

Confounding & Mediation

January 28, 2020

Biostatistics 208

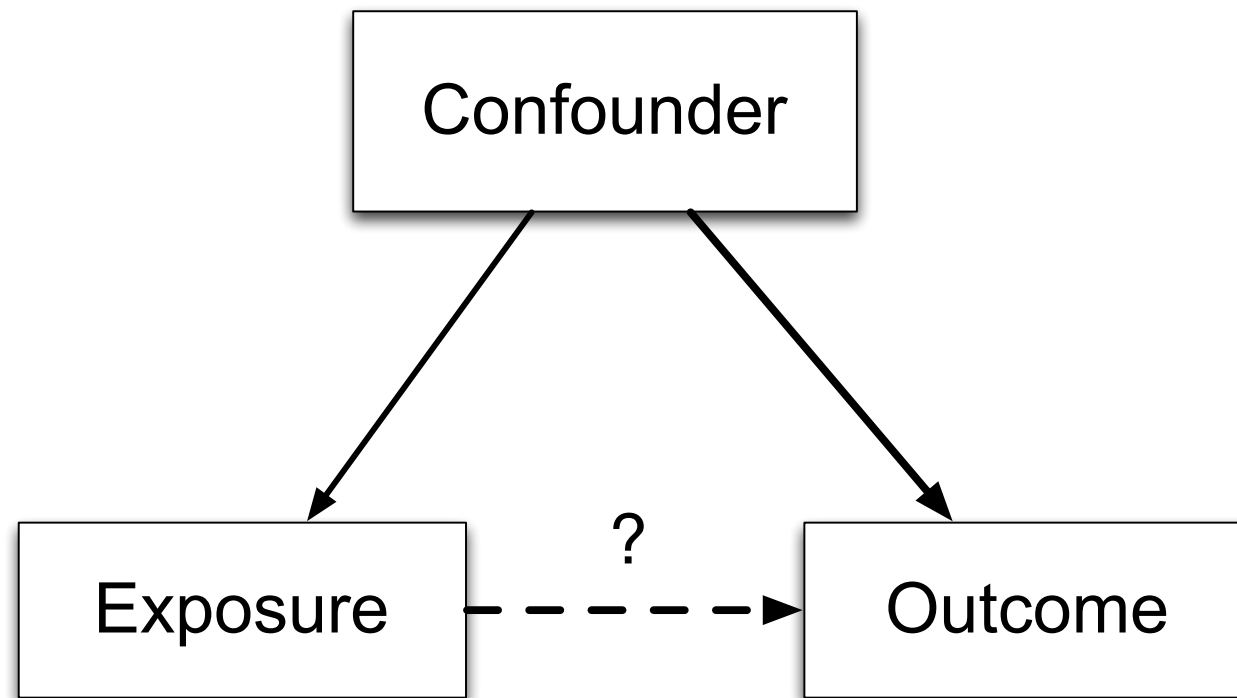
Confounding & Mediation

- **Context:** use regression to investigate a predictor of primary interest
- **Goal:** estimate (possibly causal) association between predictor & outcome
 - primary predictor often referred to as *exposure/treatment/intervention*
- Association is typically assessed via estimated coefficients for concerned predictors
- **Approach:**
 - identify potentially important causal relationships (e.g. via a DAG)
 - estimate association of interest controlling for possible *confounding*
 - if applicable, assessment of *mediation* and *interaction* effects

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
- Possible control strategies include
 - randomization, restriction, matching, instrumental variables and adjustment/stratification
- Our focus: *adjust for confounding effects using regression models*
 - similar to stratification methods for frequency tables, but allows adjustment for multiple confounders (continuous and/or categorical)
 - how to adjust adequately
 - when it will work
- Alternative analytic techniques include propensity score adjustment/matching, inverse probability weighting, marginal structural models, targeted maximum likelihood, ... (Biostatistics 215)
- **Note**: *we don't use statistical tests to detect confounding* - possible bias from confounding must be addressed in study design, analyses & conclusions whether or not relevant associations achieve statistical significance

Prototype DAG (directed acyclic graph) for a single confounder:



Review: Potential outcomes and causal effects

- Suppose we could observe outcomes for each individual under both exposure *and* no exposure, holding everything else constant
- For an outcome y & exposure e , these potential (counterfactual) outcomes are denoted

$$y(e = 1), \quad y(e = 0)$$

- Within-person differences between potential outcomes represent *individual causal effects*
 - by definition, these are free of confounding influences
 - *Problem*: in general, only one of the potential outcomes is observable on an individual
- Overall causal effect can be summarized by a marginal average causal effect (ACE):

$$ACE = E[y(e = 1)] - E[y(e = 0)]$$

- Two common approaches to estimating ACE : randomization, and statistical modeling (e.g. regression, propensity scores, etc.)

Causal effects, RCTs, and linear regression modeling

- Randomized controlled trials (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 study data potentially achieved by accurately *modeling* all confounding effects
 - *within modeled strata*, exposed, unexposed assumed exchangeable
- Adjusted estimate unbiased for causal effect (*ACE*) if all necessary confounders (i.e., *minimum sufficient adjustment set*, or *MSAS*) included and *accurately modeled*
 - Recall from lecture and lab 2, adjusted regression coefficient for a predictor of primary interest in a linear regression model has both a marginal and conditional interpretation (not necess. true for nonlinear models like logistic reg.)

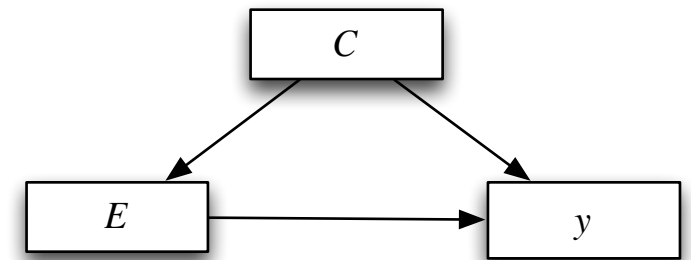
Why regression adjustment works to remove confounding

- 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, β_1 & $\beta_2 > 0$ and E, C are positively correlated
- Because E and C are correlated, model must include C to estimate β_1 (the effect of exposure) without bias

Simulation code

- Example simulation in Stata - follows the hypothesized DAG:

- generate a binary confounder C
- generate a continuous exposure E , posit. correlated with C
- generate a continuous outcome $y = \beta_0 + \beta_1 E + \beta_2 C + \varepsilon$
- assume $\beta_2 > 0$ (i.e. Y, C are positively associated)



```
program define regsim
drop _all
set obs 50
* generate a binary confounder with equal numbers in each group
gen confounder = 0
replace confounder = 1 if _n<=25
* generate exposure
gen exposure = 62 + 7*confounder + rnormal(0,4)
* generate an error variable for the outcome (mean 0, SD 30)
gen ro = rnormal(0,30)
* generate the outcome as a function of both exposure & confounder
gen outcome = -25 + exposure*2.5 + confounder*30 + ro
end
```


Why adjustment works to remove confounding

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

Positive assoc. between E & C

```
. 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

Positive assoc. between C & Y

```
. 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

Positive assoc. between E & Y

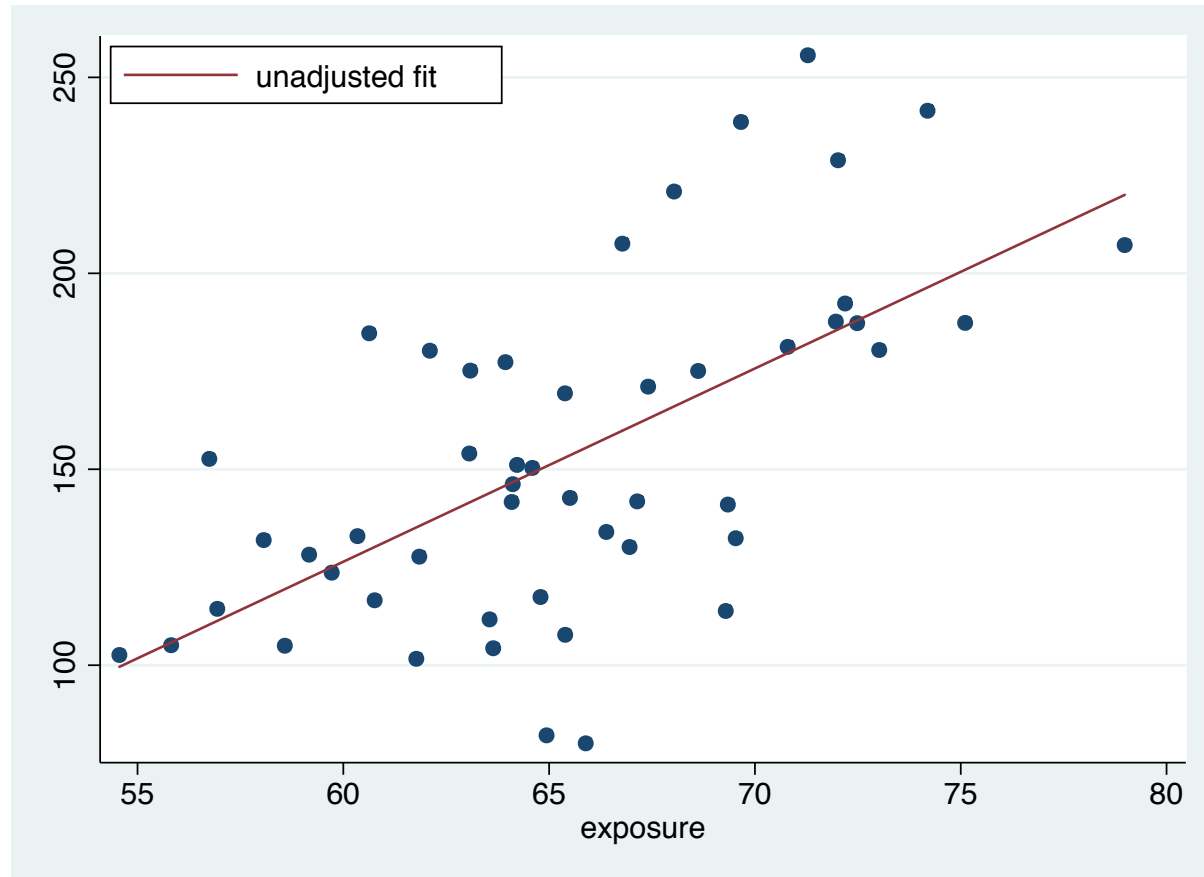
```
. corr exposure outcome
```

(obs=50)

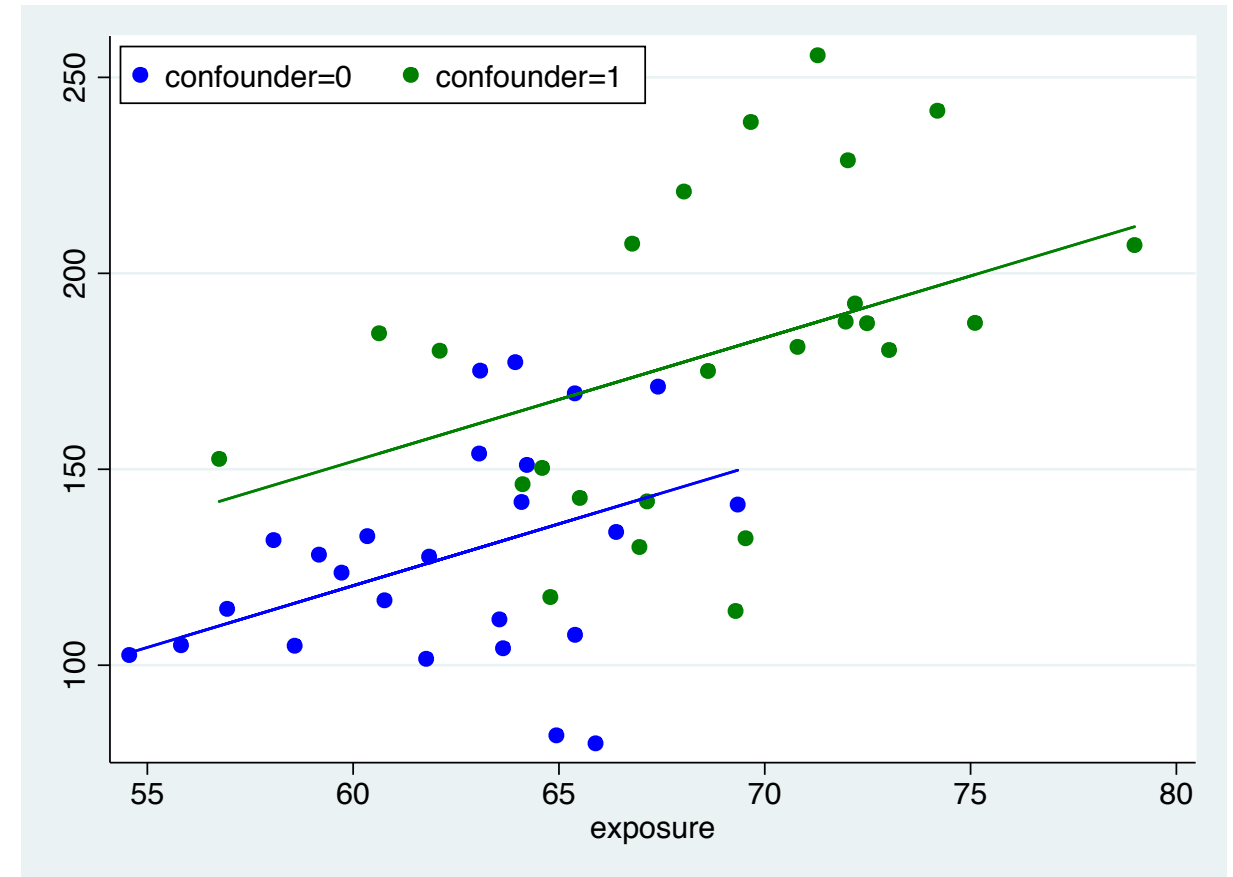
	exposure	outcome
exposure	1.0000	
outcome	0.6211	1.0000

Why adjustment works to remove confounding

Unadjusted



Adjusted



$$\text{Model : } E[y | E, C] = \beta_0 + \beta_1 E + \beta_2 C$$

Why adjustment works to remove confounding

- Unadjusted effect of E partly reflects increasing prevalence of C with increasing exposure
- 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

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

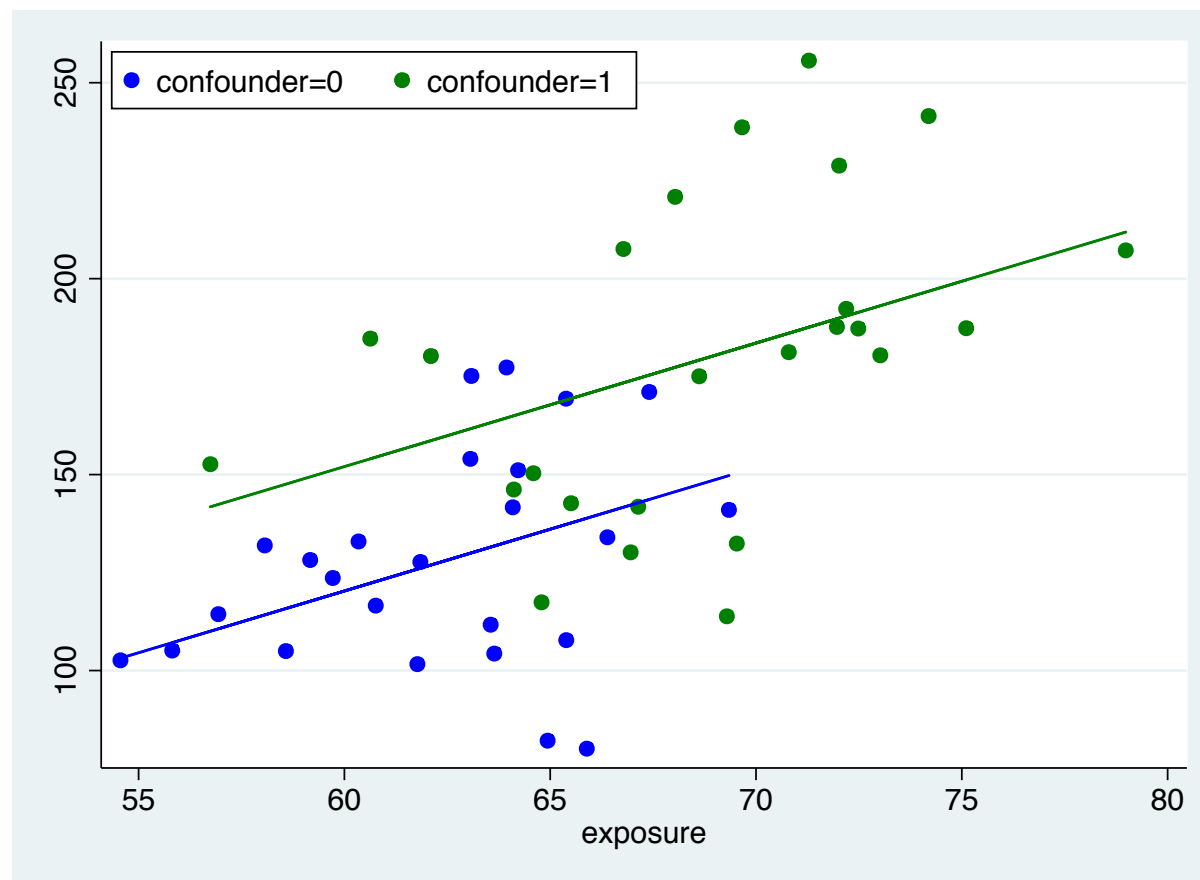
Interpretation: after we model the effect of the confounder, the estimated effect of exposure on the outcome is unbiased (and attenuated from the unadjusted effect), and the 95% CI includes the true value of 2.5.

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 and appropriate modeled)
 - 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 week's topic: how to check and relax this assumption



Range of confounding patterns

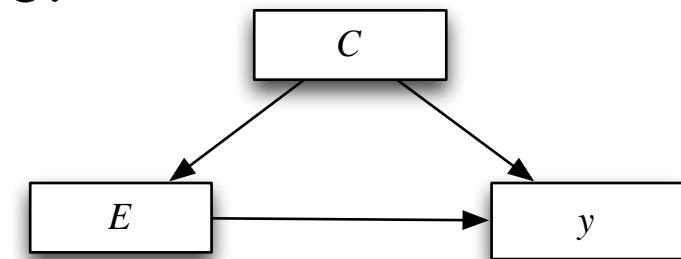
For an outcome y , binary exposure E & continuous confounder C :

Model for unadjusted effect: $E[y|E] = \alpha_0 + \alpha_1 E$

Unadjusted effect: $E[y|E = 1] - E[y|E = 0] = \alpha_1$

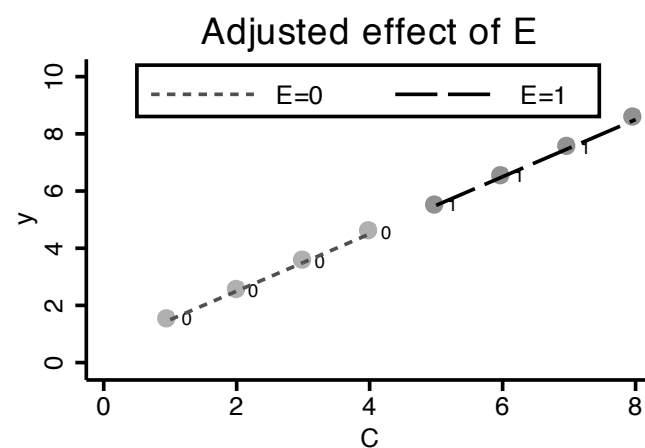
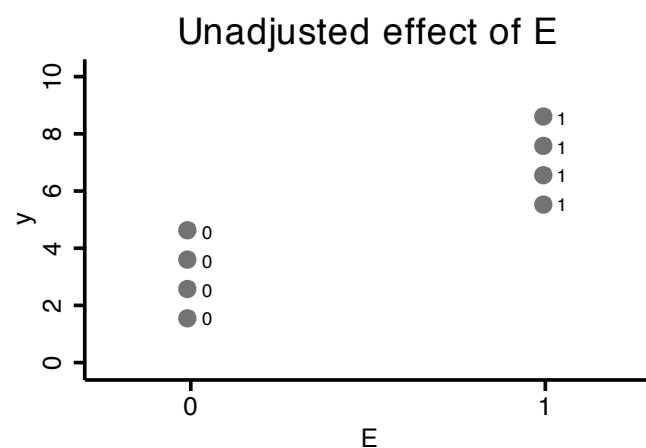
Model for adjusted effect: $E[y|E, C] = \beta_0 + \beta_1 E + \beta_2 C$

Adjusted effect: $E[y|E = 1, C = c] - E[y|E = 0, C = c] = \beta_1$



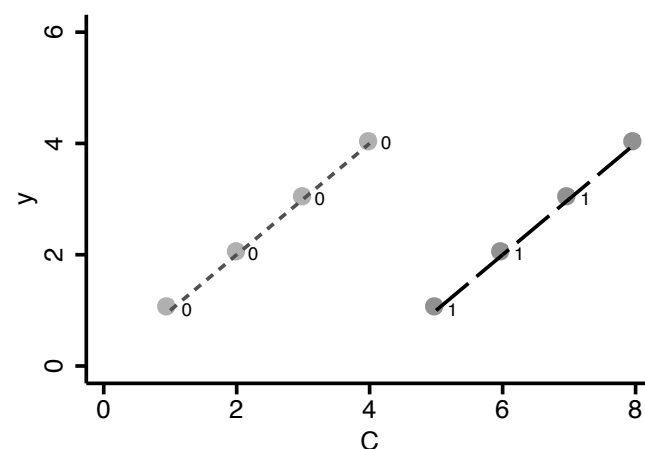
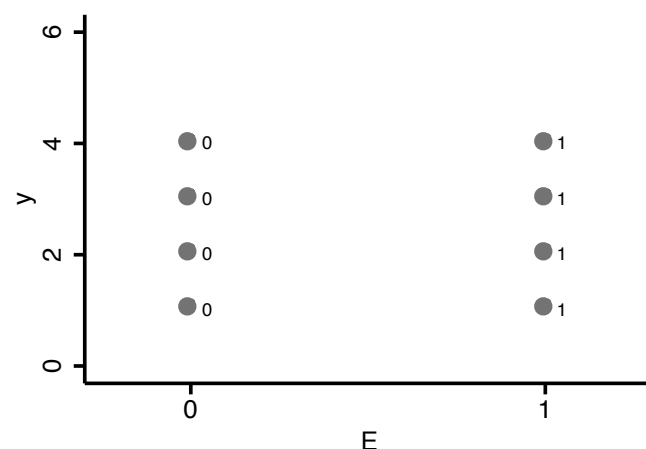
Possible confounding if : $\alpha_1 \neq \beta_1$

Examples :



A: positive confounding

$$\alpha_1 > 0; \beta_1 = 0$$



B: negative confounding

$$\alpha_1 = 0; \beta_1 < 0$$

Positive confounding: two scenarios

Positive confounding may arise between predictors that are

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

Negative confounding: two scenarios

Negative confounding may arise between predictors that are

1. *Positively correlated, with opposite effects on outcome*
2. *Inversely correlated, with similar effects on outcome*

Negative confounding

- Example: needlestick injuries
 - Post-exposure prophylaxis (PEP) with AZT not marginally 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

- Example: BMI decreases with age in HERS cohort (-0.13 kg/m^2 per year on average), but increases in both age & BMI are associated with higher systolic blood pressure (SBP)

Unadjusted association of BMI and SBP in HERS

```
. 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.

- BMI decreases with increasing age in this pop.
- BMI and age positiv. assoc. with increasing SBP

Summary: negative confounding

- Negative confounding can mask an independent association in unadjusted analysis
- Implications for predictor selection: univariate screening, automated forward selection techniques for choosing adjustment predictors may miss negative confounders
 - backwards deletion approaches preferred

Interpreting adjusted regression effect estimates as causal

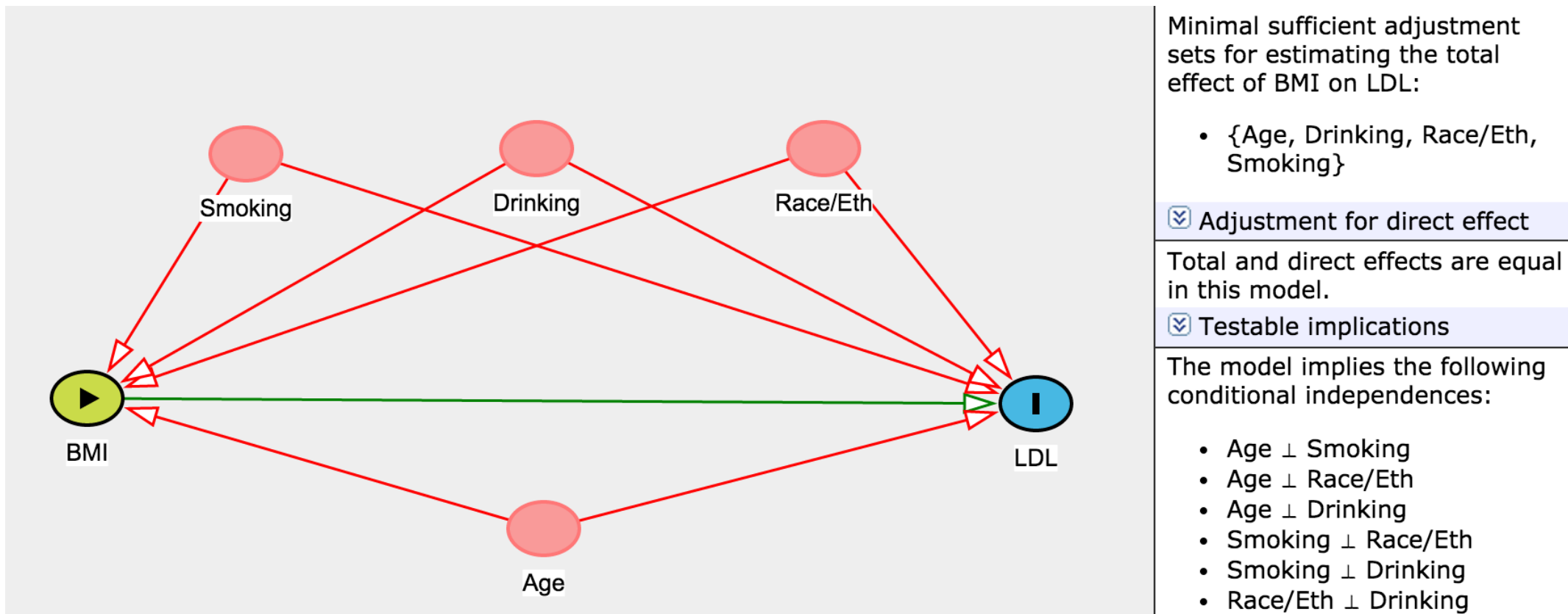
- Example: Association between BMI & LDL in HERS

- Unadjusted estimate:

```
. regress LDL BMI, noheader
```

LDL	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
BMI	.4151123	.1303648	3.18	0.001	.1594893	.6707353
_cons	133.1913	3.7939	35.11	0.000	125.7521	140.6305

- Goal: estimate adjusted association, accounting for possible confounders



Example: Association between BMI & LDL in HERS

- Adjusted estimate:

```
. 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.)

Example: Association between BMI & LDL in HERS

- If the following conditions hold
 - All confounders are accounted for
 - The model is correct, and confounder distributions are comparable at diff. BMI levels
- Adjusted regression estimate for effect of BMI based on the linear regression model provides an estimate of the average causal effect (ACE) on LDL
 - The mean change in LDL per unit increase in BMI, for fixed levels of (i.e. conditional on) the adjustment variables (output on previous slide)
 - The adjusted marginal mean change in LDL per unit increase in BMI:

```
. margins, dydx(BMI)
```

Average marginal effects

Model VCE : OLS

Expression : Linear prediction, predict()

dy/dx w.r.t. : BMI

Number of obs = 2745

		Delta-method				
		dy/dx	Std. Err.	t	P> t	[95% Conf. Interval]
BMI		.3591038	.1341047	2.68	0.007	.0961472 .6220605

– *marginal and conditional effects equal in linear model case*

Example: Association between BMI & LDL in HERS

- **Note:** focus in this example centers on BMI $\rightarrow$ LDL association
 - Conclusions about causal effects are limited to that association and attendant assumptions
 - Adjusted coefficients for other variables in the model (slide 24) do not necessarily share a causal interpretation

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
 - 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)

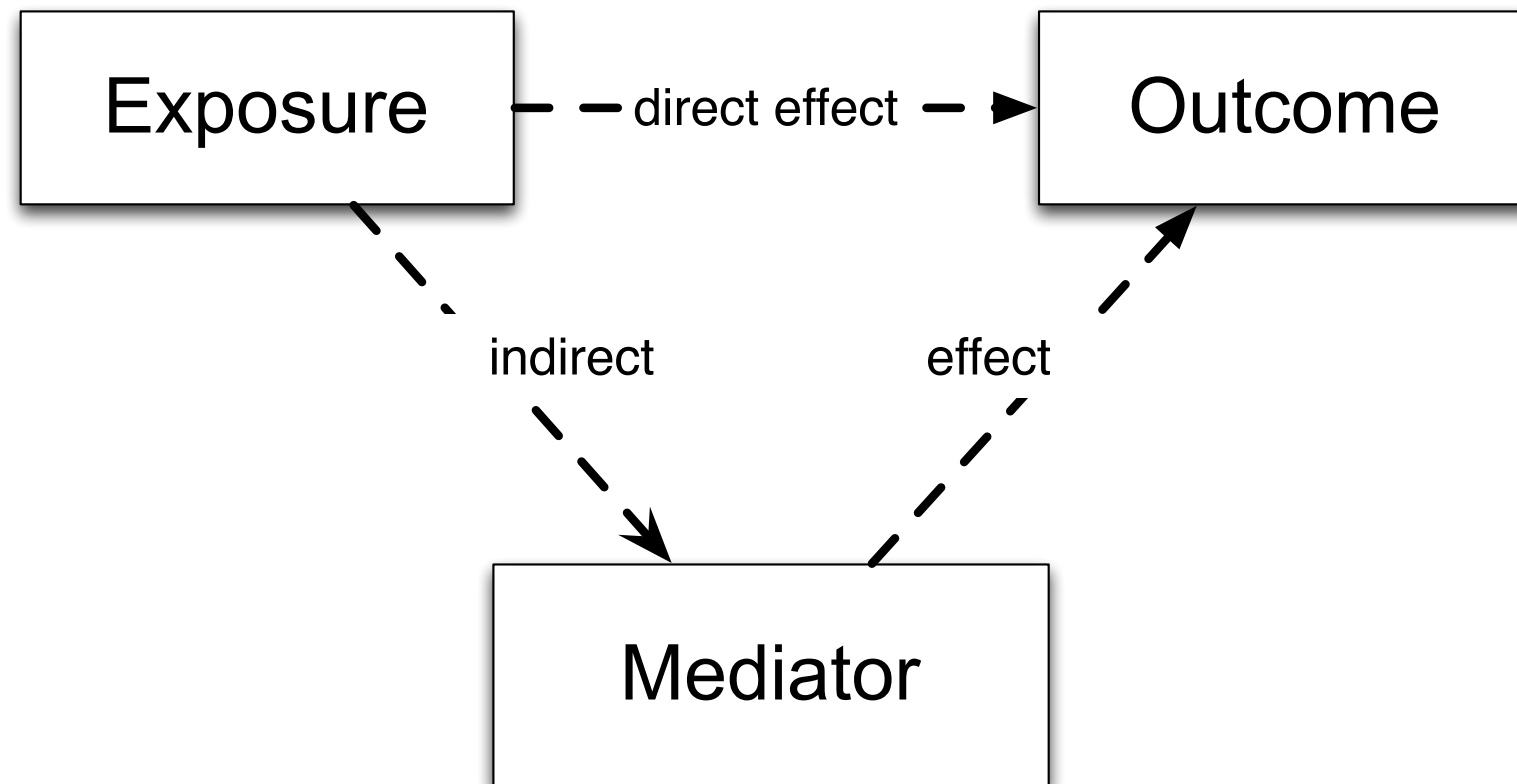
Confounding: summary

- 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

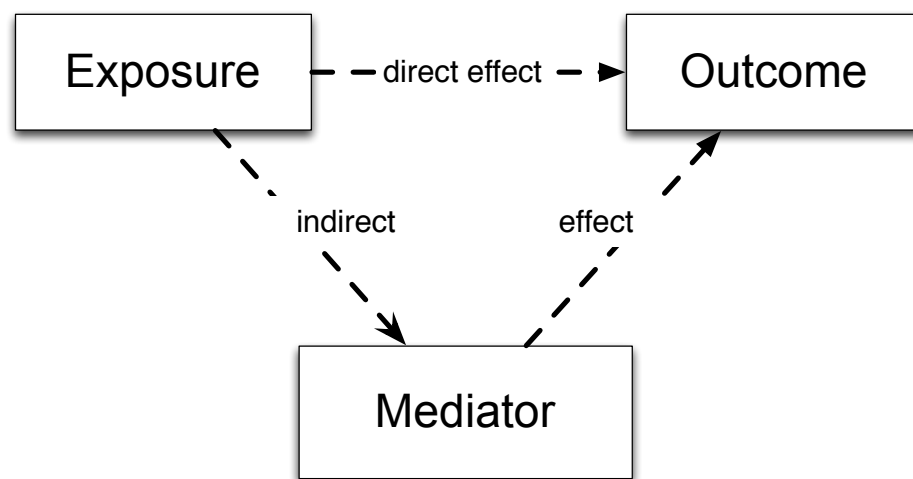
- *Confounder*: a common cause of both exposure and outcome (or a surrogate for a common cause)
- *Mediator*: on causal pathway from predictor to outcome
- In regression models, mediation and confounding behave alike
 - must be distinguished on substantive grounds
- Goal in mediation analysis: estimate *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



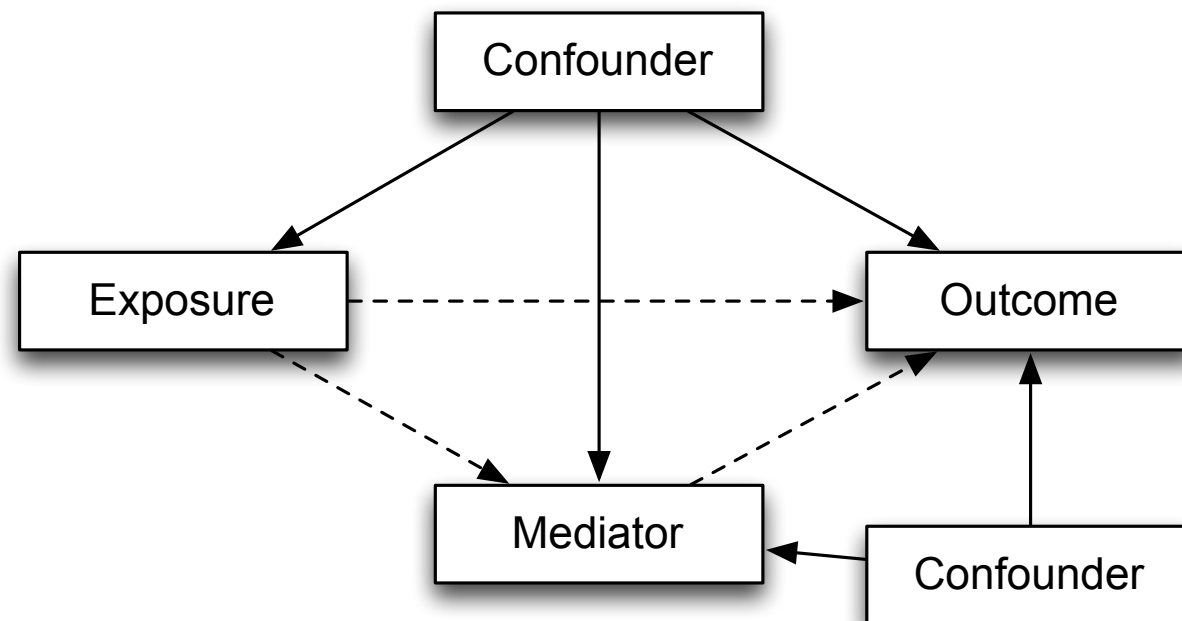
Examining mediation via linear regression

- 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

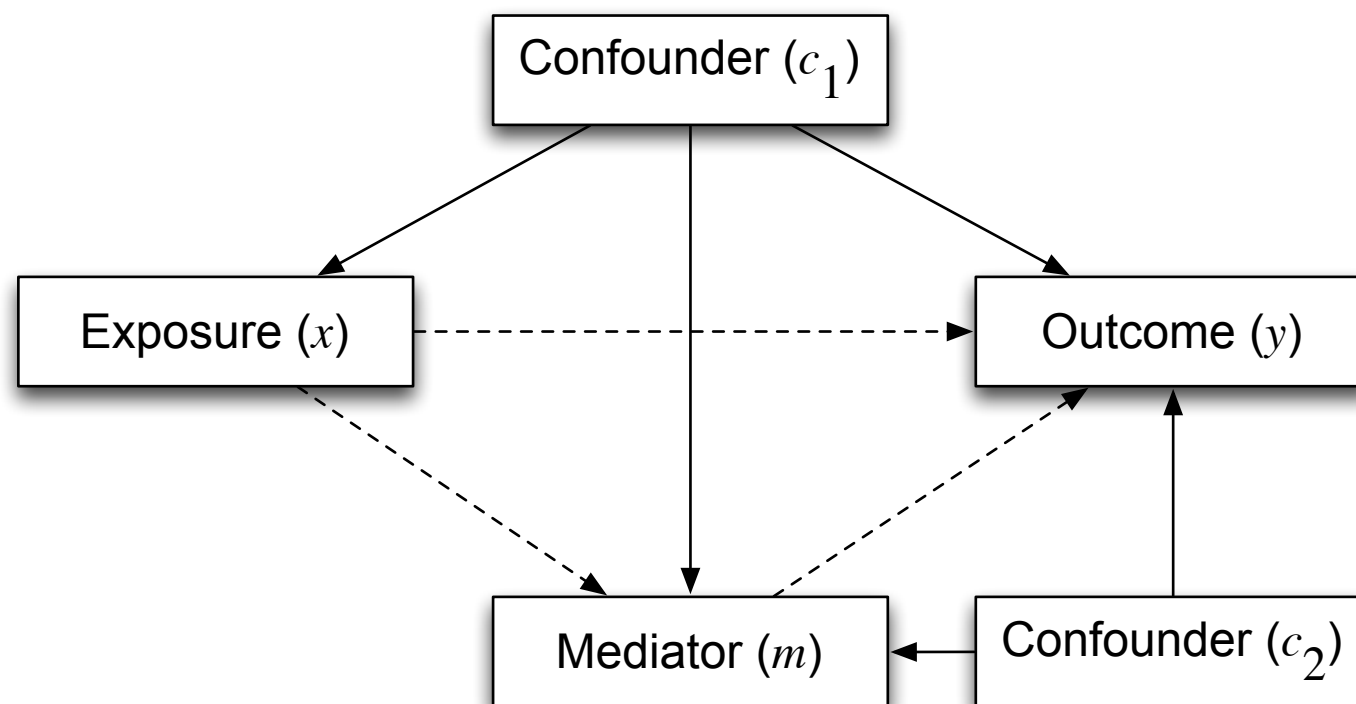
- Three regression models:
 1. *dependence of mediator on exposure and confounders*
 2. *dependence of outcome on exposure and confounders*
 3. *dependence of outcome on exposure, confounders, and mediator*
- *Note: Models 1-3 should be estimated using the same set of observations (i.e., with complete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



Examining mediation

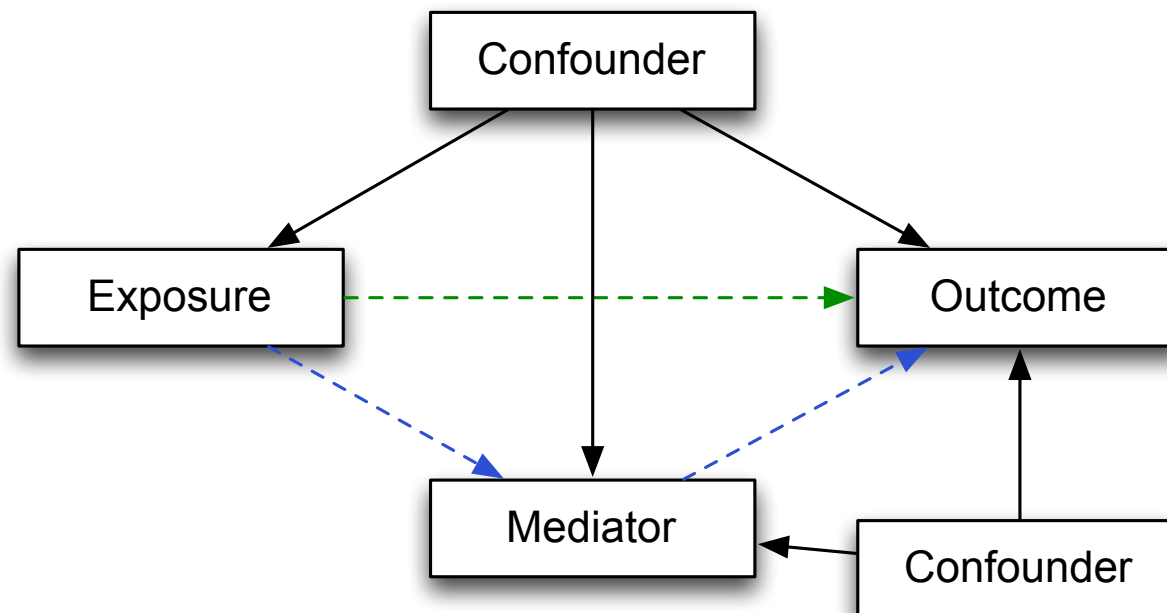
- Three models verifying mediation

1. $E[m|x, c_1] = \alpha_0 + \alpha_1 x + \alpha_2 c_1$
2. $E[y|x, c_1] = \gamma_0 + \beta_{overall}x + \gamma_1 c_1$
3. $E[y|x, m, c_1, c_2] = \beta_0 + \beta_{direct}x + \beta_2 m + \beta_3 c_1 + \beta_4 c_2$



Overall and direct effect estimates

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



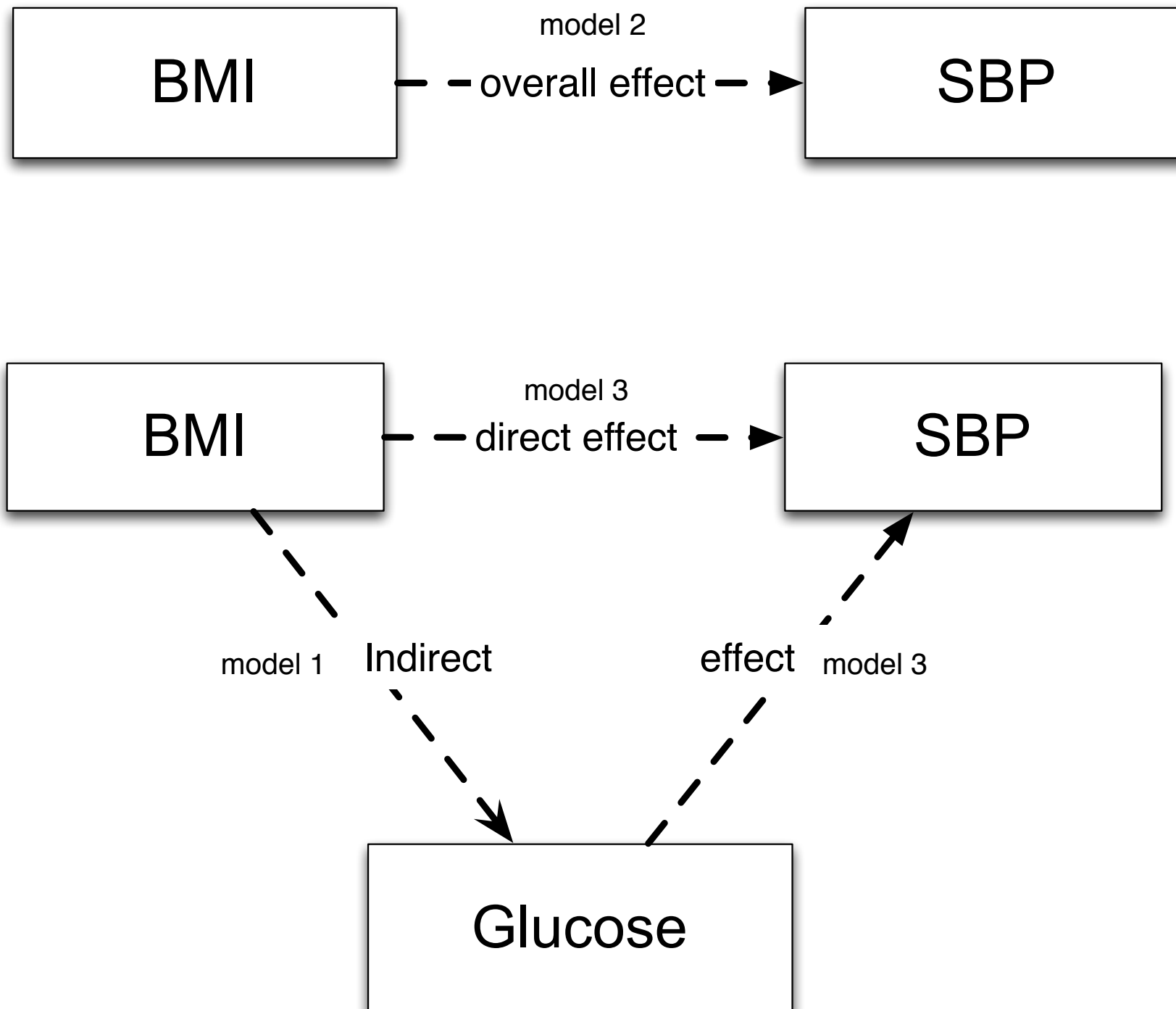
- *indirect effect* of exposure on outcome (i.e. estimates the degree to which exposure is mediated)

Indirect effect estimates

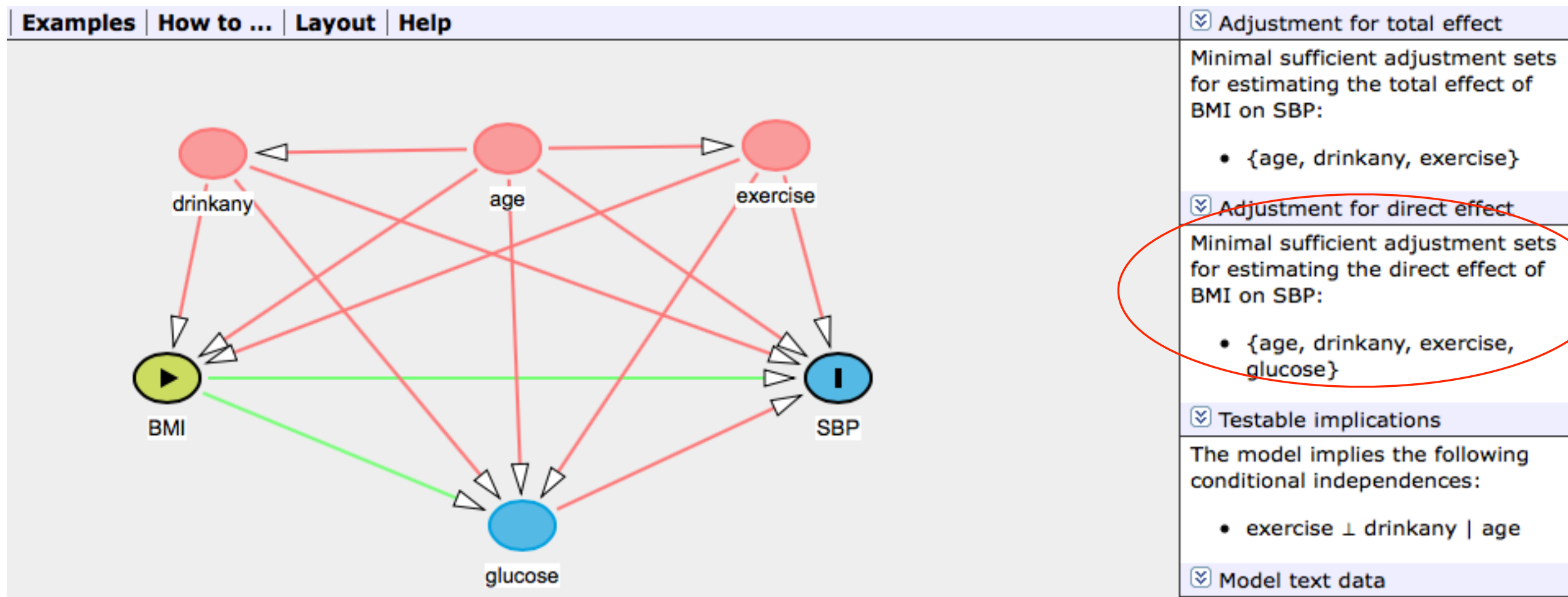
- Mediator, outcome both continuous (i.e. linear regression model applies): we can use either¹
 - difference of exposure effects in Model 2 and Model 3 ($\beta_{\text{overall}} - \beta_{\text{direct}}$),
 - exposure effect in Model 1 $\times$ mediator effect in Model 3 ($\alpha_1 \times \beta_2$)
- If mediator and/or outcome is binary
 - use downloadable mediation package `medeff` (to be illustrated in lab)
- For *linear models only*, can also quantify mediation by *proportion explained (PE)*, defined as $(\beta_{\text{overall}} - \beta_{\text{direct}})/\beta_{\text{overall}}$

1. Mackinnon DP et al. A Simulation Study of Mediated Effect Measures. *Multivariate Behav Res.* (1995)

Example: Mediation of BMI $\rightarrow$ 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]**

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 b_overall-b_direct
```

```
.08344789
```

```
. display link1*link2
```

```
.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 can be unreliable (they are correlated)
- 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 (ideally, avoiding multiple comparison issues)

Ref: Vittinghoff E, Neilands TB. Sample Size for Joint Testing of Indirect Effects. Prev Sci. 2015;16:1128-35.

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.

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)

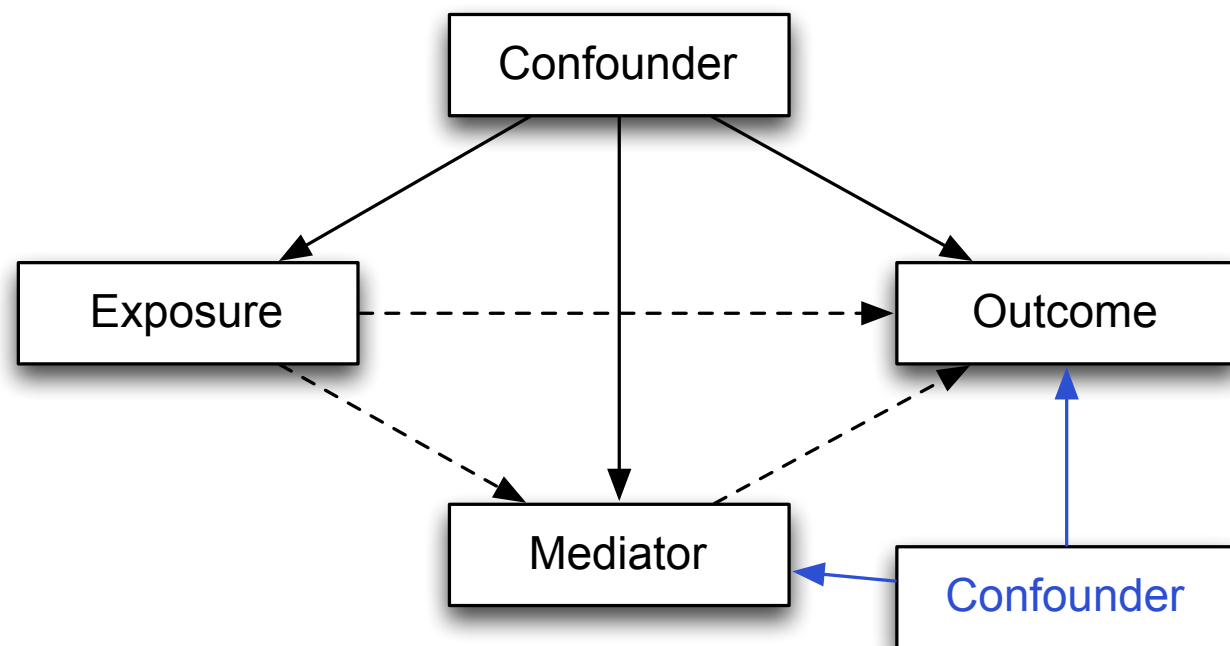
```
(N)    normal confidence interval
```

```
(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 effects²
 - In the presence of such confounding, the mediator is a *collider* on front door path from exposure to outcome

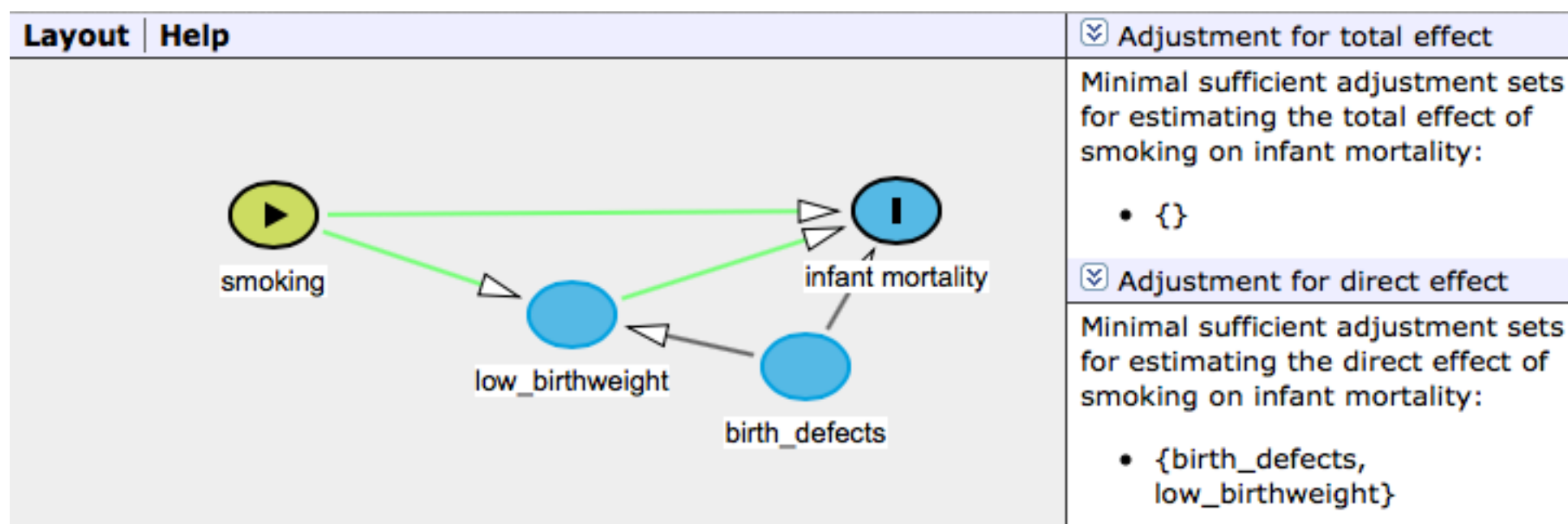


2. Cole SR, Hernán MA. Fallibility in estimating direct effects, Int J Epid, 2002;31:163-5

Confounding of mediator/outcome relationship

- Example: low birth weight as mediator of effects of smoking on infant mortality³

The “paradox” refers to the observation that low birthweight babies with mothers who smoke have been observed to do better than those with low birthweight & non-smoking mothers



Possible explanation:

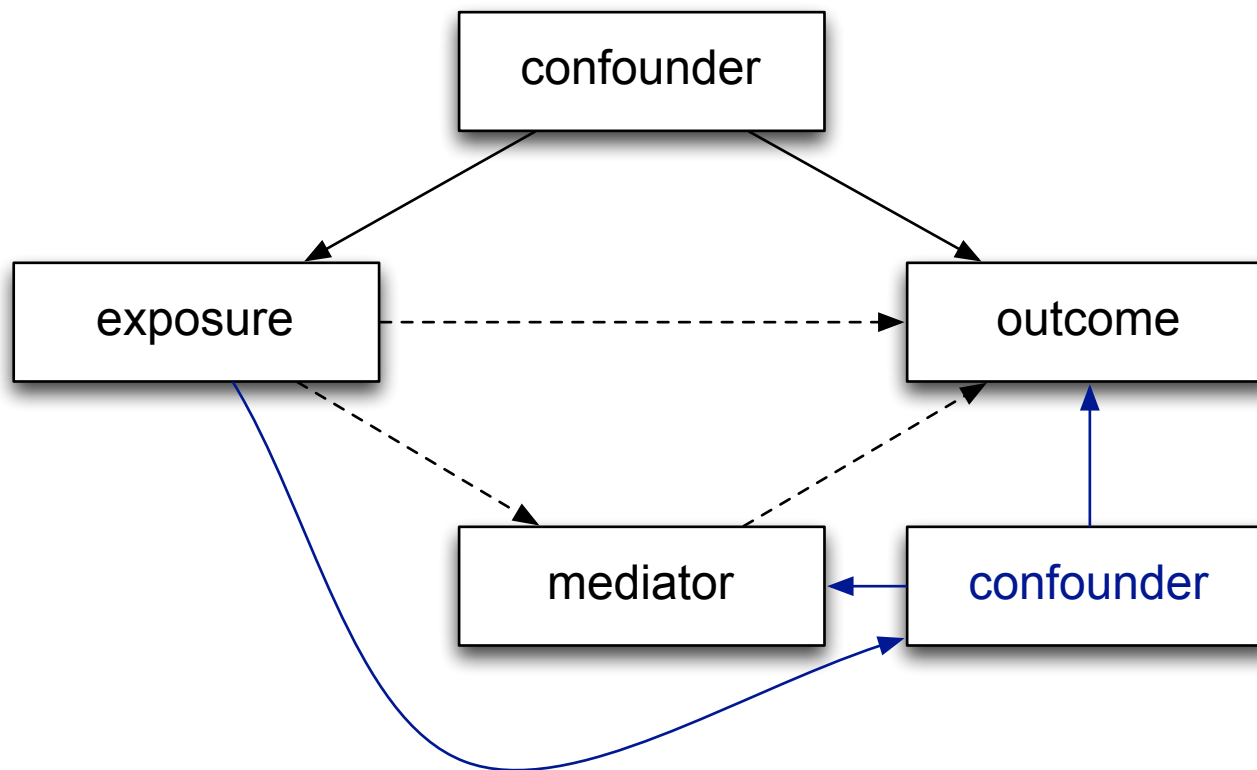
- low-birth-weight in infants from mothers who smoke might be a consequence of either smoking or of a birth defect
- not smoking & low birth weight together indicative of the presence of a birth defect

Controlling for birth defects resolves the paradox

3. Whitcomb BW, Schisterman EF, Perkins NJ, Platt RW. Quantification of collider-stratification bias and the birthweight paradox. *Paediatr Perinat Epidemiol.* 2009;23:394-402.

Confounder/mediators of mediator/outcome relationship

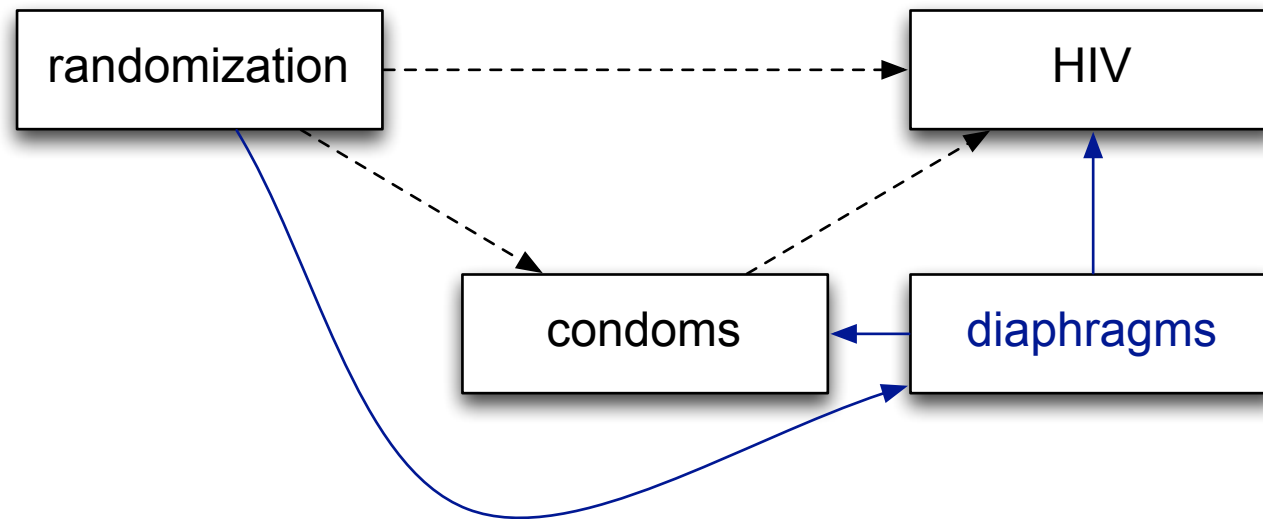
- If a confounder of mediator/outcome relationship is **also a mediator of exposure effects**, regression adjustment fails to yield unbiased estimates (special methods such as inverse probability weighting needed) ⁴
- In this case, regression adjustment for the confounder/mediator removes some of the effect of exposure



4. 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 only



- 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 ⁵
 - ─ *Direct effect of randomization not mediated through condoms of interest*
 - ─ Diaphragm use as a possible confounder-mediator

5. Rosenblum M, Jewell NP, van der Laan M, Shiboski S, van der Straten A, Padian N. Analyzing Direct Effects in Randomized Trials with Secondary Interventions: An Application to HIV Prevention Trials. *J R Stat Soc Ser A Stat Soc.* 2009;17:443-465.

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 `medef f`)
 - 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)
- Methods for *causal mediation analysis* introduced in Lecture 9

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
- Good general reference:

VanderWeele TJ. Mediation Analysis: A Practitioner's Guide. Annual Rev. Public Health. 2016;37:17-32.