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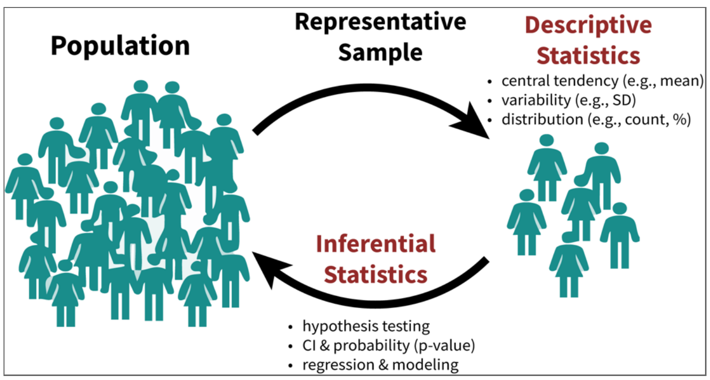
WEEK 5: STATISTICS

In Week 4, we used visualization to 'analyze' our data. While visualizing data is often the first step in analysis, it only allows us to eyeball patterns and relationships. This week, we move beyond visual inspection to statistical analysis. Statistics allow us to perform two main types of analysis:

1. ***Describe*** what we observed in our data, and
2. ***Infer*** what our data tells us about the broader population



Adapted from **Numiko**

Today we'll learn to conduct these statistical analyses in R. This lesson will cover:

1. Build summary tables and figures for our dataset using `dplyr`, `tidyr`, and `ggplot2`
2. Run basic statistical tests (t-tests, ANOVA, correlations) and interpret output
3. Fit linear models with `lm()` and interpret coefficients
4. Estimate marginal means using `emmeans` and run pairwise comparisons

CLEAN THE ENTIRE WORKSPACE

In [53]:

```
rm(list=ls())
```

LOAD REQUIRED LIBRARIES

In [54]:

```
ReqdLibs = c("readxl","dplyr","tidyr","gt","sjPlot",
             "ggplot2","ggthemes","ggpubr","ggExtra",
             "lme4","emmeans","janitor","broom","car","IRdisplay")
invisible(lapply(ReqdLibs, library, character.only = TRUE))
```

THEME DEFAULTS

In [55]:

```
thm = theme(
          strip.text.x=element_text(size=20,face="bold"),
          strip.text.y=element_text(size=20,face="bold"),
          legend.text=element_text(size=16,face="bold"),
          legend.position = "top",
          legend.title=element_text(size=16,face="bold"),
          title =element_text(size=14, face='bold'),
          text = element_text(colour = "black",size=18),
          plot.title = element_text(colour = "black",size = 22, face = "bold"),
          axis.ticks.length = unit(0.3,"cm"),
          axis.line = element_line(colour = "black",linewidth=0.85),
          axis.ticks = element_line(colour = "black",linewidth=0.85),
          axis.text = element_text(colour = "black",size=24),
          axis.title=element_text(size=25))
```

1. SUMMARIZE OUR SAMPLE IN TABLES AND FIGURES

READ DATA

In [56]:

```
# Read in demographics data
demo <- read_excel("SubjectInfo.xlsx")

# Display structure of demo data
head(demo)
```

A tibble: 6 × 8

Subject No	Age	Reported Weight (kg)	Reported Length (cm)	Gender	Level Slow	Level Walk	Weight from force plates(kg)
<chr>	<dbl>	<dbl>	<dbl>	<chr>	<dbl>	<dbl>	<dbl>
Sub1	26	86	185	M	885.5529	891.5307	90.57511
Sub2	28	77	178	F	767.7686	760.2377	77.88004
Sub3	21	52	170	M	530.6408	558.2018	55.49656
Sub4	25	73	168	M	NA	NA	NA
Sub5	34	86	173	M	878.6303	898.5188	90.57845
Sub6	19	54	160	F	553.4936	558.2845	56.66555

CLEAN UP VARIABLE NAMES IN THE DEMO TABLE

In [57]:

```
demo_clean = clean_names(demo) %>% filter(subject_no!="Sub4")
head(demo_clean)
```

A tibble: 6 × 8

subject_no	age	reported_weight_kg	reported_length_cm	gender	level_slow	level_walk	weigh
<chr>	<dbl>	<dbl>	<dbl>	<chr>	<dbl>	<dbl>	
Sub1	26	86	185	M	885.5529	891.5307	
Sub2	28	77	178	F	767.7686	760.2377	
Sub3	21	52	170	M	530.6408	558.2018	
Sub5	34	86	173	M	878.6303	898.5188	
Sub6	19	54	160	F	553.4936	558.2845	
Sub7	21	59	163	F	605.0228	605.9027	

LET'S TRY AND REPRODUCE WHAT THE AUTHORS TOLD US ABOUT THE SAMPLE

Methods

Subjects and experiment

Twelve healthy participants (6 female, 6 male, mean ± SD age 24 ± 5 years, weight 70 ± 12 kg, and height 173 ± 8 cm) were recruited using flyers at Cleveland State University and word-of-mouth. They performed the experiment after providing informed consent. The experimental

In [58]:

```
# Create summary statistics
demo_summary <- demo_clean %>%
  summarise(
    sex = paste0(sum(gender == "M", na.rm = TRUE), "F, ",
                 sum(gender == "F", na.rm = TRUE), "M"),
    age =    paste0(round(mean(age, na.rm = TRUE),0), " ± ",
                    round(sd(age, na.rm = TRUE),0)),

    weight = paste0(round(mean(reported_weight_kg, na.rm = TRUE),0), " ± ",
                    round(sd(reported_weight_kg, na.rm = TRUE),0)),

    # weight = paste0(round(mean(weight_from_force_plates_kg, na.rm = TRUE),0), " ±
",
    #                 round(sd(weight_from_force_plates_kg, na.rm = TRUE),0)),

    height = paste0(round(mean(reported_length_cm, na.rm = TRUE),0), " ± ",
                    round(sd(reported_length_cm, na.rm = TRUE),0)),
  )

# Create gt table
tb <- demo_summary %>%
  gt()
```

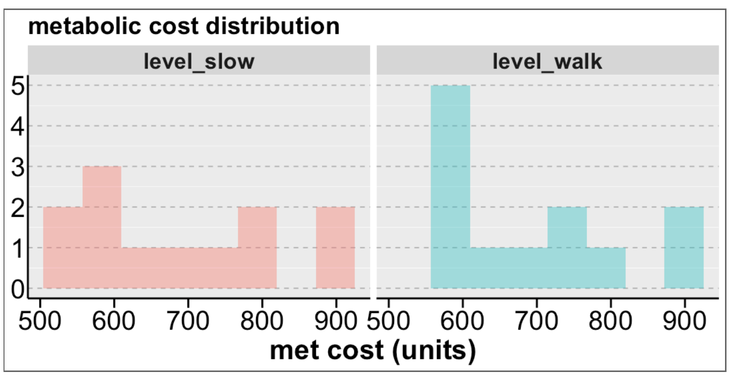
```
# Display inline
tb %>%
  as_raw_html() %>%
  display_html()

# display_markdown("#### We were able to reproduce means and SD! We also found out
that the weight summary was actually from force plate data.")
```

sex	age	weight	height
6F, 6M	24 ± 5	66 ± 12	173 ± 8

LET'S LOOK AT HISTROGRAMS FOR LEVEL-SLOW AND LEVEL-WALK

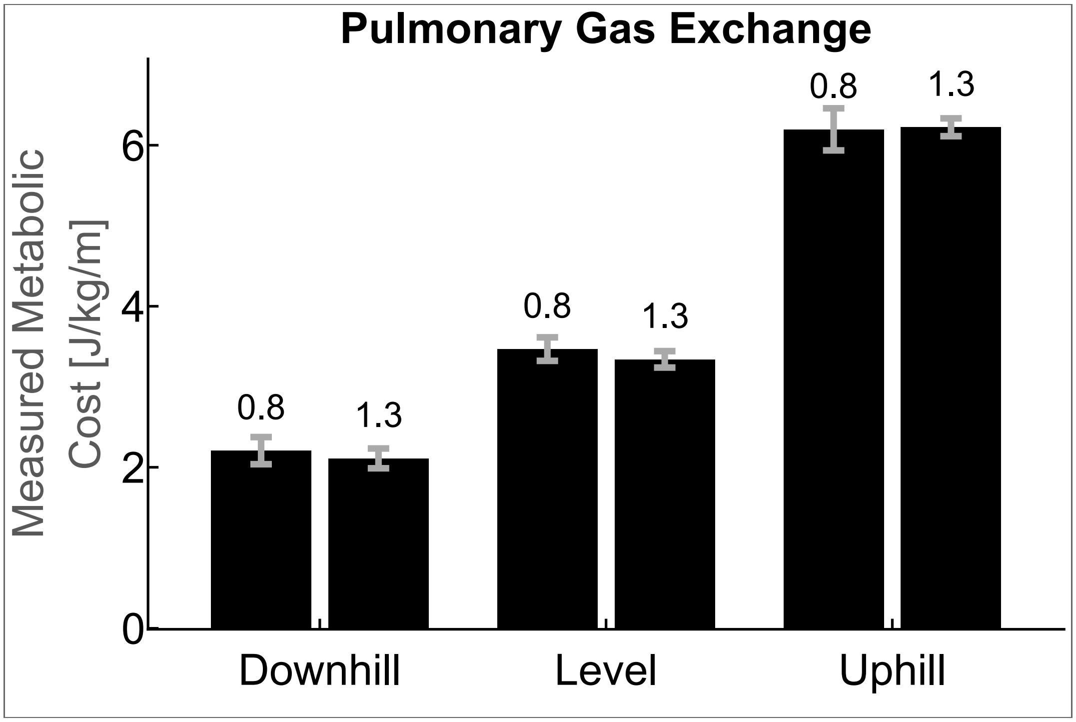
In [59]:

```
options(repr.plot.width = 9, repr.plot.height = 4.5)

# just for plotting, let's pivot the level_slow and level_walk to look at their
distributions together
demo_hist = demo_clean %>% pivot_longer(cols = c(level_slow, level_walk), names_to =
"MetCost_cond",values_to = "MetCost_val")

# visualize distributions using geom_histogram
ggplot(data = demo_hist, aes(x = MetCost_val, y = after_stat(count), fill =
MetCost_cond)) +
geom_histogram(bins = 8,alpha = 0.4, show.legend = FALSE) +
labs(title = "metabolic cost distribution", x = "met cost (units)") +
facet_grid(~MetCost_cond) +
theme_cleveland() + thm
```



2. RUN BASIC STATISTICAL TESTS

So you recall the figure we reproduced in Week 4. Let's try to run some basic comparisons using these data.



In [60]:

```
# Read in calculated dataset
data_calc <- read.csv("calcData.csv")
# data_calc = data_calc %>% filter(Sub!="Sub4")
head(data_calc)
```

A data.frame: 6 × 20

	Sub	cond	incline	speed	R	VT	VE	VO2	ag
	<chr>	<chr>	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<int
1	Sub1	walk	downhill	0.8	0.9019424	0.7859465	20.33910	728.5032	26
2	Sub1	walk	downhill	1.3	0.8661119	0.8409442	25.83893	1005.2468	26
3	Sub1	walk	level	1.3	0.8340541	1.0104406	32.61279	1368.0726	26
4	Sub1	walk	uphill	0.8	0.8661413	1.2505480	40.73454	1687.3811	26
5	Sub1	walk	uphill	1.3	0.8315770	1.4630620	58.84244	2540.5691	26
6	Sub1	walk	level	0.8	0.8727268	1.0036957	26.00091	992.9288	26

PAIRED T-TESTS

Now, you can see that this really is a 3x2 repeated measures design (3 inclines and 2 speeds) but let's begin by running a simple paired t-test to compare the measured metabolic cost between the fast and slow speeds. And then interpret the output.

In [61]:

```
# first make sure there in fact are complete paired observations
table(data_calc$speed)
```

```
# See the data structure
data_calc %>% count(Sub, speed)
```

```
0.8 1.3
 36  35
```

A data.frame: 24 × 3

Sub	speed	n
<chr>	<dbl>	<int>
Sub1	0.8	3
Sub1	1.3	3
Sub10	0.8	3
Sub10	1.3	3
Sub11	0.8	3
Sub11	1.3	3
Sub12	0.8	3
Sub12	1.3	2
Sub13	0.8	3
Sub13	1.3	3
Sub2	0.8	3
Sub2	1.3	3
Sub3	0.8	3
Sub3	1.3	3
Sub4	0.8	3
Sub4	1.3	3
Sub6	0.8	3
Sub6	1.3	3
Sub7	0.8	3
Sub7	1.3	3
Sub8	0.8	3
Sub8	1.3	3
Sub9	0.8	3
Sub9	1.3	3

In [67]:

```
# remove non-complete cases
data_calc2 = data_calc %>% filter(Sub!="Sub12")

# Compare C_meas between speeds 0.8 and 1.3
```

```
slow_speed <- data_calc2 %>% filter(speed == 0.8) %>% pull(C_meas)
fast_speed <- data_calc2 %>% filter(speed == 1.3) %>% pull(C_meas)

# Paired t-test
result <- t.test(slow_speed, fast_speed, paired = TRUE)
result
```

```
	Paired t-test

data:  slow_speed and fast_speed
t = 0.20974, df = 32, p-value = 0.8352
alternative hypothesis: true mean difference is not equal to 0
95 percent confidence interval:
 -0.5181596  0.6371173
sample estimates:
mean difference 
     0.05947885 
```

ANOVA

SIMPLE ANOVA

Here's a simple one-way ANOVA that treats all observations as *independent*.

In [37]:

```
simple_anova_model <- aov(C_meas ~ incline, data = data_calc2)
summary(simple_anova_model)
```

```
            Df Sum Sq Mean Sq F value Pr(>F)    
incline      2 186.33   93.17   293.2 <2e-16 ***
Residuals   63  20.02    0.32                   
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

REPEATED MEASURES ANOVA

But you and I know that these observations are *NOT independent*.
This violates a key assumption of the ANOVA. So to account for the repeated measures structure, we change the simple ANOVA by adding an `Error()` term specified as `Error(Sub/incline)` which tells R that incline measurements are nested within subjects and hence not independent.

In [38]:

```
rm_anova_model <- aov(C_meas ~ incline + Error(Sub/incline),
                      data = data_calc2)

summary(rm_anova_model)
```

```
Error: Sub
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals 10  9.073  0.9073

Error: Sub:incline
          Df Sum Sq Mean Sq F value   Pr(>F)
incline    2 186.33   93.17   329.7 4.89e-16 ***
Residuals 20   5.65    0.28
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Error: Within
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals 33  5.296  0.1605
```

CORRELATIONS

We can also examine relationships between continuous variables. Here we test the correlation between adjusted VO2 and metabolic cost using Pearson correlation and visualize it with a scatterplot.

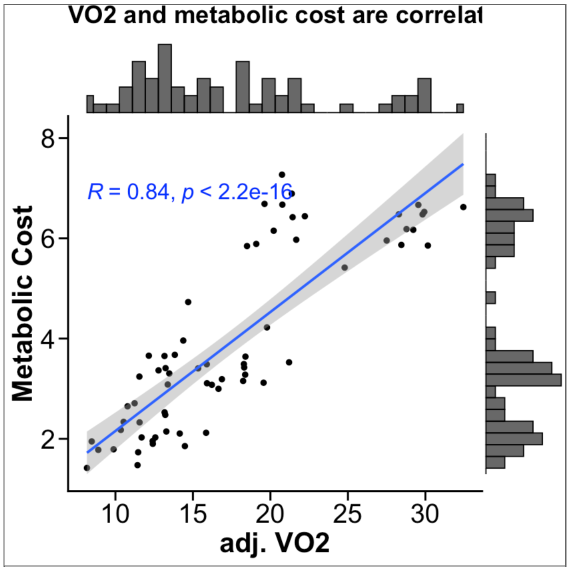
In [39]:

```
data_calc2 %>%
 with(cor.test(C_meas, adjVO2, method = "pearson"))
```

```
        Pearson's product-moment correlation

data:  C_meas and adjVO2
t = 12.541, df = 64, p-value < 2.2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.7551194 0.9012101
sample estimates:
      cor
0.8430697
```

In [40]:

```
options(repr.plot.width = 7, repr.plot.height = 7)

cor_plot <-

ggplot(data = data_calc2, aes(x = adjVO2,y = C_meas)) +
 geom_point(size = 2) + geom_smooth(formula = 'y~x',method = "lm") +
 # the stat_cor function from ggpubr is a neat way to add correlation values on the
plot itself
 stat_cor(method = "pearson", size = 7, col = "blue") +
 labs(title = "VO2 and metabolic cost are correlated", y = "Metabolic Cost", x =
"adj. VO2") +
 theme_classic2() + thm

# cor_plot
ggMarginal(cor_plot, type = "histogram")
```



3. FIT LINEAR MODELS

In this case, because we intend to establish mean differences, fitting a linear model actually is much like running a t-test or ANOVA as we did above. Except it allows us to set interaction terms and do so with missing observations.

In [69]:

```
lm_fit <- lm(C_meas ~ incline * speed, data = data_calc)
summary(lm_fit)
```

```
Call:
lm(formula = C_meas ~ incline * speed, data = data_calc)

Residuals:
     Min       1Q   Median       3Q      Max 
-2.54476 -0.24414 -0.05276  0.25556  1.45122 

Coefficients:
                    Estimate Std. Error t value Pr(>|t|)    
(Intercept)          2.36053    0.49798   4.740 1.21e-05 ***
inclinelevel         1.31083    0.69995   1.873   0.0656 .  
inclineuphill        3.79515    0.69995   5.422 9.27e-07 ***
speed               -0.19237    0.46596  -0.413   0.6811    
inclinelevel:speed  -0.06201    0.65176  -0.095   0.9245    
inclineuphill:speed  0.24403    0.65176   0.374   0.7093    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.5581 on 65 degrees of freedom
Multiple R-squared:  0.9095,	Adjusted R-squared:  0.9026 
F-statistic: 130.7 on 5 and 65 DF,  p-value: < 2.2e-16
```

In [70]:

```
#ooh, btw, check this out:
display_markdown("#### BTW, if you ran an ANOVA of this linear model, it would give
you the exact same estimates as your original ANOVA!")
anova(lm_fit)
```

BTW, IF YOU RAN AN ANOVA OF THIS LINEAR MODEL, IT WOULD GIVE YOU THE EXACT SAME ESTIMATES AS YOUR ORIGINAL ANOVA!

A anova: 4 × 5

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
	<int>	<dbl>	<dbl>	<dbl>	<dbl>
incline	2	203.36432749	101.68216375	326.4114629	1.256380e-34
speed	1	0.07587859	0.07587859	0.2435790	6.232965e-01
incline:speed	2	0.07828560	0.03914280	0.1256529	8.821346e-01
Residuals	65	20.24849430	0.31151530	NA	NA

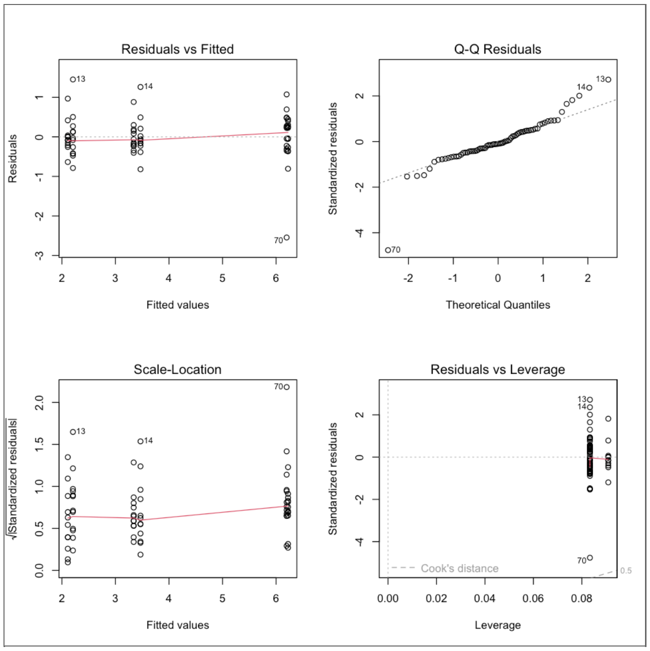
MODEL DIAGNOSTICS

1. Residuals vs Fitted: Checks if relationship is linear and variance is constant.
    - *We want random scatter around horizontal line at 0.*
2. Q-Q Plot: Checks if residuals are normally distributed
    - *We want points to follow the diagonal line closely*
3. Scale-Location: Checks if variance is constant across fitted values (homoscedasticity)
    - *We want horizontal line with random scatter (no systematic shape)*
4. Residuals vs Leverage: Identifies influential points
    - *Ideally, we want all points to be within Cook's distance lines*

In [73]:

```
options(repr.plot.width = 8, repr.plot.height = 8)

par(mfrow = c(2,2))
plot(lm_fit)

# WE can also print out the exact values and indices of the influential observations
cooksd <- cooks.distance(lm_fit)
influential <- which(cooksd > 4/nrow(data_calc))
print(paste("Influential observation #", influential, "C Meas is",
cooksd[influential]))
```

```
[1] "Influential observation # 13 C Meas is 0.111746052521325"
[2] "Influential observation # 14 C Meas is 0.0843345595874919"
[3] "Influential observation # 16 C Meas is 0.0610988460034664"
[4] "Influential observation # 70 C Meas is 0.343605624121243"
```



PUBLICATION-READY REGRESSION TABLES

Here, I am usign the `sjPlot` package to show you an example of a publication-ready regression table. There are many ways/packages to acheive the same type of table, e.g., `gt` and `gtsummary` packages.

In [ ]:

```
regression_table = tab_model(lm_fit, show.stat = TRUE,
                             dv.labels = "Measured Metabolic Cost")
display_html(regression_table$page.complete)

# saving the table as DOC or HTML
tab_model(lm_fit, file = "linear-model-fit.docx")
```

	Measured Metabolic Cost			
Predictors	*Estimates*	*CI*	*Statistic*	*p*
(Intercept)	2.36	1.37 – 3.36	4.74	**<0.001**
incline [level]	1.31	-0.09 – 2.71	1.87	0.066
incline [uphill]	3.80	2.40 – 5.19	5.42	**<0.001**
speed	-0.19	-1.12 – 0.74	-0.41	0.681
incline [level] × speed	-0.06	-1.36 – 1.24	-0.10	0.924
incline [uphill] × speed	0.24	-1.06 – 1.55	0.37	0.709
Observations	71			
R^2 / R^2 adjusted	0.910 / 0.903			

4. POST-HOC PAIRWISE COMPARISONS WITH MARGINAL MEANS

	Fast (0.8)	Slow (1.3)	Row Mean
Downhill	2.21	2.11	2.16
Level	3.47	3.34	3.41
Uphill	6.20	6.22	6.21
Col Mean	**3.96**	**3.89**	**3.93**

EMMs allow us to follow up significant main effects by testing which specific groups differ from each other, with appropriate corrections for multiple comparisons.

ESTIMATE MARGINAL MEANS

Note that ours is an interaction model, and we know that the effect of `speed` is not significant. So really we would like marginal means for incline.

In [48]:

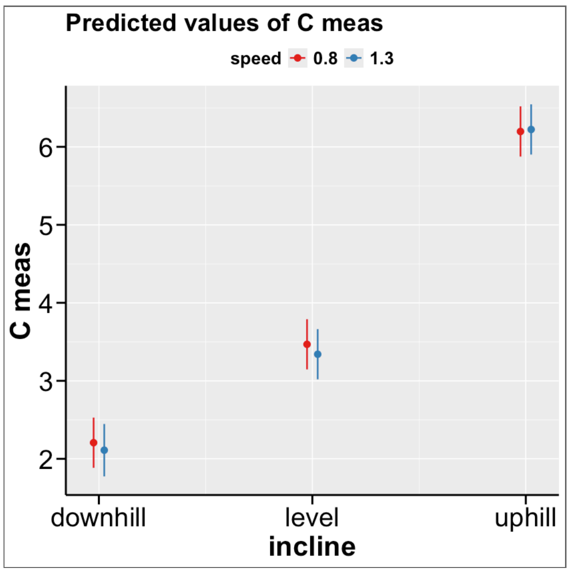
```
# Estimated marginal means for speed - the same as were compared by the t-test
speed_means <- emmeans(lm_fit, ~ speed)
speed_means

display_markdown("------------")
# Estimated marginal means for incline - the same as were compared by the ANOVA
incline_means <- emmeans(lm_fit, ~ incline)
incline_means

display_markdown("------------")
# Estimated marginal means for both factors - "splits" them by these two factors
speed_by_incline_means <- emmeans(lm_fit, ~ speed + incline)
speed_by_incline_means

display_markdown("------------")
# Plot estimated marginal means
plot_model(lm_fit, type = "pred", terms = c("incline", "speed")) + thm
```

```
NOTE: Results may be misleading due to involvement in interactions


 speed emmean     SE df lower.CL upper.CL
   0.8   3.96 0.0930 65     3.77     4.14
   1.3   3.89 0.0944 65     3.70     4.08

Results are averaged over the levels of: incline
Confidence level used: 0.95
```

```
NOTE: Results may be misleading due to involvement in interactions


 incline  emmean    SE df lower.CL upper.CL
 downhill   2.16 0.116 65     1.93     2.39
 level      3.40 0.114 65     3.18     3.63
 uphill     6.21 0.114 65     5.98     6.44

Results are averaged over the levels of: speed
Confidence level used: 0.95
```

```
 speed incline  emmean    SE df lower.CL upper.CL
   0.8 downhill   2.21 0.161 65     1.88     2.53
   1.3 downhill   2.11 0.168 65     1.77     2.45
   0.8 level      3.47 0.161 65     3.15     3.79
   1.3 level      3.34 0.161 65     3.02     3.66
   0.8 uphill     6.20 0.161 65     5.88     6.52
   1.3 uphill     6.22 0.161 65     5.90     6.54

Confidence level used: 0.95
```

```
Some of the focal terms are of type `character`. This may lead to
  unexpected results. It is recommended to convert these variables to
  factors before fitting the model.
  The following variables are of type character: `incline`

Ignoring unknown labels:
• linetype : "speed"
• shape : "speed"
```



RUN PAIRWISE COMPARISONS AMONG THE VARIOUS INCLINES

In [49]:

```
# With adjustment for multiple comparisons
pairs(incline_means, adjust = "tukey")

display_markdown("------------")
pairs(incline_means, adjust = "bonferroni")
```

```
 contrast          estimate    SE df t.ratio p.value
 downhill - level     -1.25 0.163 65  -7.645  <.0001
 downhill - uphill    -4.05 0.163 65 -24.864  <.0001
 level - uphill       -2.81 0.161 65 -17.413  <.0001

Results are averaged over the levels of: speed
P value adjustment: tukey method for comparing a family of 3 estimates
```

```
 contrast           estimate    SE df t.ratio p.value
 downhill - level      -1.25 0.163 65  -7.645  <.0001
 downhill - uphill     -4.05 0.163 65 -24.864  <.0001
 level - uphill        -2.81 0.161 65 -17.413  <.0001

Results are averaged over the levels of: speed 
P value adjustment: bonferroni method for 3 tests 
```

TESTING ONLY FOR HYPOTHESIZED EFFECTS

If you had a hypothesis going in, for example, only uphill will differ from the other two conditions. The other two conditions will not differ from one another, then you can run this comparison by manually specifying contrasts.

In [50]:

```
# check levels (what comes first, second and third)
factor(data_calc$incline) %>% levels()
# incline_means
contrast(incline_means,
         list("Uphill - Downhill" = c(-1, 0, 1),    # Compare uphill to downhill
              "Uphill - Level" = c(0, -1, 1)))      # Compare uphill to level
```

'downhill' · 'level' · 'uphill'

```
 contrast           estimate    SE df t.ratio p.value
 Uphill - Downhill      4.05 0.163 65  24.864  <.0001
 Uphill - Level         2.81 0.161 65  17.413  <.0001

Results are averaged over the levels of: speed 
```

THE END