

Confounding & Mediation

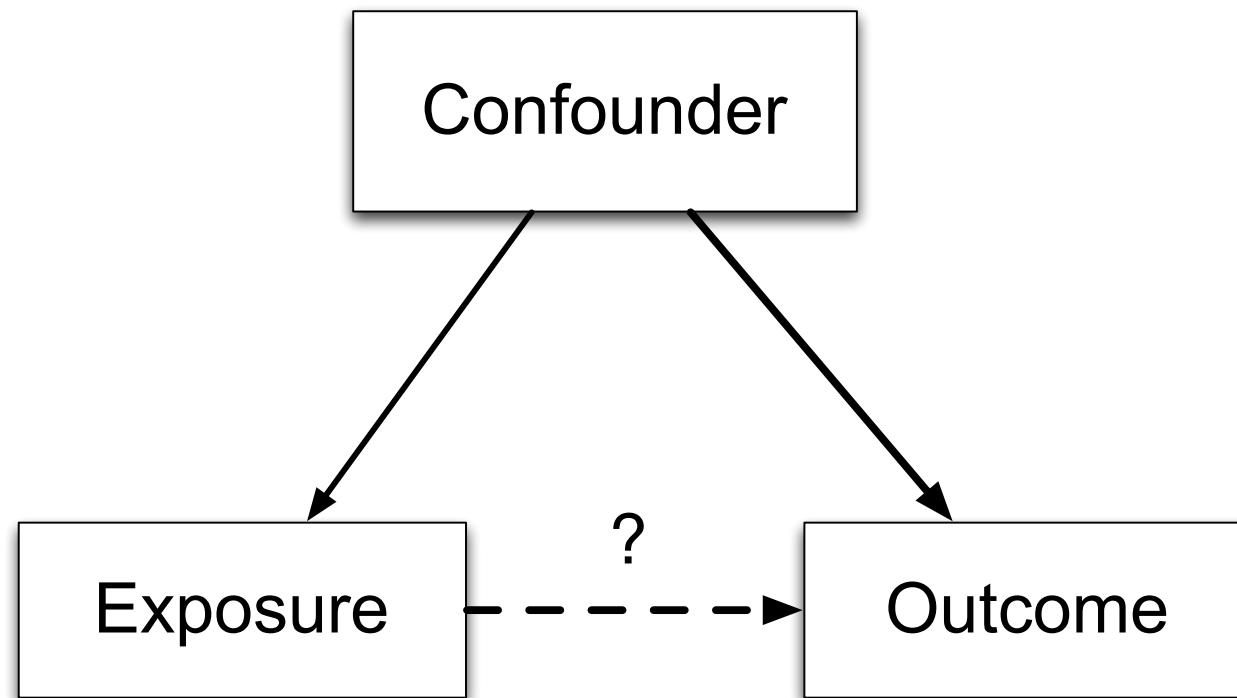
January 28, 2014

Biostatistics 208

Confounding

- *Confounder*: a common cause of both exposure (treatment) and outcome, or a surrogate for a common cause (e.g., age)
- Estimation of causal effects of an exposure/treatment from observational data must account for possible confounding to avoid bias
- Standard analytic strategies include
 - restriction, stratification, matching, propensity scores, inverse probability weighting
- Our focus: *adjust for confounding effects using regression models*
 - how to adjust adequately;
 - when it will work

Confounding DAG



Potential outcomes and causal effects

- Suppose we could observe outcomes for each individual under exposure *and* no exposure, holding everything else constant
- Within-person differences between these *potential* (aka “counterfactual”) *outcomes* are what we mean by causal effects
 - by definition, free of confounding influences
- Problem: in general, only one of these outcomes is observable

Causal effects, RCTs, and linear regression modeling

- RCTs use randomization to break the confounder → exposure link, so treatment is unconfounded
 - treatment groups are *exchangeable*, at least in large trials
 - causal effect estimated by comparing average outcomes between treatment groups
- Exchangeability in observational data potentially achieved by accurately *modeling* all confounding effects
 - *within modeled strata*, exposed, unexposed exchangeable
- Adjusted estimate unbiased for causal effect if all necessary confounders (i.e., *minimum sufficient adjustment set*, or *MSAS*) included and *accurately modeled*

Why adjustment works to remove confounding

- A simple example based on simulated data
 - continuous exposure E
 - binary confounder C , correlated with E
 - continuous outcome $y = \beta_0 + \beta_1 E + \beta_2 C + \varepsilon$
 - in this example, $\beta_2 > 0$ and E, C are positively correlated
- Because E and C are correlated, model must include C to estimate β_1 – the causal effect of exposure – without bias

```
. tab confounder, sum(exposure)
```

confounder	Summary of exposure		
	Mean	Std. Dev.	Freq.
0	62.316344	3.7140415	25
1	68.662241	4.9223134	25
Total	65.489293	5.3755658	50

```
. tab confounder, sum(outcome)
```

confounder	Summary of outcome		
	Mean	Std. Dev.	Freq.
0	127.6001	27.565811	25
1	179.34827	39.539979	25
Total	153.47418	42.673949	50

```
. corr exposure outcome  
(obs=50)
```

	exposure	outcome
exposure	1.0000	
outcome	0.6211	1.0000

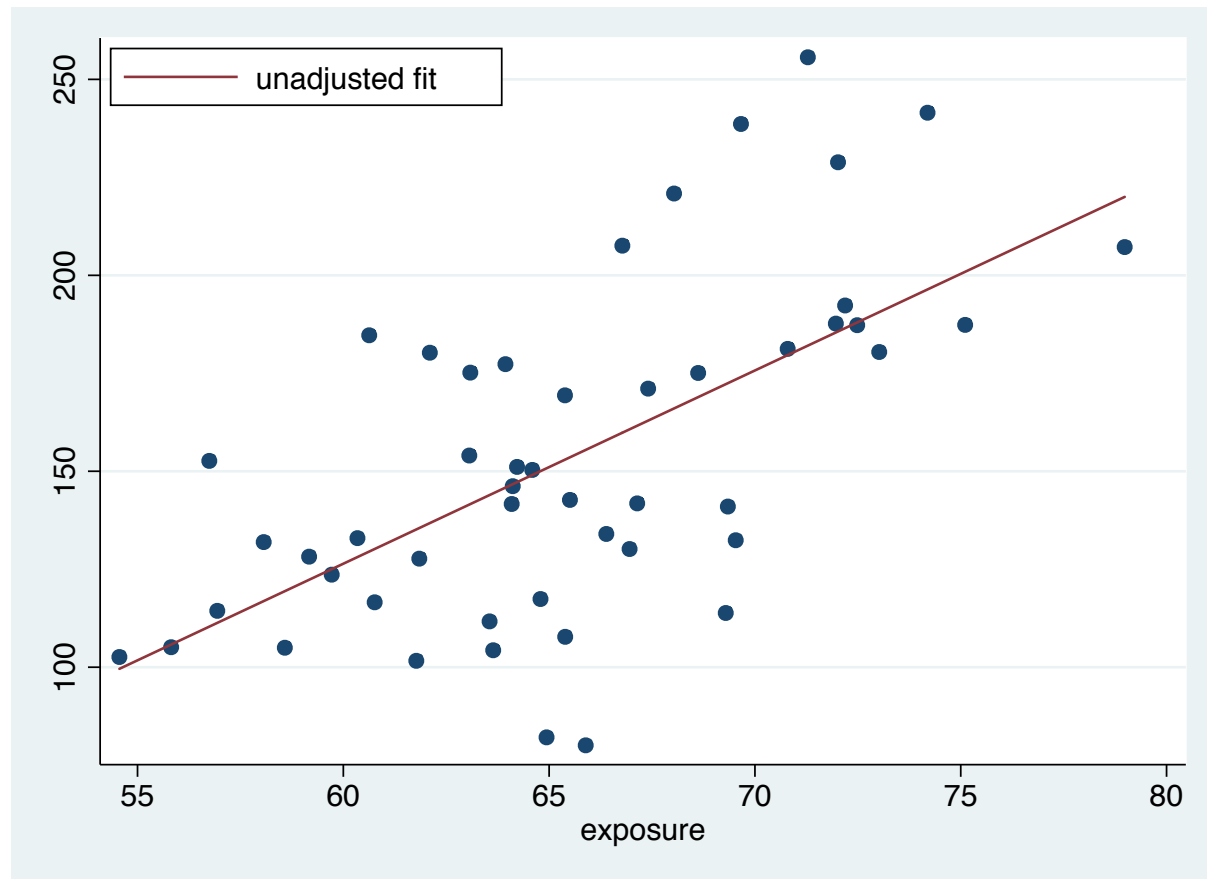
Why adjustment works to remove confounding

- Unadjusted effect of E partly reflects higher prevalence of C among exposed ($E = 1$)
- Adjustment fixes the problem by modeling effect of C
- Model allows us to estimate effect of E within strata with $C = 0$ and $C = 1$
- No confounding by C within strata, because C does not vary

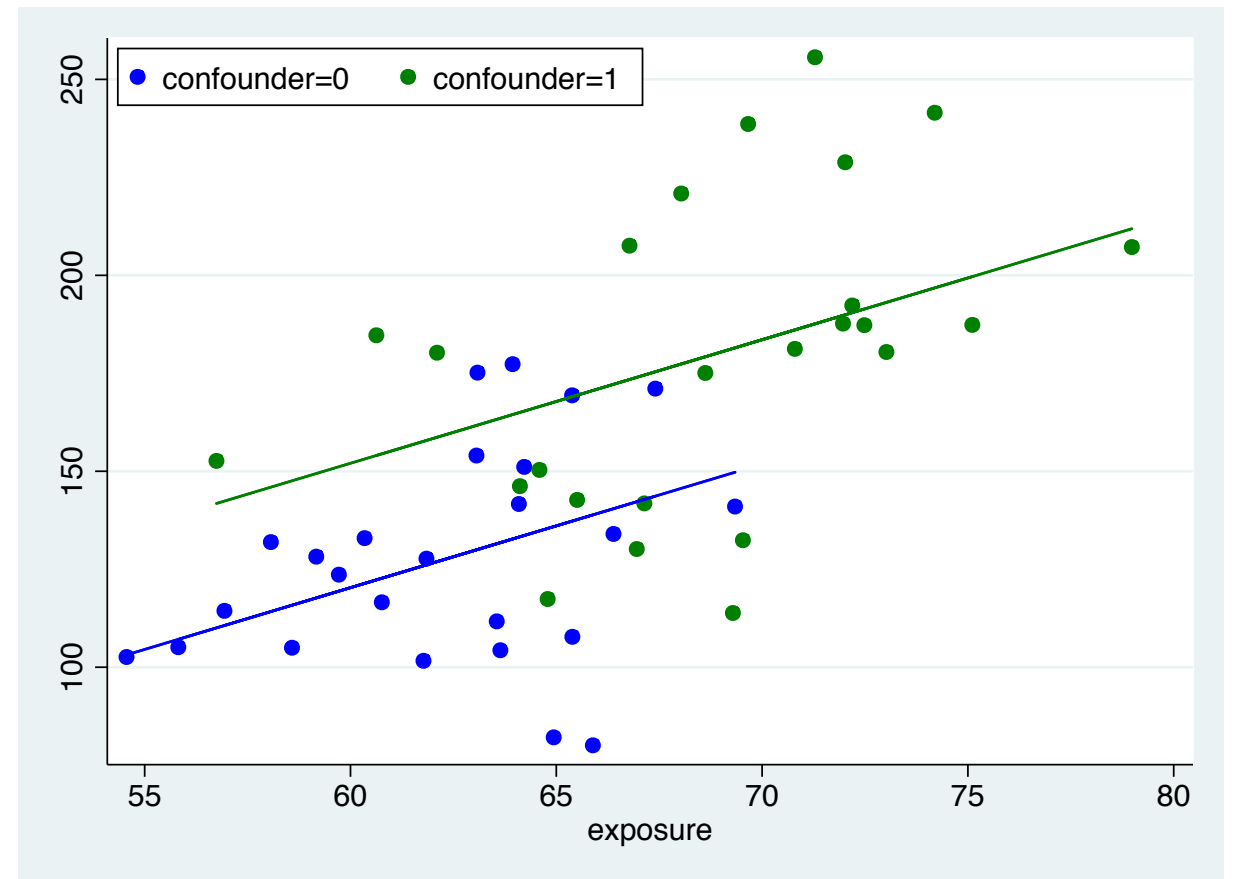
Why adjustment works to remove confounding

simulated data example based on the set-up in slide #6 (assumed: $\beta_1 = 2.5$)

Unadjusted



Adjusted



Unadjusted model

```
. regress outcome exposure
```

Source	SS	df	MS	Number of obs = 50		
-----+-----				F(1, 48) = 30.15		
Model	34425.9351	1	34425.9351	Prob > F = 0.0000		
Residual	54806.2966	48	1141.79785	R-squared = 0.3858		
-----+-----				Adj R-squared = 0.3730		
Total	89232.2317	49	1821.06595	Root MSE = 33.79		

outcome	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
-----+-----						
exposure	4.930837	.8979918	5.49	0.000	3.125303	6.736371
_cons	-169.4428	59.00268	-2.87	0.006	-288.0757	-50.81

Interpretation: without modeling the effect of the confounder, the estimated effect of exposure partly reflects the effect of the confounder. Here, confounding is bad enough that the 95% CI for the exposure effect excludes the true value of $\beta_1 = 2.5$.

Model adjusting for the confounder

```
. regress outcome exposure i.confounder
```

Source	SS	df	MS	Number of obs = 50		
Model	42543.0681	2	21271.5341	F(2, 47)	=	21.41
Residual	46689.1635	47	993.386458	Prob > F	=	0.0000
Total	89232.2317	49	1821.06595	R-squared	=	0.4768
				Adj R-squared	=	0.4545
				Root MSE	=	31.518

outcome	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
exposure	3.152571	1.043347	3.02	0.004	1.053628	5.251514
1.confounder	31.74228	11.10442	2.86	0.006	9.403068	54.0815
_cons	-68.85659	65.32243	-1.05	0.297	-200.2684	62.55518

Interpretation: after we model the effect of the confounder, the estimated effect of exposure on the outcome is unbiased (and attenuated), and the 95% CI includes the true value of 2.5.

Confounding: interpretation of results

- Unadjusted estimate for exposure
 - estimates an observable trend in whole population
 - usually lacks a causal interpretation
- Adjusted estimate
 - unbiased for causal effect (provided confounding is adequately controlled for)
 - adjustment can impact precision of estimates

Confounding and interaction

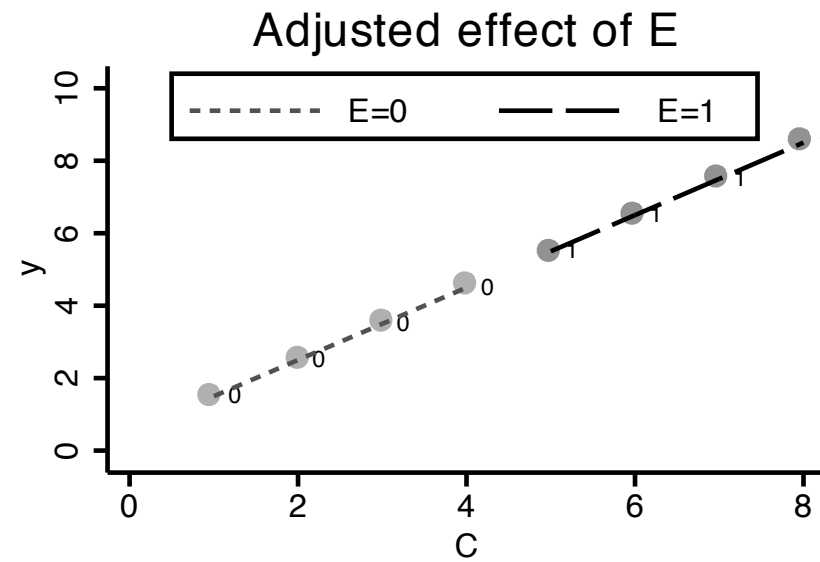
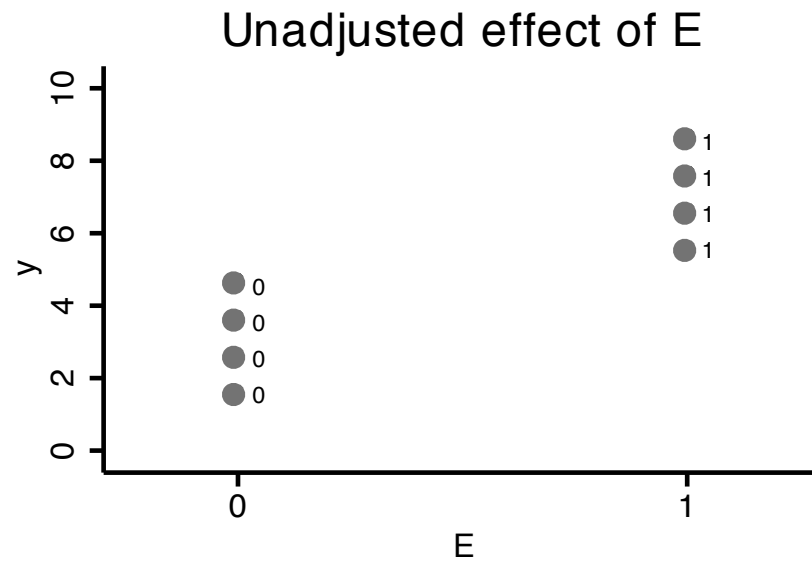
- Regression lines for strata with $C = 0$ and $C = 1$:
 - slopes estimate exposure/outcome association within each stratum
 - assumed parallel: no interaction – E has same effect within both strata
- Next big topic: how to check and relax this assumption

Behavior of adjusted regression coefficients

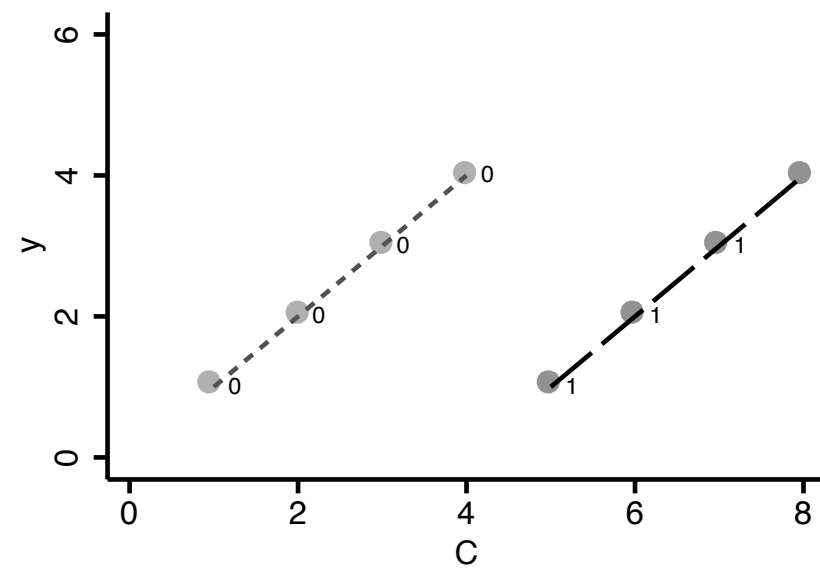
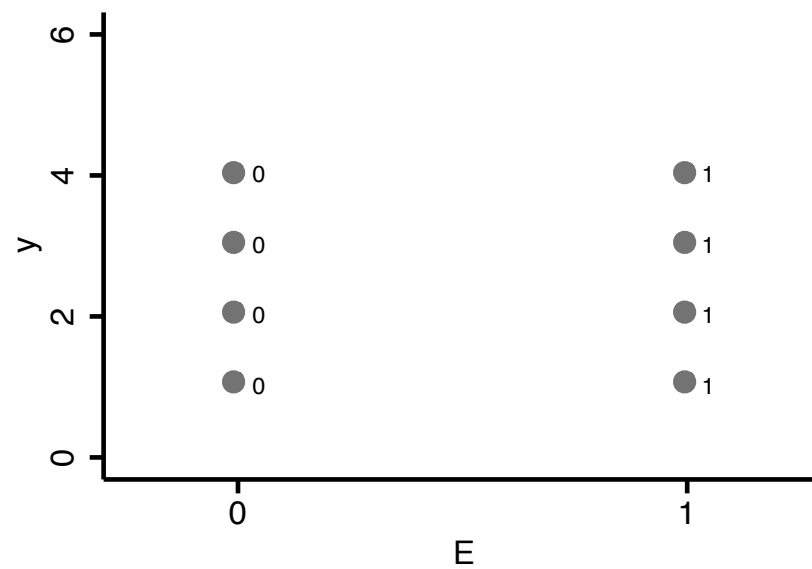
- Adjusted coefficient for exposure is attenuated when exposure and confounder are:
 1. Positively correlated, with similar effects on outcome
 2. Inversely correlated, with opposite effects on outcome
- This is the *typical pattern* for confounding

Note: there is a new Stata command that allows the change-in-coefficient criterion to be used in model selection

Range of confounding patterns



Positive Confounding



Negative Confounding

Negative confounding

- Example: needlestick injuries
 - Overall, post-exposure prophylaxis (PEP) with AZT not associated with protection against HIV transmission
 - * PEP associated with severity of injury
 - * severity increases transmission risk
 - PEP protection unmasked after controlling for severity
- *Confounding by indication* masks protective effect

Negative confounding: two scenarios

Negative confounding may arise between predictors that are

1. *Positively correlated, with opposite effects on outcome:* Injury severity, PEP, and HIV transmission
2. *Inversely correlated, with similar effects on outcome:* BMI decreases with age in HERS cohort (-0.13 kg/m^2 per year on average), but both are associated with higher systolic blood pressure (SBP)

Unadjusted association of BMI and SBP

```
. regress SBP BMI
```

Source	SS	df	MS	Number of obs	=	2758
Model	3573.21875	1	3573.21875	F(1, 2756)	=	9.91
Residual	993677.692	2756	360.550687	Prob > F	=	0.0017
				R-squared	=	0.0036
				Adj R-squared	=	0.0032
Total	997250.911	2757	361.715963	Root MSE	=	18.988

SBP	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
BMI	.2063226	.065539	3.148	0.002	.077812	.3348332
_cons	129.1723	1.907635	67.713	0.000	125.4318	132.9129

BMI and SBP adjusted for age

```
. regress SBP BMI age
```

Source	SS	df	MS	Number of obs	=	2758
Model	33945.7704	2	16972.8852	F(2, 2755)	=	48.54
Residual	963305.14	2755	349.657038	Prob > F	=	0.0000
Total	997250.911	2757	361.715963	R-squared	=	0.0340
				Adj R-squared	=	0.0333
				Root MSE	=	18.699

SBP	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
BMI	.3034786	.0653778	4.642	0.000	.1752842	.431673
age	.5056204	.0542507	9.320	0.000	.3992443	.6119965
_cons	92.69495	4.341354	21.352	0.000	84.18231	101.2076

Interpretation: effect of BMI on SBP is larger – **0.30 mmHg per kg/m² increase** in BMI – after adjustment for age, compared to unadjusted effect of 0.21 mmHg per kg/m².

Summary: negative confounding

- Negative confounding can mask an independent association in unadjusted analysis
- Not all that uncommon
- Implications for predictor selection: univariate screening, forward selection procedures may miss some negatively confounded predictors
 - ─ backwards deletion preferred

Interpretation of multiply-adjusted coefficients

```
. regress LDL BMI age10 nonwhite smoking drinkany
```

Source	SS	df	MS	Number of obs = 2745		
Model	42279.1873	5	8455.83745	F(5, 2739) = 5.97		
Residual	3881903.3	2739	1417.27028	Prob > F = 0.0000		
Total	3924182.49	2744	1430.09566	R-squared = 0.0108		
				Adj R-squared = 0.0090		
				Root MSE = 37.647		

LDL	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
BMI	.3591038	.1341047	2.68	0.007	.0961472	.6220605
age10	-1.897166	1.130776	-1.68	0.094	-4.114426	.3200947
nonwhite	5.219436	2.323673	2.25	0.025	.663108	9.775764
smoking	4.750738	2.210391	2.15	0.032	.4165362	9.08494
drinkany	-2.722354	1.498854	-1.82	0.069	-5.661352	.2166445
_cons	147.3153	9.256449	15.91	0.000	129.165	165.4656

Interpretation: each kg/m² increase in BMI is independently associated with an increase of 0.36 mg/dL in LDL (95% CI 0.10-0.62, $p=0.007$), adjusting for age, race/ethnicity, smoking, and alcohol use. Analogous interpretations hold for the other effect estimates.

Confounding is difficult to rule out

- Were all important confounders adjusted for?
- Were they measured accurately?
- Were their effects modeled adequately – no un-modeled nonlinearities or omitted interactions?
- Was there enough “overlap” between exposed and unexposed to avoid extrapolation?

Getting the model right

- Non-linearities and interactions
 - default linear, no-interaction model may be too simple, allow residual confounding
 - departures hard to detect in small samples
- Avoiding extrapolation may require *restriction* of sample and scope of inference to subset of exposed with comparable unexposed controls (e.g., treatment effect in the treated)
- Difficulty of getting the model right a primary motivation for methods based on *propensity scores* (Biostat 215)

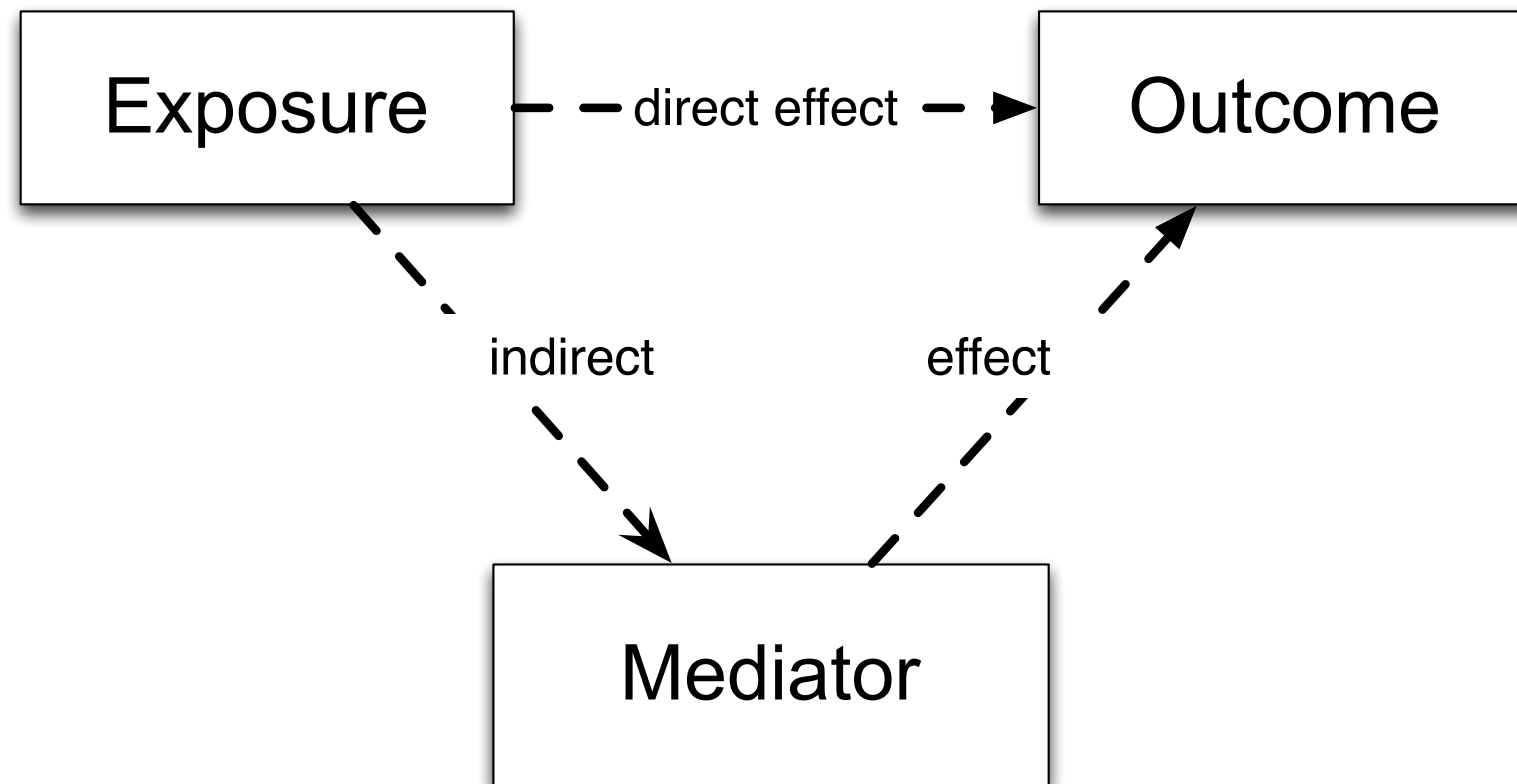
Confounding: summary

- Ideally, regression controls for confounding by jointly modeling effects of exposure and confounders
- Unbiased estimation of causal effects depends on getting the model right
- Bigger samples don't reduce un-modeled confounding, but do facilitate correct modeling of well-measured confounders
- Residual confounding is difficult to rule out

Mediation

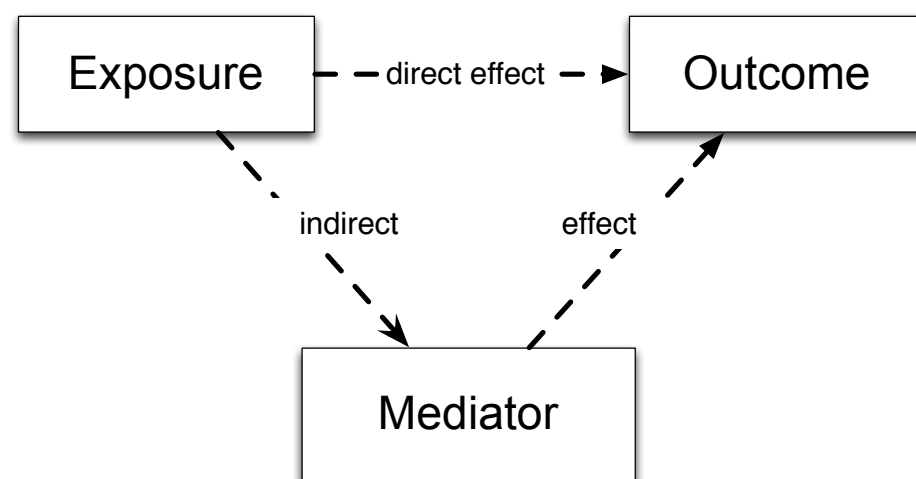
- *Confounder*: a common cause of both exposure and outcome (or a surrogate for a common cause)
- *Mediator*: on causal pathway from predictor to outcome
- In regression models, mediation and confounding behave alike
 - must be distinguished on substantive grounds
- Goal in mediation analysis: estimate *indirect* effect of exposure via mediator, *direct* effect via other pathways
- Examples of mediation
 - direct effect of cigarette smoking on coronary artery disease not mediated by blood pressure
 - mediation of the effect of treatment with bisphosphonates on fracture risk by changes in bone mineral density
 - mediation of effects of smoking on infant mortality by low birth weight
 - mediation of effects of assignment to a barrier contraceptive method (diaphragms) on HIV infection risk by condom use

Mediation DAG



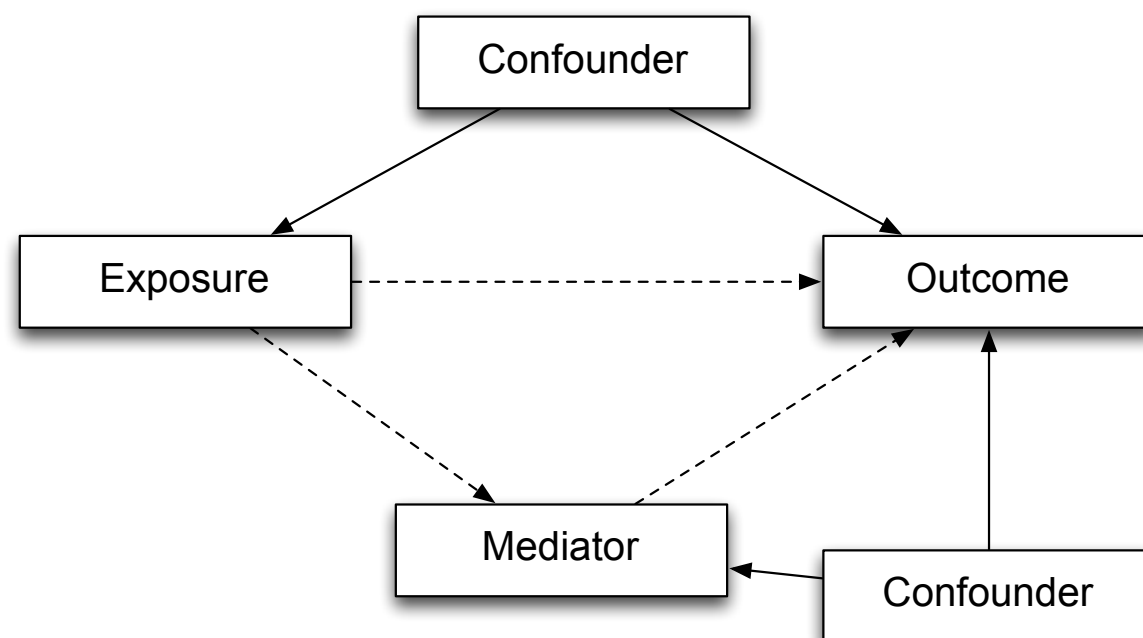
Examining mediation

- Use a series of regression models to figure out whether:
 - exposure has indirect effect via mediator
 - * exposure independently affects mediator
 - * mediator affects outcome independently of exposure
 - exposure has a direct effect via other pathways
 - what proportion of overall effect of exposure is indirect



Examining mediation

- Three regression models:
 1. *dependence of mediator on exposure and confounders*
 2. *dependence of outcome on exposure and confounders*
 3. *dependence of outcome on exposure, confounders, and mediator*
- *Note: Models 1-3 should be estimated using the same set of observations (i.e., with complete – possibly imputed – data on outcome, exposure, mediator, and confounders)*



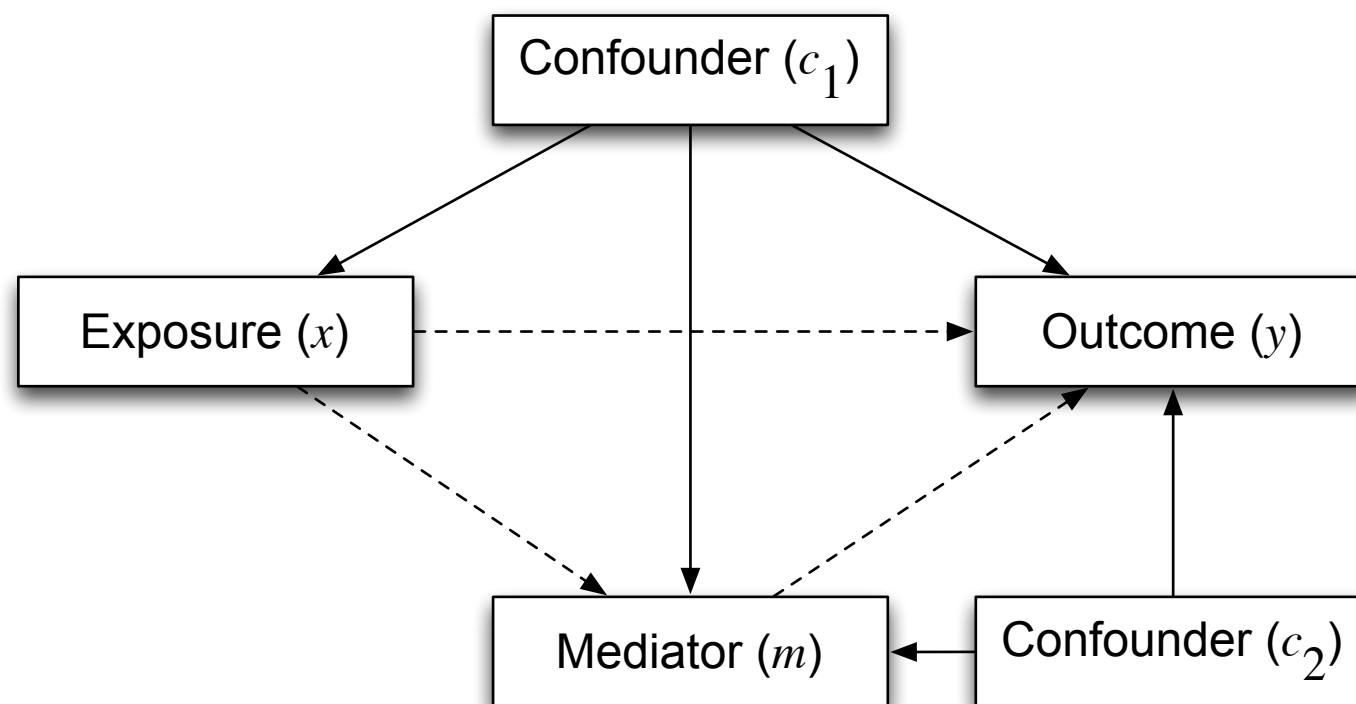
Examining mediation

- Three models verifying mediation

1. $E[m|x, c_1, c_2] = \alpha_0 + \alpha_1 x + \alpha_2 c_1 + \alpha_3 c_2$

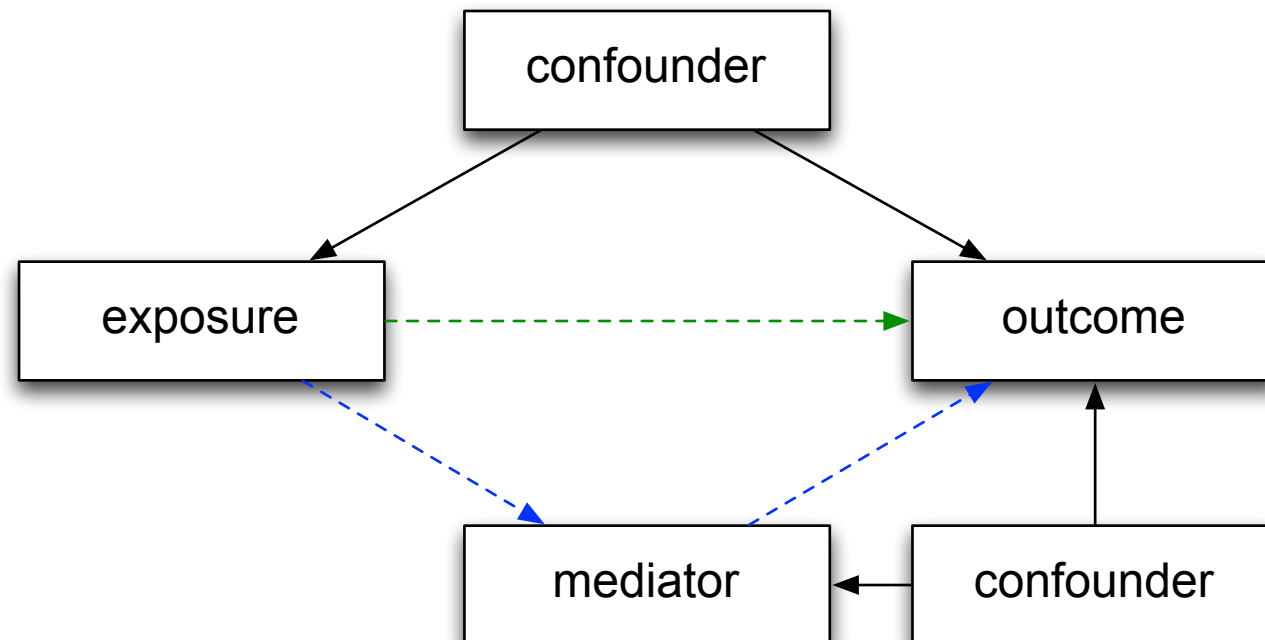
2. $E[y|x, c_1] = \gamma_0 + \beta_{overall}x + \gamma_1 c_1$

$$E[y|x, m, c_1, c_2] = \beta_0 + \beta_{direct}x + \beta_2 m + \beta_3 c_1 + \beta_4 c_2$$



Overall and direct effect estimates

- Model 2: *overall effect* (dashed lines) of exposure on outcome, including effect via mediator
- Model 3: *direct effect* of exposure on outcome, via other pathways not involving mediator

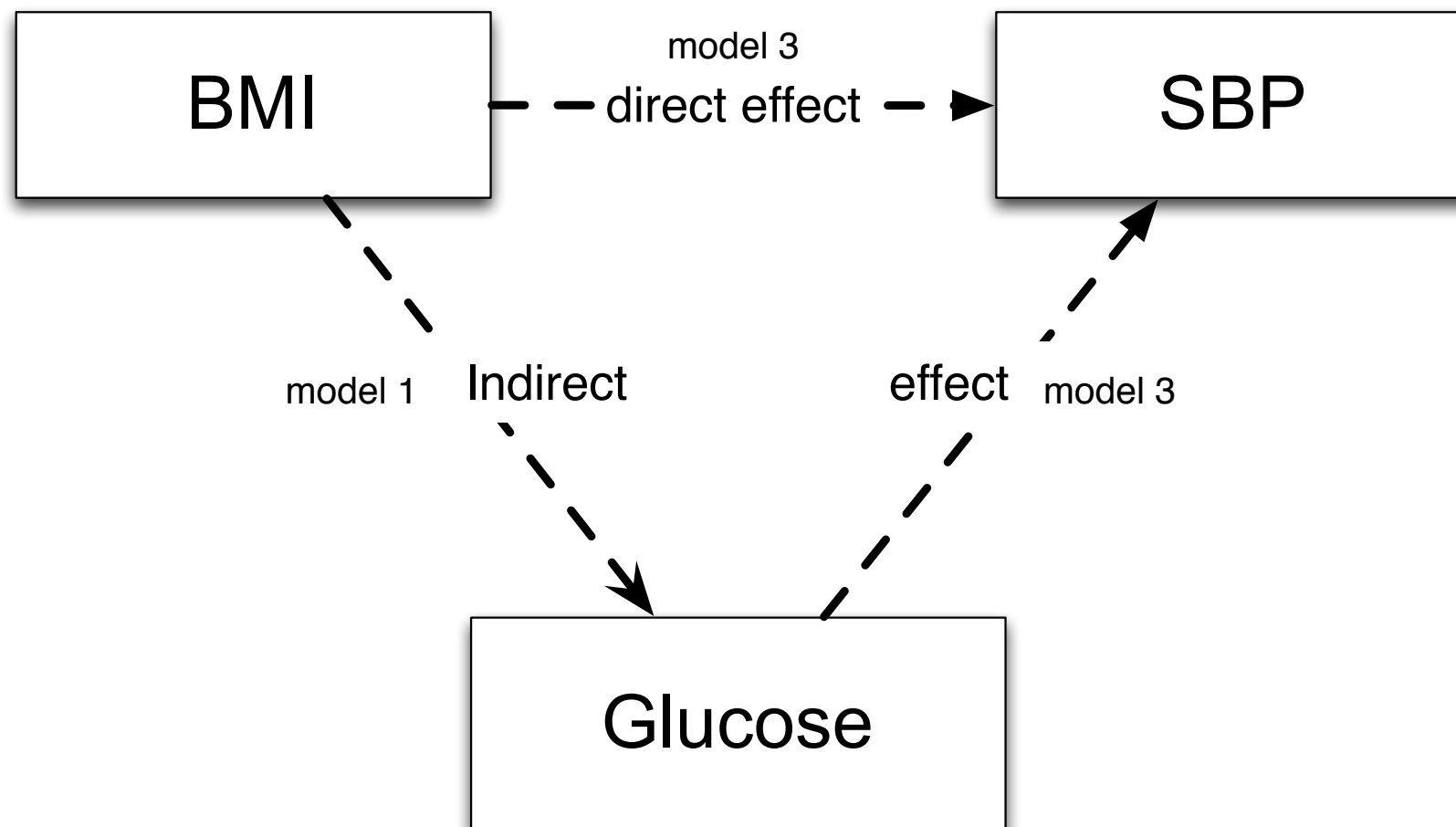
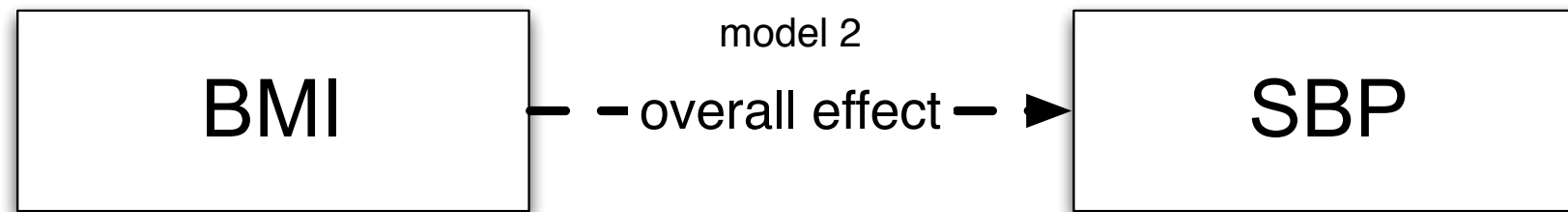


- *indirect effect* of exposure on outcome estimated from models 1 & 2 (i.e. estimates the degree to which exposure is mediated)

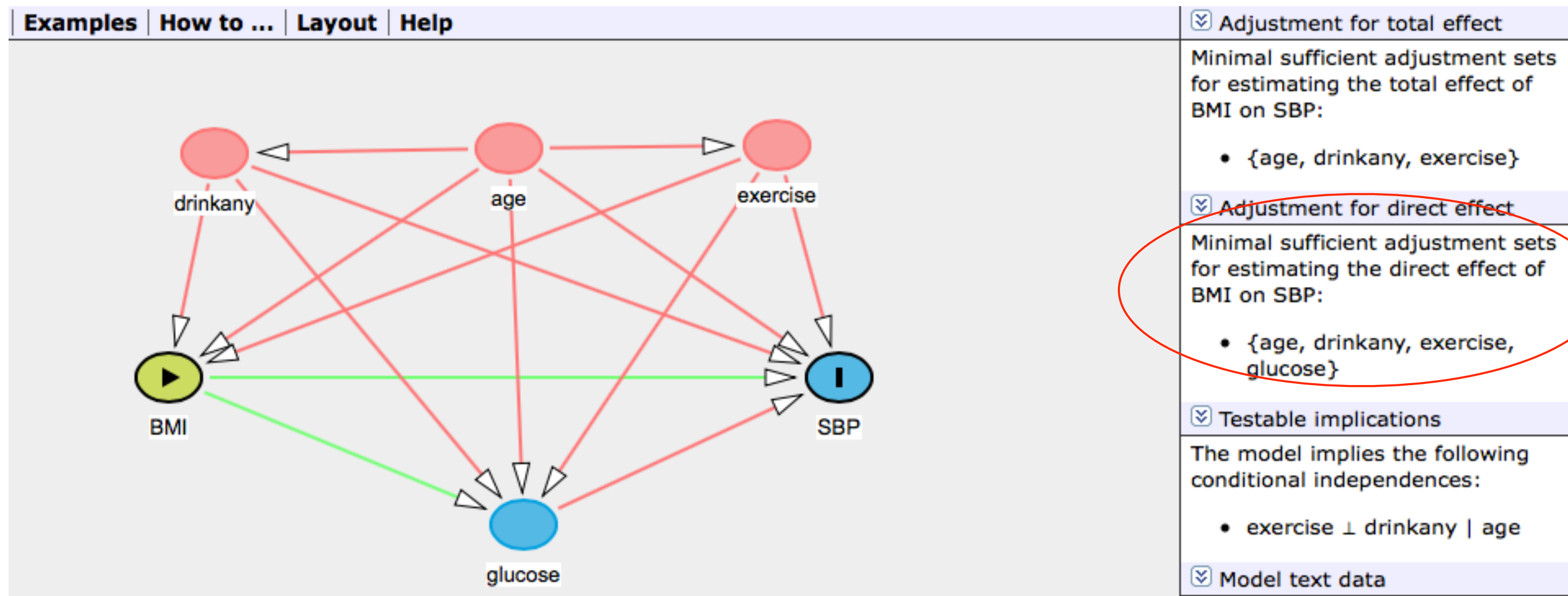
Indirect effect estimates

- Mediator, outcome both continuous (i.e. linear regression model applies): we can use
 - exposure effect in Model 1 $\times$ mediator effect in Model 3 –
 - difference of exposure effects in Model 2 and Model 3
- Mediator and/or outcome is binary
 - use downloadable mediation package `medeff`
- Alternatively: quantify mediation by *proportion explained (PE)*, defined as $(\beta_{\text{overall}} - \beta_{\text{direct}}) / \beta_{\text{overall}}$

Mediation of BMI → SBP by glucose



Mediation of BMI → SBP by glucose



Model 1: BMI predicts higher glucose

* **model 1**

. regress glucose BMI age exercise drinkany

Source	SS	df	MS
Model	321116.455	4	80279.1137
Residual	3426880.46	2751	1245.68537
Total	3747996.91	2755	1360.43445

Number of obs = 2756 F(4, 2751) = 64.45 Prob>F = 0.0000 R-squared = 0.0857 Adj R-squared = 0.0843 Root MSE = 35.294

glucose	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
BMI	1.6902	.1256441	13.45	0.000	1.443833	1.936566
age	-.2144159	.102671	-2.09	0.037	-.4157359	-.0130959
exercise	-2.774221	1.400946	-1.98	0.048	-5.521234	-.0272081
drinkany	-7.15434	1.385168	-5.16	0.000	-9.870414	-4.438266
_cons	82.01119	8.38539	9.78	0.000	65.5689	98.45349

* save for calculating indirect effect

. scalar link1 = _b[bmi]

Interpretation: each unit increase in BMI results in an increase of 1.7 mg/dL (95% CI 1.4-1.9, $p < 0.0005$) in average glucose, assuming the model includes an MSAS for this relationship

Model 2: overall effect of BMI on SBP

* **model 2**

. regress SBP BMI age exercise drinkany

Source	SS	df	MS	Number of obs	=	2756
Model	42202.7194	4	10550.6799	F(4, 2751)	=	30.40
Residual	954711.522	2751	347.041629	Prob > F	=	0.0000
				R-squared	=	0.0423
				Adj R-squared	=	0.0409
Total	996914.241	2755	361.856349	Root MSE	=	18.629

SBP	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
BMI	.245644	.0663176	3.70	0.000	.1156067	.3756813
age	.4933303	.0541919	9.10	0.000	.3870694	.5995913
exercise	-2.911396	.7394488	-3.94	0.000	-4.361327	-1.461465
drinkany	-2.022715	.7311205	-2.77	0.006	-3.456316	-.5891144
_cons	97.07514	4.425984	21.93	0.000	88.39656	105.7537

* save for calculating PE

. scalar b_overall = _b[BMI] // for use in calculating PE

Interpretation: the overall effect of a 1-unit increase in BMI is to increase average SBP by 0.25 mmHg (95% CI 0.12-0.38, $p < 0.0005$), again assuming an MSAS

Model 3: direct BMI effect via other pathways

* **model 3**

. **regress SBP BMI age exercise drinkany glucose**

Source	SS	df	MS
Model	50555.9327	5	10111.1865
Residual	946358.308	2750	344.130294
Total	996914.241	2755	361.856349

Number of obs = 2756 F(5, 2750) = 29.38 Prob>F = 0.0000 R-squared = 0.0507 Adj R-squared = 0.0490 Root MSE = 18.551

SBP	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
BMI	.1621961	.0681763	2.38	0.017	.0285141	.2958781
age	.5039164	.0540069	9.33	0.000	.3980183	.6098145
exercise	-2.774428	.7368653	-3.77	0.000	-4.219294	-1.329563
drinkany	-1.669494	.7315689	-2.28	0.023	-3.103974	-.2350138
glucose	.0493716	.1002102	4.93	0.000	.2972212	.690211
_cons	93.02612	4.483349	20.75	0.000	84.23505	101.8172

* save for calculating indirect effect

. **scalar link2 = _b[glucose]**

* save for calculating PE

. **scalar b_direct = _b[BMI]**

Interpretation: the direct effect of a 1-unit increase in BMI is to increase average SBP by 0.16 mmHg (95% CI 0.03-0.30, $p = 0.017$), again assuming model includes an MSAS.

Estimates of indirect effect

* two ways of calculating indirect effect

```
. display link1*link2
```

```
.08344789
```

```
. display b_overall-b_direct
```

```
.08344789
```

* PE

```
. display round((b_overall - b_direct) / b_overall * 100)
```

```
34
```

Interpretation: the indirect effect of a 1-unit increase in BMI via glucose is to increase average SBP by **0.08 mmHg**, assuming an MSAS (note: this only works when mediator, outcome continuous). **34%** of the overall effect of BMI on SBP is mediated by its effect on glucose levels

Mediation of BMI by glucose levels

- BMI independently predicts glucose (Model 1); glucose independently predicts SBP (Model 3)
- BMI effects:
 - Overall: 0.25 mmHg per kg/m² (Model 2)
 - Direct: 0.16 mmHg per kg/m² (Model 3)
 - Indirect: 0.08 mmHg per kg/m² (Models 1 and 3)
- PE: glucose explains $(.25-.16)/.25*100 = 34\%$ of BMI

Why test for mediation?

- We don't test for confounding, but need to validate indirect pathway
- Comparing overall and direct effect estimates from Models 2 and 3 is hard (they are correlated), can be unreliable
- Testing both links in indirect pathway more reliable, powerful:
 - i.e., test exposure effect in Model 1, mediator effect in Model 3
 - both must be statistically significant (avoiding multiple comparison problems)

Assessing evidence for indirect pathway

```
. * Model 1
```

```
. regress glucose BMI age exercise drinkany
```

```
-----+-----  
      glucose |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]  
-----+-----  
          BMI |      1.6902    .1256441    13.45   0.000     1.443833     1.936566  
      ...
```

```
. * Model 3
```

```
. regress SBP BMI age exercise drinkany glucose
```

```
-----+-----  
          SBP |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]  
-----+-----  
 glucose10 |      .4937161   .1002102     4.93   0.000     .2972212     .690211  
      ...
```

Interpretation: both links in the indirect pathway are strongly supported.

Note: no multiple testing issue because both links must be significant

Bootstrap Confidence Interval for PE

- Apply bootstrap re-sampling using a simple Stata program to estimate PE
- Use ≥ 500 bootstrap samples and bias-corrected 95% CI

```
. capture program drop mediate
. program define mediate, rclass
    1. syntax varlist, outcome(varlist) mediator(varlist fv) covars(varlist fv)
    2. reg `outcome' `varlist' `covars'
    3. scalar b_overall = _b[`varlist']
    4. reg `outcome' `varlist' `covars' `mediator'
    5. scalar b_direct = _b[`varlist']
    6. return scalar pe = (b_overall - b_direct) / b_overall * 100
    7. end

. bootstrap pe=r(pe), reps(1000) notable nodots: mediate BMI, outcome(SBP)
mediator(glucose) covars(age exercise drinkany)
```

Bootstrap results	Number of obs	=	2756
	Replications	=	1000

```
command: mediate BMI, outcome(SBP) mediator(glucose) covars(age exercise drinkany)
pe:      r(pe)
```


Bootstrap Confidence Interval for PE

- Apply bootstrap re-sampling using a simple Stata program to produce the estimated PE
- Use ≥ 500 bootstrap samples and bias-corrected 95% CI

```
. * get bias corrected bootstrap CI using the estat command
```

```
. estat bootstrap, all
```

```
Bootstrap results                                Number of obs      =       2756
                                                Replications        =       1000
```

```
command: mediate BMI, outcome(SBP) mediator(glucose) covars(age exercise drinkany)
pe:      r(pe)
```

	Observed		Bootstrap			
	Coef.	Bias	Std. Err.	[95% Conf. Interval]		
pe	33.971067	2.727515	20.677067	-6.555239	74.49737	(N)
				17.10639	75.33008	(P)
				17.31348	76.9211	(BC)

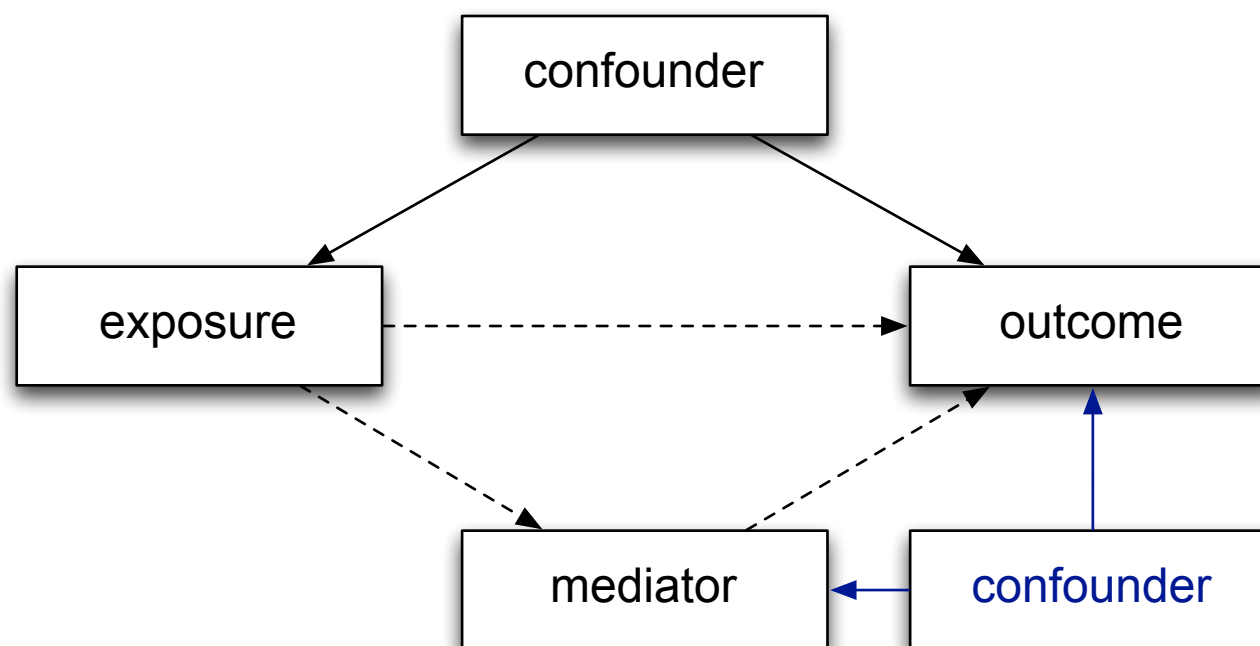
```
(N)    normal confidence interval
```

```
(P)    percentile confidence interval
```

```
(BC)   bias-corrected confidence interval
```

Confounding of mediator/outcome relationship

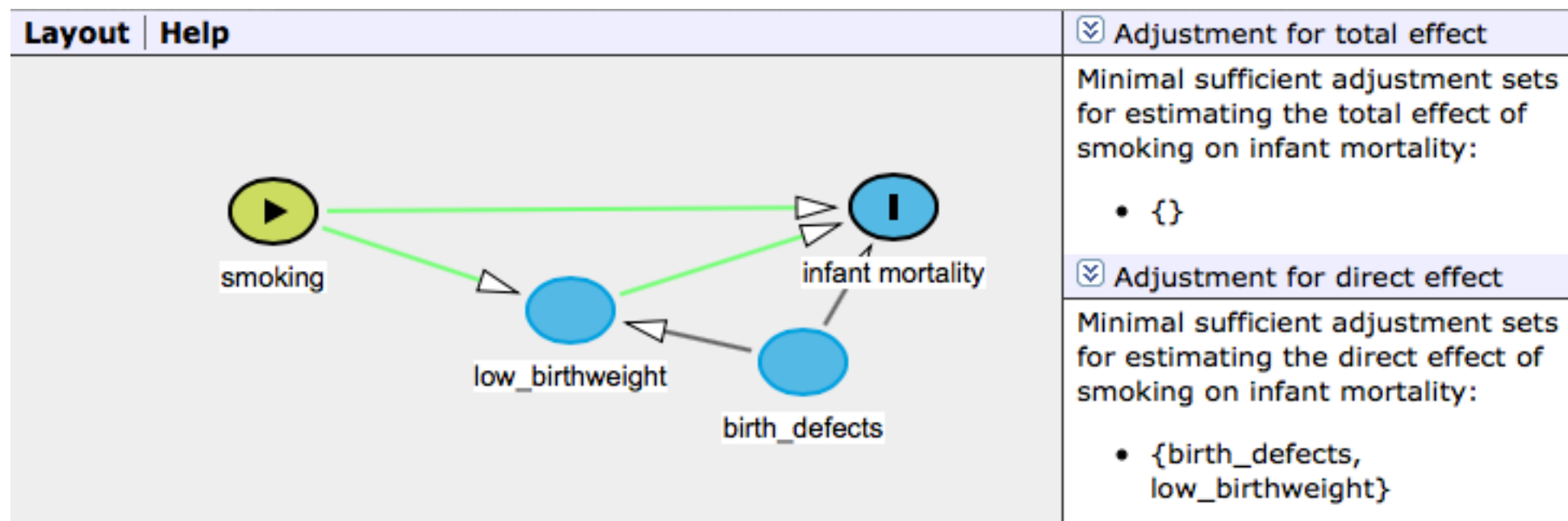
- Failure to control for confounding of **mediator - outcome relationship** can lead to biased estimates of mediation effects¹
 - In the presence of such confounding, the mediator is a *collider* on front door path from exposure to outcome



1. Cole SR, Hernán MA. Fallibility in estimating direct effects, Int J Epid, 2002;31:163-5

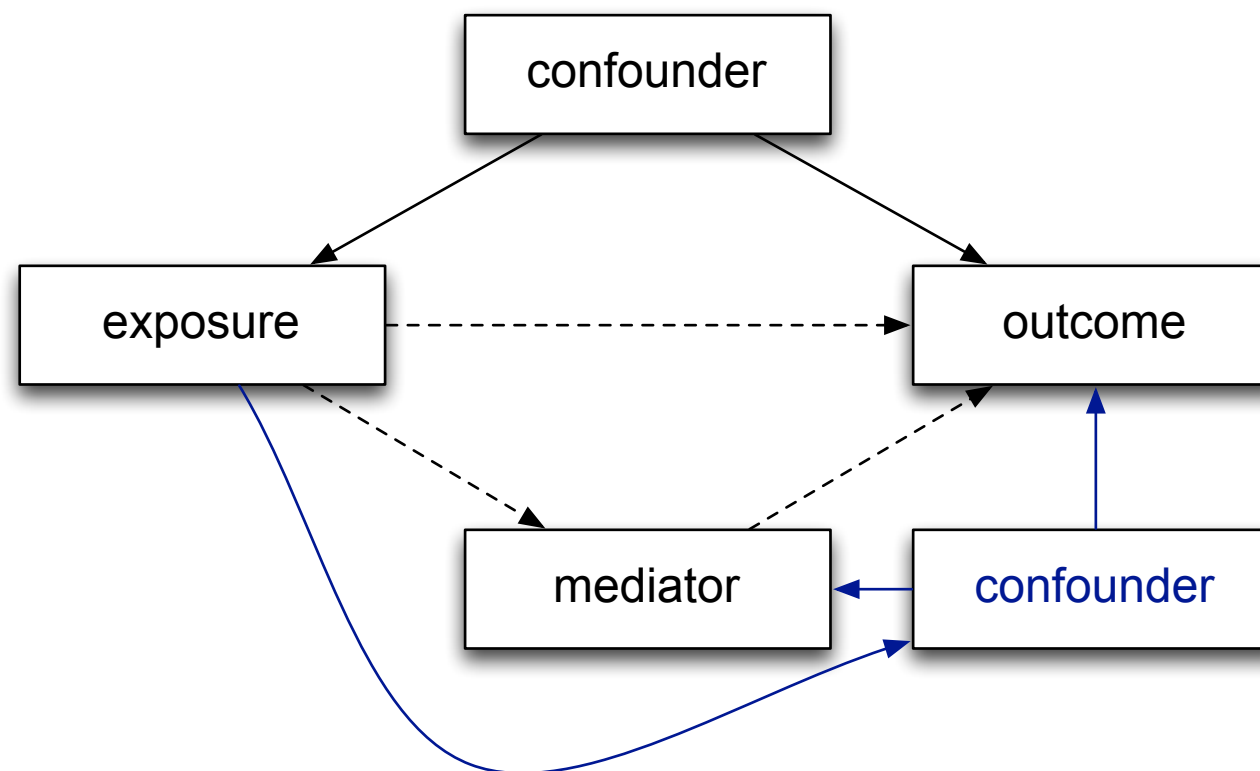
Confounding of mediator/outcome relationship

- Example: low birth weight as mediator of effects of smoking on infant mortality



Confounder/mediators of mediator/outcome relationship

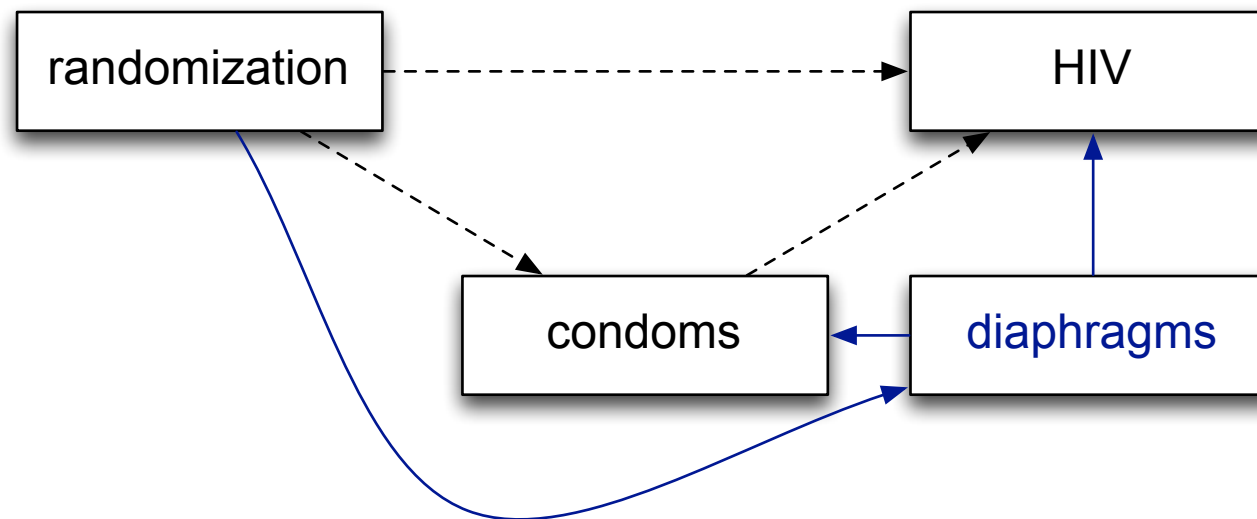
- If a confounder of mediator/outcome relationship is **also a mediator of exposure effects**, regression adjustment fails to yield unbiased estimates (special methods using inverse weighting needed) ²
 - In this case, regression adjustment for the confounder/mediator removes some of the effect of exposure



2. VanderWeele TJ. Marginal structural models for direct and indirect effects, Epidemiol, 2009;20(1): 18-26.

Confounder/mediators of mediator/outcome relationship

- MIRA study of diaphragms to prevent HIV infection in African women
 - randomization to either diaphragms+ condoms / condoms



- Comparison of outcomes by randomized assignment to the intervention (diaphragms) provides a valid estimate causal effect (none was observed)
- Possible between-group differences in post-randomization condom use
 - *Direct effect of randomization not mediated through condoms of interest*
 - Diaphragm use as a possible confounder-mediator

Additional issues with mediation analyses

- Potentially stronger conclusions about mediation possible with longitudinal data
 - Exposure affects mediator, mediator subsequently affects outcome. Example: Alendronate → BMD → fracture risk
 - However, effects may be effectively simultaneous: illness, frailty → depression → decreased cognitive function
- More complicated methods needed if
 - exposure and mediator interact; Stata package `medeff` needed
 - time-dependent covariates acting as confounder-mediators: *marginal structural models*
- Estimates of proportion explained (*PE*)
 - not really a proportion, complicated to estimate for nonlinear models (logistic, proportional hazards)

Mediation Summary

- Mediation distinguished from confounding on substantive grounds
- 3 models used to estimate overall, direct, indirect effects (and PE)
- Models must include confounders of mediator/outcome
- Estimating indirect effects often requires special methods