DANNY LENNON: Here we are. Alex, thank you so much for joining me, man. I'm looking forward to our chat.

ALEX KOLLIARI TURNER: Thank you, Danny. Pleasure to be here.

DANNY LENNON: Yeah. This is been in the making for a while because we've actually talked about this probably a couple years back nearly, and I remember you first telling me about the work you are going be pursuing, which we kind of get into, and so it's interesting to see how that's developed, and I'm fascinated to ask plenty of questions about it.

So just for people listening, just to give them some context, maybe give them a rundown of what you're doing now, what your work involve and any kind of relevant background that may aid putting some context around this discussion.

ALEX KOLLIARI TURNER: Yeah. So I'm a second year PhD student at the University of Brighton working on a project that's investigating the role of anabolic steroids on muscle gene expression, epigenetic markers, and their potential enhancement of muscle memory.

And we are as a research group very interested in the molecular mechanism of muscle memory and so the research that we are conducting at the moment is ongoing and we are in the process of recruiting participants which is why, Danny, we're talking now because I'm looking for participants and we're aiming to recruit four groups of people, sedentary controls guys who do not take any performance-enhancing drugs, individuals who take performance-enhancing drugs and have taken anabolic steroids for a minimum of 20 weeks and then guys who have taken anabolic steroids for a minimum of 20 weeks at least 2 to 4 years ago, but are currently drug-free.

And I'll explain later on why we are looking at those groups and essentially, we by biopsying those individuals we're going to get better understanding on the effect of anabolic steroids on gene expression inside the muscle, muscle epigenetic markers and their influence in muscle memory.

DANNY LENNON: Yeah. It really is fascinating. It's going to be supercool to see that work being done and as you said we'll round back to that later on. I do want to get into maybe some things that lay a foundation for understanding the actual work you're -- you're doing and the purpose of the specific research question you're trying to answer.

So may be a good place to start is going right back to looking at mechanisms of hypertrophy, and one of the things you have mentioned to me before is the specific interest in looking at myonuclei accumulation in relation to hypertrophy. Can you talk about that mechanism why it's important and what things people should understand about that?

ALEX KOLLIARI TURNER: Of course. So muscle fibers are very interesting cells in the fact that by volume they are the largest cell inside the human body. So muscle fibers are actually very, very big cells and they can achieve lengths of up to 23 inches.

So, for example, in the sartorius muscle that goes across your quadriceps, a single muscle fiber could be 23 inches long and that makes them hundreds times greater by volume than other cells in the body. And so if you're thinking of these muscle fibers as incredibly long, cylindrical shaped cells that contain contractile proteins that eventually slide past each other that result in movement, there is actually a transport problem in the cell because if the cell was to only have one nucleus that was responsible for making these contractile proteins in the cell, those proteins would never be able to diffuse the entire distance of the cell.

So muscle fiber is a very interesting and unique -- there's only a few other tissues in the human body that are like this where they actually contain many, many nuclei within them as a single cellular body. So they're multinucleated, and the technical term for that is that they all exist in the syncytium.

Now for muscle growth to occur three times, you have to have muscle protein synthesis outweigh muscle protein breakdown as a net balance over a time course, and there is two ways in which that can occur. The nuclei that are imprisoned inside the muscle fibers, the so-called myonuclei that are already there, they can increase their protein synthetic rate, and the protein will be made and it was around a certain fixed area around the myonucleus and that area is called a myonuclear domain.

So it's regarded that a nuclei can only supply proteins for a very small volume that it surrounds, the so-called myonuclear domain, so the pre-existing myonuclei that are already there can increase the amount proteins that they are making and they can fill out their myonuclear domain, but there is actually regarded that there is a ceiling to that growth because proteins can diffuse so far. So once the myonuclear domain is filled, the nucleus is

then not going to be able to supply proteins any further.

And so the second way in which the muscle fiber can then grow is by activating these stem cells that surround the muscle fiber that are called satellite cells, and these cells once activated through hard training or anabolic steroid usage will divide in two, and one of the cell will be capable of donating its nucleus into the fiber to become a new myonuclei, and the other nucleus will then remain as a satellite cells so that you don't deplete your stem cell pool inside your muscle through time.

So once it gets activated, these nuclei will go into the fiber and then the more nuclei you have inside your actual muscle fibers, the propensity for growth increases because you now have the ability to make more proteins through time, and therefore it's highly likely that muscle protein synthesis would outweigh muscle protein breakdown. So that's the mechanism of muscle growth in terms of myonuclei accumulation.

DANNY LENNON: And one of the kind of related concepts that we're going to dive into and kind of relates to some of your work is this idea of muscle memory, and I think this perhaps means slight different things to different people. So for the context we're talking about here with muscle hypertrophy and myonuclei accumulation, how should we define what exactly we're talking about when it comes to muscle memory? And what evidence do we have for that this occurs or doesn't occur?

ALEX KOLLIARI TURNER: So muscle memory must be when I think about muscle memory is sort of the analogy of kind of like riding a bike where you might've learned to ride a bike as a child and then you haven't ridden a bike for a particularly long time, and then you can suddenly ride a bike again, and that's a skill acquisition and retention of skill acquisition.

That is not what we are interested in, and that's more believed to be a sort of memory inside the nervous system or it's becoming apparent is there is actually a residual memory inside muscle fibers themselves that results in a muscle memory in the aspect of if you accumulate muscle mass due to an exposure to anabolic stimulus so that could be resistance training or anabolic steroids, if you then subsequently lose that muscle mass because that anabolic stimulus is removed, the hypertrophic stimulus is removed, your muscle fibers might shrink in size, but then when you get re-exposed to a second hypertrophic or anabolic stimulus, your muscle fibers will grow at a much faster rate, and you reacquire that muscle mass on a second occasion much faster.

And there is a lot of anecdotal evidence for this, and there is also research studies where they have shown this where they have given people resistance exercise program to follow. Their muscle mass has increased. Then they have removed them from resistance exercise. They've lost their muscle mass, but then when they re-exposed them to resistance exercise on the second occasion, their muscle fibers and their muscle mass returns and the muscle, fibers increase in size at a much faster rate.

So it might be the case of you can lose your muscle mass, but you can regain your muscle mass as long as you then go back to resistance exercise, which is important implications for sarcopenia populations and also for the prescription of when people should resistance exercise during their lifetime because it would make sense that you should try and build as much muscle mass as you can in your younger years to fill up your fibers with nuclei so that when you get older and your satellite cells and your stem cells inside your muscle fibers decrease in time, you then have already gotten myonuclei at an elevated level to help you retain muscle mass in later life, which is good for sarcopenia prevention.

And so there's originally the muscle fiber growth and atrophy, people used to think that when a muscle fiber grew and the number of nuclei inside that fiber increased, that when a muscle fiber decreased in size and experienced atrophying conditions, the nuclei were destroyed and they disappeared, and that explained why the muscle fiber shrank.

And there was a large amount of evidence in the scientific literature of what they would do is induce hypertrophy in an animal model. The muscle fibers will increase in size. They get more nuclei and then they'd induce an atrophying condition, and the muscle fibers would shrink, and then they would take out the muscle fibers, and they would grind them up and homogenate, and they would search the homogenate for markers of nuclear degradation, and they would find them such as caspase activation and DNA fragmentation. And so people used to think that, ah, well, when a muscle fiber increases in size, nuclei go up, and when it decreases in size, nuclei go down.

But, actually, what seems to be that that's actually not the case, and because those studies were relying off grinding up whole muscle fibers and looking at homogenates ,they were not specifically looking at the myonuclei within the fibers. And there was some groundbreaking research that was conducted a few years ago by Christine Gunderson and his lab in Oslo, and essentially, they looked in a live mice single muscle fibers and they specifically labeled the myonuclei inside the muscle fiber. And they witnessed what happened to the myonuclei when they then subjected the mice to an atrophying condition, and they found that although the muscle fiber would shrink in size, the myonuclei that were present did not disappear. And so they then conducted later research where they discovered that those myonuclei could then be turned back on and enabled muscle fibers to regrow in size.

And that’s also been shown now in another animal model in something called tobacco hawkmoth. It has been shown in insects and the tobacco hawkmoth has a muscle called intersegmental muscle that is used to generate crawling of its larvae and the emergence of the adult from the pupae, and essentially, that muscle undergoes atrophy at a certain time course during the lifecycle of this insect, and it will lose 40% of its muscle mass.

And they've now observed as this muscle decreases by 40% because it’s no longer required to be used as the adult emerges, they can see that the muscle fibers will shrink, but the myonuclei that are present inside those fibers don't go down, and so that previous evidence of where they found markers of nuclear degradation inside the muscle is not actually because the myonuclei were being degraded. It's actually because there's numerous other cellular cells inside the muscle as a whole.

So, for example, the satellite cells could be go -- undergo a nuclear degradation, and so you'd find their markers of nuclear degradation, but there is also other cells such as endothelial cells and parasites, and they are involved in blood capillaries and vessels inside the muscle. There is also fibroblast inside muscle that are responsible for making the extracellular matrix and there is also macrophages, which are white blood cells. So, all of those cells when the muscle is subjected to an atrophying condition might actually die off and so you might see markers of nuclear degradation inside the muscle, but the nuclei that incorporated inside don't disappear.

And Christine Gunderson has written a fantastic review paper on this, and essentially, he says that you can imagine that the number of nuclei inside the muscle is kind of like a temperature gauge on a very old-fashioned mercury thermometer where inside the thermometer when it gets hotter, the mercury

moves upwards and it will push the small floating temperature gauge upwards on the thermometer, and then as the temperature cools down, the mercury will slowly fall back down, but the temperature gauge that was resting at the high temperature will remain.

And the same way you can think about the myonuclei accumulate through time. Muscle fibers shrinks as breakdown outweigh synthesis and myonuclei then remain, and then those residual myonuclei can then be used to facilitate muscle growth later down the line. And so it can be the speed of growth upon restimulation is faster than the speed of growth in the first instance, and ultimately, that seems to make sense from an evolutionary scale because if you were to accumulate muscle mass seasonally and then lose all of that muscle mass, it would make sense that it would be easy to then reacquire that muscle mass upon a second occasion as opposed to have to reacquire that muscle mass slowly through that first time and that's kind of makes sense that you can retain this, this, this memory of Myonuclei being accumulated into the muscle to enable second phases of growth to be faster than first phases of growth, and that would make sense from an evolutionary scale to retain that beneficial adaptation inside the muscle even if you're subjected to an environmental condition of atrophy such as low protein intake for during the year.

DANNY LENNON: And I really like that you have kind of laid out that clear mechanism for an observation that people have either made themselves or heard other people make or have seen talked about in other places where this idea of returning to resistance training after a period of the training whether that was through injury or just not doing any resistance training or low calorie/protein intake where someone has lost muscle over a significant period of time, that growth rate on returning to typically a more anabolic environment plus resistance training leads to greater or a faster growth rate in the

muscle than building that muscle in the first place.

And so with this understanding of essentially this myonuclear domain theory, where does the discussion around anabolic steroids come in here? And why is that being something that you want to examine within some of the trials you're running?

ALEX KOLLIARI TURNER: So anabolic steroids are involved in this topic of discussion because they have been demonstrated both in animal and human models to bind to their receptor, the androgen receptor inside these satellite cells and cause the satellite cells to proliferate and then subsequently donate their nuclei into muscle fibers. And so anabolic steroids work throughout the body by binding to this -- the androgen receptor, and once they've done that in satellite cells, myonuclei accumulation will occur. And so someone is conducting resistance exercise and they are getting that stimulation for satellite cells donation of myonuclei in addition to the donation of nuclei from anabolic steroids, then muscle growth will be enhanced. So anabolic steroids both cause myonuclei accumulation to result in muscle growth and satellite cell proliferation, but they will also cause muscle growth just by stimulating protein synthesis inside the muscle itself. So the nuclei that are already present will be making more proteins and then after you taking steroids should increase the number of nuclei inside the muscle fibers as well.

And there was a study that was conducted in 2013 out of Christine Gunderson's lab by the Ingrid Egner is the lead author on that study, and they exposed mice to anabolic steroids for two weeks. It was only testosterone propionate and they observed a 66% in the increase in the number of nuclei and a subsequent 77% increase in fiber cross-sectional area.

And then they removed the drug, which was a pellet, testosterone propionate pallet for three

weeks and also for three months in separate mice cohorts, but in both of those time frames, they realized that and they observed that fiber size decreased in the group that was exposed to anabolic steroids compared to the control, and actually fiber size was exactly the same after three weeks and three months both in the control group and the group that was exposed to anabolic steroids, but the group that was exposed to anabolic steroids had much higher myonuclei numbers.

And then when they exposed both of those groups to a hypertrophic stimulus, they discovered that the myonuclei rich number muscles of the group that was previously exposed to steroids over a six-day period had a 31% increase in muscle growth whereas the control group did not grow significantly in that six-day period. And then when they remeasured the muscle mass of 14 day period, the control group had gone up in muscle mass, but the previously treated group of steroids had gone up even more and actually was 20% higher than the control group.

So this mice study shows that anabolic steroids incorporate nuclei. They cause an increase in fiber cross-sectional area, but then when you remove anabolic steroids and the period of atrophy happens and this hypertrophic stimulus is removed, fiber cross-sectional area will decrease, decrease to the same level as a control, and then when reexposed to a hypertrophic stimulus, the muscle will increase in size at a much faster rate because it has more myonuclei in it compared to a muscle that has a low number of myonuclei inside.

And so, essentially, we have an animal model there that shows that muscle memory mechanism occurs through anabolic steroid exposure, but we don't have a human model, and that’s sort of where our research is trying to come in because there's only been one study that's looked at previous uses of anabolic steroids where they biopsied both the trapezius

muscle and the vastus lateralis in the leg, and it was conducted in Umeå University in Sweden by a character called Anders Eriksson and Fawzi Kadi was also involved in this research.

And they found that people who had taken anabolic steroids had elevated numbers of myonuclei within the trapezius muscle, and this is where some very interesting findings were discovered because they biopsied guys that didn't lift any weights and roughly 20% -- 30% of their nuclei expressed the androgen receptor and 50%, sorry, 50% of their nuclei were expressing the androgen receptive guys that don't lift any weights, and then if you lift weights, the nuclei in your trapezius muscle that express the androgen receptor goes up to nearly 70%, and if you are currently on steroids, it goes up to nearly 80%.

So within the trapezius muscle, there seems to be this effect where if you take anabolic steroids, you increase the number of androgen receptors you have inside that muscle, so it becomes a great propensity for growth. And then they found out that in individuals who used to take steroids many years ago actually had and retained these highly elevated numbers of myonuclei. And so it seems to be that steroids do in humans cause nuclei to accumulate and those nuclei do then not disappear. That seems to be what we're seeing so far in the literature.

DANNY LENNON: At least for muscular hypertrophy, someone that has taken anabolic steroids at some point is going to always maintain an inherent advantage over at least themselves an ulterior universe where they had not taken those steroids because they've been able to accumulate more nuclei than they otherwise would and even going through a period of atrophy because of that increased number of nuclei future efforts to gain muscle will allow them to build more than they otherwise would. So that inherent advantage of these hypertrophy will extend forever for that -- for

that person beyond the time course of just taking the steroids.

ALEX KOLLIARI TURNER: That's what we would hypothesize, but we don't know in humans what myonuclei decay rates are [indiscernible 00:24:06] because in mice it seems to be that once the nuclei get incorporated, they do not disappear, but in humans we don't have the evidence yet to conclusively say that once a nucleus is incorporated from steroid usage that it then doesn't subsequently disappear for a time. The only evidence we have is the observational evidence from Kadi and Eriksson's research that past steroid users are indeed retaining high numbers of myonuclei.

So, and there seems to be the trapezius muscle is one of the -- is a good muscle to be able to use as an experimental model in humans because we know that it accumulates myonuclei in steroid users. It has a high proportion of those nuclei expressing the steroid receptor. So if we could observe in steroid users after they have finished a steroid cycle how much nuclei do you have inside your muscle fibers and then re-observe them months down the line, if they're still retaining those high numbers, then at least in the timeframe that we've observed, you could say that the nuclei incorporated from steroids remain and do not go down.

But Gunderson in his review paper makes a point that there is very little research in humans who are trying to determine myonuclei decay rates and he thinks that a nucleus could last for roughly 15 years as there was some research done where they were looking at radioactive markers from people that had been exposed to the nuclear bombs from World War II and he believes that because from that research of looking at the exposure to nuclear biomarkers they think that potentially 15 years is how long a myonuclei could be retained for in someone's muscle, but we don't -- we don't know that for sure.

And that -- that's why in our research the plan is that once we're going to biopsy people soon after they've concluded a steroid cycle and they are not going on testosterone replacement therapy, they are going to be going through post psychotherapies, so they would be convert -- they would be reverting back to kickstarting and utilizing their internal testosterone production so there is the anabolic stimulus of anabolic steroids has gone away, and then we will biopsy them soon after that and then biopsy them again 4 to 5 months later to see how much muscle mass have they lost or retained, and what's happening to the nuclei inside the muscle fibers. Are they going down in any -- in anyway?

DANNY LENNON: Maybe now is a good time to actually jump to the study you are actually carrying. I know you've given some information there and from, from an overview level just to really clarify for people, can you maybe remind us, number one, of those groups that you're looking to recruit for why you are picking those specific groups and really the question you're trying to answer through this?

ALEX KOLLIARI TURNER: Yes. So we -- we've already had one year of sampling and we've biopsied 12 participants and we are in 2019 now aiming to biopsy a lot more. We have dates scheduled on 16th and 17th of March 2019 and 13th, 14th, and 15th April 2019 where we will be doing more biopsies and so if you're interested in this research, you can come and be involved. And we are looking for sedentary controls, the guys that don't lift any weights, guys who are not exposed to performance-enhancing drugs and have never taken performance-enhancing drugs or self-declare that and they train at least cumulative resistance training of 8 hours regularly within a week.

And then we're looking for guys who train that same amount except they are exposed to anabolic steroids and they have been exposed

at some point in their life for 20 weeks, and they will be coming to us. When -- when they come to be biopsied, they would have finished a steroid cycle. So if someone is in this -- in the next couple months finishing a steroid cycle and planning on going on, on to post psychotherapy, the people we've been in discussion with tend to be guys that want to regain their fertility to get their other half's pregnant, then that's the kind of prime candidates that we're looking for or if someone in the past four, five months has finished a steroid cycle and they're not on testosterone replacement therapy, they no longer gotten analytic stimulus exogenously, then they can be involved, and then that group would then be invited back for sampling 4 to 5 months time to see how their muscle fibers have changed.

And then, additionally, we to sort of extend that group, we're looking for guys who've taken anabolic steroids 2 to 4 years ago, and they've exposed themselves to 20 weeks worth of usage, and they have not been on since to then see how much are their nuclei numbers because then we can sort of get another group who've taken anabolic steroids quite a while ago, and if they're still retaining a high number of myonuclei compared to other groups, then it adds to evidence of may be myonuclei numbers that accumulate from steroids usage do not decline in humans through time and that's -- that's the fundamental question is we know anabolic steroids accumulate myonuclei, but the question is do they go down while people go off anabolic steroids over short time frame, so that's the four months returning group and then over a long timeframe guys who took 2 to 4 years ago.

DANNY LENNON: It's so fascinating, and I think it's an amazing opportunity for people that find this discussion interesting to get involved in actual real cutting-edge science. So it's something that--

ALEX KOLLIARI TURNER: Yes. I mean, we will reimburse people's travel expenses. So I've been in contact with some

guys who were in Scotland and, you know, they -- we might be able to work something out for them to come and visit particularly if you are current -- if you have recently finished a steroid cycle or if you -- if you have took steroids 2 to 4 years ago, then definitely they can come and get involved.

And I would say that we focused heavily here on a discussion on myonuclei accumulation and decay rates because that's fundamentally the biggest thing we're looking at, but once we have the -- the muscle tissue, we'd actually be storing some of it in a genetic preservant and that genetic preservant will preserve all of the RNA inside the muscle and also preserve all of the proteins and the DNA. And so we would be able to look at muscle transcripts. So what transcripts have been expressed both mRNA and then all other lovely other types of RNA, small interfering RNAs and etc., but also would be able to look at what proteins are all or the differences in the proteins inside the muscle of these guys, and then also by preserving muscle nuclear DNA, we would be able to look at the epigenetic markers and -- and no one's looked at gene expression as far as I know inside a human who is using steroids. So that's particularly interesting.

The group that looked at previous steroid users I mentioned from Anders Eriksson's group in 2006, they actually found that guys that had previously taken steroids actually expressed 80 different proteins inside their muscle fibers compared to the guys that were not exposed to steroids and that's particularly interesting because there's, you know, what proteins are different inside the muscle fibers of guys who have taken steroids compared to not and we will get a better understanding of just how anabolic steroids cause muscle growth by looking at their transcripts inside the muscle.

And there was one very interesting study conducted in mice last year by Rossetti, a group in the States, and they had a group of mice

where they exposed them to weekly Nandrolone decanoate injections. So that's an anabolic steroid, and they inhibited at the same time the mTOR pathway with Rapamycin and they discovered that even if you inject a mice with Nandrolone and you inhibit the mTOR pathway, which is regarded as a master regulator pathway for protein synthesis, the limb muscles, so the legs of these mice still grew in size. So somehow steroids is increasing muscle mass. Nandrolone seems to what we have there evidence is that Nandrolone increases muscle mass inside mice independent of the mTOR pathway.

So there could be a redundancy system that anabolic steroids can increase muscle mass outside of just this master regulator and protein synthesis and that group hasn't published anything yet to follow up on that study because that's very interesting finding particularly, I mean, that's just not only is interesting if you're -- if you're taking steroids and want to know more about how they mechanistically cause muscle growth, but that's got implications for sarcopenia populations as well. So, I mean, that's finding that would be very interesting to pursue further once you have the transcripts of guys who've very recently finished steroids and so we're just going to get better understanding of what anabolic pathways are upregulated at the molecular level, and how they then decline through time.

DANNY LENNON: Yeah. It's so cool. There's so much going on and actually, I have some questions about some of those things you mentioned particularly as they may relate to enhancing our knowledge in certain other areas, but just to clarify for may be people listening who are based in the UK are eligible for one of those groups you mentioned and want to get involved with this cool project. Number one, what is the kind of like time requirements that you need them in the lab, any sort of things in terms of reminder of the dates that you want them there and then

anything else that need to be aware of how to get involved and best way to contact and set that all up?

ALEX KOLLIARI TURNER: Yeah. So this is happening in the University of Brighton's Eastbourne campus because that's just where the Sport Science Department is based. The postcode is BN20 7SN, and you would be required to if you control someone who is not exposed to anabolic steroids or previous user, you would only be required to visit on one occasion.

We would need you for roughly 4-ish hours and you'd come in, and we'd do the paperwork, obtain consent, describe to you again the aims of the study, everything you'd be subjected to on that day, and you then donate blood, urine, and saliva. We then measure your muscle mass. We've got three different techniques. We're just going to do all three and we're going to go through a bioimpedance scale, a [indiscernible 00:34:29] and some research grade skinfold calipers. And then you have a muscle biopsy of the trapezius muscle and then once that's -- once that's completed, you'll then be done.

So it's a relatively short-time commitment. Particularly, if you are -- if you are a current user, then you will have that once and then if you decide to remain off anabolic steroids, then you then come back in four to five months' time and have that whole procedure done again. And yeah, we can look into reimbursing your travel expenses, particularly if you are a current or a past user as those are the groups that we're most, most interested in.

DANNY LENNON: Awesome. And for everyone who is interested and maybe you're driving or something, that I'll link up all of this in the show notes, which I will talk about after the show, and please do if you have any interest in getting involved, go and do. It's a super exciting project to get involved.

ALEX KOLLIARI TURNER: Sure, Danny, the best way to get hold of me is I've put my email in the -- I'll give it to you and you can put in the show notes, my academic email. Send me an email if you're interested, genuinely interested, you want to take part, I'll speak to you on the phone shortly afterwards and we can arrange your visit for either the 16th or 17th of March 2019 or April 13th, 14th, 15th 2019. If you are listening to this, those dates have already taken place, and you still want to get involved, get in touch with me because we have new dates announced over time. Also if you just -- if you don't want to take part, but you are interested in helping out with the project, I've got an advert that can be put up in Jim's. We'll have to do that and I've also got an advert that can be shared on social media. So if you are interested in that as well, send me an email. I can send you some links where you can easily then share that and that will greatly help me out as well.

DANNY LENNON: Awesome. I encourage people to do that in the name of science. So please do. Alex, you mentioned something there about just a moment ago about some of this RNA transcripts and previous before we chatted, you talked about how some of these kind of [indiscernible 00:36:27] technologies may be used in potential testing protocol. So I'm interested to kind of open up the general conversation around drug testing and how some of this may play into that. So what some of the first things just to get everyone on the same footing that you would want them to know about essentially how drug testing works?

ALEX KOLLIARI TURNER: Anabolic steroids, when you look at the World Anti-Doping Agency publications of how many people get caught taking drugs with the existing drug tested sport, anabolic steroids are the most commonly detected drugs. So about of all the posters that happen about 50% of them are because of steroids and of that 50%, testosterone is the most commonly detected anabolic steroid. And you can think about

anabolic steroids being divided into two groups: the endogenous steroids that your body makes naturally, so that'd be testosterone, prohormones and the like, and then the exogenous steroids, which have been designed by pharmaceutical companies with the aim of disassociating the anabolic effect of steroids to the androgenic or masculinizing effect that usually results in disadvantageous side effects.

So those would be your oral steroids: Turinabol, Winstrol, Dianabol and some other injectables: Equipoise, Nandrolone, except Trenbolone and the like and there's many others. So I'm only just mentioning a few of them, but in testosterone drug detection, essentially, if someone was to take testosterone, what we try what -- the research what the drug testers are looking at is how it changes hormone ratios inside their urine.

So in the normal individual, they will excrete testosterone to an epimer of testosterone, which just means it's the same molecule, but there's just some inversion of some of its chemical groups called epitestosterone and that's normally excreting in the normal human about a 1:1 ratio, but if you were to take exogenous testosterone, it will increase the amount of testosterone that's getting placed inside your urine and your epitestosterone numbers that your body produces naturally by self will remain the same. So your testosterone to epitestosterone ratio will increase, and the World Anti-Doping Agency regards as if your ratio goes above 4 to 1, then you have tested positive for testosterone.

And to prove that testosterone inside your urine has come from an outside source, they'd then conduct a carbon isotope ratio test where they will essentially look at the carbon isotopes by combusting that testosterone found in your urine and there is a distinct isotope ratio for testosterone that has been come from a plant and testosterone that's come from a human. So if that testosterone found inside your urine has

an isotope signature of a plant, then that means it's being made in a pharmaceutical laboratory because most of these drugs are made from something called Stigmasterol, which comes from soybeans. And so it's going to have a isotope ratio of a plant and if that is found in your urine, then you're regarded to have used exogenous testosterone.

But what we're finding that and what was discovered about a decade ago was that there is actually a genetic differences in people in how they excrete testosterone in their testosterone to epitestosterone ratio because essentially testosterone, when you take it, it goes through first pass metabolism in the liver, which would deactivate testosterone and then second pass metabolism, which will add a chemical group to that testosterone to enable it to dissolve in water because it's a fat-soluble chemical and what we're finding is that there's genetic polymorphisms in the ability for people to add these chemical groups to testosterone to result it being placed in the urine.

And there is a class of enzymes called UGT enzymes, and particularly, we're interested in an enzyme of UGT2B17, and this is like most of the genes in your body, it was in two copies, and you know one copy came from your mother, one copy came from your father, and you find that some people can have both copies, so they're positive/positive for this gene; some people can only have one, so they're positive/negative, and then some people can have no copies of this gene, so they're negative/negative. And in these individuals that are negative/negative for this gene, they lack the ability to add this chemical group testosterone to put inside the urine and the studies have shown that after people have been injecting testosterone of 500 mg of testosterone in [indiscernible 00:40:55] single administration, which is quite a large dose, they actually end up only 40% of those people that they've subjected them to that single dose actually went above a 4 to 1 T to E ratio.

So many of those individuals will never get flagged by testing positive for testosterone because they actually never put the testosterone in the urine to go above the 4 to 1 ratio. So that's quite an interesting finding and from that it was realized that we cannot have a blanket population level 4 to 1 cutoff, and they've done studies now where they've looked at how geographically it varies by who contains this gene. So it seems to be that they've looked at the Scandinavian populations and Asian populations only, but it seems that it's a high likelihood in Asian populations that they lack both copies of the gene.

And so from that what is now done for endogenous testosterone doping is you actually look at somebody's individual T to E ratio through time. So someone that is registered as a testing athlete has urine test regularly, statistically, what they will do is they will say, what was your T to E ratio now? What was it and your previous drug tests? And if your T/E ratio ever goes above 4:1, then you're regarded as testing positive and they'll start to look at the carbon isotope ratios, but if you're one of these people who has a genetic polymorphism, your T/E ratio naturally might be 0.1 to 1, and if you then take steroids, it might go up to 0.4 to 0.5 to 1.

So you're never going to go above the 4:1 cutoff, but the deviation of 0.1 to 0.5 is still FIFO deviation, and so the statistical program will realize your T/E ratio has jumped up fivefold and that for you is consistently you're normally at 0.1 to 1 ratio through time and then suddenly it jumps up to 0.5, then that statistical deviation is too high for you given your natural own T/E ratio, and therefore you're regarded as being flagged as an anomalous result, and then that anomalous result would then be reviewed by research -- researchers in the field who will then look at your T/E ratios through time and if you're consistently for easy sake 0.1 to 1 for 10 months period and then you spike to 0.5 to 1

and then back down to 0.1 to 1 for another couple of tests, then that is an anomalous result. You've gone up fivefold. Yes, you didn't go above the population cutoff of 4:1, but for yourself the deviation is too much. So that would be flagged as an anomalous finding and then the isotope ratio test would then be conducted, and that would then confirm if exogenous testosterone was present inside your urine.

And so the idea was that can we add markers so that whole body of hormonal profiling is called the Athlete Biological Passport, and this is the steroid module. There's a whole separate module for blood doping with EPO and that's the hematological module where they look at changes in the number of red blood cells and immature red blood cells you have through time. But the steroid module looks at T/E ratio and a few other hormone ratios and whether or not you go statistically outside your own reference range, it's created for you based on the numbers that you consistently deliver through time and that's how we prove endogenous doping. Yeah.

DANNY LENNON: Yeah. It's super interesting just as you say that as we discover some more of the stuff that this nuance has brought into testing and we have to rethink how we're going to assess certain athletes to essentially trying catch some of these things in things that lie outside the fat part of the Bell curve for lack of a better term and--

ALEX KOLLIARI TURNER: Exactly, yes, yes.

ALEX KOLLIARI TURNER: It kind of reminds me of something that [indiscernible 00:44:38] are talking here of the recent Jon Jones case and many people listening will know obviously may be a huge MMA fan, I'm keen to get more of your insights on this because this is an area where we probably don't have a lot of answers and in time it may be one of those things where how certain findings are interpreted, and what they

mean, and how we go about analyzing things will change as we learn a bit more.

So maybe before I get your thoughts on that just to kind of get people on a similar footing, Jon Jones, UFC athlete who was flagged originally in I think mid-2017 for oral Turinabol, since served a drug ban for that, came back, was competing and in subsequent tests in recent times had been flagged for a long-term metabolite of Turinabol. And I'll let you get into the specifics of that, and this kind of whole crazy cases ensued where potentially even though one of these numbers as outsider or flags that something has been taken or is outside a reference range that potentially that doesn't mean that there is a new doping violation, and this is kind of a new ground at least for Turinabol.

So from an overview, what were some of your thoughts and can you maybe lay out some of the details for people that are as interested as me in this particular case?

ALEX KOLLIARI TURNER: Yes. So Jon Jones was taking -- well, possibly, he's tested positive for an exogenous steroid. So the way you identify that is by diagnostic unique metabolite inside his urine, and that metabolite can only ever get inside his urine because somehow he's ingested the parent compound. And he was tested and I've got the list up, Danny, actually found the list of how often he was tested. So December 8, 2015 he was negative. March 4, 2016 he was negative. April 4, 2016 negative. April 23rd, 2016 negative. June 20, June 16, 2016 he tested positive for Clomiphene, and he then was punished for that. And then December 21st, 2016 negative. February 2017 negative. April 4, 2017 negative. May 10 negative and this is then where the Turinabol comes into it because July 6, 2017 he was negative. July 7, 2017 he was negative. And then July 28, 2017 he was positive for 20 to 80 peak grams of the M3 metabolite.

And so Turinabol was discovered by Gregory Rodchenkov who was the mastermind behind the Russian anti-doping scandal in this movie -- and the star of the movie Icarus on Netflix where they discuss the Russian anti-doping scandal. He discovered this long-term metabolite of Turinabol called the M3 metabolite that they say in their literature could stain someone's urine months afterwards someone has been exposed to the drug. And what happened was Jon Jones was regarded as somehow ingesting Turinabol from some source and was therefore, he did end up, he didn't fight for two years. So the end of 2017, he didn't fight till the end of 2019. Sorry. It's a whole year, 2018.

But what was happening with Jon Jones is that they keep on finding reoccurring amounts of these picogram amounts of M3 metabolite in his urine and he would test negative, and then he would test positive for a very small amount of the M3 metabolite. Test negative again and then test positive again for the picograms of M3 metabolite and then negative again. And there is no literature where somebody has been given oral Turinabol, and then we've looked at the excretion dynamics of this long-term metabolite. The research is only ever come from looking at the urine of athletes who have declared to have taken Turinabol or have other short or medium term, long term metabolites inside their urine.

And Jon Jones for some reason seems to be sporadically excreting these long-term metabolite in a pulsatile matter and there is evidence from other drugs, other chlorinated drugs, particularly Clomiphene that if somebody -- this is a dose administrated study published where if you give somebody the drug regularly over a period of a few months and then take it away as you then look in the following six months, they will excrete sometimes one day in the urine they will excrete in the picogram amount, and in the following day there might be none. The

following week there might be nothing. Following three weeks there might be nothing. And then they will suddenly excrete even more picogram amount of the -- of the Clomiphene metabolites than they did previously. So it goes up and down in the urine through time.

And the authors of that paper seem to believe it's because there is some sort of fat storage of this molecule going on and it's being pulsatile and excreted in a sporadic manner. You also have to think about people's individual excretion dynamics of drugs is going to be different based on their genetic background like we've already seen with testosterone, and so what we really need is a dose administration study where somebody is given Turinabol to then observe both the excretion dynamics of short, medium, and long-term metabolites months after they've not been exposed to the drug anymore to see if it is indeed excreted in a pulsatile manner.

And that's being conducted right now as we speak by the World Anti-Doping Agency, and I'm sure maybe next year the data will be published, but because Jon Jones has never excreted a short or a medium-term metabolite and is only ever excreted the long-term metabolite in this pulsatile sporadic manner where you find in his urine a few days, a few weeks later you don't, and then a few days, a few weeks later you do, that is regarded that he is storing the long-term metabolite in his fat cells and that is being released close to a fight because of the weight cut and he may well have some unique excretion pattern based on his genetic background anyway.

But we don't have enough data to be able to know how humans excrete metabolite, but that given the evidence of never finding a short or a medium-term metabolite, and the evidence from Clomiphene being excreted in a pulsatile manner similar to the dynamics that you see with Jon Jones is why the experts that have reviewed this case the UFC have independently

employed have decided that he is not being re-exposed to a new and he has not been had a new exposure to the drug. It's all because of the exposure that occurred back in July of 2017.

DANNY LENNON: Yeah. And this is something I've talked to with a few people kind of online and to a certain degree have had some pushback in essentially making some of the claims that it seems unlikely at least that some of the more conspiracy type theories people have or just saying well, he is a [float 00:52:02] cheater and each of these recurrences is just proving he is taking something as opposed to show there is no clear evidence that this hypothesis that has been come up to explain why these picogram amounts are reoccurring. There is nothing definitively showing that, but again comes down to mechanistically that make some degree of sense. We don't have definitive--

ALEX KOLLIARI TURNER: Exactly.

DANNY LENNON: Conclusions either way. So it's a game of probabilities and my point to these people will probabilistically it would seem more likely that this kind of hypothesis of the -- this pulsatile effect and this long-term metabolite hanging around potential being in fat tissue and being released over time even though we don't know for sure that seems more likely than any of the reasons we could suggest that which he is continually doping and getting caught up for that because so far none of those explanations people have offered seem to make any sense either pragmatically, it would make, it would mean be like just the dumbest athlete ever or even physiologically some of the explanations aren't possible.

ALEX KOLLIARI TURNER: Because there's never been a short or a medium-term metabolite found in his urine and he's only ever been in this pulsatile excretion of this long-term M3 metabolite, it would make sense if you weigh out the evidence of the fat storage argument, that would explain his excretion dynamics of the

drug and other reasons to explain excretion dynamics of this drug always involve re-exposure to the drug, and by default, you would therefore witness short and medium-term metabolites of the drug because he is being tested on a nearly 3 to 2-week basis in some of these cases where two weeks he has no, no, nothing there. One week he then has picogram amounts of the M3 and then nothing the week afterwards or two, three weeks afterwards. So the fact that you're never seeing the short and the medium-term, only the long-term, fits the excretion dynamic idea of the fat cell storage and subsequent release and not to the idea of re-exposure.

But having said all this, we don't have enough data of actual giving Turinabol to people and witnessing excretion dynamics. So we are to explain the excretion dynamics we observe from Jon Jones left with excretion dynamics that we do observe from other drugs like the Clomiphene where you do observe this exactly same pattern and the reasoning behind that was the fat cell storage and release. But the fact that we're having research being conducted at the moment on giving Turinabol to people will help shine light on this because if that research and I hope they're doing this. They haven't probably published the research methods.

They're just saying this is what's happening, but if they're giving Turinabol to people, they then hopefully are then collecting their urine on a daily basis, weekly basis, monthly basis for as long as possible is feasibly possible for the study because if in all of the participants, then they collected the urine 4, 5, 6, 7, 8, 9, 10 months after their exposure to Turinabol, if not a single one of those participants is pulsatily secreting the M3 metabolite, then that would then for Jon Jones' results come across something very, very fishy.

However, if -- if you do find in those people that they are excreting the M3 metabolite in a pulsatile manner, then you then have direct

evidence that indeed this does happen in humans, but they -- because the drug testing becomes so precise with this picogram amount and because it's so difficult to do this research because ethically you can't give people anabolic steroids, it's very difficult to get the ethical approval to do that that we just need to wait for this WADA research to be published, but you see what I am saying that how like if we do find that is secreting in a pulsatile manner, then that's exactly what was observed in Jon Jones are true, but if in none of those individuals there is no pulsatile excretion, then something else is going on with Jon Jones.

DANNY LENNON: Right, yeah. We'll get at least an answer to that hypothesis that's been put forth and people will always come back one way or another.

ALEX KOLLIARI TURNER: Yes, but given the current data, that's the best guess we have is actually fat cell storage and release.

DANNY LENNON: Yeah. That's our best working hypothesis to explain why we're seeing this, but it's just -- it's just fascinating to see in real-time a case like this where something completely new that we can't fully explain is coming up, and it's just interesting to me to see people try and work through this and going to observations and trying to come to hypotheses and whilst this is all playing out with someone's real kind of life and livelihood on the line as well, it just adds to the bit of drama.

So, Alex, we're -- we're running close to time where I want to wrap up here. So before I get to the kind of final question, again, where can people find you online if they want to keep up-to-date with anything that you're doing with social media, any of the work you're doing, maybe also a reminder of where they can contact you, where is the best places for them to go on the Internet?

ALEX KOLLIARI TURNER: So best place to find me would just be to basically email me. So look in the show notes

for my email address. If you're interested in taking part, definitely do email me. If you want to just help share the advert for the study, then email me and I can provide you the premade Facebook post or Instagram post that can be shared. I can provide an advert that can be put up in Jim's. And we're recruiting people now because we have sampling dates March 16, 17 and April 13, 14, 15, and where you will be able to come and visit, but if you are listening to this podcast after those dates just happen, still get in touch because we always have new dates, and even if you can't make those dates, do get in touch, and I am happy to share more information of the study with you over email and also the ability for you to share online.

But one thing, Danny, I did want to touch upon why we entered into the podcast is obviously some people think to talk about steroids is rather controversial, but I think I hold the opinion and the most people also hold the opinion of if you're competing in a sport that does drug testing where you are breaking the rules if you take anabolic steroids, then obviously, that's not -- not good, but if you are competing in a sport in which there is no drug testing, and if you're not breaking any of the laws inside the country that you reside, and you decide to take anabolic steroids, then that is a lifestyle choice.

And we can -- we see in the scientific literature that and we know some anecdote and you can read published research by people called Harrison Pope and Gen Kanayama, and they will say, and we know from national surveys that have been conducting recently that the biggest demographic of individuals is using steroids at the moment especially United Kingdom is young men, and it's on the increase. And more and more guys are turning to use anabolic steroids not because they are doping in drug tested sport, not even because they are competing in untested bodybuilding or untested power lifting, but because they decide to make that lifestyle choice because they want

to increase their muscle mass. And if that's the decision they'd decide to make, then that's their own choice.

And I think the best thing we can try and do for these people is try and provide harm reduction surface where they can gain more information and education about how the drugs work inside their body in addition to what they can do to mitigate the negative health consequences of using the drugs because Gen Kanayama and Harrison Pope have published research where estimates that they have from American surveys is that 30% of men who take anabolic steroids become dependent on the drugs and that is not addicted in the sense where they have to, you know, search for their next injection to get high, but they become dependent on the drugs because they once they go off the drugs, they have a low testosterone environment, and they have shut down their own internal testosterone, and if they are not educated well enough on how to handle that environment to restart their own internal testosterone, then they find it very difficult because of the symptoms of low testosterone that experience they want to continue using the drugs, but also they might not want to go off taking anabolic steroids because of the loss in muscle mass that could occur.

And anabolic steroids do have side effects and the issue is the longer the people take them and the higher the doses that they expose them to, the high likelihood that they are going to have mitigating health consequences because of them, and so the anabolic steroids will affect their cholesterol profile. It would increase their bad cholesterol, decrease their good cholesterol, it will increase their red blood cell count and the hematocrit, and make the blood thicker, which could result in kidney damage and also increase their liver enzyme values, but all of those marketers of those detrimental biomarkers that can get elevated on anabolic steroids are just increased the people take long dose -- large amounts of anabolic steroids or

the long periods of time, and if participants who -- people who decides to take steroids, they are not getting their bloodwork done to monitor these markers. They are increasing the likelihood of having health damage because of the decision they've decided to make.

So I think as a society now we should try and adopt a harm reduction practice for these people because people are going to be taking anabolic steroids anyway, and if it is on the increase, the best thing we can try and do is provide an education service to these people so they know how to take them safely if they decide to do that and also provide them with the ability to monitor their health while they are doing it so that if any negative health biomarkers are elevated, they are then consciously aware of that and can make the subsequent correct mature decision to reduce those negative elevated biomarkers.

So an ability for a user to go to a clinic where they could not only get needles, but they could also get a cardio echogram, ECGs, they could get the bloodwork done to see what their cholesterol profile is, the liver enzyme profile is, the red blood cell count is and then someone could then interpret those results for them, and -- and provide them on evidence-based advice on how to go about reducing those negative biomarkers. So those -- these individuals are then unknowingly subjecting themselves to something that later down the line deteriorates their health because they've been on steroids for a very long period of time, they've never checked their red blood cell count has been elevated for four, five years and then they end up having kidney damage down the line because they didn't know whereas it'd be much better than they knew this information, and then they can make a choice about what they want to do moving forward.

DANNY LENNON: That's why I think some of the work that you're trying to do is actually extremely valuable for people who have been taking steroids or have

done in the past or even planning to in the future because it's just another piece of the puzzle that's giving answers to certain things that are going on and it's going to be of benefit to people who are taking anabolic steroids. So I think that's one part of that in addition to all the great things you just outlined there.

So, Alex, that brings us to the final question we always round up the podcast on can be to do with completely anything that you wish and it's if you can advise people to do one thing each day that would have a positive impact on any area of their life, what would that one thing be?

ALEX KOLLIARI TURNER: Think about five years ago now, I started to buy a small little diary that contains just a couple of lines for each day and basically at the end of each day or the following day or sometimes I just do on a Sunday, I just write a couple of sentences about what I did each day and now I can look back and I've got -- I can look back three years ago what I did on one day in the middle of June just randomly and it's quite interesting you can look back and see all these things that you remember that you did and have a record of events that you found enjoyable, periods of your life that were not enjoyable, and what was going on and why that was the case. So something I quite enjoy doing, and I will continue to do.

DANNY LENNON: That's awesome, man. I appreciate that and I appreciate taking so much time out today to chat through some of this and I found it extremely fascinating and it's -- it's been great to hear your insights and to put up with my questioning as well and thanks for being part of it, man.

ALEX KOLLIARI TURNER: No, thank you, Danny. I really appreciate it and if there's anyone out there who wants to either take part in the study, please just email me or if you want to help share the advertisement equally email me, and I appreciate very much being on it, Danny. So thank you for everything you do.