Helpful information on Caffeine Paper:

**What are they trying to find out?**
If caffeine decreases muscle growth.

**How did they go about the study?**
- Used three models: 1. Muscle cells, 2. Mice, 3. Rats
- Also used LKB1 Muscle specific Knockout Mice (explained later).
- Used electrical stimulation on cells and rodent’s (mice and rats) muscles to induce contractions.
- Electrically stimulated for an equivalent of 6 sets of 10 repetitions.
- Measured cells by microscope images, cutting them up and measuring proteins within.
- Measured animals by weight and activity, but also by removing their muscles, grinding them up, and measuring proteins within (animals did not feel anything).
- Simply: More protein = more muscle growth (roughly).

**Definitions:**
*mTOR*: Molecule that promotes muscle growth. Inhibits AMPK.

*AMPK*: Molecule that inhibits muscle growth. Inhibits mTOR.

*Akt*: Molecule that regulates both mTOR and AMPK – increases mTOR, decreases AMPK. So, in general, considered muscle promoting molecule.

**Figure 1**
This figure shows the amount of AMPK, Akt molecules, as well as smaller molecules controlled by mTOR in the absence or presence of varying concentrations of caffeine (x-axis) and insulin (dark shaded bars) – all in muscle *cells*.

If bars are higher, then there is more of the molecule.

*Insulin*: Growth stimulating hormone.
*p-S6K*: Muscle growth machinery molecule.
*p-S6*: Muscle growth machinery molecule.
*p-4EBP1*: Muscle growth machinery molecule.

Note: Focus on the bars, not the black marks above the bars – unless you are familiar with protein quantification.

**Figure 2**
This figure shows the effect caffeine has, at varying concentrations (x axis), on the growth of muscle cells.

In images, blue represents muscle nuclei (where muscle growth signals begin) and green represents the muscle protein, itself.

Figure 2A shows caffeine at physiological doses (normal, day to day intake).
Figure 2B shows caffeine at super-physiological doses (far above normal, day to day intake).
Figure 2C shows caffeine (physiological, except the last dose – 4mM) on the formation of new muscle cells.

**Figure 3**
This figure represents caffeine injections and electrical stimulation on mouse muscles – muscles were collected/taken/removed and measured either immediately after stimulation (acting as a muscle contraction/exercise) or 8 hours later.

Again, it shows the quantities of the AMPK molecule and Akt molecule – this time, in muscle tissue, not cells exclusively.

They used Wild Type (WT)/control mice and LKB1 knockout mice (see below).

*White bar/SAL-REST*: Saline injection (no caffeine) into mouse and no stimulation.
*Light horizontal bar/SAL-STIM*: Saline injection (no caffeine) into mouse and electrical stimulation to force muscle contraction like exercise.
*Black bar/CAF-REST*: Caffeine injection, no stimulation.
*Thick horizontal bar/CAF-STIM*: Caffeine injection, electrical stimulation of muscle to force muscle contraction like exercise.

*WT* = Wild Type - normal, unaltered mice.
*KO* = Knockout – mice with the LKB1 molecule removed.
*LKB1* = LKB1 is a molecule that *activates* AMPK (Reminder: AMPK inhibits muscle growth).
*\* above bars* = significant vs control (SAL-REST)
*# above bars* = significant vs compared group (WT vs KO)
*AMPK*: Molecule that inhibits muscle growth. Inhibits mTOR.
*Akt*: Molecule that regulates both mTOR and AMPK – increases mTOR, decreases AMPK. So, in general, considered muscle promoting molecule.

Note: Ignore 3C, unless you know how to read protein quantification.

**Figure 4**
In this figure, you are looking at varying mTOR associated (muscle promoting) molecules with injection of caffeine and/or with electrical stimulation of the mouse’s muscle to act like exercise – they removed muscles and measured molecule amount immediately (0hr) and 8 hours after stimulation (8hr), in wild type and LKB1 knockout mice.

They used Wild Type (WT)/control mice and LKB1 knockout mice (see below).

Figure 4E represents the amount of a particular molecule incorporated into proteins – this quantifies the exact amount of protein being produced, not just the responsible molecules that initiate muscle protein synthesis.

*White bar/SAL-REST*: Saline injection (no caffeine) into mouse and no stimulation.
*Light horizontal bar/SAL-STIM*: Saline injection (no caffeine) into mouse and electrical stimulation to force muscle contraction like exercise.
*Black bar/CAF-REST*: Caffeine injection, no stimulation.
*Thick horizontal bar/CAF-STIM*: Caffeine injection, electrical stimulation of muscle to force muscle contraction like exercise.

*WT* = Wild Type - normal, unaltered mice.
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*\* above bars* = significant vs control (SAL-REST)
*# above bars* = significant vs compared group (WT vs KO)
*p-S6K*: Muscle growth machinery molecule.
*p-S6*: Muscle growth machinery molecule.
*p-4EBP1*: Muscle growth machinery molecule

Note: Ignore 4D and 4E protein picture unless you know how to read protein quantification.

**Figure 5**
This is a figure that shows the actual muscle change (growth, same, decrease?), in rats when they surgically cut the tendon to an accessory/helping muscle, making one muscle (plantaris) work much harder to compensate (this is the stimulus, like weight lifting). They also injected either water or caffeine. All rats were wild type – no knockouts.

Here, they measure the plantaris (stimulated muscle) change in size, weight, the AMPK molecule activation amount, Akt molecule activation amount, and 4E-BP1 activation amount.

*Hypertrophy*: Muscle growth.
*AMPK*: Molecule that inhibits muscle growth. Inhibits mTOR.
*Akt*: Molecule that regulates both mTOR and AMPK – increases mTOR, decreases AMPK. So, in general, considered muscle promoting molecule.
*p-4EBP1*: Muscle growth machinery molecule
*Plantaris*: Muscle being measured.
*White bar/SHAM*: Surgery to the other leg of the rat, but no tendons cut – should be intact.
*Gray bar/OVLD*: Overload via surgery and cut of the accessory muscles so the plantaris must do all the work – increasing its stimulus for growth.

**Table 1**
Bodyweights, fat mass, food/calorie intake of all the rats given only water (control) vs caffeine group. This shows if the animals were the same before measures or different.

**Conclusions?**
You decide, then join me at 7pm ET tonight on YouTube and Instagram. 😊