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Twin-Singleton Comparisons Across Multiple Domains of Life

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Trailer

In this chapter, we address the question whether individuals born from a multiple pregnancy differ from singletons. The answer to this question is important for health-care professionals and researchers, as well as multiples themselves and their family members.

First, we review findings from the literature with respect to twin – non-twin differences in early life and conclude that a multiple pregnancy increases the risk of congenital problems and mortality for the unborn and newborn children.

Next, we provide an overview of the outcomes of comparing adult twins to their singleton siblings across a wide range of traits assessed in the Netherlands Twin Register (NTR). In a within-family design, comparing twins to siblings from the same family, we correct for familial confounding. Overall, hardly any evidence was found for the presence of twin-sibling differences for the five domains explored, which included body composition and physical development, personality and psychopathology, behavioral and sociodemographic traits, physiological parameters and physical disease, and cognitive function. With the exception of minor differences in body composition, twins do not seem to differ from singletons, when taking family factors into account.

In conclusion, while being a twin can be seen as special, adult twins are similar to ordinary siblings across most domains of life.

Definitions

1. Twin – An individual born after the same pregnancy of multiple zygotes leading to a multiple birth.
2. Singleton – An individual who was the single (surviving) zygote during a pregnancy and therefore not part of a multiple birth.
3. Barker hypothesis – The theory of fetal origins of adult disease.
4. Congenital disorder – A condition present at birth that results from genetic or chromosomal disorders, exposure to certain medications or chemicals, or certain infections during pregnancy.
5. Within-family design – A comparison of outcome (dependent) variables between members of the same family who may differ on the predictor (independent) variables.

Learning Objectives

- To gain insight into the risk of congenital problems and mortality in multiple pregnancy
- To understand the challenges in studying twin-singleton differences and how the study of multiples and their singleton siblings from the same family can resolve some of these problems
- To increase knowledge regarding potential differences between multiples and singletons in their development, behavior, and health

4.1 Introduction

Over the past decades, twin and higher-order multiple pregnancy rates have increased in many countries worldwide [14, 82, 112]. While the increased number of infertility treatments is often stated as the reason for this increase, the increase in maternal age is also a significant contributor [57]. Compared to singletons, that is children born from a single pregnancy originating with a single zygote, children born from a multiple pregnancy are born on average 3 weeks earlier [33], and are about 4 cm shorter and 1 kilo lighter at birth [70]. Multiples, that is, twins, triplets, and higher-order multiples, may be at increased risk of complications during pregnancy, delivery, and in the postnatal period. The fetal origins hypothesis and the developmental origins of health and disease hypothesis, often referred to as the Barker hypothesis, poses that low birth weight reflects intra-uterine growth retardation that may affect later development and increases the risk of disease development and earlier mortality [4]. Numerous studies in the general population have indeed found evidence for an association of low birth

weight with diseases including hypertension, cardiovascular disease, and diabetes [55], and with all-cause mortality [48]. An important question is whether this hypothesis extends to twins, with twins compared to singletons having a higher risk of disease development and early mortality due to their on average lower birth weight, which would have consequences for the health care of multiples.

Twins do not only differ from singletons in birth weight. They also shared a womb, and grew up with a sibling who is exactly of the same age and often the same sex and even, depending on the zygosity, alike in physical appearance. Growing up with such a close companion may lead to differences in behavior as it may encourage or discourage certain behaviors and lifestyles through mechanisms of social interaction [18]. Twins also tend to have older parents than non-twin children, and for a range of behavioral and cognitive outcomes the children of older parents do somewhat better than children of younger fathers and mothers [102, 113].

The above inference raises the important question of whether twins are similar to singletons, both with regard to health and non-health-related traits. The answer to this question is relevant to health-care professionals but also to researchers making use of the twin design in studies to unravel the influence of genetic and environmental factors on traits of interest. The generalization of findings based on twin research rests on the assumption that twins do not differ from non-twin individuals. For example, in studies of gene-by-environment interaction, twins should not differ from singletons regarding their exposure to the environment under study. While this seems a reasonable assumption, for example in case of the death of parents, this may be different for some other life events such as divorce or having an intimate relationship with others, because twin relations may discourage such relationships [66, 67, 69]. Importantly, in addition to health-care professionals and scientists, twins themselves and their family members are interested in the question whether and how twins are different from singletons.

In this chapter, we first discuss findings from the literature with respect to twin – non-twin differences in early life, e.g., birth weight, pre- and perinatal mortality differences, and congenital problems. In the second part, we provide the results for adult twin – non-twin comparisons across a wide range of traits assessed in the Netherlands Twin Register (NTR), including a large series of biomarkers.

4.2 Congenital Disorders and Infant Mortality

When the proportion of multiple births in Europe increased from 1.9% (1984–1988) to 3.1% (2004–2008), the prevalence of congenital anomaly from multiple births also increased [15]. A multiple pregnancy carries extra risk for fetuses and neonates. Twins grow slower during the third trimester than singletons [23], experience more intrauterine growth restriction [19], and are more likely to have a low birth weight [72]. This may influence both twins but may also be limited to one of the members in a twin pair, in the case of selective fetal growth restriction [35]. Compared to singletons, multiple birth children show a substantially higher rate of overall perinatal mortality [91, 99] and stillbirth [24, 42], though some studies report lower perinatal mortality rates in preterm twin pregnancies, possibly due to increased medical surveillance in the case of a multiple pregnancy [3, 47].

In about 60% of twin pregnancies, malpresentation occurs, with one or both of the twins not optimally positioned for birth [40]. Surgical delivery and assisted interventions during vaginal delivery are common in multiple pregnancies [45, 88, 99] and multiple birth is a risk factor for low Apgar scores [97, 98]. While preterm delivery and low birth weight explain part of the higher perinatal mortality and morbidity rates in twins, the risk of adverse outcomes is still higher when comparing twins with normal birthweight to singletons of the same birthweight [112].

The second-born twin generally faces the greatest risks as obstetric complications, such

as placental separation, cord prolapse, uterine atony, prolonged intertwin delivery time, and cervical spasm, may occur after delivery of the first-born twin [88] and can cause fetal distress, low Apgar scores, and neonatal morbidity [41, 75].

Prematurity and low birthweight are also associated with neurodevelopmental disorders and cerebral injury. Cerebral palsy (CP) is reported five to ten times more often in twins compared with singletons [80, 81, 89]. The risk of CP is affected by birth asphyxia that causes cerebral impairment [81] and by neonatal death or stillbirth in the co-twin [13].

There is considerable evidence that babies from multiple pregnancies have a higher risk of total congenital anomalies than singleton babies [30, 34, 73, 81], with reports of a relative risk of 1.29 for congenital anomaly in multiple births relative to singletons [14]. Hall [39] estimated that probably 10% of monozygotic twins are born with a congenital anomaly. The most common anomalies in twins and singletons for which twins have a higher risk than singletons are cardiovascular anomalies [34, 49, 60, 62, 87, 108]. Higher rates in twins are also reported for anomalies of the central nervous system, the digestive system, in particular gut atresias, the genitourinary track, and musculoskeletal systems [34, 60]. In addition, neural tube defects have been reported more often in twins [109], and the prevalence of clubfoot is twice that of the general population [110]. Twins, however, do not seem to have an excess risk of oral cleft compared to singletons [37, 110]. With respect to chromosomal abnormalities, study results differ. Some have shown lower rates of chromosomal abnormalities in twins compared to singletons [16, 25, 49, 73, 108], while others showed no differences [34, 60]. Some chromosomal anomalies and imprinting disorders are more prevalent in MZ twins with discordant presentation. For example, in Beckwith-Wiedemann syndrome the majority of affected twins have an unaffected MZ co-twin who may have only some features of the disease, and it has been suggested that a methylation failure in the twinning process is involved [11].

Several explanations have been proposed for the higher rates of congenital anomalies and malformations in twins, including disturbances in early embryonic development, especially in MZ twinning [34, 49, 63], hemodynamic instability in monozygotic placentation [79], contribution of artificial reproductive technologies and other treatments of infertility [10, 14, 34], as well as maternal age at pregnancy [76]. In the majority of the cases, congenital anomalies occur in discordant pairs where only one twin is affected [17]. The etiology is poorly understood but may involve epigenetic factors [92] as was found in a study of monozygotic twin girls who were discordant for a caudal duplication anomaly [74]. The coding region of the *AXIN1* was sequenced in both twins and while no mutation was detected, this region was significantly more methylated in the affected twin than in the unaffected twin.

Several conditions are unique to multiple pregnancies such as monochorionic-monoamniotic condition, twin-twin transfusion syndrome (TTTS), and some rare malformations such as conjoined twins, *fetus in fetu,* and acardiac malformation [56, 65]. Many congenital anomalies in twins are more common in MZ twins than in DZ twins [73] and within the MZ twins, more common in monochorionic than dichorionic twins [34]. Together with TTTS, congenital anomalies are an additional risk for mortality and adverse neurodevelopmental outcome in monochorionic twins [38, 61, 77].

A very rare condition, which may occur in MZ twins, involves the reversal of the internal organs known as *situs inversus partialis* or *totalis* [58]. Several case reports of MZ twins with *situs inversus* are reported in the literature [2, 12, 31, 50, 90]. This may be related to the phenomenon of "mirror twins," when the features appear asymmetrical in co-twins. For example, left-handedness in one twin and right-handedness in the other twin may be the expression of an anatomical mirror image at the level of the nervous system [31]. Other explanations include conjoined twinning, a late division of the embryo leading to MZ

twinning, and a malrotation of the viscera during early embryonic life [31, 58].

In conclusion, a multiple pregnancy increases the risk of congenital problems and mortality of the unborn and newborn. Still, the majority of twins are born healthy in countries with good health-care systems and develop normally. This however, does not exclude the possibility that twins differ from singletons in more subtle ways. In the next part, we therefore explore possible differences between twins and their singleton siblings.

4.3 Adult Twin-Sibling Comparisons Across a Wide Variety of Traits

4.3.1 Background and Procedure

While a twin pregnancy carries, as described above, a number of risks, the majority of twins are born healthy. One important question is whether such twins differ from singletons in their development and health at later age. To provide insight into the potential differences between multiples and singletons, it is essential to choose the correct reference group to which to compare the multiples. By selecting a group of singletons from the general population, this may introduce a bias as this population would also include individuals from one-child families, confounding the effects of being born after a singleton pregnancy with those of having no siblings. Even when choosing singletons from families with more than one child, this does not correct for potential differences across twin and non-twin families, such as parental behaviors or parental genotypes. Hence, differences observed at the population level between twins and non-twins in so-called between-family comparisons should be interpreted carefully. Differences may reflect true effects, but they may also be confounded by between-family differences in, e.g., family structure, urban-rural residency, and multiple other factors, including the maternal genotype, which is known to be associated with DZ twinning [64].

One way of eliminating these problems is by comparing twins to their own singleton siblings. This design optimally matches controls (siblings) and cases (twins), as twins and siblings come from the same family, and largely share their genetic background and family environment [20]. Within-family designs are becoming common in molecular genetics, where it is recognized that gene-outcome associations found among unrelated individuals may reflect between-family variation in genetic and environmental factors. A within-family comparison reduces confounding by these factors but does require statistical approaches that take into account the dependencies in the data such as paired-sample tests. Alternatively, differences due to genetic and environmental factors can be assessed in genetic structural equation models that simultaneously model the mean and the covariance structure in the data [83]. This approach is often taken in behavioral genetic studies, when the aim of the study is to estimate genetic and non-genetic contribution to the observed variance in a trait of interest. These studies, however, do not always report the outcomes of twin-sibling comparisons and sometimes assume that twins are similar to non-twin siblings.

In the following, we employed the within-family design in an adult sample from the Netherlands Twin Register. The Netherlands Twin Register (NTR) is one of the larger twin registers and also includes family members of twins, collecting data on twins as well as siblings, parents, spouses, and offspring. The NTR conducts longitudinal survey and experimental studies with the help of registered twins and their family members. Information on young twins is obtained from parents and teacher reports, while adolescent and adult twins and their registered family members provide the data themselves [123]. Here, we compare adult twin individuals to their singleton siblings for a wide range of variables collected in survey and biobanking studies. To this aim, we selected at random one twin and a sibling from the same family, with the sibling of the same sex as the twin. We also selected only those siblings of twins who did not differ more than 6 years in age from the twin.

For more detailed information on the methodology, please see the Appendix. The focus of most previous NTR studies has been on the quantification of genetic and non-genetic influences for a wide domain of traits, and while siblings were often included in the study design, relatively few studies reported the outcomes of twin-sibling comparisons. We identified the NTR studies that explicitly tested and reported the outcomes of twin-sibling comparisons and added these results to our discussion of twin-sibling differences in traits from various domains.

4.3.2 The Outcomes of Twin-Sibling Comparisons Across Multiple Domains in the NTR

Tables 4.1 and 4.2 summarize the findings for the twin-sibling comparisons for survey and biobank data, respectively.

Body Composition and Physical Development

Our findings for body composition as presented in Tables 4.1 and 4.2 show that adult twins differed significantly from their singleton siblings in height and body mass index, with twins being somewhat smaller and lighter than their non-twin brothers and sisters. This was seen when data were obtained in the survey and a trend was also present for the data collected during the home visit, when weight was measured. In line with the trend for lower body mass index, twins also tended to have a smaller waist circumference at the time of the home visit.

Our results are in line with another large study in childhood and adolescence in the NTR, where twins were shown to be shorter and have a lower BMI than their siblings [28]. In a subset of this sample, they found the expected the twin-sibling difference in birth length and birth weight, with the effect still present at the age of 1 year but found no evidence for twin-sibling difference for height, weight, and BMI at age 4. For this sample, no significant twin-sibling differences in body composition were seen at the young adult age, though there was a trend for twins to have a somewhat lower weight and shorter leg length. Additional components of physical development have also been examined. While differences in growth hormone levels were seen, with average lower levels of DHEAS and IGF-I levels in twins compared to their siblings [26], male twins did not differ from their siblings in testis size [29] and female twins did not differ for age at menarche [8].

These results and those of previous studies indicate that twins, who are more often born after a shorter gestation period and weigh less at birth than their singleton siblings, remain somewhat shorter and lighter well into adulthood, but in other aspects develop in the same way as their siblings.

Personality and Psychopathology

Table 4.1 presents the data for our matched twin-sibling comparison for five personality traits. Few differences are apparent, with only a trend for a personality trait called "openness to experience," where twin seems to score somewhat lower than the siblings. For sensation-seeking traits, twins and siblings are similar, and twins also did not differ from siblings in their perception of support, life satisfaction, or loneliness. Several NTR studies previously tested and reported on adult twin-sibling differences in personality, in large samples that did not employ within-family designs, and reported no twin-singleton differences for the traits studied, which included sensation seeking [95], neuroticism [106] life satisfaction [96] or trait anger (Distel et al. [21].

With respect to psychopathology, Table 4.1 shows twins did not differ from their siblings in ADHD symptoms in adults. They also did not differ in borderline personality total scores, confirming previous findings in an overlapping sample [21], nor for anxious depression. Depression, anxiety, and a combined anxious depression score were the subject of several previous studies in large samples of adult twins and singleton siblings, but no matter the definition, the two groups did not differ [66, 68, 93]. Adult twins and singleton siblings were also similar in the prevalence of burnout

Table 4.1 Outcomes of within-family twin-sibling comparisons for body size, personality, mental health, demographics, and lifestyle data collected in adult participants (survey 8) from the Netherlands Twin Register. Twins and siblings from the same family were selected to be of the same sex and of similar ages (not more than 6 years apart in age)

	Pairs	Twin		Sibling		Twin-sibling comparison results	
	N	Mean	SD	Mean	SD	T-test value	P-value
Continuous traits							
Age (years)	685	30.41	12.86	32.14	12.20	−15.347	0.000
Height (cm)	655	173.73	8.93	174.40	8.50	−2.480	0.013
Body mass index (BMI, kg/m^2)	634	22.73	3.12	23.89	3.82	−7.365	0.000
NEO neuroticism	613	29.38	7.60	29.90	7.29	−1.400	0.162
NEO extraversion	613	42.97	5.94	42.50	6.00	1.510	0.132
NEO openness to experience	613	36.59	5.78	37.37	5.82	−2.734	0.006
NEO agreeableness	613	45.54	4.97	45.01	4.84	2.206	0.028
NEO conscientiousness	613	44.76	5.78	45.04	5.54	−0.948	0.344
SSS total sensation seeking	411	11.22	2.41	11.13	2.45	0.711	0.478
SSS thrill adventure seeking	605	9.16	3.65	8.83	3.69	1.942	0.053
SSS experience seeking	418	16.00	4.46	16.33	4.58	−1.205	0.229
SSS boredom susceptibility	593	18.22	4.79	18.30	4.82	−0.303	0.762
SSS disinhibition	593	13.45	3.70	13.18	3.52	1.566	0.118
UNC-FSSQ confidant support	561	23.28	2.81	22.98	3.06	1.782	0.075
UNC-FSSQ affective support	559	13.81	1.97	13.72	1.99	0.745	0.457
SWLS general satisfaction with life	649	27.67	4.91	27.34	5.17	1.299	0.194
TILS loneliness	633	3.90	1.20	3.96	1.26	−0.920	0.358
CAARS ADHD index	599	8.11	4.12	8.44	3.93	−1.554	0.121
PAI-BOR total borderline personality	603	15.16	8.37	15.92	8.22	−1.775	0.076
PAI-BOR affect instability	603	4.40	3.00	4.59	3.00	−1.225	0.221
PAI-BOR identity problems	603	3.82	2.72	4.05	2.90	−1.562	0.119
PAI-BOR negative relationships	602	4.14	2.85	4.62	2.75	−3.244	0.001
PAI-BOR self-harm	602	2.81	2.40	2.65	2.31	1.242	0.215
ASR anxious-depressed scale	526	4.93	5.15	5.29	5.46	−1.183	0.237

(continued)

Table 4.1 (continued)

		Twin		Sibling		Test statistics	
Dichotomous traits	***N***	**N yes**	**% yes**	**N yes**	**% yes**	**Chi-square**	**p-value**
Being in good subjective health	657	590	89.8	575	87.5	1.675	0.196
Ever been in contact with mental health services	532	128	24.1	160	30.1	5.339	0.021
Regular sport participation	634	415	65.5	399	62.9	0.945	0.331
Regular alcohol use (2 or more times per week)	618	233	37.7	248	40.1	0.912	0.340
Current smoker	613	217	35.4	203	33.1	0.929	0.335
Ever tried hash	591	167	28.3	178	30.1	0.578	0.447
Being in a steady relationship, when 30 years or older	312	275	88.1	280	89.7	0.271	0.603
Living together with partner, when 30 years or older	312	258	82.7	268	85.9	1.095	0.295

Abbreviations: *NEO* NEO Five-Factor Inventory, *SSS* Sensation Seeking Scale, *UNC-FSSQ* Duke-UNC Functional Social Support Questionnaire, *SWLS* Satisfaction With Life Scale, *TILS* Three-Item *Loneliness Scale*, *CAARS* Conners' Adult ADHD Rating Scales, *PAI-BOR* Personality Assessment Inventory-Borderline Features scale, *ASR* Adult Self-Report

[68, 69]. Earlier NTR studies did not show evidence for differences between twins and siblings for obsessive-compulsive symptoms in adults [36] nor, in sample of young adults, for autistic traits [43]. A comparison of younger twins and siblings with respect to psychopathology showed no differences in ADHD symptoms between adolescent twins and singleton siblings [84]. Table 4.1 also shows twins and singleton siblings were similar in their reports of being in good health and in ever having been in contact with mental health services.

Considering the overall picture of these findings, twins do not seem to differ from their siblings in personality and psychopathology. Any differences found were very small.

Behavioral and Sociodemographic Traits

Table 4.1 also presents the results of the twin-sibling comparison for various health behaviors, which show twins to be similar to their singleton siblings in their reports of regularly sport participation, regular alcohol drinking, current smoking behavior, and ever having tried hash. This is in line with NTR studies, in which the prevalence of problem drinking [71] and cannabis use initiation [22] was similar in twins and their siblings. Likewise, previous NTR studies which examined aspects of childhood behavior have not found differences between twins and siblings for bullying and victimization in 9-year-old twins and siblings [101] and for truancy during secondary education [1].

With respect to sociodemographic traits, Table 4.1 indicates there are no differences between twins and siblings for being in a relationship, and for living together with a partner. As age may play a role, we limited our comparison for relationship status to those aged 30 or older. A previous study by [67] showed that at the age of 27 years MZ female twins were less often in a relationship than siblings. NTR studies on other sociodemographic traits did not find any twin-sibling differences for employment status [68] and

Table 4.2 Within-family twin-sibling comparison for biomarkers as assessed in the Netherlands Twin register. Twins and siblings from the same family were selected to be of the same sex and of similar ages (not more than 6 years apart in age)

	Pairs	Twin		Sibling		Correlation of trait difference with		Twin-sibling comparison results using standardized scores[a]	
	N	Mean	SD	Mean	SD	Difference in age	Difference in BMI	T-test value	P-value
Age (years)	382	36.30	12.28	37.37	11.98	1.00	0.084	−6.586	0.000
Height (cm)	378	174.37	9.02	175.58	9.13	−0.041	−0.089	−3.855	0.000
Body mass index (BMI)	374	24.37	4.05	25.29	4.37	0.084	1.00	−3.399	0.001
Waist circumference (cm)	373	83.30	11.26	85.72	12.23	0.062	0.809***	−3.153	0.002
Fasting total cholesterol (mmol/l)	323	4.85	1.02	4.94	0.94	0.103	0.074	−0.911	0.363
Fasting HDL (mmol/l)	323	1.40	0.35	1.41	0.37	0.053	−0.258***	−2.118	0.035
Fasting LDL (mmol/l)	323	2.93	0.92	2.95	0.84	0.089	0.096	−0.223	0.824
Triglycerides (mmol/l)	323	1.16	0.57	1.27	0.63	0.033	0.277***	−1.450	0.148
Fasting glucose (mmol/l)	321	5.30	0.73	5.41	0.88	−0.000	0.266***	−1.140	0.255
Fasting insulin (μIU/ml	315	8.41	4.83	9.82	7.94	0.055	0.392***	−1.539	0.125
Hba1c (%)	318	5.26	0.51	5.33	0.64	0.116	0.051	−1.388	0.166
White blood cell count (10^{12}/L)	346	6.33	1.72	6.58	1.94	0.020	0.165**	−1.690	0.092
Red blood cell count (10^{12}/L)	347	4.68	0.48	4.66	0.46	0.022	0.164**	1.470	0.143
C-reactive protein (mg/L)	353	2.69	4.18	3.47	5.00	0.030	0.299***	−1.726	0.085
Interleukin 6 (pg/mL)	347	1.42	1.27	1.97	5.24	0.093	0.043	−1.758	0.080
AST (U/L)	293	20.83	6.92	20.68	7.22	0.075	0.113	0.701	0.484
ALT (U/L)	275	10.93	7.39	11.02	9.19	0.085	0.072	0.314	0.754
GGT (U/L)	293	28.03	24.47	29.87	24.00	0.081	0.184***	−0.399	0.690
Creatinine (U/L)	293	85.52	13.95	85.28	14.26	−0.058	0.046	0.420	0.674
Telomere length	289	2.78	0.69	2.66	0.49	−0.209***	−0.045	3.491	0.001

Abbreviations: *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma-glutamyltransferase
*<0.05, **<0.01, ***<0.001
[a]Age and sex standardized scores were used in the paired sample t-tests for height, BMI, and waist circumference. For all other variables, age, sex, and BMI standardized scores were used

the prevalence of being in a creative profession [100]. However, compared to their singleton siblings, twins more often lived in highly urbanized areas in two younger age cohorts, though no difference was seen for the oldest age cohort [107]. It is possible that in the younger cohorts the age difference between twins and siblings was of importance, as the on average older sibling may already have had the financial means or the motive of an own increasing family to move to less populated areas.

Overall, twin status does not affect choices related to health behavior and most other behaviors, though particular aspects of social behavior such as being in a relationship and residential choices deserve more attention to provide definite answers.

Biomarkers and Disease

The results for our twin-sibling comparisons for biomarkers can be seen in Table 4.2. For total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride levels, there were no significant differences between twins and siblings. With respect to glucose metabolism, looking at the data the pattern seems to suggest a trend for siblings to have higher values, in particular for insulin. However, differences in BMI between twins and siblings are strongly related to differences in glucose metabolism parameters and when comparing scores after the effects of BMI are taken into account, twins and siblings were similar in glucose metabolism. We further compared twins and siblings on C-reactive protein (CRP), white and red blood cell count, and IL6 level and found no significant differences for any of these variables. This is in line with a previous small-scale study including 222 twins and 85 siblings in which no differences between twins and siblings were observed for the cytokine response to ex vivo amyloid-beta stimulation [86]. Table 4.2 also summarizes the twin-sibling data for liver enzymes and creatinine, which were similar for the two groups. This extends the findings by van Beek et al. [5], who in a larger sample that included this subset, also reported no differences in the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) but did not comment on gamma-glutamyl transferase (GGT).

While the majority of our comparisons did not reveal any twin-sibling differences, there was a trend for twin and siblings to differ in telomere length, with shorter telomere length in the siblings compared to the twins, even when correcting for age effects using standardized scores. Further studies would be needed to determine whether this represents a meaningful twin-sibling difference.

A series of previous NTR studies focused on cardiovascular functioning in adult twins and their siblings. No differences were seen for blood pressure, whether measured in a laboratory setting [46], ambulatory during everyday life [53], or defined as hypertensive state [32]. For cardiac functioning, operationalized as heart rate or heart rate variability, again no significant differences between twins and their siblings were found [52, 54]. Twin-sibling differences were also not present for respiration rate [52] or cortisol [51].

With respect to other physical diseases, NTR studies in adult women showed twins did not differ from their singleton siblings in the occurrence of polycystic ovary syndrome based on survey information [104], nor in the presence of cervix smear abnormalities as determined by cervical screening [103]. Twin-sibling differences were also not reported for asthma in a study including more than 11,000 adult male and female twins and siblings, but surprisingly siblings reported somewhat higher rates of allergy than twins (23.3% vs. 18.2%, [105]).

In summary, these studies show that, while there may be a few exceptions pointing to a disadvantage in singletons, twins and their singleton siblings are generally very similar in biomarkers and disease.

Cognitive Function

We did not apply a within-family test for cognitive function in this study, but here we summarize the results from previous NTR studies. A number of studies in young twins have shown differences in cognitive abilities. In samples of twins and siblings with average age between 9 and 13, twins scored lower

on full scale, verbal and performance IQ [94] and on IQ, reading performance, and verbal working memory [59]. De Zeeuw et al. [111] tested cognitive function in the largest sample yet, including 1375 twins and 1375 siblings and again showed lower scores for specific aspects of cognitive function (IQ, reading performance, and verbal working memory) at age 9 in twins compared to their siblings. However, twins are often not the first-born children in a family. After taking birth order into account, the cognitive disadvantage of twins dissipated. Lower scores for cognitive function were only seen when comparing the twins to older siblings. When comparing the scores of twins at age 9 with the scores of their younger siblings at age 9, twins no longer differed from their singleton siblings. Twins did not differ from older or younger siblings with respect to for visuospatial working memory, verbal, and spatial short-term memory. Interestingly, twins scored higher on physical education than their older and younger singleton siblings [111]. Note that not all studies on cognition in young twins showed lower scores in twins compared to their singleton siblings. A recent study including more than 11,000 twins and 262 of their siblings at age 7.5 showed that reading ability in twins was comparable to that in siblings and to national norms [7]. Two smaller NTR studies provided information on the twin-sibling comparison for adult cognitive function: Van den Berg et al. [9] found no twin-sibling differences for reading vocabulary, and Posthuma et al. [85] showed no difference in intelligence between twins and siblings.

Overall, while lower scores for cognitive ability in twins have been reported, these effects seem related to the position (birth order) in the nuclear family. Those first born in the family more often score higher on cognitive tests than those later born, an effect which is independent of whether they are born from a singleton or multiple pregnancy. As not all studies in children showed lower scores across the cognitive domains and studies in adults did not show any twin-singleton differences, a twin-singleton difference for cognitive function seems modest and likely limited to specific domains.

4.4 Concluding Remarks

A twin or multiple pregnancy carries an increased risk of prenatal mortality and congenital abnormalities. However, the majority of children born from a multiple pregnancy are healthy at birth and develop into healthy individuals. Still, multiples could differ from singletons in other ways, due to their lower gestational age and birthweight or due to growing up with a sibling of the same age and often same sex and looks, i.e., the close companionship hypothesis. Our studies indicate that twins do not differ from their singleton siblings across a wide range of behavioral and lifestyle parameters, biomarkers, or diseases.

The one aspect on which twins differed from non-twins was body composition. Twins remained smaller and lighter compared to their singleton siblings, even as adults, attaining about 1 point lower BMI lower than singletons. As very few physiological differences were seen, the difference in body composition seems to be a lasting and likely beneficial aspect of being a twin.

Very little evidence was found for the close companionship hypothesis: twins did not differ from their singleton siblings across a wide range of behavioral and psychological traits. Twins did do better than singletons in physical education classes at school, which could indicate the effect of always having a playmate during childhood. In adults, no differences in regular sport participation are seen, suggesting that the effect, if confirmed, may only be present at younger ages. We did not test for effects of zygosity and we cannot exclude the possibility that specific twin effects may still occur for specific groups, especially monozygotic twins, as implied by the finding of [70] that MZ female twins were less often in a relationship than others. However, as our study included a large number of monozygotic twins the effects are likely to be small and limited to specific situations and groups.

We did not investigate effects of fertility treatment. Many of the studies presented here

were conducted in twins who were born before ART and IVF became frequently used in the Netherlands, but it may have occurred in the younger twins participating in the studies. Still, many of these twins will not have had a sibling, as fertility problems occurred in these families, and the number of IVF twin-sibling pairs will thus be very limited. A previous study by the NTR matching IVF with DZ naturally conceived twins showed no differences in growth, attainment of motor milestones, or in behavioral development, leading to the conclusion that for nearly all aspects, development in these groups of children is similar [6].

While twins and siblings born in the same family do not differ, it is still possible that individuals born in twin families differ from singletons born within non-twin families due to the genetic background of their parents. This may be especially so in DZ twin families, as we know that mothers of DZ twins are somewhat taller and heavier than mothers of monozygotic twins [44], and genes found to be related to twinning are also related to increased body mass [64]. In a study of 5-year-old Dutch twin children, female twins were as tall as singleton children, while male twins were still somewhat shorter than children from the general population and twins overall had a lower BMI than the general population [27]. In a separate study, Estougie et al. [28] reported no differences in height between young adult twins and their siblings and their height was comparable to the general population. For BMI, no differences were observed between 18-year-old twins and 18-year-olds from the general population, whereas the siblings of twins had increased BMI values. A Finnish study among 17-year-old twins reported that twins were as tall as singletons, but that boy twins were still leaner, though an American twin study showed no twin effects on weight and height at age 8 [78]. It is possible that, when it comes to body weight and body height, twins on average reach their full potential at a later age than singletons. Alternatively, twins may never reach their full potential for height and weight. As a large number of the genetic variants involved in body composition are now known, this hypothesis could be tested in the near future. Other factors may also differ within families for twins and non-twins. Maternal age at birth and parity are higher on average for DZ twins, and twins differ from singletons in gestational age. Also, the rearing environment is different for two children than for a single child, though this is not specifically limited to multiples but is the case for any family including multiple children.

Another aspect that may deserve additional attention is the position of multiples in the family. For a number of cognitive traits, the differences between twins and siblings could be explained by position within the family. This has not been systematically studied for other traits, which may reveal similar position-in-family effects. In addition to the position in the family, whether twins were born first or second, also requires more attention. In this study, there was an equal distribution of twins born first and second by design. As birth complications more often occur for the second born twin, this may be another factor leading to small differences between twins and singletons.

Our results regarding a large number of traits and common disorders are fairly optimistic, indicating that twins do not differ from singletons. By comparing the twins to their non-twin siblings, we avoid confounding by between-family factors. We note, however, that our work did not look at rare disorders and that despite the large number of participants in the Netherlands Twin Register some forms of bias may be present. Parents of twins who presented with serious complications at birth may decide against participation. Adolescent and adult twins with health problems, whether mental or physical, may be less inclined to enroll in the longitudinal study or may drop out during the study. In addition, the decision to enroll and continue participation is also influenced by other factors such as educational attainment. Still, twins are born in all strata of society and tend to be motivated to take part in medical and scientific studies and, though we have seen that NTR participation is related to educational attainment, participants with lower educational levels are also present in the sample.

In conclusion, with the exception of congenital disorders and body composition, twins

do not seem to differ from singletons, when taking family factors into account. While being a twin can be seen as special, for most traits twins are just like ordinary siblings.

4.5 Quotes by Twins

In the ninth NTR survey to adult participants [123], twins were asked whether they liked being a twins. The following quotes are a selection from answers provided by more than 5000 multiples. Their comments are in line with the findings in our chapter.

They highlight that a multiple pregnancy carries risk:

- "I did not grow up as twins. Twin sister died at birth."

Many of the multiples view their multiple status as something special, though not everyone responds positively.

- "I like it as long as people see us as separate individuals. It is in any case not ordinary and I do like that."
- "Sad for all those singletons…honestly."
- "Quite nice. I have different (closer) contact with my twin brother than with my other brothers."
- "Quite nice; as twin you are never alone."
- "Being a twin has two sides. A very nice side because we got along very well as sisters and because being a twin is also somewhat special. The disadvantage of being a twin is that I have the feeling I am constantly compared to my twin sister and that I do this also."
- "Still somewhat special. We do have another connection with each other than with my other brothers (4)."
- "Super great! I feel that there is someone who always has my back and who understand me. I think this is because we are also friends."
- "In general it is nice, because we understand each other very well and are strongly connected. But sometimes it is also a bit suffocating since you were and are always compared to each other and we also compare ourselves and never want to do under for the other."
- "Nice. You have a special connection with each other which you do not have with brother or sister. A bit difficult to learn to make friends and keep them, because I always had my sister and never had to do this."

However, comments also indicate that the multiples do not see much difference with ordinary brothers and sisters, especially not as adults.

- "We are a dizygotic twin pair, so I do not see it as very special. I do not connect more with him than with my other brothers."
- "We have a very different life. I view him just like my other brother."
- "It's OK. Not very special. The connection with my younger sister is no less, my younger sister and my twin sister are just as important for me. The one is not more important than the other. Sometimes it is nice to be a twin, sometimes less nice. It has (in the past) advantages and disadvantages."
- "Quite nice, not very special."
- "Quite nice but not as special as people often expect."
- "It is nice that you can race to see who finishes his study first, but noting more."
- "It is somewhat more special than other brothers/sisters, but for the rest not much difference."
- "In the past special to do everything together, now I do not notice much. It is just like an ordinary brother."
- "In the past very nice, now it just seems as if we are normal sisters."
- "OK. In the past I did a lot together with my twin brothers Now it is just like with my other brother or sister."
- "Normal. No differences with people who are not a twin."
- "Not different than having my other 2 brothers."

4.6 Review Questions

Open Questions:

1. Question: How would two major hypotheses explain differences between twins and singletons?

Answer: A somatic and a social hypothesis. First, twins are born earlier and have smaller body size at birth. This may be associated with a range of later outcomes. Second, growing up with a close companion since birth and mechanisms of social interaction may lead to differences in behavioral outcomes between twins and non-twins.

2. Question: What type of design optimizes testing for differences between twins and non-twins?

Answer: A so-called within-family design in which outcomes in twins are compared to outcomes in their non-twin siblings.

3. Question: What is the empirical evidence for twin non-twin differences (as a function of developmental stage)?

Answer: Multiples have an increased risk of congenital problems but with exception of being lighter and having a lower body mass index; there are few differences between adult twins and singletons.

4.6.1 Multiple-Choice Questions

1. For which class of diseases/disorders do we observe prominent differences between twins and non-twins?
   (a) Psychiatric diseases
   (b) Congenital anomalies
   (c) Cardiovascular disease
   (d) Neoplasia

Answer: (b)

2. What is the average pregnancy duration for twins?
   (a) 32 weeks
   (b) 35 weeks
   (c) 37 weeks
   (d) 40 weeks

Answer: (c)

3. Which of the following is true about multiple pregnancy in comparison to singleton pregnancy?
   (a) Shorter gestational age
   (b) More intrauterine growth restriction and lower weight at birth
   (c) Higher risk of complications during pregnancy and malpresentation at birth
   (d) All the above

Answer: (d)

4. Which disorders are more prevalent in MZ twins in comparison to singletons and DZ twins?
   (a) Down syndrome
   (b) Beckwith-Wiedemann syndrome
   (c) Cri du chat syndrome
   (d) Huntington's disease

Answer: (b)

5. In adulthood, what is the domain in which some twin-singleton differences are found?
   (a) Body composition and physical development
   (b) Personality and psychopathology
   (c) Cognitive function
   (d) Behavioral and sociodemographic traits
   (e) Physiological parameters and physical disease

Answer: (a)

4.7 Appendix: Within-Family Twin – Non-Twin Comparisons Across a Wide Range of Traits Assessed in the Netherlands Twin Register

4.7.1 Participants and Selection Procedure

To compare twins to their siblings, we made use of the data from two separate projects: (1) the eighth NTR survey on health, lifestyle and personality, which was sent out to adult participants between 2004 and 2009 [120, 123]; (2) a large-scale blood collection project carried out between 2004 and 2009 in which participants were visited at home to obtain blood samples and health information [125].

For each of the two datasets, we followed the same procedure. We first selected all twins and their singleton siblings with known age, sex, birth year, and, in the case of twins, zygosity. Known half-siblings and non-biological siblings were excluded. Next, we randomly selected one of the twins in case a twin pair both participated and assessed whether there was a same-sex singleton sibling in the family who was born within 6 years from the twin. This sibling was then selected, if at the time of participating he or she was within 6 years of the age of the twin. In the case multiple singleton siblings of a twin met the criteria, we selected the same-sex sibling closest in birth year. We made two exceptions to this procedure. In the case of an opposite-sex twin pair, when a male twin-sibling pair could be formed in the dataset, we selected the male pair. This was done to maximize the presence of male pairs in the analyses as fewer males participated than females. When both older and younger siblings within the 6-year time frame were present, preference was given to the sibling younger than the twin, as twins more often have older siblings than younger siblings. Even applying these criteria, our sample selections included more female pairs than male pairs and more older than younger singleton siblings.

4.7.2 NTR Survey 8

As part of a longitudinal survey study, this survey was sent out to adult twins registered with the NTR. It was completed by 10,176 multiples and 2,142 siblings and collected information on a wide range of traits [120]. After applying the selection criteria, the sample for the present analyses consisted of 685 twin-sibling pairs, 177 (26%) being male. Average age (sd) of the twins at the time of survey completion was 30.4 (12.9) and of the siblings 32.1 (12.2) years. In 142 (21%) of the pairs, the twin was older than the sibling. We compared the twins and their singleton siblings on the following continuous traits: self-reported height and body mass index (BMI, calculated as $\text{weight}_{(kg)}/\text{height}^2_{(m)}$); the big five personality dimensions (openness, conscientiousness, extraversion, agreeableness, neuroticism) as measured with the NEO Five-Factor Inventory [117, 121]; borderline personality components measured with the Personality Assessment Inventory-Borderline Features scale (PAI-BOR; [124]); sensation seeking score and its subscales thrill and adventure seeking, boredom susceptibility, disinhibition and experience seeking as measured with the Sensation Seeking Scale [119, 126]; attention deficit hyperactivity disorder (ADHD) as measured with the Conners' Adult ADHD Rating Scales (CAARS; [116]); the anxious depression scale of the Adult Self-Report which combines elements of depression and anxiety [114]; dimensions of social support (confidant and affective) as measured with the Duke-UNC Functional Social Support Questionnaire; and life satisfaction as measured with the Satisfaction With Life Scale [118]; loneliness as measured with the Three-Item Loneliness Scale [122]. In addition, we compared twins and siblings on categorical traits with outcomes operationalized as yes versus no, including the following traits: being in good health (reports of good or excellent

health were coded as yes), ever been in contact with mental health services, being a current smoker, drinking alcohol regularly (reports of drinking alcohol 2 or more times per week were classified as yes), ever tried hash, being in a relationship (data only included for those age 30 years and older), and living together with a partner (data only included for those aged 30 years and older).

4.7.3 NTR Biobank

Between 2004 and 2010, 9530 individuals provided a blood sample and health-related information as part of a large-scale biobank project [125]. When conducting the selection procedure as described above, this resulted in 382 twin-sibling pairs, of which 144 (38%) were male-male pairs. The average age of the twins was 36.3 (12.3) and of the siblings 37.4 (12.0). In 265 pairs, the singleton sibling was older than the twin. We compared twins and their singleton siblings on the following variables: lipid profile (total cholesterol, HDL, LDL, and triglyceride levels), glucose metabolism (glucose, insulin, and HbA1c levels), white and red blood cell counts, C-reactive protein as indicator of general inflammation, liver enzymes alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyltransferase (GGT), creatinine as measure of kidney function and telomere length. For lipid profile and glucose metabolism, data were only included if the participant had kept to the instruction to be fasting at the time of blood collection (see [125]).

4.7.4 Analyses

All analyses were conducted in IBM SPSS Statistics version 25. To compare the twins with their singleton sibling, we conducted a paired-sample t-test for continuous traits and a McNemar chi-squared test for categorical traits. As age and BMI may be important factors in the physiological parameters, we here correlated the differences in age and BMI for the twins and siblings with their differences in physiological parameters and present the test outcomes for age, sex, and BMI standardized residuals. Considering the large number of comparisons conducted, we consider a trend when p-values are between 0.010 and 0.001 and p-values <0.001 as significant.

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Appendix: Within-Family Twin – Non-Twin Comparisons Across a Wide Range of Traits Assessed in the Netherlands Twin Register

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