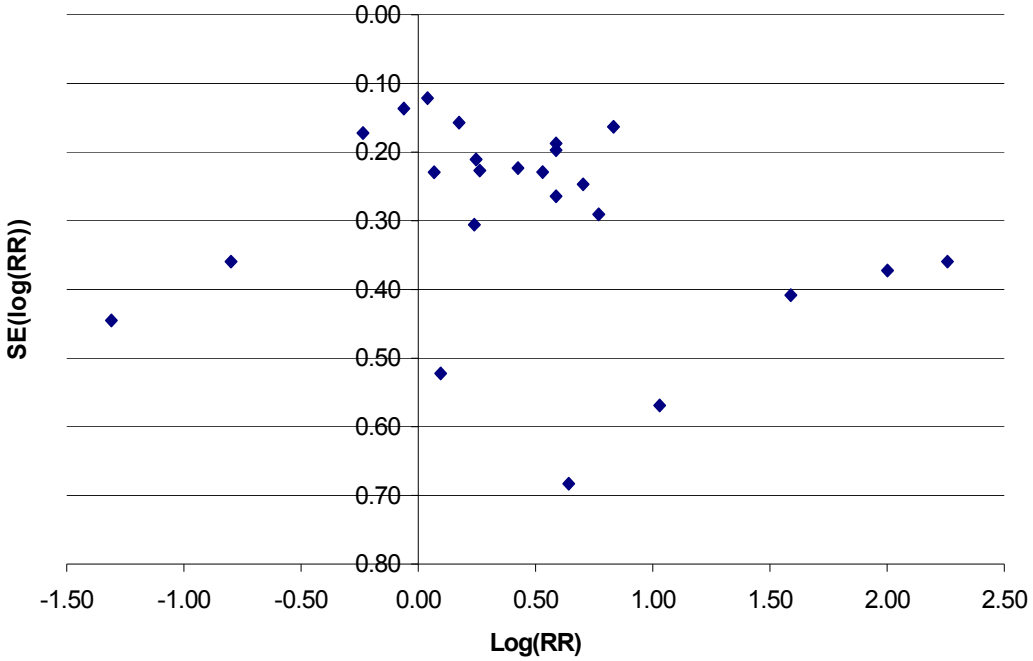


Figure 5.8. Funnel plot on intermittent sun exposure.



5.9.3 Chronic sun exposure Figures

Figure 5.9a. RR estimates and CI on chronic sun exposure (first group of studies).

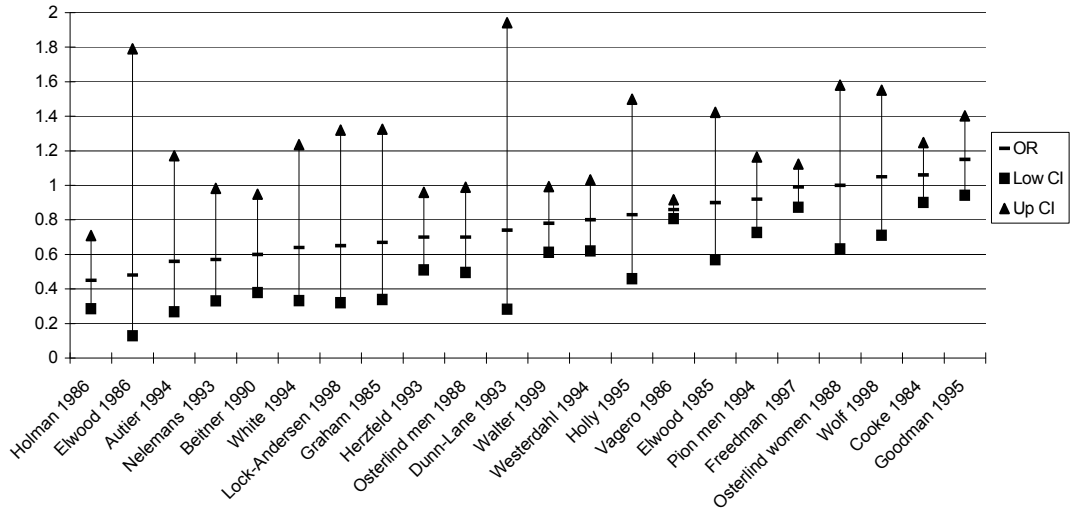


Figure 5.9b. RR estimates and CI on chronic sun exposure (second group of studies).

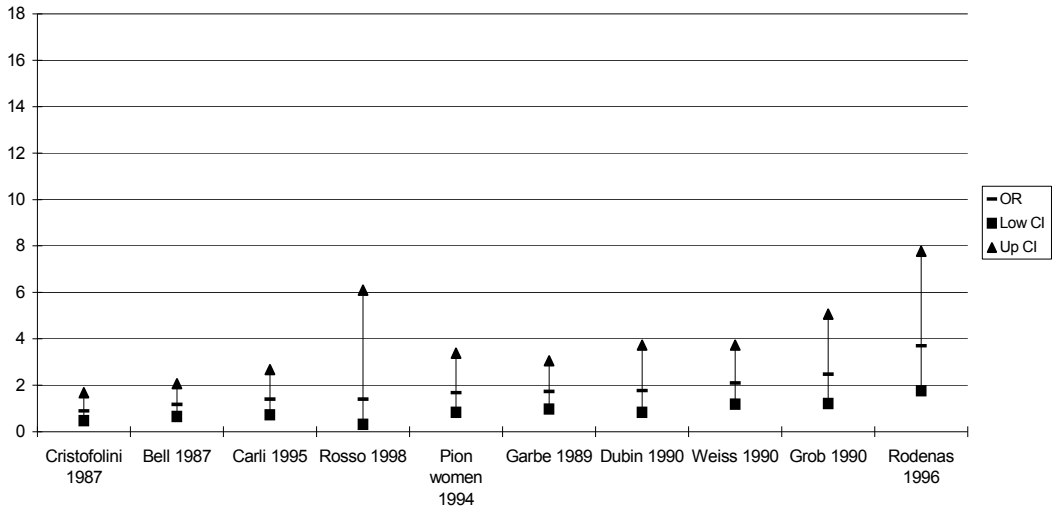
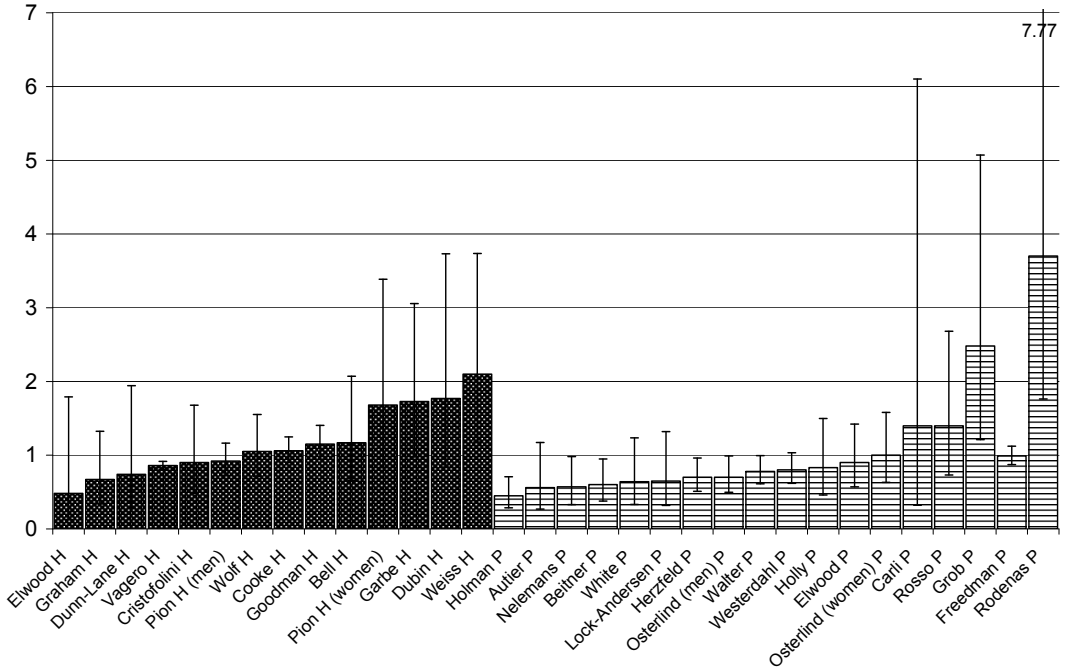
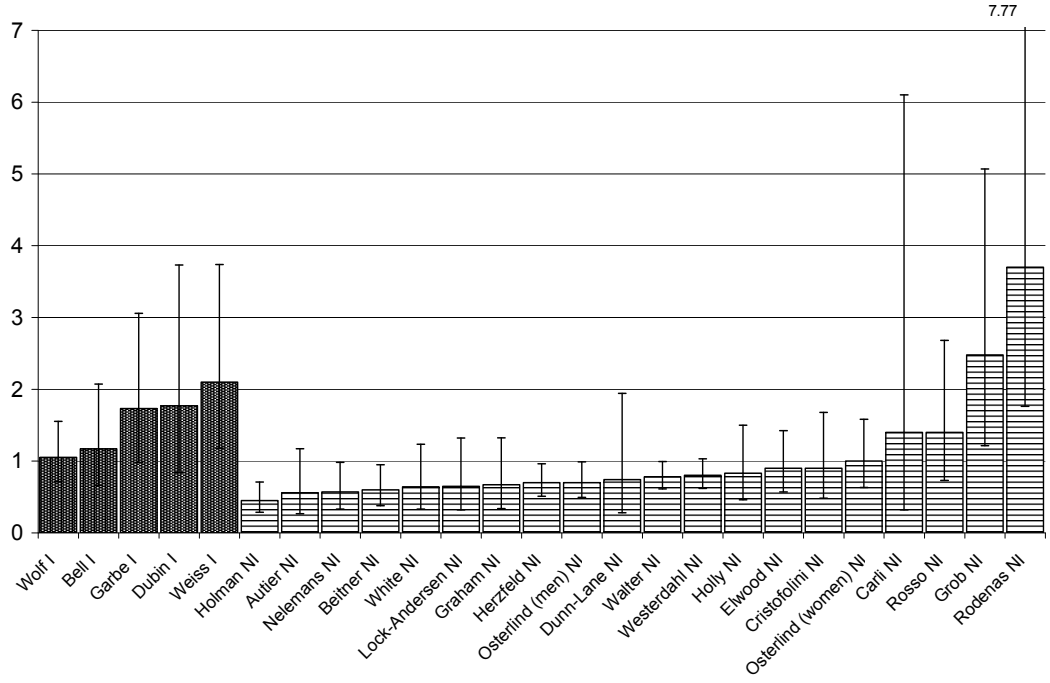


Figure 5.10. RR and CI for chronic sun exposure by type of controls.



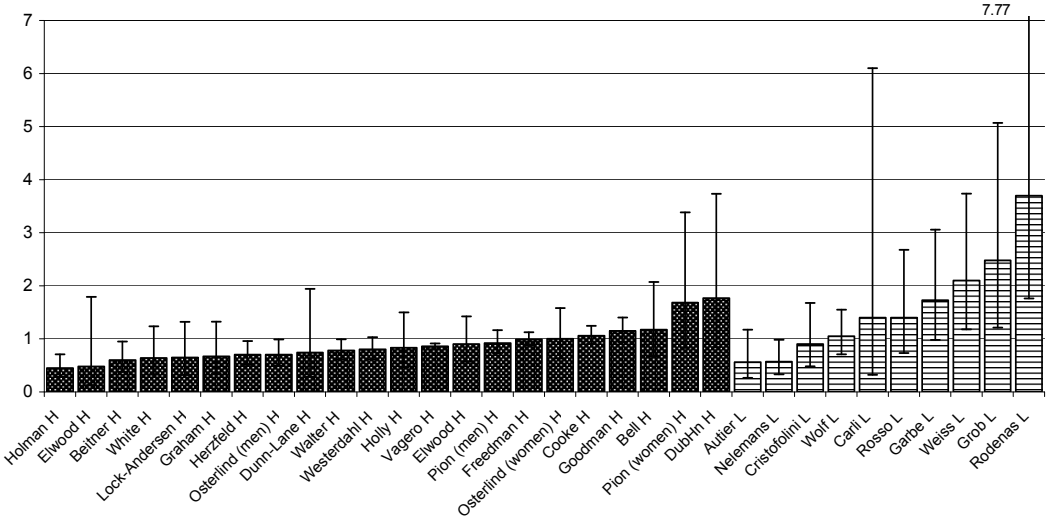
Hospital-based studies are indicated with dark bars and studies with controls drawn from Population or other sources with grey bars.

Figure 5.11. RR and CI for chronic sun exposure by inclusion of subjects with dermatological problems.



Studies that wrote to have Included subjects with dermatological problems are indicated with dark bars and the others, which Not Include them, with grey bars.

Figure 5.12. RR and CI for chronic sun exposure by type of country.



"High incidence" countries are indicated in dark and "Low incidence" countries with grey bars.

Figure 5.13. Funnel plot chronic sun exposure.

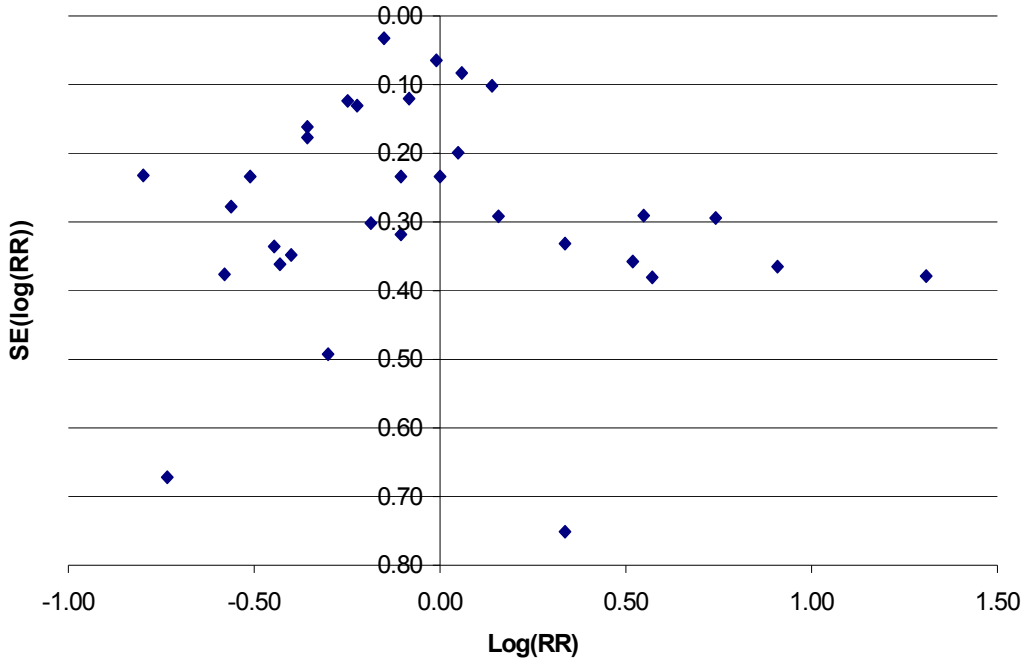
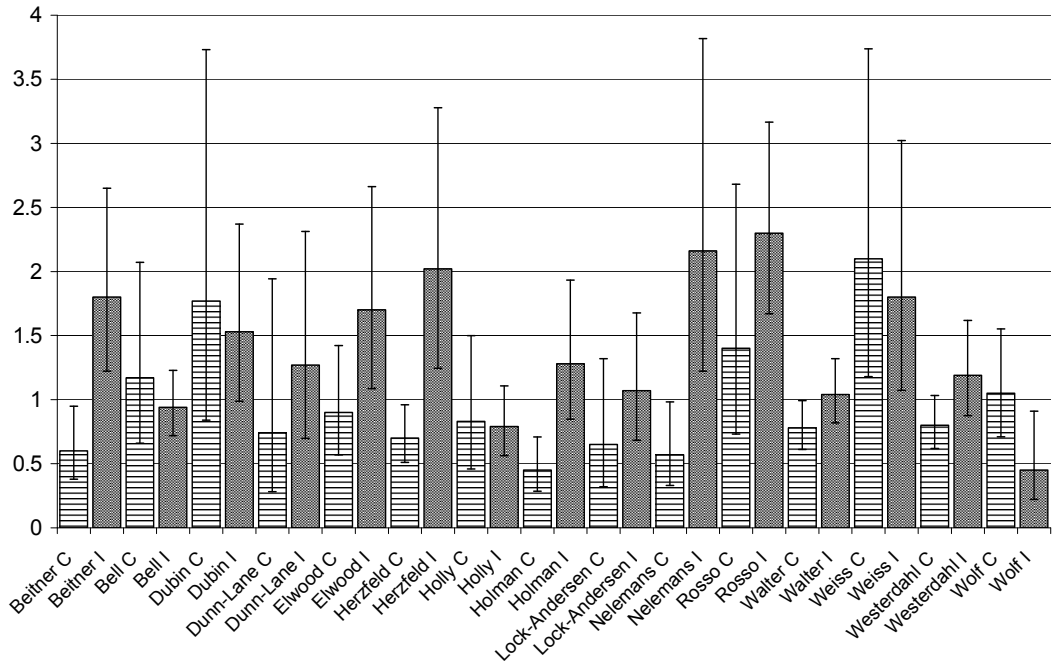
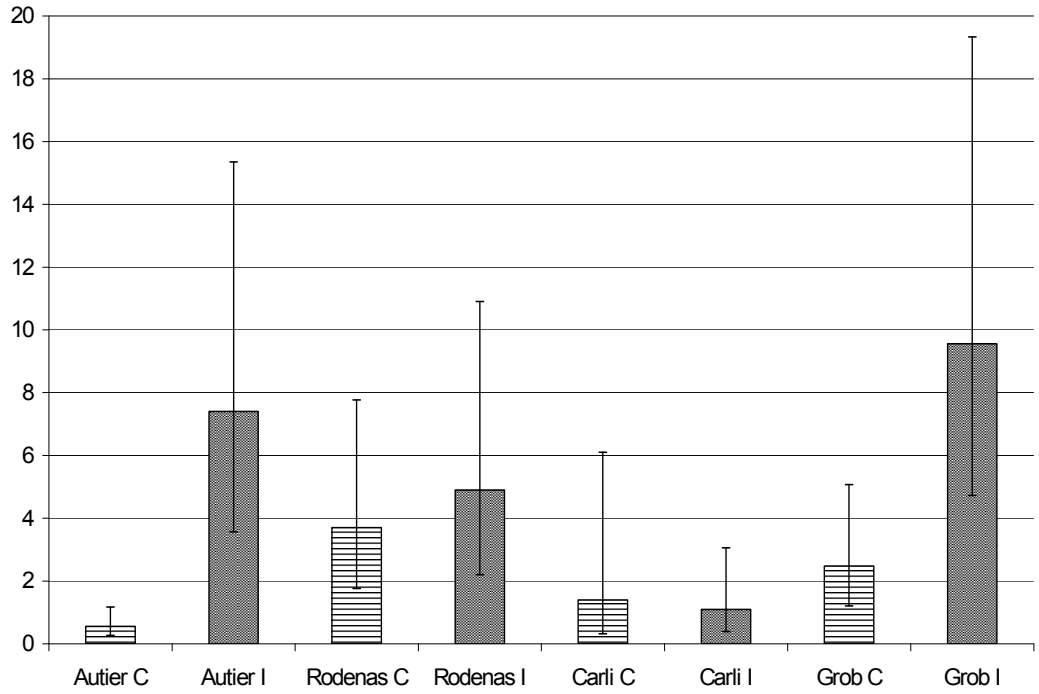


Figure 5.14a. RR and CI for Chronic and Intermittent sun exposure (first group of studies).



Intermittent sun exposure estimates are indicated in dark and chronic sun exposure estimates with grey bars

Figure 5.14b. RR and CI for Chronic and Intermittent sun exposure (second group of studies).



Intermittent sun exposure estimates are indicated in dark and Chronic sun exposure estimates with grey bars.

5.9.4 Sunburns in childhood

Figure 5.15a. RRs and CI on sunburns in childhood (first group of studies).

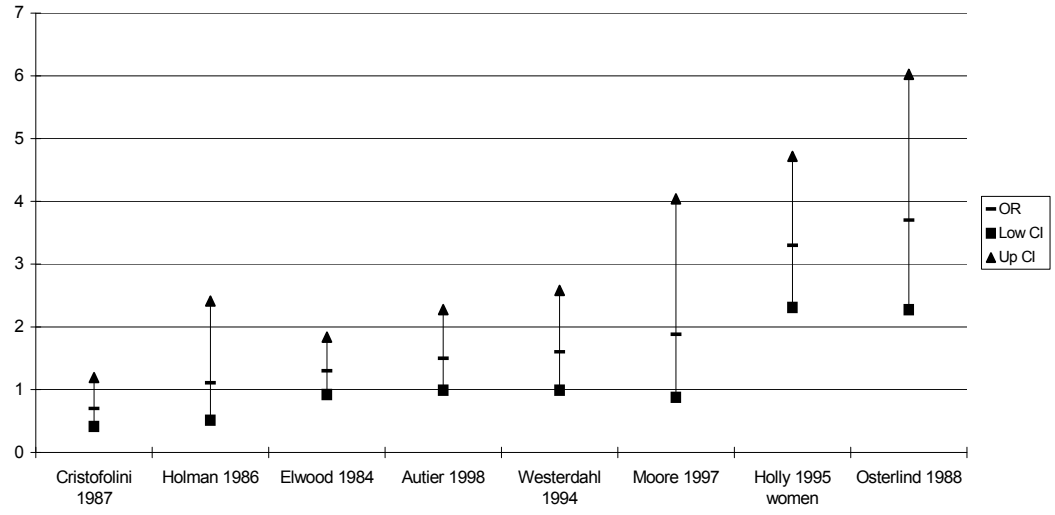


Figure 5.15b. RRs and CI on sunburns in childhood (second group of studies).

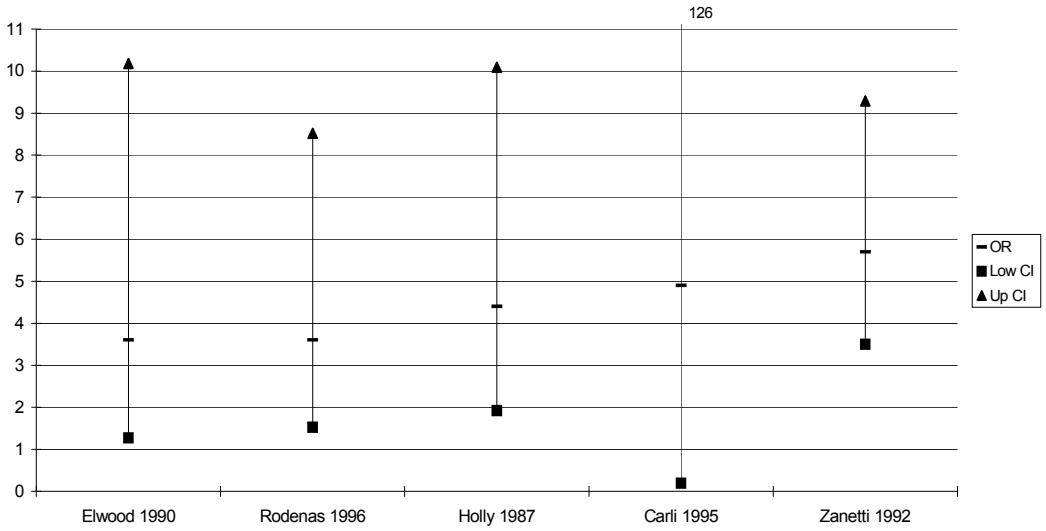
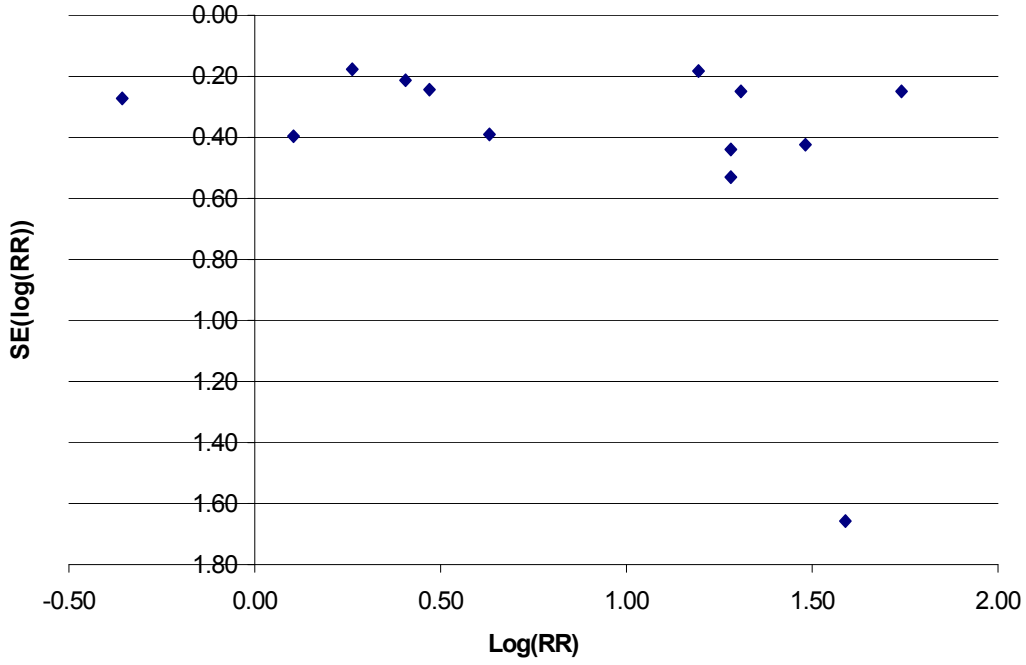


Figure 5.16. Funnel display of sunburns in childhood.



5.9.5 Sunburns in adulthood Figures

Figure 5.17a. RRs and CI for sunburns in adulthood (first group of studies).

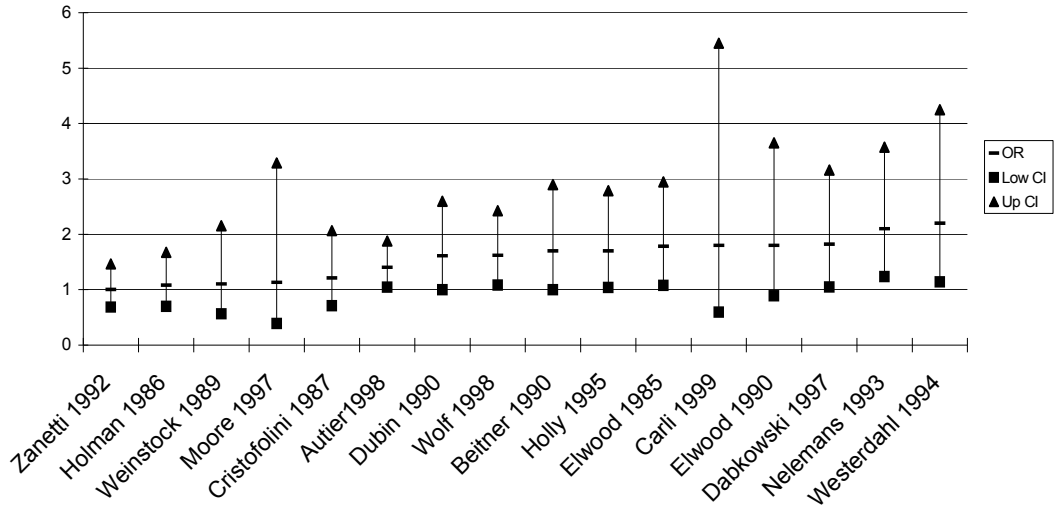


Figure 5.17b. RRs and CI for adulthood sunburns (second group of studies).

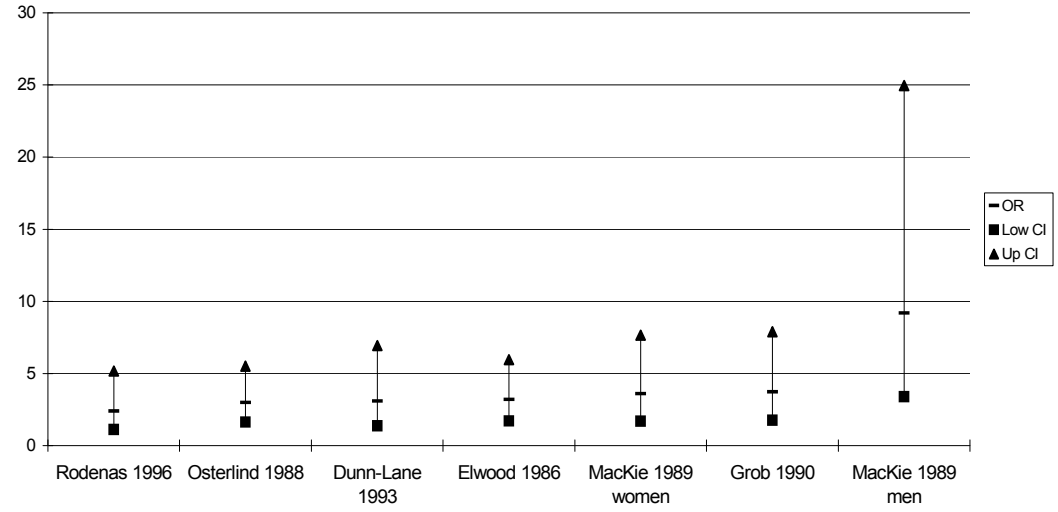
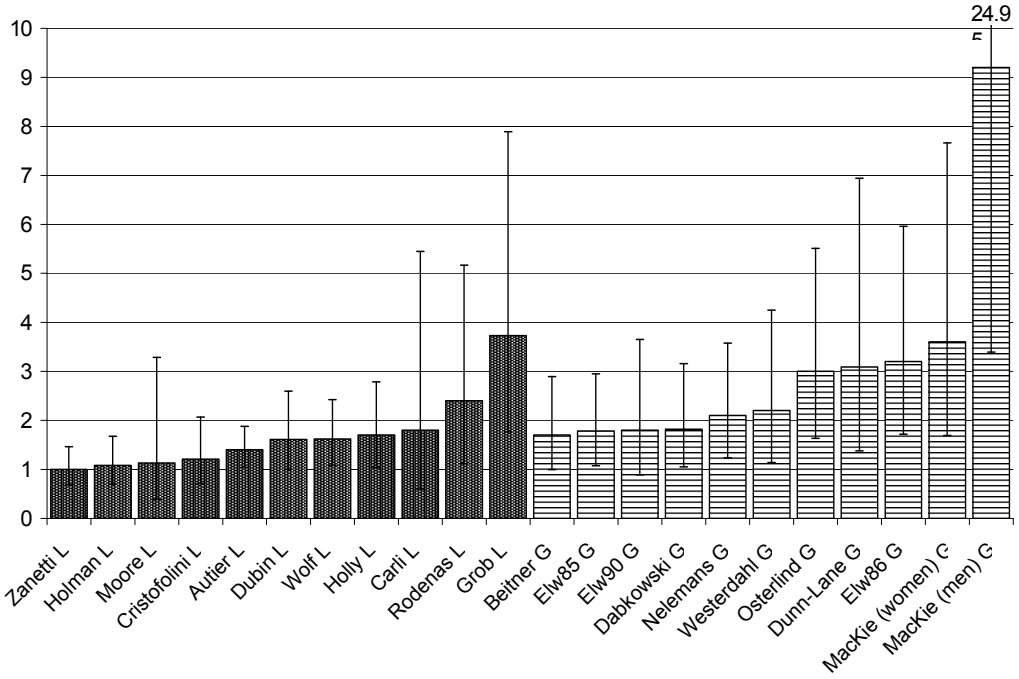


Figure 5.18. RR and CI for sunburns in adulthood by latitude.



Studies conducted at latitude Lower than 50 are indicated with dark bars and at latitudes Greater than 50 with grey bars.

Figure 5.19. Funnel plot for adulthood sunburns.

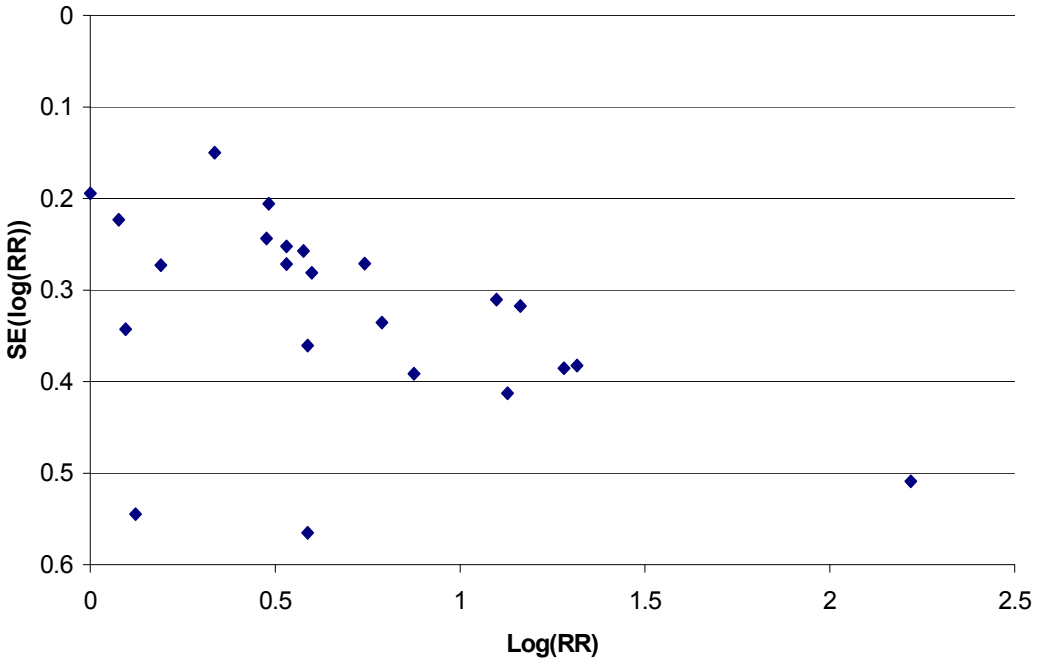


Figure 5.20. Log Relative Risk (y) vs its standard deviation (s), for sunburns in adulthood.

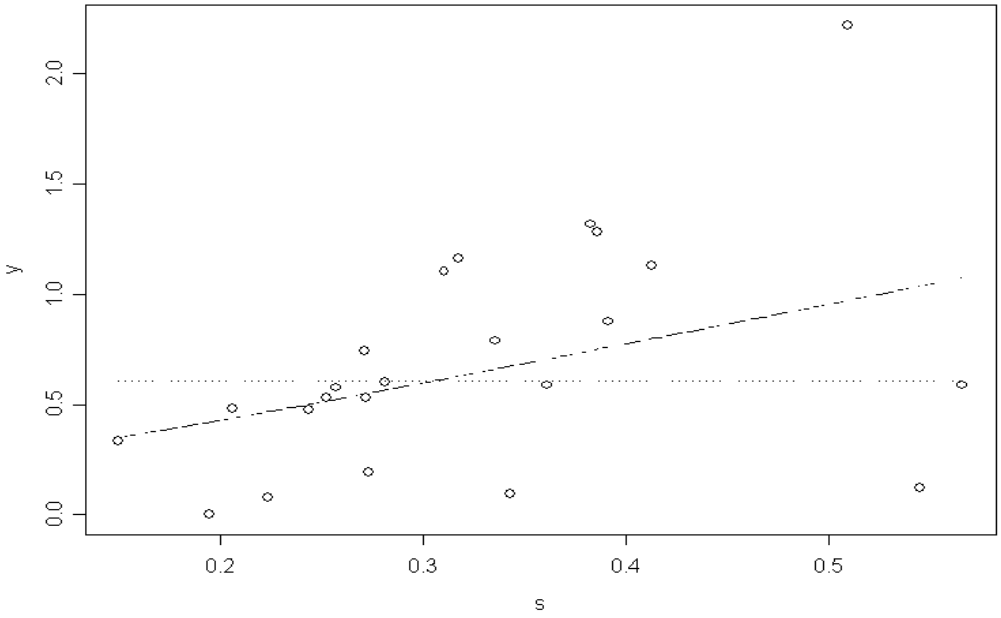
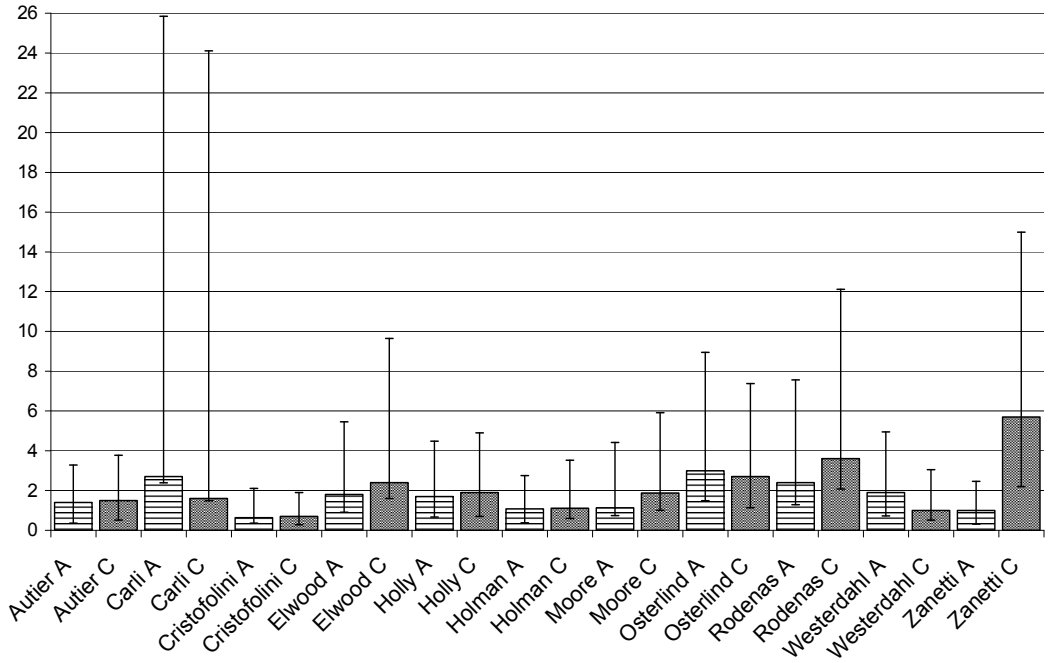


Figure 5.21. RR and CI for sunburns in Adulthood and Childhood.



RR for sunburns in Childhood are indicated in dark and RR for sunburns in Adulthood with grey bars.

5.9.6 Family history Figures

Figure 5.22a. RR and CI for family history (first group of studies).

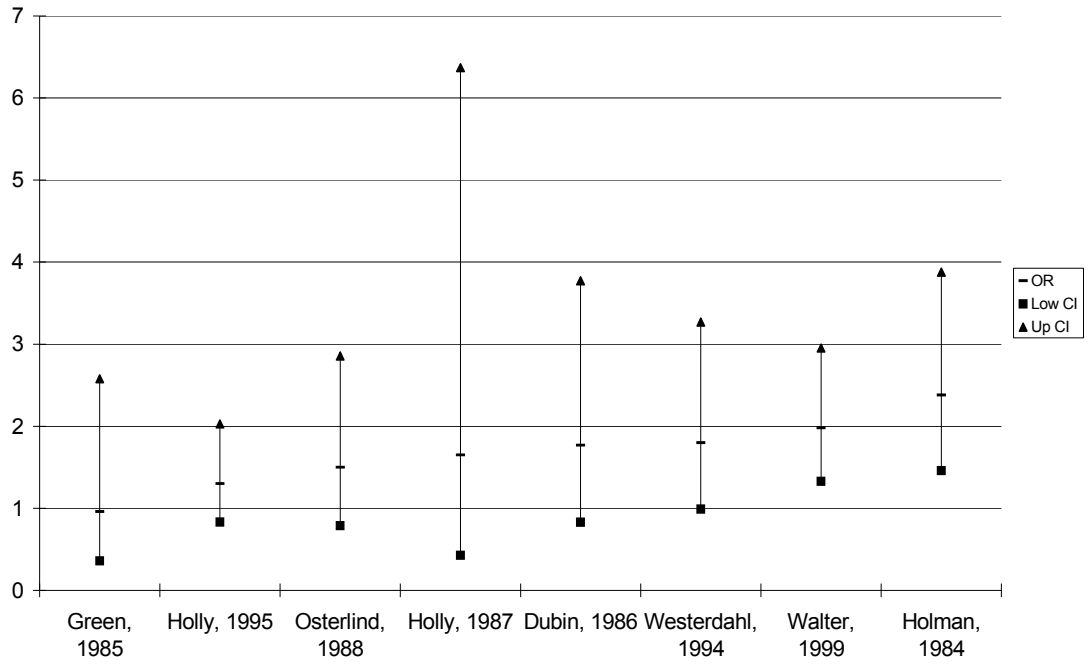


Figure 5.22b. RR and CI for family history (second group of studies).

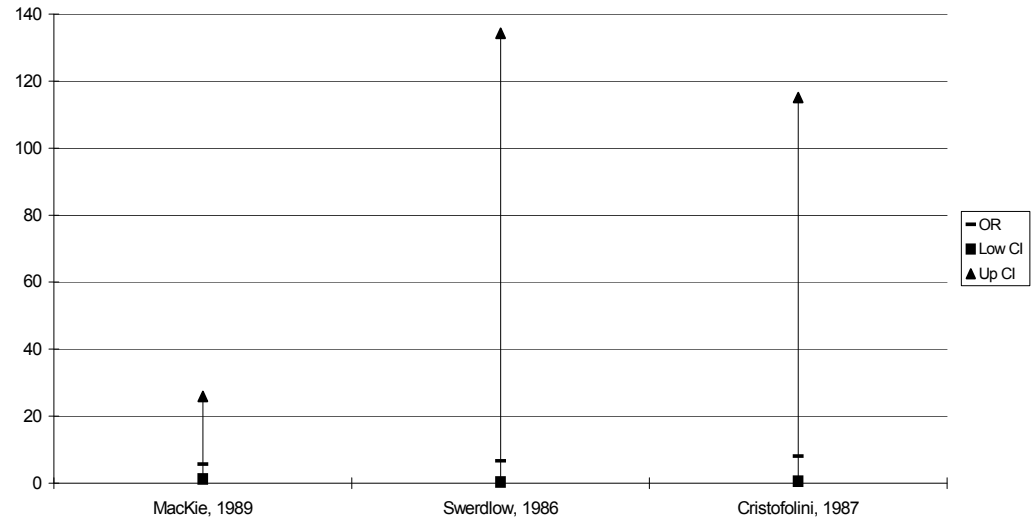
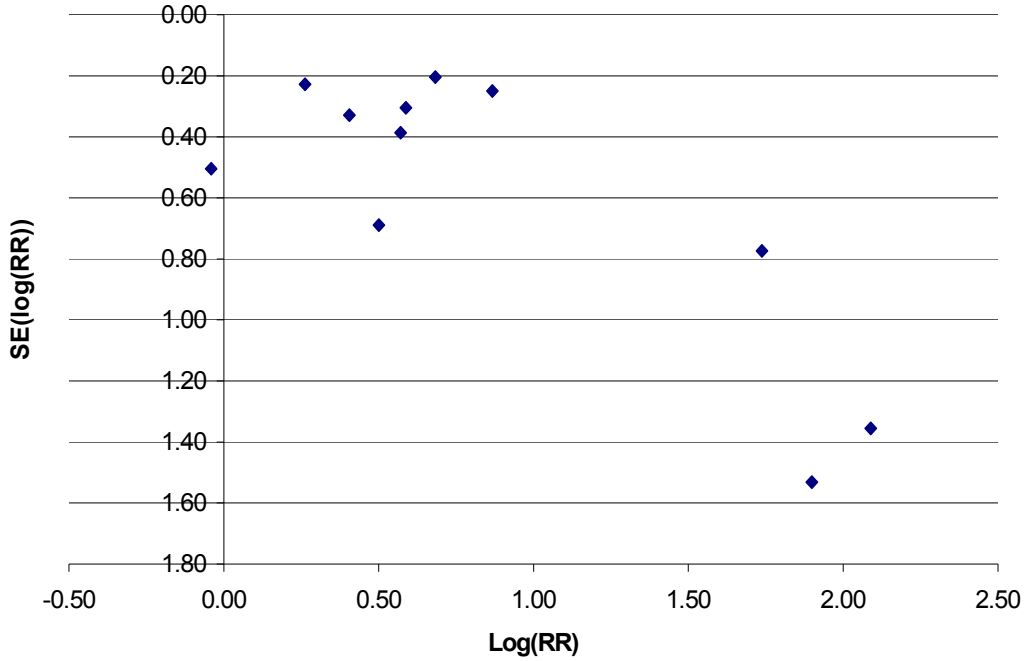


Figure 5.23: Funnel plot for family history



5.9.7 Indicators of photodamage Figures

Figure 5.24a. RR and CI on pre-malignant cancer lesions (first group of studies).

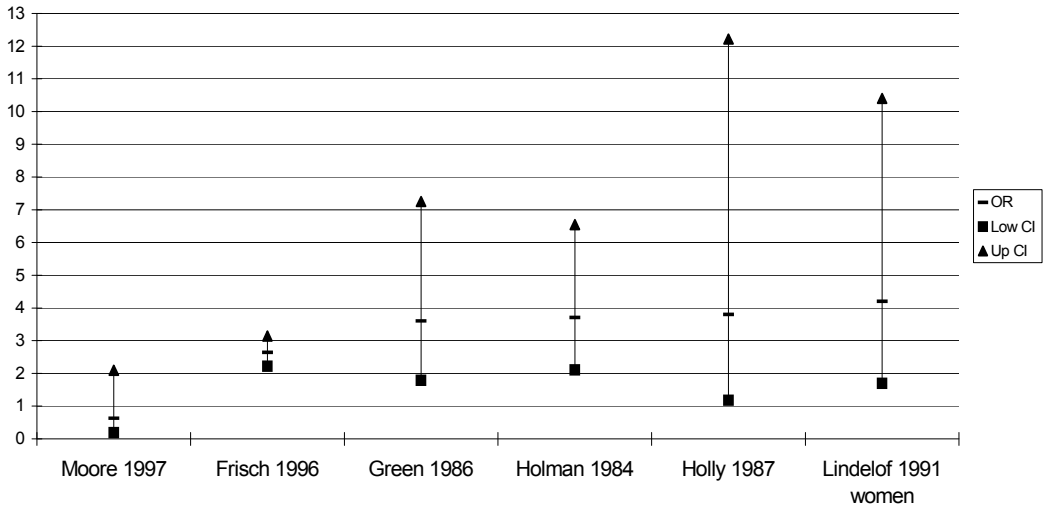


Figure 5.24b. RR and CI on pre-malignant cancer lesions (second group of studies).

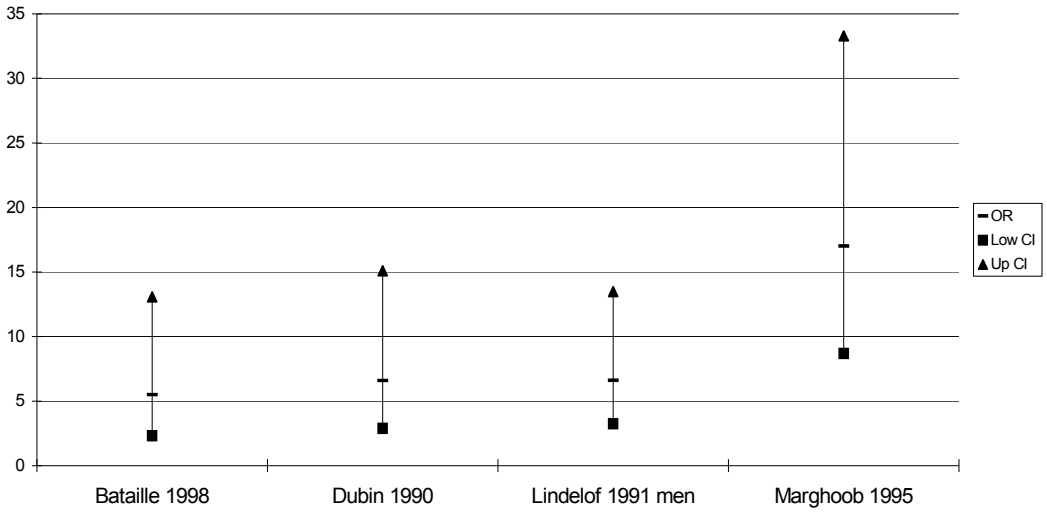


Figure 5.25. RR and CI on other indicators of photodamage.

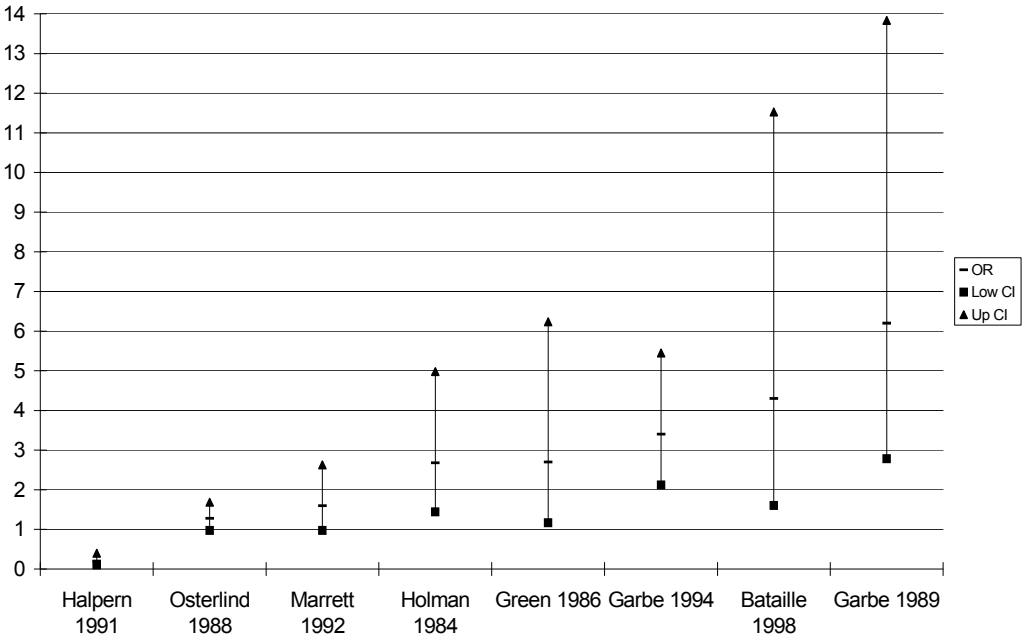
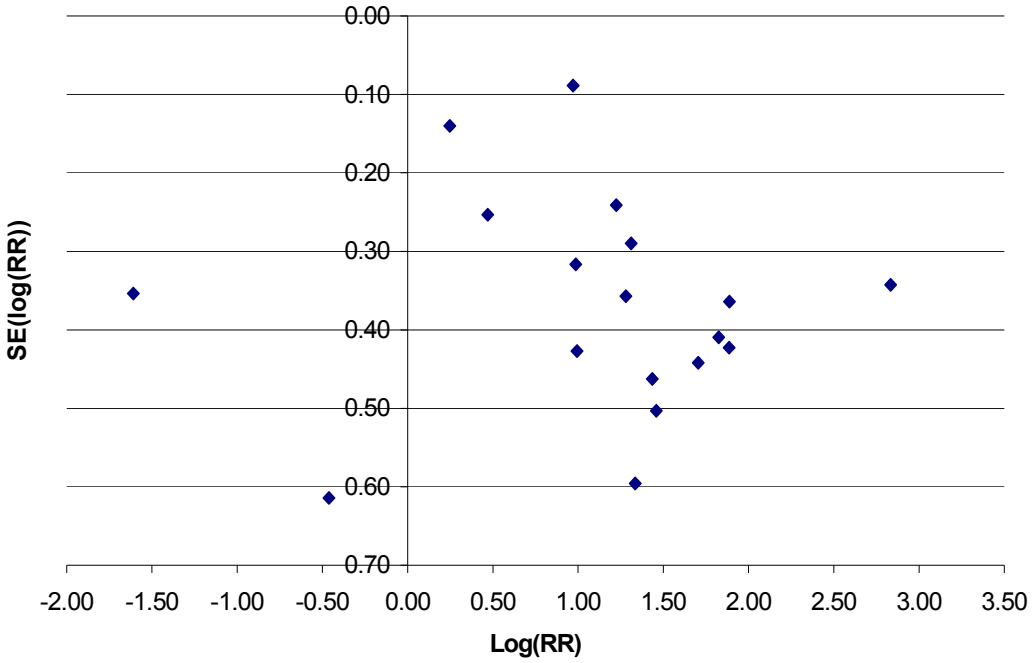


Figure 5.26. Funnel plot on indicators of photodamage.



5.9.8 Freckles density Figures

Figure 5.27a. RR and CI for high freckle density (first group of studies).

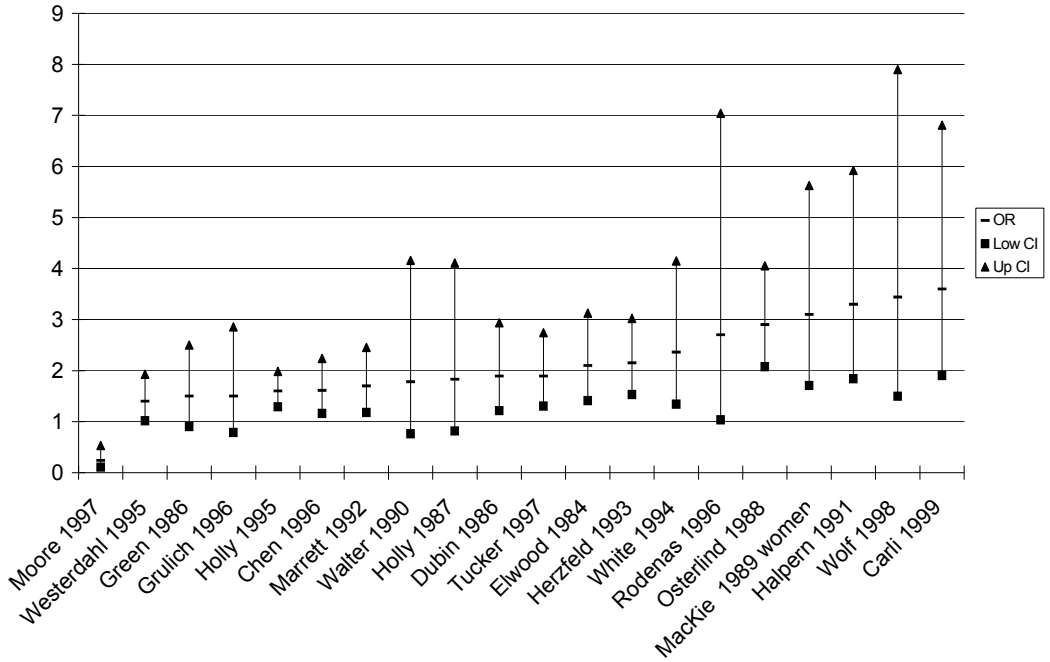


Figure 5.27b. RR and CI for high freckle density (second group of studies).

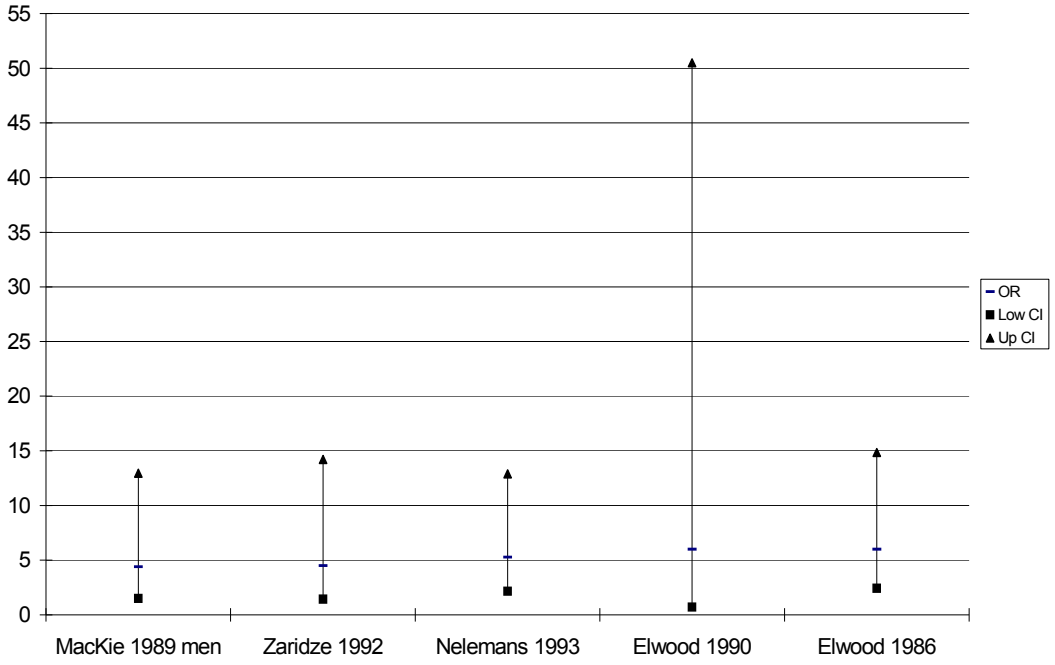
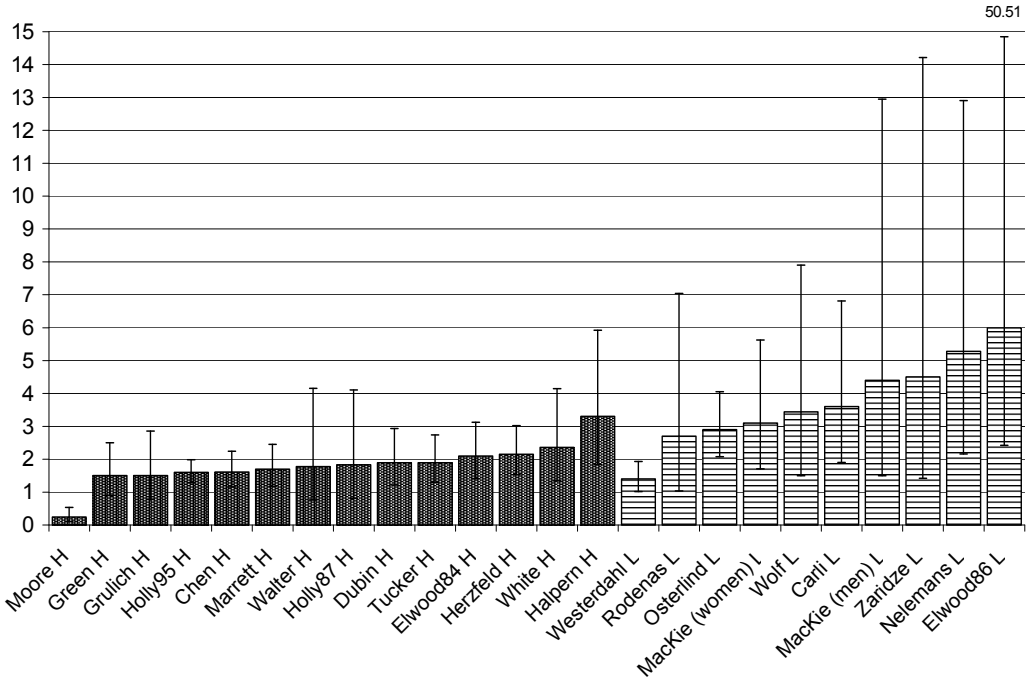
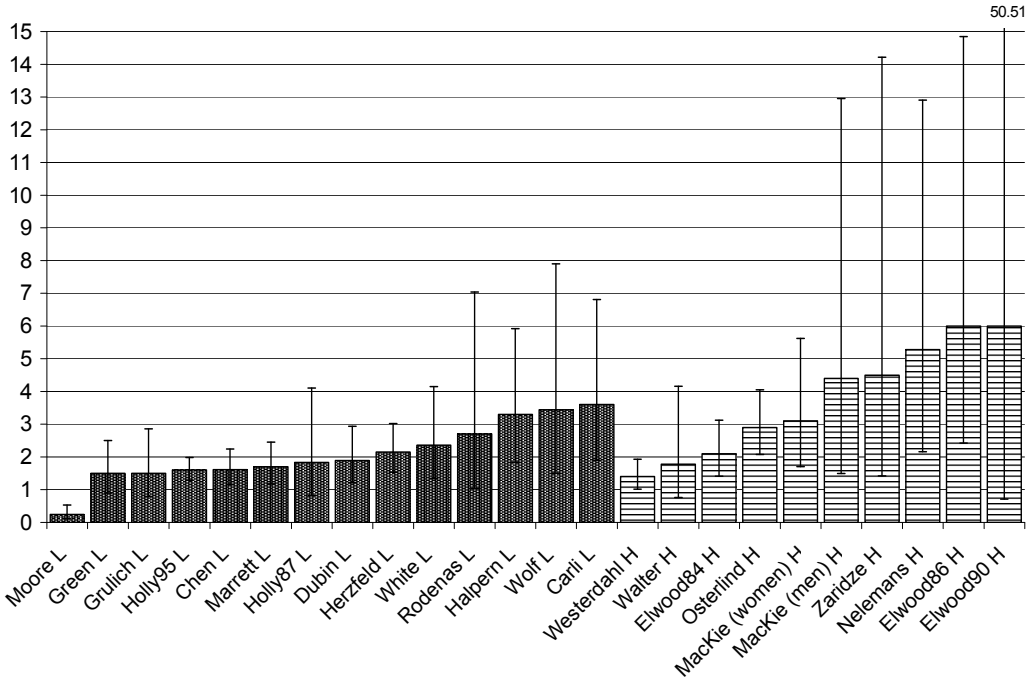


Figure 5.28. RR and CI for Freckles density by type of country.



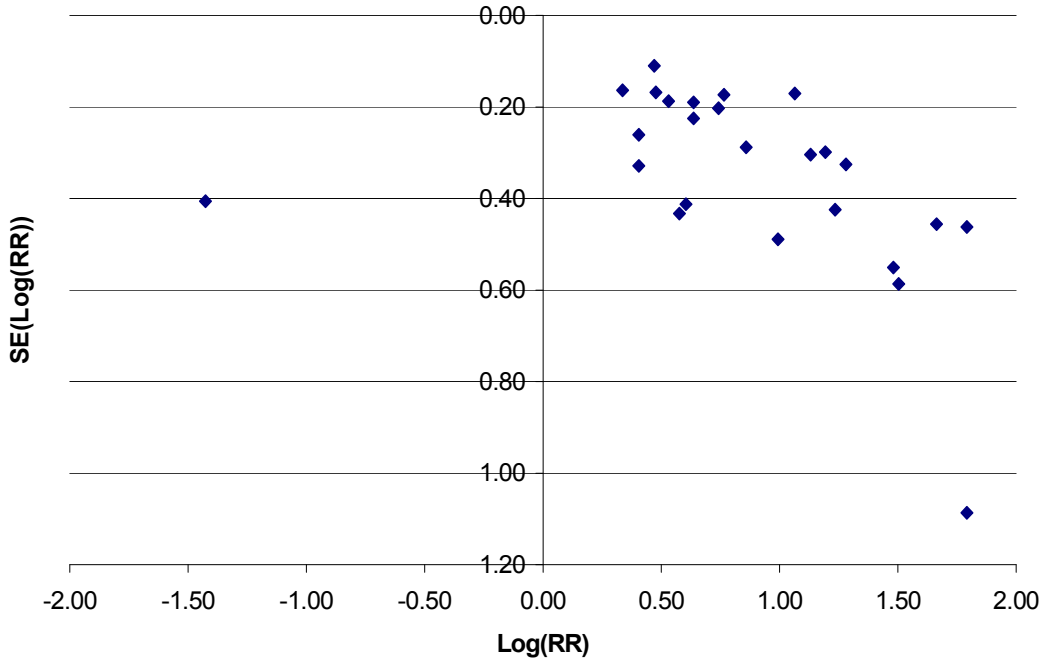
Studies conducted in “High incidence” countries are indicated with dark bars and studies conducted in “Low incidence” countries with grey bars

Figure 5.29. RR and CI for Freckles density by latitude.



Studies conducted in countries at Lower latitude (<50) are indicated with dark bars and studies conducted in countries at High latitude with grey bars.

Figure 5.30. Funnel plot for high freckles density.



5.9.9 Eye colour Figures

Figure 5.31a. RR and CI for blue versus dark eye colour (first group of studies).

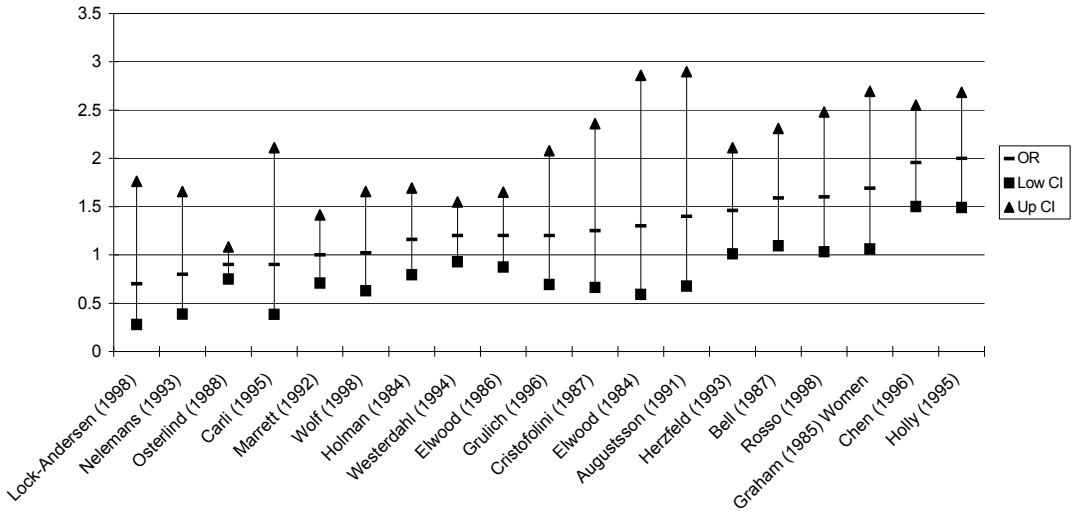


Figure 5.31b. RR and CI for blue versus dark eye colour (second group of studies).

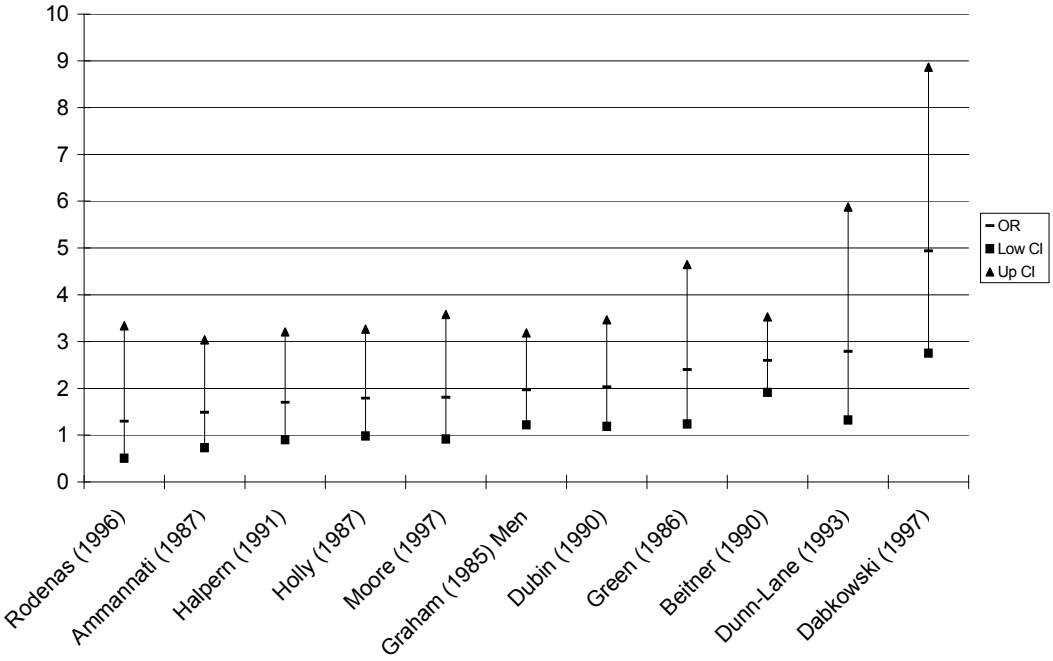
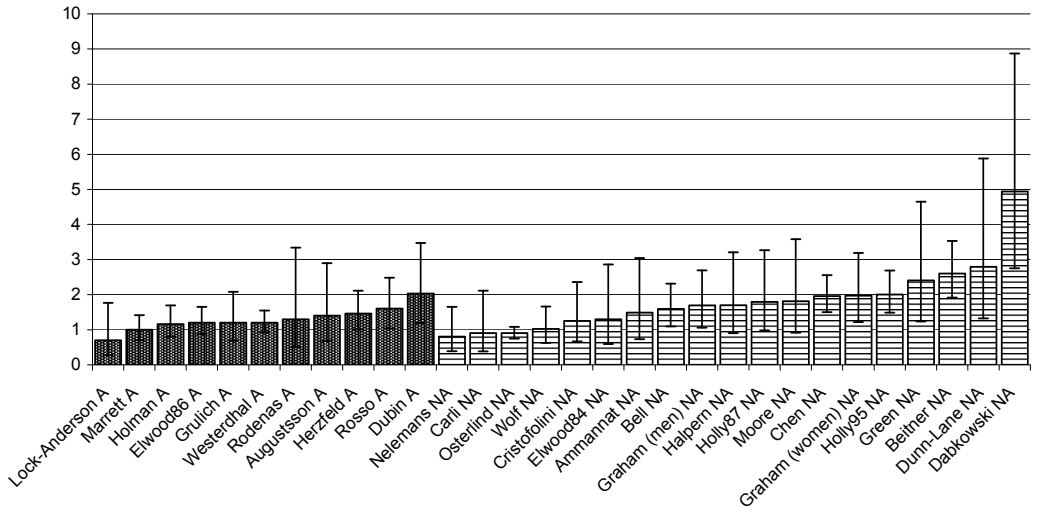
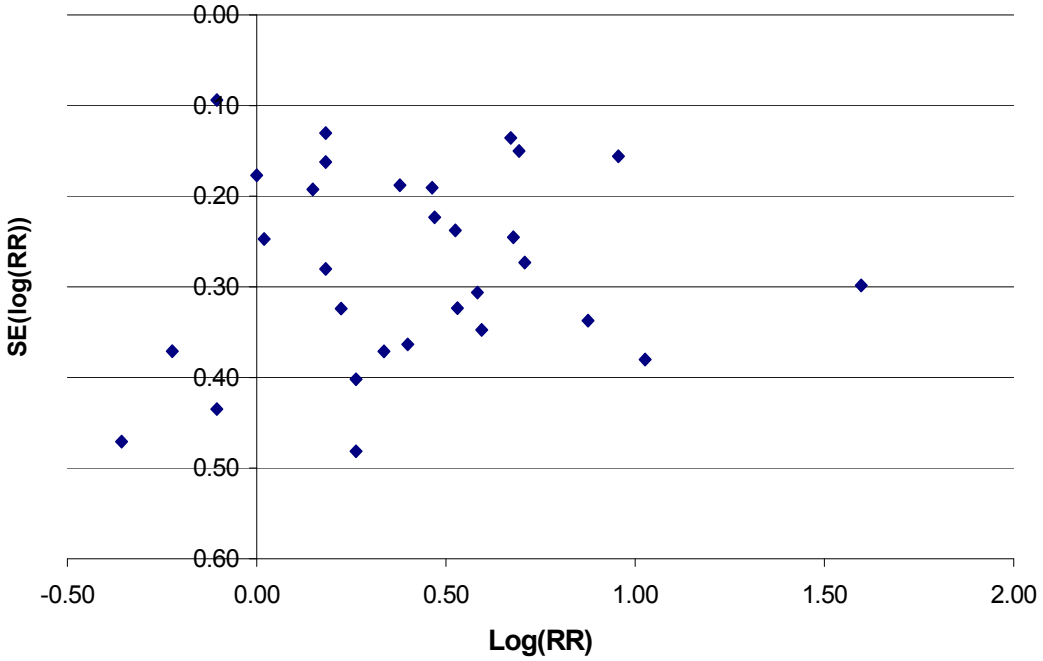


Figure 5.32. RR and CI for blue eye by adjustment for phenotype.



Estimates Adjusted for phenotype are indicated with dark bars and Not Adjusted estimates with grey bars.

Figure 3.33. Funnel plot for blue eyes.



5.9.10 Hair colour Figures

Figure 5.34a. RR and CI for red versus dark hair colour (first group of studies).

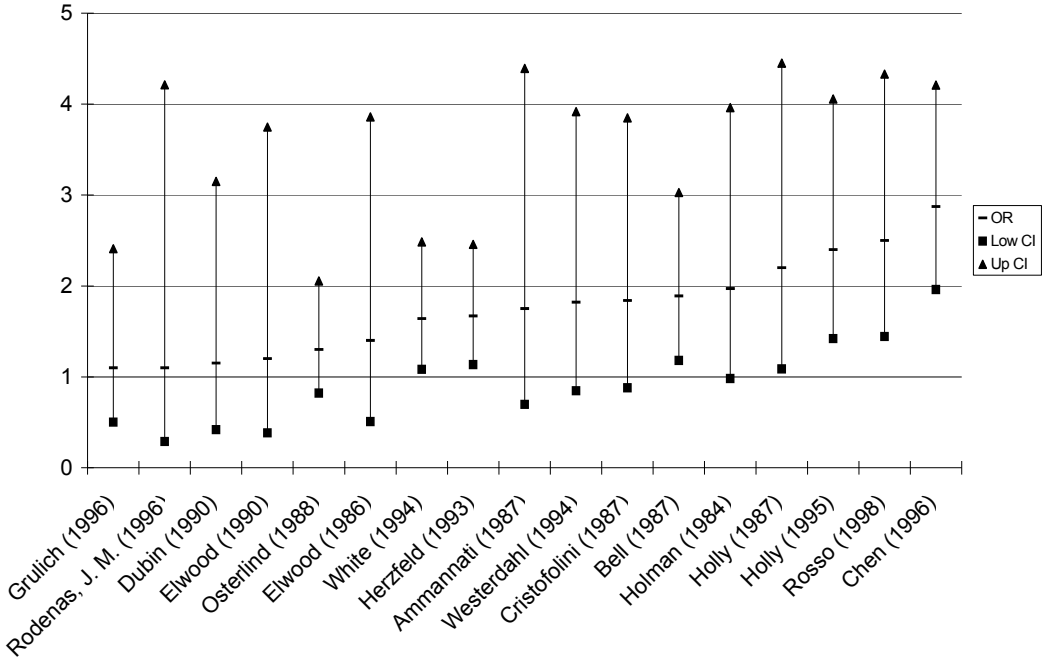


Figure 5.34b. RR and CI for red versus dark hair colour (second group of studies).

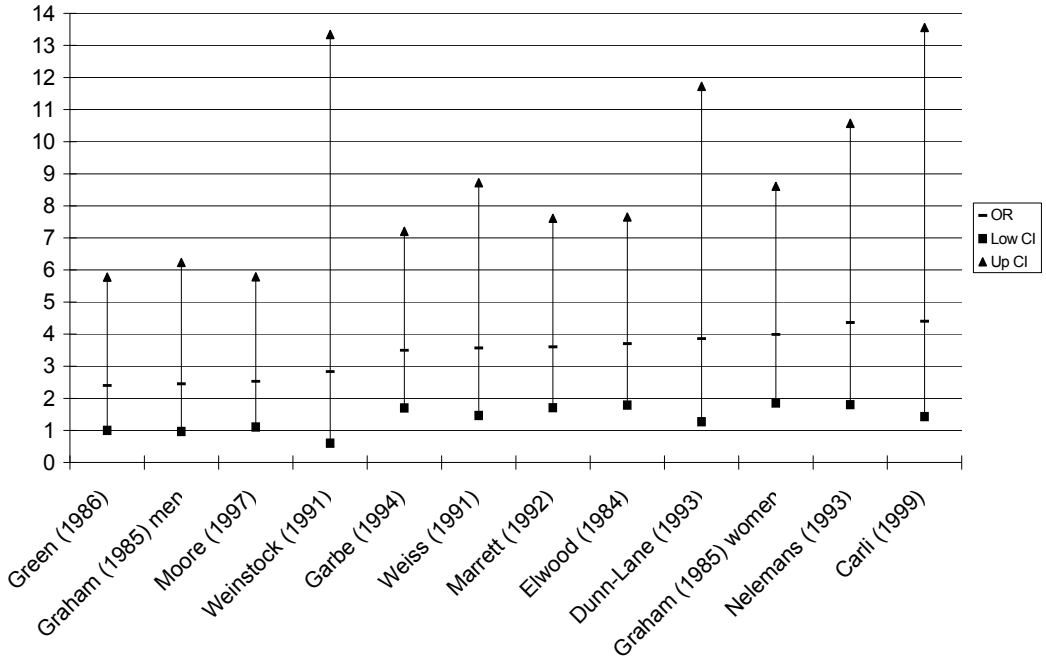


Figure 5.34c. RR and CI for red versus dark hair colour (third group of studies).

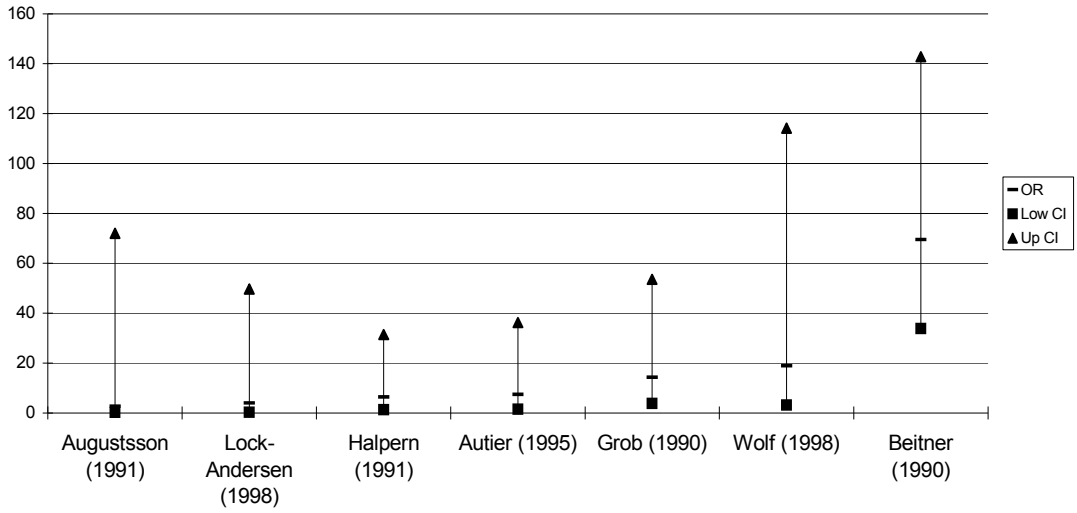
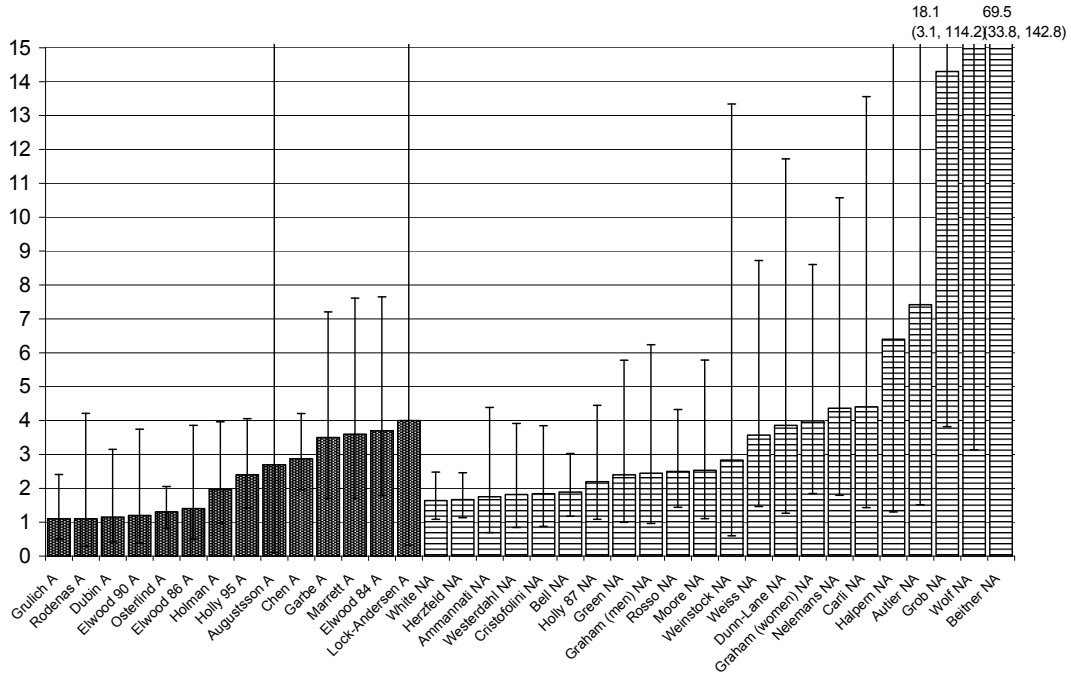
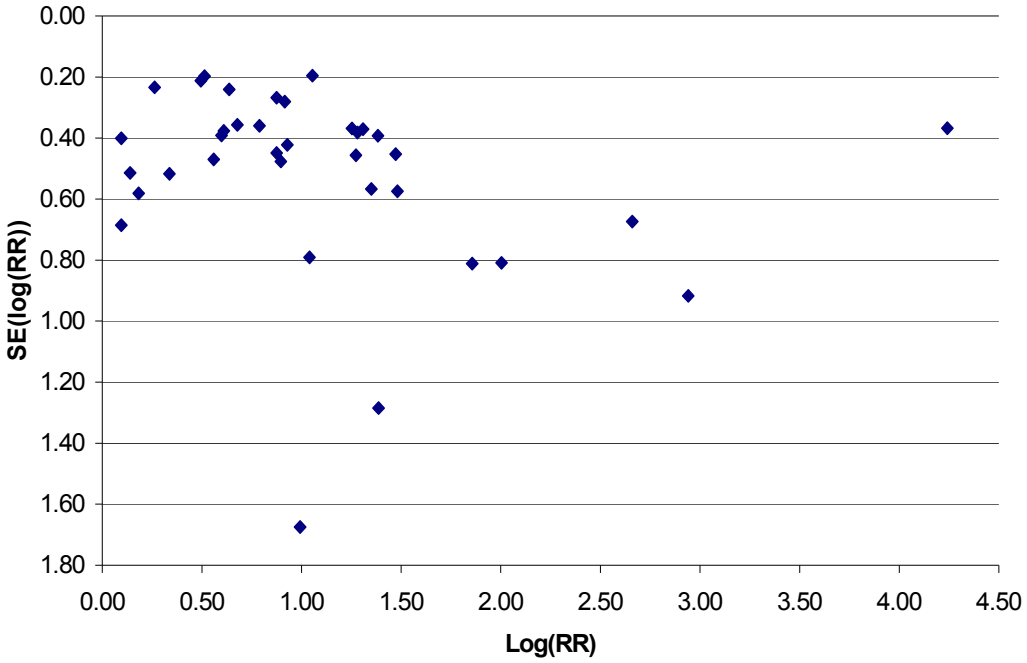


Figure 5.35. RR and CI for red hair by adjustment for phenotype or phototype.



Estimates Adjusted for phenotype or phototype are indicated with dark bars and Not Adjusted estimates with grey bars.

Figure 5.36. Funnel plot for red hair.



5.9.11 Skin colour Figures

Figure 5.37a. RR and CI for "light" versus "dark" skin colour (first group of studies).

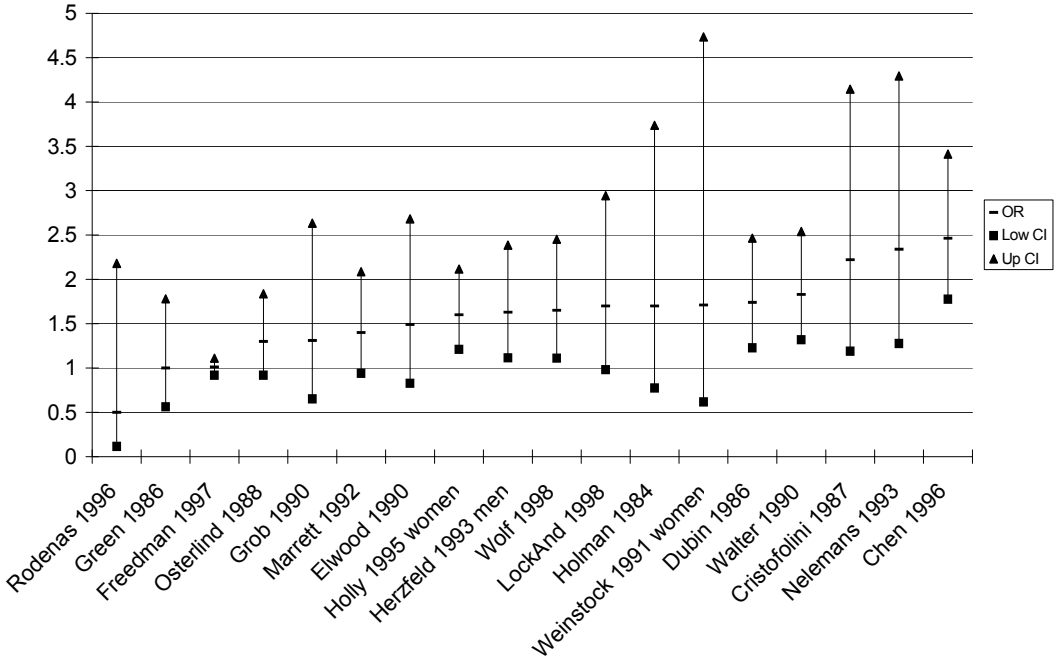


Figure 5.37b. RR and CI for "light" versus "dark" skin colour (second group of studies).

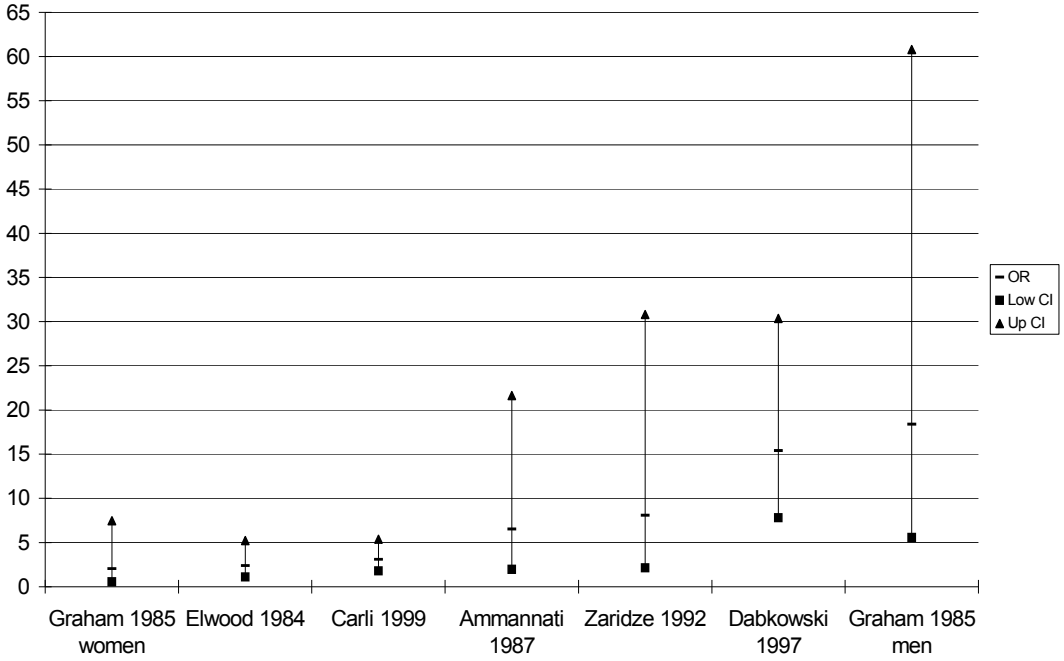
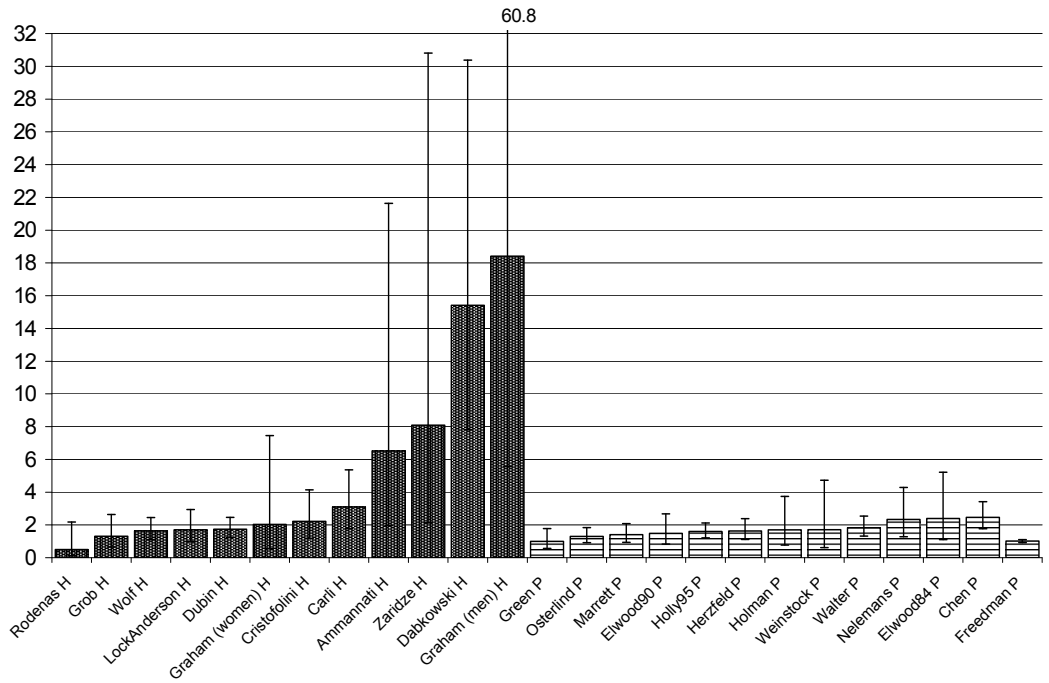
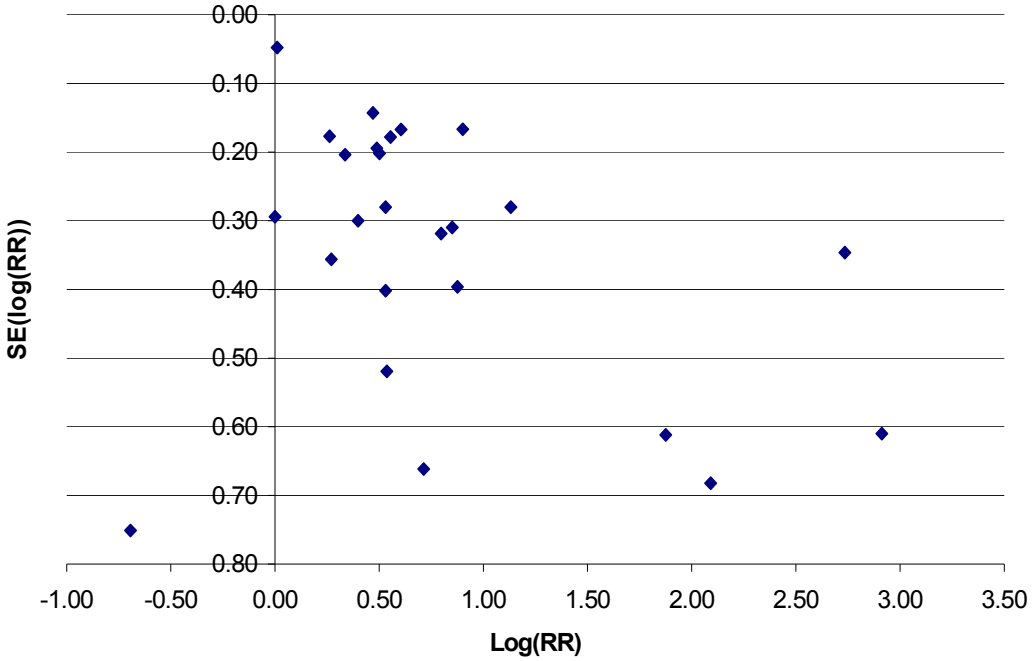


Figure 5.38. RR and CI for light skin colour by type of cases.



Studies whith cases drawn from hospital are indicated with dark bars and studies with cases drawn from population with grey bars.

Figure 5.39. Funnel plot for light skin versus dark skin colour.



5.9.12 Skin phototype Figures

Figure 5.40a. RR and CI for skin phototype (first group of studies).

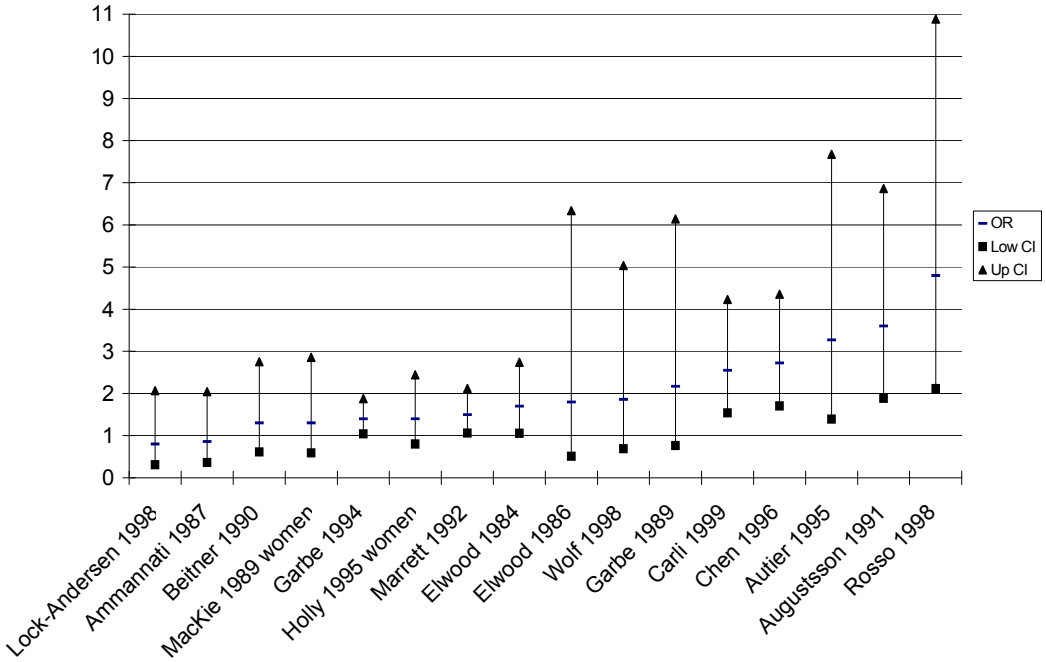


Figure 5.40b. RR and CI for skin phototype (second group of studies).

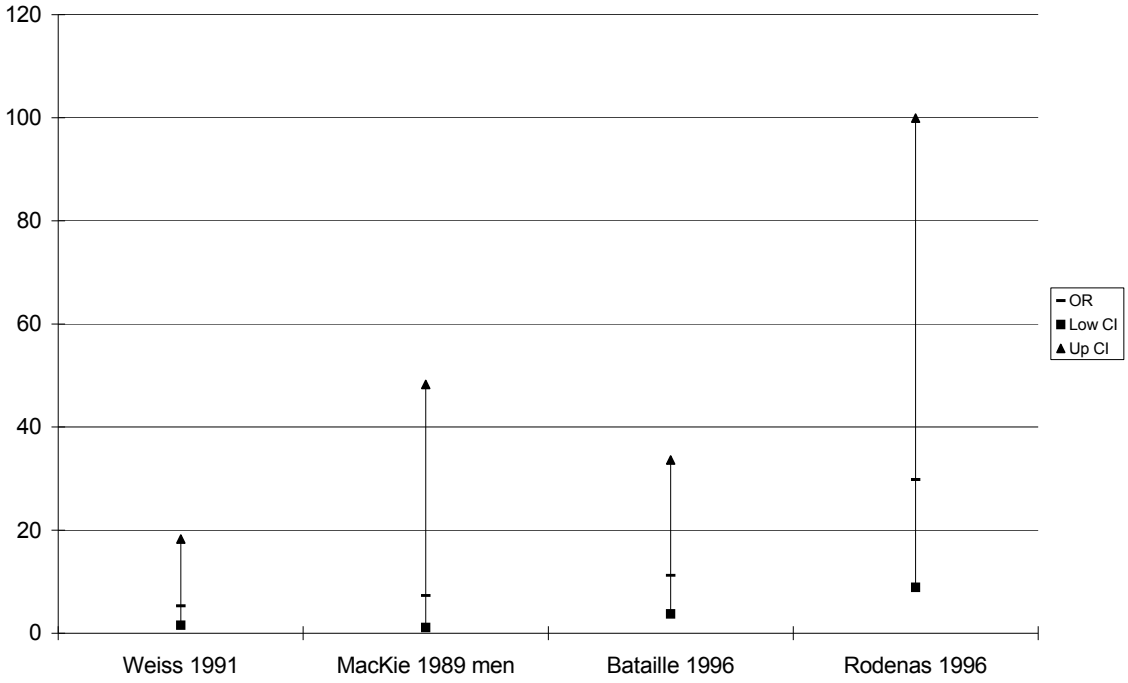
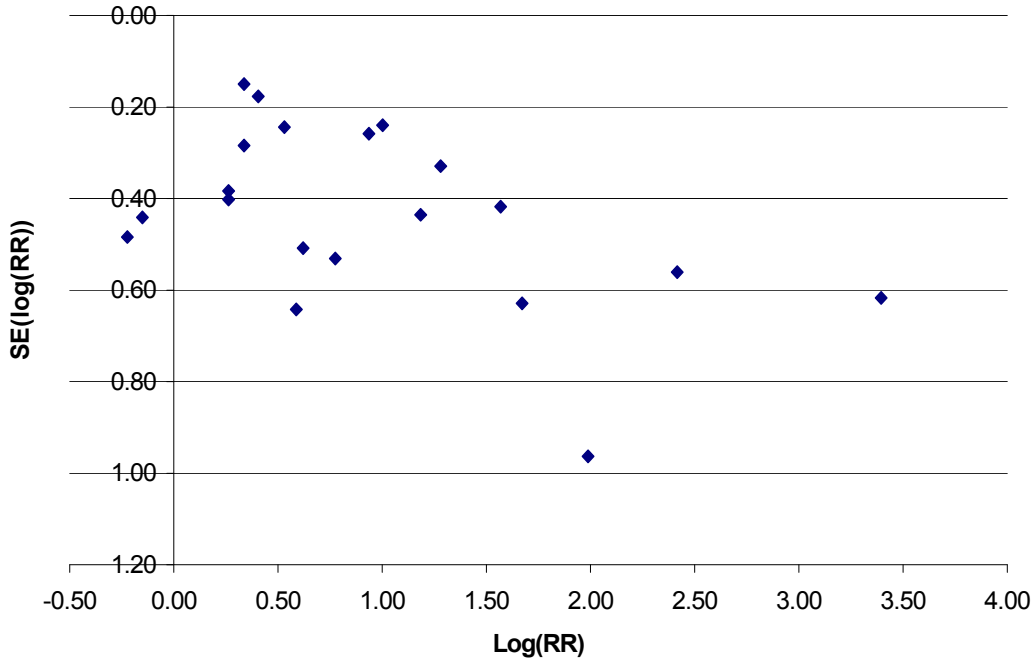


Figure 5.41. Funnel plot for skin phototype.



CHAPTER 6. CONCLUSIONS

6.1 Introduction

Previous reviews on epidemiological studies mostly had a narrative style. Meta-analytic techniques allow a more systematic way of assessing information from independent publications. The explosion of information in the scientific literature has amplified the require to synthesize research results.

In cancer epidemiology meta-analysis plays a special role because of the need to summarize the evidence for risks that are small, but that may have large public interest. Most epidemiological studies do not permit the detection of anything but a large effect associated with a fairly common exposure. Thus meta-analyses can assess weak risk factors that have a large public health impact summarizing information from several studies.

A published meta-analysis on breast cancer and vegetable and fruit consumption was described to present an approved methodology for meta-analysis on epidemiological studies. Several meta-analyses of published data on melanoma were carried out in order to summarise and investigate all its main risk factors.

Meta-analysis was not applied merely as a statistical method, which combined published results, because non-experimental studies, such as case controls studies, do not allow for the assumption that the variation between studies is only attributable to statistical sampling error. It is unlikely that this so-colled homogeneity assumption is fulfilled. Part of the variation in the estimates is prabably due to differences in definitions, in measurements of the exposure, features of the studies and of the populations. An important function of these meta-analyses was the exploration of sources of variation in study results, which should be viewed as strength of this work. A systematic investigation of between-study heterogeneity, as a function of differences in design features, types of analyses and populations

characteristics, helped to explain the controversy between study results and provided interesting considerations for cancer epidemiology.

6.2 Breast cancer

A large and consistent body of epidemiological evidence indicated a strong protective effect of higher intakes of vegetables and fruit for a wide variety of forms of cancer. This meta-analysis aimed to show that there is a considerable influence attributed to diet also for a hormone related cancer as cancer of the breast.

Relative risk estimates for breast cancer were extracted from twenty-six published studies, from 1982 to 1997, reporting data on fruit and vegetables consumption. Meta-analysis confirmed the association between intake of vegetables and, to a lesser extent, fruit and breast cancer risk. Increasing vegetable consumption might reduce the risk of breast cancer. Vitamin C and beta-carotene were also found associated with a decreased risk for breast cancer. However colinearity between and confounding by antioxidants is a fundamental issue as intakes of these micronutrients tend to be positively correlated.

Several factors were analysed to investigate between-study heterogeneity and the use of validated questionnaires was found slightly significantly associated with a change in the estimate. Some other factors seem to influence variation in the estimates, even if they were found not statistically significant. They are related to the design of the study, the kind of controls used in case-control studies, the adjustment for confounders and study location.

It is important to bear in mind that measurements of dietary intake do not in any way eliminate the possibility that some other factors in fruits and vegetables are responsible or that they simply reflect evidence of a protective effect of vegetarism for breast cancer. Either measure may be a marker for other substances or other factors. Vegetarianism, for example, may be associated with a high consumption of soy-based foods which have been shown to be

associated to significantly lower levels of a class of estrogens normally associated with breast cancer risk in postmenopausal women.[345] Specific dietary habits could reflect education and consequently socio-economic status, which in turn, may be closely related to reproductive history. In my analysis adjustment for parity did not significantly explained between-study heterogeneity, however, the pooled estimate calculated on RRs not adjusted for parity indicates a significant protective effect of high vegetables consumption wereas the pooled estimate from adjusted RRs did not. Any way these findings are only indicative because the number of available studies was too small to have enough power to test the relevance of all possible confounding factors. Problems of confounding by some yet unrecognised factors could not be ruled out and the role of residual confounding is an important issue in the epidemiology of weak associations.

A relationship between breast cancer and diet was also demonstrated by a recent paper that showed that tall adult height, which is positively associated with breast cancer risk, may partly determined by several dietary factors, from those vegetables and fruit, during childhood and adolescent. Furthermore it was shown that obesity increases the risk of breast cancer in postmenopausal women by around 50%, probably increasing serum concentration of free oestradiol.[346] Fruit and vegetables consumption may be implicated globally in several factors that affect diet and the style of life. Much research has surrounded the hypothesis that a high intake of fat increase risk of breast cancer. Many studies, however, do not support this hypothesis.[347] However the food pattern may be of special interest as high fat or energy intake is often associated with a low consumption of fruits and vegetables. Therefore, it is possible that a lower intake of fat simply implies a high fruit and vegetable consumption. An adjustment for energy-intake to address the issue of diet composition rather than absolute intake can be conceived.[91;92;122;125-127;130] However, in this analysis, there seems to be no particular influence on the estimated relative risks due to energy adjustment.

Smith-Warner et al.[348] published recently a meta-analysis on primary data from cohort studies. The authors evaluate the negative association between fruit and vegetable consumption and breast cancer that was found in many case-control studies. Strict inclusion criteria, within the Pooling Project[17;348], were adopted to select a homogeneous subset of studies suitable to investigate relationship between diet and cancer. Considering only prospective studies, because case-control studies are easily affected by recall and selection bias, they found a not significant protective effect of fruit and vegetable consumption on breast cancer risk. Similar results were found in my work when only cohort studies or population-based case-control studies were considered, even if in my meta-analysis the difference with the other studies was not statistically significant. It is interesting to note in Smith-Warner's analysis that when the estimates are adjusted for fat intake, the pooled RR for highest versus lowest quartiles of total fruit and total vegetables consumption became statistically significant (p=0.04).

Another remarkable meta-analysis[84] was published quite recently. Summary estimates for several fruit and vegetables groups were shown and 53% of the 70 risk estimates reported in the summary show at least a 25% reduction in breast cancer risk for the highest versus lowest consumers. As in my work evidence was more consistent for vegetables than for fruits.

In conclusion, it is not possible to recommend dietary supplementation to reduce breast cancer risk, based on the evidence from this meta-analysis, but high consumption of fruit and vegetables is suggested. In line with European Code Against Cancer,[349] fruit and vegetables should be taken with each meal whenever possible, because high consumption of them could lead to a reduction in all type of cancer and probably for breast cancer too.

6.3 Melanoma

Cutaneous melanoma is potentially a curable cancer if it is discovered in an early stage of development. The positive aspect of melanoma is that it is readily detectable simply by examining the skin, unlike other cancers, which are usually hidden from detection until they are relatively large or metastatic disease has occurred. Thus, pooled estimates from this meta-analysis could be essential in selecting groups of people at greatest risk. Selective screening of individuals who have these risk factors should reduce the morbidity and mortality of cutaneous melanoma.

A systematic meta-analysis of epidemiological data (110 independent published studies included in the analysis, from 1966 to 2002 for pigmented lesions and from 1984 to 1999 for the other risk factors) was carried out for all risk factors of cutaneous malignant melanoma: exposure to ultraviolet sun radiations, sunburns, indicators of actinic damage, family history of melanoma, phenotype characteristics, pigmented lesions and skin type.

Even though differences in definitions, in population features, methodological variability, problems with confounders and unexplained heterogeneity, from these series of published studies conducted over the past few decades, a great deal was learned about the relationship between all main risk factors and melanoma.

The pooled estimate for one or more affected first-degree relatives suggested a considerable risk (pooled RR=1.79; 95% C.I.: 1.46; 2.19). The pooled estimate was highly reliable because no between-study heterogeneity or indications for publication bias were found. Thus, given the implications of a positive family history, verification of family history is essential. Family members of all patients with cutaneous melanoma should be examined for suspicious moles and it has been suggested to practice periodic mole surveillance for life.[350;351]

Improvement of the methodology of epidemiological studies, together with interesting laboratory results, led to general acceptance of solar ultraviolet light as the major

environmental cause of melanoma.[24;352] From this work it emerged that epidemiological evidence suggested that the effect of long continued heavy exposure, such as that received by persons with outdoor occupations, is different from the effect of intermittent exposure, received typically in recreational and vacation activities. It is this latter exposure that is likely to be the major factor (pooled RR=1.58; 95% C.I.: 1.26; 1.99) associated with melanoma in developed countries and it is reduction in this exposure that public health programs should address. Considerably larger total doses of sun exposure, but given by relatively constant occupation exposure over a long period of time, were associated with a decreased risk of melanoma (pooled RR=0.93; 95% C.I.: 0.84; 1.04). In fact, if the overall pooled estimate does not show a significant protective effect, this suggestion came from studies with a better design and a quite detailed information on sun exposure. Intermittent and constant exposure may be intrinsically different, with conflicting effects, so that the risk for an individual depends on the balance between these two exposures. Intermittent sun exposure is intrinsically related to sunburn, which also presents very high estimates (pooled RR=1.84; 95% C.I.: 1.54, 2.20). Sunburn in childhood showed an even greater risk (pooled RR=2.23; 95% C.I.: 1.54; 3.23), even if not significantly different from the previous estimate.

Even if sunburn seems to have a considerable effect on melanoma, we must be careful about using apparently simple but possibly misleading messages such as avoiding sunburn. Actually it may well be that avoidance of sunburn is only effective on melanoma if intermittent ultraviolet exposure is reduced. Furthermore these messages may increase the use of sunbed tanning or sun blockers, to allow greater outdoor exposure without burning, but this may not be helpful in terms of melanoma. The public health message should be the simple one that sunbathing (with or without sunscreen) is hazardous behaviour, there is no such thing as a safe tan, and whenever possible, it is better to stay in shade.[353] However, given the finding that regular frequent exposure to sunlight is associated with a lower relative risk of

melanoma compared with having only occasional exposure, population-based recommendations are not easy to give. It has been postulated that reduction of exposure to sunlight, particularly only an internediate reduction, may actually increase the risk of melanoma by changing someone from a regular frequent exposure pattern to an episodic exposure pattern. This should be taken into account in public health programs considering that phenotypical factors play also an important role. Furthermore, it is worthy to remember that sun exposure shows a protective effect against many other types of cancer.[315-319] The ideal case would, therefore, be to find a way of obtaining the beneficial effects of UVB exposure without suffering the increased incidence of skin cancer. However it is suggested that standing outside 15 minutes a day three times a week lets the skin produce enough vitamin D most of the year.

Subjects who exhibited some indicators of photodamage, which are intrinsically related to sun exposure, also showed a considerable risk (RR=2.98; 95% C.I.: 2.04; 4.36). Among them, those revealed pre-malignant and cancer lesions had a very high risk (RR=4.98 with 95% C.I.: 2.74; 6.82), compared to people who do not have any pre-malignant or cancer lesion. A lower risk, even if still significant, was observed for subjects that presented other indicators of actinic damage (RR=1.96 with 95% C.I.: 1.06; 3.61).

Interrelationship between the external agent of ultraviolet exposure and personal characteristics of the subject is the central issue in many aetiological researches in melanoma. In this analysis RR estimates for total sun exposure, adjusted for phenotypic characteristics or phototype, were slightly significantly (p=0.09) higher than unadjusted ones and this trend was seen for all measures of sun exposure.

Among phenotypic characteristics, number of common naevi and large atypical naevi were very important risk factors for melanoma occurrence. Risk for people with many common naevi was almost ten times the risk for people with few naevi (pooled RR=6.89;

95% C.I.: 4.63; 10.25, for “100+” compared to “0-15”). Subjects with five atypical naevi presented a risk ten times higher than the people with none atypical nevus (RR=10.49; 95% C.I.: 5.05; 21.76).

People with few or none freckles or a brown colour for hair, eyes or skin, showed less than half the risk of people with many freckles (RR=2.11; 95% C.I.: 1.76; 2.53), red hair (RR=2.79; 95% C.I.: 2.15; 3.61), blue eyes (RR=1.52; 95% C.I.: 1.30; 1.76), and fair skin (RR=2.02; 95% C.I.: 1.60; 2.55). Skin type also played a relevant role and subjects with type I, who do not burn and tan easily, presented a RR more than two fold higher that IV type, who burn and do not tan (RR=2.31 and 95% C.I. 1.73, 3.05).

Conflicting results in the literature are mainly a reflection of differences in epidemiological methods, which despite using increasingly sophisticated analyses were still relying on the data, which were basically biased. Meta-analysis always suffers from methodological problems that inaevitably arise when combining data from several epidemiological investigations. In fact if many research papers publish modest estimates, those RRs may well be due to same biases in all the studies. If the same systematic biases are present across a range of studies, the only effect of meta-analysis is to reinforce them, generating spurious statistical results. Thus meta-analysis can lead to insights when study design, exposure assessment or exposure levels, study populations, etc., are found to relate to study outcome. An important function of meta-analysis is the investigation of between-study heterogeneity which is an opportunity to understand study variation. These points were carefully considered and discussed to understand their impact on the findings.

Interesting correspondences were found looking at some methodological features of the studies such as type of study, source of data in case-control studies, matching, categorization of exposures, inclusion of subjects with dermatological problems or family history and features of the population or study location.

Relative risk estimates on naevi assessed on arms, obtained from studies with controls drawn from population were significantly ($p=0.05$) lower than estimates on studies with cases and controls drawn from hospitals.

Similarly for atypical naevi, studies with controls from hospitals were significantly ($p=0.02$) lower than case-control studies with population based controls. Moreover case-control studies presented significantly ($p<0.001$ for atypical naevi) lower and much more precise estimates compared to cohort studies. Contrary to expectations, estimates from case-control studies are usually more reliable than the ones obtained from the other types of designs because assessment of naevi counts is more precise. Cohort studies usually express results with broad categories that presented significantly ($p=0.01$) higher estimates than more articulated categorizations of atypical naevi. Furthermore cohort studies had significantly ($p<0.001$) younger population than case-control studies and this difference may play an important role.

Among papers on total sun exposure, case-control studies with hospital-based controls again showed significantly ($p=0.002$) lower estimates than case-control studies with population-based controls, but in this case they are probably less trustworthy, because for sun exposure the problem of recall bias could be important. In fact the hospital-based case-control studies included subjects with family history of melanoma and the majority included subjects with dermatological problems or any tumour. An indication in this sense came also from studies conducted after 1990, when much professional and common opinion was aware of the relationship between melanoma and sun exposure; recent studies presented estimates significantly ($p=0.005$) higher than studies published before 1990. Further work, to include in the meta-analysis papers published before 1984, may be useful to verify this finding.

Unlike the studies on naevi and on the other estimates on sun exposure, significantly ($p=0.06$) lower estimates were found in studies with population-based controls evaluating

chronic sun exposure. A similar indication of a protective effect of chronic sun exposure came from studies that did not include subjects with dermatological problems (significantly different from the other studies: p=0.01) and from countries that conducted studies with a better design (significantly different from the others countries: p=0.02) and a quite detailed information on sun exposure. Thus, in this case, estimates from studies with population-based controls are probably more reliable than estimates from studies with hospital-based controls.

In this meta-analysis matching was found to be another important design characteristic that may be considered a marker of well-designed studies and explained some of the between-study heterogeneity (for indicators of actinic damage p=0.01).

Latitude of the study seems to be an important factor for sunburns (p=0.002) and for high density of freckles (p=0.04). At high latitude, sunburns and high density of freckles probably play a special role, increasing the risk of developing melanoma. Furthermore an association between them is more than likely so that subjects with a high density of freckles have a phenotype that induces more easily sunburns.

A suggestion for an association among host factors also came from heterogeneity analysis that showed significantly lower estimates for hair colour (p=0.06) and eye colour (p=0.06) adjusted for phenotype and/or phototype, compared to unadjusted.

In this analysis I could not devote much attention to the distribution of melanoma in different body sites, however future works should be addressed in this direction. Fascinating suggestions on the aetiology for melanoma come from a very recent study.[203] A new interesting hypothesis was proposed on the relationship between sun exposure and naevi, based on a "divergent pathway" model for melanoma occurring in different body sites. Melanoma, for people with a low tendency to develop naevi, would tend to be on sun exposed body sites and would be associated with chronic sun exposure. On the other hand naevi-prone

individuals would not require much exposure, beyond that required for initiation, for the development of melanoma on non sun-exposed body sites.

At the moment primary prevention behaviours should include protective clothing, using shade, limiting sun exposure, and avoiding sunbathing, as is suggested also by the European Code Against Cancer (2003)[349]. We will probably have to wait for more specific biological markers for proving previous exposures that we will have much further advancement in our knowledge of the relationship between sun exposure and the development of melanoma in those people at risk.

Future studies should focus on developing effective strategies for determining the effectiveness of skin cancer screening.

6.3.1 Need for screening on melanoma

Efforts at primary and secondary prevention of coetaneous malignant melanoma have been ongoing for the past 15-20 years in many parts of the world. Primary prevention consists of public education programs that should promote sensible sun exposure, over the long-term. Secondary prevention is aimed at preventing deaths from melanoma and encourages early detection and thus removal of thin melanomas.

Melanoma fulfils the criteria for a potentially successful screening program because the disease is a serious public health problem, there is a simple diagnostic test (skin examination), and there is improved survival with early detection. Actually the consequence of melanoma is directly related to the stage at diagnosis, and since melanomas take long time to reach an advanced stage, early detection can save many lives.[175]

A NIH consensus conference in 1992 concluded that “there is sufficient evidence to warrant screening programmes for melanoma in the United States” and that “melanoma meets most of the criteria for initiating screening”.[354] Argument against introducing melanoma

screening have been based on cost and the lack of reliable data on the efficacy of any screening test. Girgis published a cost-effectiveness analysis showing that a melanoma screening programme could be cost-efective, particularly if five yearly screening is implemented by family practictioners for men over the age of 50. [355]

The best evidence for the effectiveness of early diagnosis programmes involves the educational initiatives introduced in Western Scotland by Mackie and colleagues. Their data showed a remarkable increase in the proportion of thin melanomas and a decrease of thick melanomas, diagnosed after the programmes were introduced.[356]

On the other hand, there is one major problem with melanoma screening. It is that the clinical signs and symptoms, which are regarded as characteristics of early melanoma, are not very specific. An important general population survey in Australia has shown that in the previous 12 months they had noted one or more changes in naevi, which would be regarded as suspicious; about one third of them sought medical advice. Therefore a very high proportion of the normal population will probably require further assessment. This has considerable implications in terms of health care costs and unwanted effects.[355]

The ideal to measure the benefits of screening would be to conduct a large scale randomised study design to evaluate skin cancer screening, as it is for any other type of cancer screening.[357] However there are consistent logistic problems with such trials, involving hundreds of thousands of people, millions of dollars, and years of follow-up.[323;358]

Several studies demonstrate the usefulness of periodic surveillance, combined with total cutaneous photography, of individuals at high-risk for developing cutaneous melanoma.[351;359] At the moment secondary prevention is based to early detection, diagnosis and treatment of individuals at greater risk by virtue of a persistently changed or changing mole, a previous cutaneous melanoma, and/or a family history of cutaneous melanoma.

Investigation of the literature on all main risk factors for melanoma, through this meta-analysis, provides further insight into the mechanisms underlying the disease, interesting considerations about how best to approach the conduct of future epidemiological studies, and last, but not least, pooled estimates to identify all individuals at high risk.

6.4 Future studies

This research showed that greater attention to heterogeneity and improved reporting of the variance among study results help investigators to understand exposure disease relationships. Country of the study, type of study design, confounders considered in the analysis are some of the factors that influence significantly the estimates. New approaches, such as reliable and robust Bayesian meta-analyses, in which evidence from observational studies may be incorporated either within a prior distribution or modelled explicitly using a hierarchical modelling framework, need to be implemented with less complex computational techniques to deal with all these factors that induce heterogeneity.

In the meta-analysis on breast cancer and diet I could not evaluate influence of diet at young ages because there was not much information on that. In fact recall of childhood and adolescent diet is poor because hypotheses relating early diet to breast cancer risk decades later is difficult to test unless novel sources of data or methods for measuring diet in the distant past will improve. However the possibility that aspects of diet during childhood or adolescent may be associated with the risk of breast cancer decades later would be warranted.

Because of the extensive epidemiological studies of the last decades, we know many of the most important melanoma risk factors and exposures. We have much less understanding of the interactions of host risk factors and exposures: skin/hair pigmentation/sun sensitivity with differing sun and UV exposures; common nevi and dysplastic nevi with types or extent of sun and UV exposure, etc. This meta-analysis seems to

suggest a possible interaction among host factors but not between naevus density and sun exposure, for example. However relationship among nevi, sun exposure and phenotypic factors is certainly quite complex. Actually individuals who are prone to burning (red hair, dense freckling, very sensitive skin) may avoid sun exposure and develop fewer naevi than might be expected. Moreover it was suggested that the relation between sun exposure and melanocytic naevi might have parabolic dose-response curve. Anyhow the number of studies in these meta-analyses was too lo to produce reliable results on the interactions among various risk factors and until we complete large studies to address these questions, we will not fully understand the mechanisms of melanoma development.

In line with previous reports[361], this work suggests that genetic susceptibility and sun exposure are likely to act multiplicatively on melanoma risk. A recent area of research integrates the rapidly advanced knowledge of the genetic factors influencing melanoma with the epidemiological evidence. Furthers efforts should be done in this directions to lead to a much fuller understanding of the biology underlying melanoma.

Ultraviolet light has been conclusively shown in a large number of epidemiological studies to be a factor in the increase in incidence. However, in analytic studies, individual sun exposure has been particularly difficult to quantify and neither is easily documented. As was seen in heterogeneity analysis, sun-related behaviour is complex and its reporting is subject to multiple biases and misclassification. There is strong need for future studies to use standardized exposure measures and methodology technique to obtained more information also about dose-response curves. Epidemiological studies should incorporate biologic samples to assess host/environmental interactions with variables less subject to misclassification and differential recall bias. Opportunities to study population groups with well-defined exposures could be very valuable, as they have been with other carcinogenic agents. In fact in

heterogeneity analysis country was one of the factor that significantly explained between study variations.

Looking at sunburns in childhood and adulthood I did not find a significantly effect of age, however definitions were very heterogeneous. Furthermore a recent systematic review[362] on ecological studies evaluating migrant population suggest that sunlight in childhood may be a strong determinant of melanoma risk. Therefore a more detailed evaluation of age-specific effect of sun exposure is suggested.

Although it is known that increased exposure to sun is a risk factor for melanoma, researchers are not sure which wavelength of ultraviolet radiation—UVA, UVB, or both—causes the cancer. Many people think that sunscreen could be effective in preventing sunburn and therefore melanoma. For this project sunscreen was not analysed because there is not a considerable number of studies on its effect and they are highly contradictory. A prospective study to determine sunscreen efficacy needs to be undertaken to overcome all the problems of previous studies.[363]

It may be possible now to consider both primary and secondary prevention strategies, since we have identified the major environmental factor for melanoma, and many host factors conferring markedly increased risk. A variety of primary and secondary preventive strategies for controlling the problem of sun exposure have been attempted. Evaluation studies looking at the extend to which such programs produce behavioral changes would still be useful.

Future studies should also focus on developing effective strategies for determining the effectiveness of skin cancer screening. Refining definitions of specific high-risk groups would greatly improve secondary prevention. Selective screening of a high-risk group would be less costly than population screening, as fewer subjects would have to be seen, but the rate of positive results would be higher with a more favourable cost-effectiveness ratio. Future studies should focus on developing a screening program that minimizes melanoma mortality

while meeting “reasonable” cost-effectiveness constraints. Further analyses should be completed on age and gender specific recommendations for melanoma screening, the frequency of screens, and the possible impact on screening recommendations of a score build based on the pooled estimates to identify high-risk people. Selection of high-risk individuals for whom a randomized trial of preventative measures might be undertaken should be considered. The feasibility, acceptability and reliability of a target strategy based on identifying, advising and possibly screening those at high risk would need to be studied before undertaking an appropriate intervention trial to see if such strategy was effective.

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