



Skeletal muscle as a protagonist in the pregnancy metabolic syndrome

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ABSTRACT

The pregnant woman normally shows clinical manifestations similar to a metabolic syndrome (MS), due to her metabolic and hemodynamic adaptations in order to share nutrients with the child. If those adjustments are surpassed, a kind of pregnancy MS (PregMS) could appear, characterized by excessive insulin resistance and vascular maladaptation. Skeletal muscle (SKM) must be a protagonist in the PregMS: SKM strength and mass have been associated inversely with MS incidence in non-pregnant patients, and in pregnant women muscular activity modulates metabolic and vascular adaptations that favor better outcomes. Of note, a sedentary lifestyle affects exactly in the other way. Those effects may be explained not only by the old paradigm of SKM being a great energy consumer and store, but because it is an endocrine organ whose chronic activity or deconditioning correspondingly releases myokines modulating insulin sensitivity and cardiovascular adaptation, by direct or indirect mechanisms not well understood.

In this document, we present evidence to support the concept of a PregMS and hypothesize on the role of the SKM mass, fiber types composition and myokines in its pathophysiology. Also, we discuss some exercise interventions in pregnancy as a way to test our hypotheses.

Background to the theory

Normal gestation demands metabolic and hemodynamic adjustments, in order to share nutrients between mother and child. Those adaptations represent a transient excursion into a kind of Metabolic Syndrome –MS– [1], a chronic condition related to physical inactivity and inadequate eating habits, which induces insulin resistance (IR), fat accumulation and arterial hypertension, increasing the risk of diabetes and cardiovascular disease [2]. The skeletal muscle (SKM) has a paramount role in those adjustments during pregnancy, not only as a store/consumer of energy through which the necessary IR can be achieved in pregnancy, but also as an important modulator of other systems' functions.

The role of SKM in insulin resistance in pregnancy

The aforementioned transient excursion is modulated by a crosstalk between the immune and the endocrine systems to increase depots during the first half of pregnancy, and to reach a relative IR in the third trimester. The immune modulation is beyond our scope but is extensively reviewed by PrabhuDas et al [3]. Of note, in the early

implantation high amounts of interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF α) are secreted by endometrial and immune cells recruited to the site of implantation, to control infections and to promote an adequate decidualization. In association with other interleukins and chemokines, they have a role in local angiogenesis to ensure a well perfused placenta, which will guide the endocrine part of that crosstalk.

The endocrine part of that modulation is mainly driven by insulin sensitizers or desensitizers, acting principally in SKM: during the second half of normal pregnancy, SKM decreases the insulin-mediated glucose disposal about 50% [4,5]. That IR in SKM can be induced by three ways: 1) Defects in insulin signaling: reduced insulin receptor, or ligand binding, or insulin receptor substrate 1 (IRS-1) tyrosine phosphorylation, or decreased phosphatidylinositol 3-kinase (PI3K) activation; 2) Defects in glucose transport, especially impaired glucose transporter (GLUT4) translocation; or 3) Defects in glucose metabolism: decreased glucose phosphorylation or oxidation, or impaired glycolytic flux or glycogen synthesis [6].

Insulin sensitizers in SKM: In the normal pregnancy the most important “defect” occurs in insulin signaling, partly mediated by a reduction in the action of adiponectin (AdpN), a protein synthesized in adipocytes, placenta and SKM [7,8] which act as insulin sensitizer

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through the activation of the peroxisome proliferator activated receptor (PPARG) and AMPK in both the SKM and the liver, inducing GLUT4 translocation and fatty acid β -oxidation [9,10]. AdpN receptors have been identified predominantly in the SKM (AdipoR1) and the liver (AdipoR2), although in humans they are expressed ubiquitously. In those receptors, hyperinsulinism may negatively regulate AdipoR1/R2 mRNA levels, via activation of PI3K and inactivation of Forkhead box, class O -Foxo1- [8,11], so the hyperinsulinemia positively feeds back IR in the SKM. Most of the studies conclude that seric AdpN concentrations decline slightly during late pregnancy, even in lean women [12] while some studies have found no change [13] or even a rise [14] in AdpN levels, which can be attributable to a resistance to AdpN, similar to that described in SKM in diverse cardiac or metabolic diseases [15]. A complementary mechanism to diminish the AdpN action is a down-regulation of its transcription in adipocytes [16] or in placenta [7] exerted by proinflammatory mediators, being TNF α one of the most interesting. Later we will be back on TNF α .

Another insulin-sensitizing hormone is leptin [17], which is produced by the adipose tissue, placenta [18] and SKM [19]. Its secretion peaks in the late second or early third trimester, as greater augmentations of body fat occur. It is well known that leptin causes satiety acting on the hypothalamus through its receptor OB-Rb. Less well known is that leptin causes fatty acid oxidation in SKM [20] and increases glucose uptake by activating sympathetic nerves and the β 2-adrenergic receptor expressed in myocytes [21]. Along pregnancy, the down-regulation of the OB-Rb receptor [22] and the elevation of the tyrosine phosphatase 1B expression [20] induces leptin resistance, and then reduces energy intake. This resistance could contribute to explain why leptin have been found increased in cases of MS as well as in hypertensive disorders of pregnancy [23].

Insulin desensitizers in SKM

The action of progesterone (Pg) and estrogens (E2) in SKM has been little studied, and not in pregnant women, but evidence points out to a dose-dependent desensitization effect: as Pg and E2 increase linearly along pregnancy, they contribute to incline the balance towards the IR in the second half of normal gestation. For example, in animal endometrial cells, low levels of Pg increase the expression of insulin-like growth factor-1 (IGF-1) but higher concentrations do otherwise [24], by reducing the expression of IRS-1 and inhibiting the insulin-induced GLUT4 translocation and glucose uptake [25]. In pregnant rats, estradiol increases insulin receptor binding as well as expression and membrane translocation of GLUT4 in adipocytes [26]. However, in late pregnancy those insulin-sensitizing effects seem to be offset by the reduction in insulin binding induced by Pg, cortisol, prolactin and hPL [27]. Complementarily, high E2 levels repress GLUT-4 expression in SKM [28].

The desensitization to insulin in SKM seems to be reinforced by “the prolactin family” (i.e., human placental lactogen-hPL- and human placental growth hormone -hPGH-), although both hormones may have insulin-like effects too, under not well elucidated circumstances [5,29]. They are produced early in pregnancy and increase linearly, peaking at 32–35 weeks [30,31], and activate metabolism, growth and proliferation through AKT, JAKs/STATs and MAPKs phosphorylation [32]. Early in pregnancy, hPL stimulates the growth of pancreatic islets, increasing insulin secretion [33]. hPL induces glucose uptake, oxidation, and incorporation of glucose into glycogen, facilitating accumulation in the mother in early pregnancy and during the fed state. In mid to late pregnancy, hPL stimulates 3H-thymidine incorporation, insulin gene transcription, insulin production and glucose-dependent insulin secretion in pancreatic islet cells; these effects may contribute to post-prandial hyperglycemia and hyperinsulinemia in the pregnancy [34]. On the other hand, hPGH increases the expression of the p85 α subunit of PI3K in the SKM, so in that way could act as a dominant-negative competitor by forming a PI3K heterodimer with the p110 subunit, thereby inhibiting PI3K activity, and thus limiting insulin signaling

downstream [5]. It has been proposed that hPL could bind to the growth hormone receptor, and that in that way it could induce IR [27].

An important insulin desensitizer for the present review is TNF α , a cytokine produced in white blood cells, fibroblasts, adipocytes and placenta. It impairs insulin signaling acting as a serine/threonine kinase of IRS-1 [34] and diminishing insulin receptor tyrosine kinase activity [35,36]. Interestingly, TNF α expression is higher during hypoxia/reperfusion of the placenta [37].

The ratio of the mentioned factors, acting mainly in SKM, results in that during the first trimester the insulin sensitivity is unchanged or even increased, but progressively decreases until reaching a minimum in the third trimester [31,38].

Roles of the discussed factors in the vascular adaptation during normal pregnancy

As said, not only metabolic but hemodynamic adjustments have to be done to share nutrients between mother and child. In this part of the review we depict how the listed factors interact with the vessel wall to adjust hemodynamics correspondingly.¹

In the vascular wall, insulin sensitizers tend to be vasodilators, while desensitizers seem to be vasoconstrictors: serum AdpN has a positive association with arterial vasodilation in healthy non-pregnant humans [39], and leptin has been found related to vasorelaxation during pregnancy [40], although no mechanism for neither of both has been established in the vascular wall. Pg and E2 contribute to pregnancy-induced vasodilation at normal or even low concentrations on human vascular smooth muscle, mainly via stimulating NO production and release through the action of their receptors [41]. On the other hand, hPL and growth hormone have shown *in vivo* vasoconstrictor properties in coronary, renal and iliac vessels inhibiting NO through adrenergic mechanisms in pigs [42]. Of note, a study showed *ex vivo* endothelium-dependent vasodilator effects exerted by each prolactin family hormone in male rat aortic rings, via NO and prostacyclin [45]. Those contradictory results could be explained by the different vascular beds, animal's sex and arterial preparation, but indicate that more research is warranted. As for TNF α , it enhances vascular contraction by inhibition of the endothelium-dependent NO-cGMP-mediated vasodilation, more in pregnant than in non-pregnant animal models [46].

During the first trimester there is fundamentally vasculogenesis, principally promoted by placental vascular endothelial growth factor (VEGF) and coadjuvated by fibroblast growth factor (FGF). VEGF

¹ To better understand what will be considered about the active role of SKM and exercise in pregnancy, it is necessary to take a glance at the basics: the vascular wall maintains blood pressure and an adequate perfusion to new territories through vascular reactivity (VR) plus angiogenesis. VR is controlled by the endothelium and the adventitia through vasodilator (i.e. nitric oxide –NO–, prostacyclin or β -adrenergic) and vasoconstrictor (e.g. angiotensin, thromboxane or α -adrenergic) ways. Under basal conditions, the endothelium is anticoagulant and vasodilator; if activated, the endothelium becomes procoagulant and vasoconstrictor (37). Early in pregnancy the trophoblast invasion lowers resistance in the placental bed by modifying pre-existing arteries and inducing local angiogenesis. The systemic vascular resistance (SVR) drops while cardiac output increases, in spite that the heart rate barely rises. As a result, blood pressure slightly decreases, reaching minimums around the week 25th, from which both systolic and diastolic values grow until getting antenatal conditions. Those changes give an extra flow to be distributed by the VR in favor of the new low-resistance shunt –the placenta-. To complement VR, the vascular wall adapts to pregnancy by vasculogenesis/angiogenesis, to create the mentioned low-resistance shunt. This adaptation is promoted by the action of hypoxia-inducible factor –HIF- in numerous hypoxia-responsive genes in placenta, boosting trophoblast growing and function [43,44]. It is important to say that placental vascular system shows a linear relationship between pressure and flow, but with an arteriovenous oxygen difference that is only a half of the maximum achievable, then it can supply very well the demands due to SKM exercise.

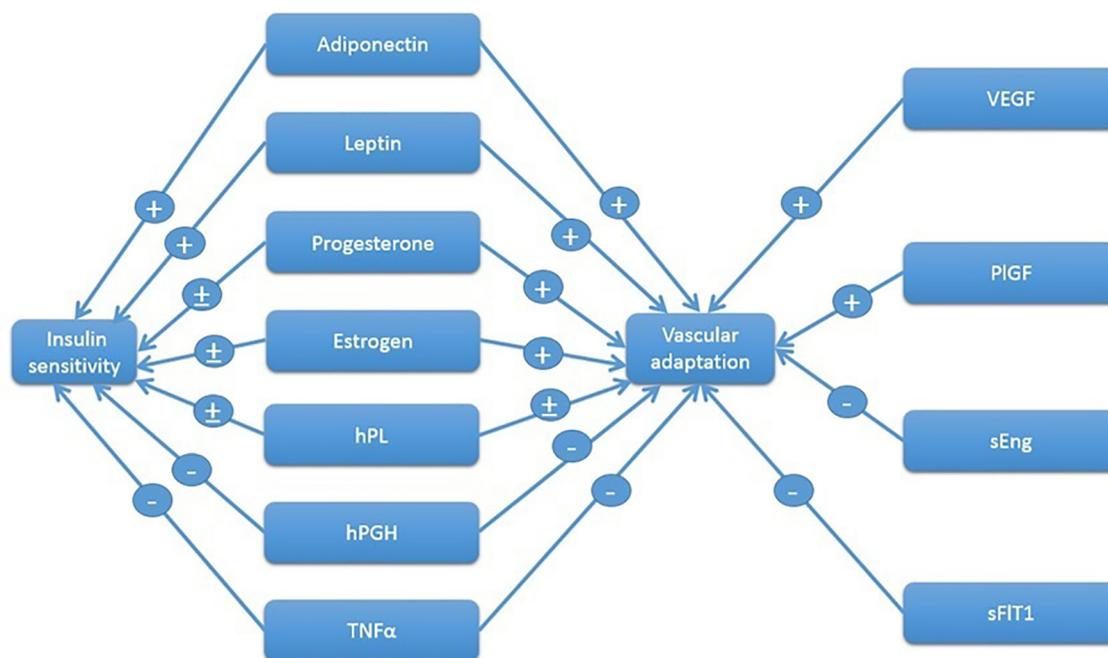


Fig. 1. A simplified model to see diverse factors affecting insulin resistance (IR, i.e., metabolic adjustments) and vascular adaptations in normal pregnancy. Under most of the conditions, each factor influences per se positively or negatively the adaptation. From a broader point of view, both adaptations are influenced by a balance of all the factors and their interactions, which are not depicted in the figure for clarity. The factors presented, although the most relevant, do not explain completely a good adaptation or a bad one to the pregnancy.

strongly stimulates placental endothelial cell proliferation, migration and tube formation [47] as well as stimulates placental artery endothelial production of NO [48]. Placental growth factor (PlGF) seems to play a synergy with VEGF for the formation of the vascular network with the development of the villous tree [49]. Towards the middle of gestation, progressive angiogenesis manifest, mediated by VEGF [50], FGF2 [51], PlGF [52], transforming growth factor- β 1 -TGF β 1- [53], PPARG [46] and leptin, which regulates angiogenesis through the OB-Rb receptor on endothelial cells, inducing phosphorylation of vascular endothelial growth factor receptor 2 (VEGFR2), which in turn induce angiogenesis [54] favoring placental development and function. Complementarily, it has been shown that hPL stimulates new capillary formation in vivo in the late-stage chick chorioallantoic membrane bioassay [55]. Of note, the placenta also produces anti-angiogenic factors, of remark soluble VEGFR1 (sFlt1) which is a receptor for VEGF, and soluble endoglin (sEng), an auxiliary receptor for TGF β super-family members [49].

Fig. 1 shows a simplified model to see the diverse factors modifying IR and vascular adaptation during normal pregnancy.

The Pregnancy Metabolic Syndrome is due to an excessive IR and a vascular maladaptation during pregnancy

If there are risk factors such as sedentary lifestyle or hypercaloric diet, the adaptations are surpassed leading to a pathologic IR and/or a vascular maladaptation where hyperinsulinemia feeds back IR, particularly in SKM, and in vascular smooth muscle it increases intercellular calcium and upregulates the angiotensin-1 receptor by posttranscriptional mechanisms [56]. Gestational diabetes mellitus (GDM), excessive gestational weight gain (EGWG) and preeclampsia (PE) are highly prevalent pathologies, the three are associated with undesired maternofetal outcomes, and share a strongly similar pathophysiology [57,58,59] and epidemiology. It is reasonable to think that the three are manifestations of a kind of MS characterized by a genetic predisposition, low-grade inflammation, IR, early weight gain and an excessive vascular response to vasoconstrictors developed during pregnancy, and

we pose to call this condition as pregnancy MS (PregMS). Analyzing the three entities as a syndrome instead of each one separately, could promote a better prevention, a more timely diagnosis, and a more complete treatment.

The insulin resistance and hyperinsulinemia may directly predispose to hypertension by increased renal sodium reabsorption [60] and reduced sodium excretion in the proximal tubule [61]. Moreover, the hyperinsulinemia stimulates the sympathetic nervous system, and IR and/or associated hyperglycemia may impair endothelial function [60]. Then hyperinsulinemia overthrows the insulin sensitizers: it downregulates AdpN, a fact which could contribute to explain the decreased values of AdpN in women with GDM and EGWG [62]. Some studies found AdpN values unchanged or higher in preeclamptic women than in normal pregnant [63] but that could be due to resistance. In addition, women with pregestational diabetes mellitus show lower E2 levels than healthy ones [64,65]. Similarly, serum levels of estradiol and Pg are significantly reduced in women with PE compared to healthy pregnant women [66]. Although Pg and E2 high levels in the third trimester of pregnancy could incline the balance towards an IR in normal gestation, the low levels in PregMS might be not a cause but a consequence of the syndrome. Similarly, in PE the placental gene expression profile of hPL is reduced, while in GDM the expression profile of hPL is diminished only in cases resulting in the birth of a large baby, but at the current stage it is not possible to differentiate whether the reduction of hPL gene expression is either causative or the consequence of fetal and/or maternal complications [67]. Of note, TNF α expression is higher in a bad perfused placenta, playing a key part to overrun IR or a vascular maladaptation until reaching pathological levels. Importantly, TNF α levels show a positive correlation with BMI and hyperinsulinemia in obese animals and humans; and as leptin, TNF α have been found increased in cases of MS as well as in hypertensive disorders of pregnancy [23].

Excessive BMI change during pregnancy is associated with an increased odds of developing gestational hypertensive disorders as well as GDM, irrespective of prepregnancy BMI [68]. That could be explained as the rapid gestational weight gain in early pregnancy may result in an

early increase in IR that leads to the “exhaustion” of the beta cell, then reducing the capacity of those cells to secrete adequate quantities of insulin to compensate for the IR induced by the progression of pregnancy, therefore leading to the development of GDM. Finally, there is an association between PE and development of GDM in a subsequent pregnancy. Therefore, while PE can work as a risk factor, PE itself can be a cause of GDM at the same time [69].

An altered profile of circulating free fatty acids is found in overweight/obese people; they can increase IR, and their correction to normal values is associated to a decrease in IR. Then, free fatty acids have been proposed as a key factor in a model integrating pathophysiologic events related to MS [70,71] including IR and hypertension [72].

The last point we will discuss, in which these three diseases are crossed, is hemodynamics. In PregMS an excess of maternal serum fatty acids and oxidative stress lead to production of oxidized lipids which cause inflammation and disrupts the endothelial function, the trophoblast invasion and the placental development. In addition, an augmented and persistent TNF α activity induces endothelial activation [37]. Those facts contribute to explain why VR alteration seems to precede the development of PE [73,74] so one can expect that early VR changes would not be restricted to PE but may be present in the other manifestations of PregMS. GDM is independently associated with impaired ventricular relaxation and systolic function [75] as it happens in PE where cardiac output is reduced. In PE additionally the plasma volume is contracted, while SVR rises due to an exaggerated response to vasopressors. The aggravated placenta might produce factors which cause an angiogenic imbalance (e.g., high sFlt-1 and/or low PlGF), increased oxidative stress (e.g., low total antioxidant status, high free radicals), and dyslipidemia [76]. Increased concentrations of the anti-angiogenic protein sFlt-1 and decreased concentrations of the pro-angiogenic proteins VEGF and PlGF are thought to damage the vascular endothelium and result in clinical PE [49]. It is well known that placental sFlt-1 and sEng are increased and PlGF reduced in PE, as well as placental tissue concentration of sFlt-1 and sEng are higher in PE and PlGF are lower in the placental tissue of women with PE [77]. sFlt-1 and sEng may act as non-signaling traps to impair pregnancy-induced adaptation in maternal placental bed vessels and contribute to systemic maternal endothelial dysfunction [78]. Increased levels of sFlt1 and sEng dysregulate VEGF and TGF β signaling respectively, resulting in endothelial dysfunction of maternal blood vessels [79,80]. Then, metabolic and vascular dysfunction underlies the pathophysiology of the three entities of the PregMS.

Statement of the theory

A hypothesis on a missing key piece in the pathophysiology of PregMS

According to the above discussion, the tissues actively involved in the pathophysiology of PregMS are the adipose, immune, pancreas and placenta, so the SKM would be nothing more than a passive store/consumer of energy and passive target of other molecules. However, during the last years there have been several lines of evidence suggesting that an active role of the SKM functions is missing in that model. Namely:

- (i) The mentioned molecules involved in the pathophysiology of PregMS can crosstalk with molecules produced by other tissues, up to now disregarded, which are involved in metabolic and hemodynamic regulation in humans under different conditions. For example, hyperinsulinemia during some periods of pregnancy may affect proteins secreted by the SKM such as musclin [81], which in turn may have an effect on the adaptations expected during pregnancy. Also, not necessarily all IL-6, TNF α , VEGF and AdpN found in PregMS, modulating IR or vascular adaptations, derive only from fat; these proteins are also expressed or released by SKM [8,82].

Other muscle-secreted proteins such as irisin modulate fat metabolism [83], and some adipokines regulate muscle metabolism, with specific impact on insulin sensitivity [9], so a crosstalk between fat and muscle is plausible. In fact, it was recently shown that irisin modulates TNF α and AdpN expression in obese mouse [84,85]. Then, large changes in adipokines seen during PregMS may affect muscle endocrine function, and vice versa. The crosstalk between molecules produced by the adipose tissue and the SKM may become more evident in the future. This leads us to propose that the molecular network involved in the pathophysiology of PregMS is more complex than the one known up to now, opening the possibility for some SKM-derived proteins to be active players in this network.

- (ii) Part of the relationship among maternal body composition, physical exercise and perinatal outcomes cannot be explained without taking into account an important role of the SKM. As an example, variables related to muscle, such as fat-free (or lean) mass and muscle fitness are related to perinatal outcomes. There is a positive relationship between maternal dominant handgrip strength measured at the second trimester of pregnancy and infant birth weight, which highlights maternal strength in pregnancy as a determinant of infant birth weight [86]. This would mean that the SKM may be a tissue mediating this “lean mass-fitness-perinatal outcomes” relationship. In this sense, SKM would not be only a passive tissue affected by other molecules, but a direct, active effector of the pathophysiological events of the PregMS.

Accordingly, from our point of view a missing key piece in the pathophysiology, treatment and prevention of the PregMS is the SKM. We hypothesize that, although largely unexplored in pregnant woman, the muscle mass, the fiber types and the myokines may be actively involved in the pathophysiology of the PregMS, because these three variables can modulate metabolic and hemodynamic aspects relevant for the PregMS, as will be shown below.

Description and testing of the theory

An active role for SKM mass, composition and myokine secretion in the PregMS

A typical muscle is made of multinucleated striated muscle cells, connective tissue, nerves, vessels and immune cells [87]. The study of dynamical and biochemical properties was the focus of most research on SKM until the end of the last century, but during the last 20 years the SKM itself was definitively recognized as a determinant of the health-disease relationship, beyond the role of exercise. This role emerged because of the bunch of complications associated to the reduction of SKM mass [88] and associations found between SKM mass and function with metabolic chronic diseases such as the MS [89,90,91,92]. Nowadays, the following lines of evidence point directly to an active role of the SKM in the pathophysiology of MS in nonpregnant women and of PregMS, through three variables: muscle mass, fiber types and myokines secretion.

i. Important role in glucose homeostasis: SKM is the largest tissue sensible to insulin. Both insulin and muscle contractions are the main stimuli for glucose uptake in SKM [93,94]. In fasting conditions and low insulin concentrations, SKM is responsible for about 10% of the glucose uptake in the body. However when glycemia, and then insulin, increase, SKM can deal with up to 70% of the glucose uptake in the body [93]. This effect is so important that alterations in glucose uptake in bulky, mainly oxidative muscles, i.e. quantitatively more important for the glucose homeostasis, such as those in the thigh [95], lead to IR in humans [96]. This highlights the role of the SKM mass and composition in the regulation of the postprandial glucose metabolism, something altered in chronic metabolic diseases, including those of the gestation, as shown in previous sections of this document.

ii. There is an inverse association between muscle mass and risk of developing MS: SKM makes about 35% of the body weight in a young, healthy adult woman. This value keeps constant or slightly decreases during pregnancy, since the weight gain at the end of pregnancy can be explained mostly because of the increase in several non-muscle maternal or fetus tissues [97,98,99]. Studies in several populations around the world have shown an inverse association between global or regional SKM mass and both IR and the risk of developing MS [89,90,100]. Also, low strength is associated with increased risks of developing MS [101]. To our knowledge there are no studies like this in pregnant woman, but in this population an equivalent relationship between muscle mass, strength and PregMS may exist as well. To test this, longitudinal studies evaluating the associations between regional and total muscle mass, as well as strength, with PregMS are warranted. Points (i) and (ii) highlight the importance of the amount of SKM mass in the pathophysiology of chronic metabolic diseases.

iii. A higher percentage of fibers type II in obese women: In humans, most of the SKM is composed of fiber types I, IIA and IIX [102,103]. Of particular importance to the present text is the fact that fibers type I and IIA are more oxidative, rich in mitochondria, with higher expression of GLUT-4, and have more ability to store lipids and more insulin sensitivity than fibers type IIX [104,105,106]. This gives to the fibers type I a metabolically healthy profile, compared to fibers type IIA and IIX.

A higher percentage of fibers type IIX and IR has been found in obese compared with lean women. Moreover, there is a positive relationship between insulin sensitivity and artery elasticity with fibers type I, but a negative relationship between fibers type I and blood pressure in adult women. Together, these results suggest a relationship among fiber types, race, obesity and IR [107,108,109,110], in which a phenotype with more fibers type II seems to be less metabolically and hemodynamically healthy in women. The measurement of muscle composition in pregnant women may be now possible by using non-invasive techniques such as proton magnetic resonance spectroscopy, as our group has proposed [111].

iv. SKM produces myokines: Myokines are proteins produced and secreted by the SKM under different stimuli, with autocrine, paracrine and endocrine functions regulating the metabolism of several tissues. Some myokines are very well-known molecules, such as interleukins, but others are new and largely unknown. These molecules contribute to explain the tight communication that SKM maintains with several tissues, such as fat, blood vessels, liver, bone, nervous system, and itself. In this sense, myokines seem to be molecular link between SKM and the adaptations to exercise. They may also be the link between SKM mass, composition and function with metabolic chronic diseases [82,92].

Then, we hypothesize that the myokines are directly involved in the pathophysiology of PregMS. To support our idea, we will discuss their metabolic (inflammation, IR, lipids and glucose metabolism) and hemodynamic functions (NO, blood pressure and angiogenesis), and highlight how they are directly connected to both MS and PregMS.

Irisin

It was discovered in transgenic mice overexpressing PGC1- α and was shown to reduce IR, hyperglycemia and weight in mice fed a high fat-diet [83]. After a large debate [112,113,114] its existence and function in humans was accepted. Its metabolic functions are mediated in part by inducing mitochondrial uncoupling in fat and SKM [83,115] and by increasing GLUT4 expression and mitochondrial content in myoblasts [115], then favoring a more oxidative, healthy phenotype in these tissues. In agreement, it is upregulated by different modes of exercise, both after a single bout or after training of moderate duration, in murine models, in healthy subjects and in patients with MS [83,116,117].

Irisin has been found reduced in obese patients, in which was also positively associated to their endothelial-dependent vasodilation dysfunction [118]. Further experiments showed that irisin increases NO

synthase phosphorylation and NO production in human endothelial cells and rats in dose and time-dependent manners [85,119]. Treatment of aortic rings of obese mice with irisin increased their NO-mediated relaxation [119]. These mechanisms may help explain the exercise-induced vasodilation and reduction in arterial stiffness seen in obese rats and humans [117,119,120]. This vascular impact of irisin could be also explained given that irisin improves the function of perivascular adipose tissue, which means a modulation of the whole vascular wall [84,85]. The administration of irisin to murine models reduced circulating levels of TNF α and reduced TNF α expression and superoxide production in aorta, so this myokine could be involved in the reduction of inflammation and oxidative stress [84,119], which could favor the irisin-induced improvement of vascular function. Then, it now seems fair to think that irisin has a beneficial vascular effect in different experimental models, including healthy humans and patients with chronic metabolic conditions.

In addition, irisin have been negatively associated with AdpN ($r = -0.4$, $p < 0.001$), and found increased in patients with MS, probably reflecting a resistance to its action or a compensatory mechanism to reduce IR in this population [91].

Interestingly, normal pregnant women have increased irisin values in their second and third trimester, while PE patients do not show those increased levels [121]. In the GDM manifestation of PregMS, measurements taken during the first trimester showed irisin levels significantly lower in women which posteriorly developed GDM, although that difference is lesser in the second trimester [122,123,124] and no statistical differences have been found in irisin levels in the third trimester [125] of women with and without GDM. As for weight gain, it has been reported a non-significant difference, less than 5.4%, in seric levels of irisin to the end of gestation between pregnant with less weight gain (from 7 to 15 kg) and pregnant with bigger weight gain (from 16 to 51 kg), although the study design did not permit to conclude if there was an overt EGWG [126]. Altogether, these results suggest an incapacity to produce irisin enough or a resistance state to this myokine at the same time that IR manifest in PregMS, which could also be associated to the lack of adequate VR seen in this condition.

Musclin: it is a 11 kDa protein which reduces glucose uptake and glycogen synthesis, both in presence or absence of insulin, in animal models and cultured myocytes [81], suggesting that it may induce IR. In agreement to this, musclin was later shown to reduce insulin-induced AKT phosphorylation in SKM [127]. High insulin activates AKT, which in turn phosphorylates FoxO1, producing a de-repression of the musclin gene, then linking the excess of insulin, typical of a state of IR, to a higher production of musclin, favoring a vicious circle that further generates more IR [81,128]. As expected if it were a myokine related to IR, it is upregulated by palmitate in myocytes [129] and is expressed predominantly in fast-glycolytic fibers [130], which are predominant in obese and diabetic patients [107] and have a kind of unhealthy phenotype, as shown before. Musclin is increased in murine models fed with a high-fat diet [131,132,133], but is reduced in serum and SKM after exercise [131]. Interestingly, musclin is also expressed in rat aortic rings in which directly favors contraction, which could contribute to explain the increase in blood pressure when applied in rats [134].

So far, all evidence points out to musclin as an unhealthy myokine. However, recent findings challenged that. First, musclin knockout mice show a reduced physical performance and low PGC-1- α , mitochondrial content and succinate dehydrogenase. It also shows more fibers type IIB in several muscles, compared to wild type mice [135]. Second, its intramuscular and circulating concentrations increase after five days of exercise in mice [135]. Third, our group found that in MS patients musclin is increased immediately after a single bout of High Intensity Interval Training -HIIT- [136]. Fourth, since the protein has a region with homology to the natriuretic peptides, which binds to NPR-C receptors, it increases ANP levels, which would lower vascular volume and blood pressure [135,137]. These results are unexpected if musclin were a myokine related to an unhealthy metabolic and cardiovascular

profile.

Then, several research groups have produced two different profiles of myosin: one in which is a central actor in producing IR, would favor hypertension and its concentrations are lowered by exercise, and a second in which myosin modulates physical performance and favors a more oxidative profile, being upregulated by exercise. Unfortunately, no papers have evaluated myosin in pregnancy, and in light of the above contradictory evidence on the function of myosin, it seems difficult to hypothesize about the behavior of myosin in normal pregnancy and PregMS. However, since some of the results showing myosin as an unhealthy myokine derived from knockout mice, they may be biased because of not well understood compensatory mechanisms. So, in our model, myosin could be considered mainly a non-healthy myokine which induces IR and hypertension.

Myostatin: it is a small dimer which inhibits AKT, causes IR and its expression is increased in obese women [138]. It is produced mainly by fibers type II [139] and works as an inhibitor of SKM mass, where it reinforces the glycolytic phenotype, and as a regulator of fat mass [138,139]. Low levels of its inhibitor, FSTL3, have been found in GDM and obese patients with PE [140,141], then favoring the function of myostatin, a probable factor involved in IR in these patients and in the whole spectrum of patients with PregMS. Higher myostatin levels were reported in women with PE [142]. These findings give this myokine the non-healthy phenotype.

Interleukin 6 (IL-6)

It was one of the first myokines identified. It is produced by both type I and II fibers, and increases glucose uptake and fat oxidation in SKM and hepatic glucose production during exercise as well as adipose tissue lipolysis [82,143,144]. Its pulsatile increase associated with exercise, opposed to the sustained, increased basal level associated to inflammatory conditions, would make the difference between the good and bad IL-6 [82]. In pregnant women around 12–15 weeks, a multi-variable regression analysis showed a risk to develop GDM [OR 1.87 (1.03–3.37) (95% CI)], associated to each 1 pg/mL seric increment of basal high molecular weight IL-6. In addition, when IL-6 seric levels exceed 2.58 pg/mL the associated risk to develop GDM increases 3.5 times [145].

Other myokines, such as chitinase-3 like protein 1 (CHI3-L1) and myonectin, which regulate metabolism and hemodynamics and are influenced by exercise [146,147,148,149,150], may also play a role in PregMS, with a healthy profile, but have not been evaluated in pregnant women yet.

After the above discussion, it seems fair to propose that the relationship among a given SKM mass, fiber type composition, and exercise-induced muscle contraction could produce a profile of myokines for non-insulin resistant, healthy, pregnant women, and a different one for obese, insulin resistant, unhealthy females with one or more manifestations of the PregMS. Then, normal pregnant women, in which the metabolic and vascular adaptations are the expected, may present the healthy profile of myokines compared with women with metabolic and vascular maladaptations, who develop PregMS and may have the unhealthy profile of myokines. All these myokines may crosstalk with the already known molecules (see Fig. 1) involved in the pathophysiology of PregMS, and then directly participate in its development and maintenance, or in its avoidance. In this sense, we propose a working hypothesis to explain the role of muscle mass, fiber types, and seven myokines, in the modulation of IR and VR as surrogates of metabolic and hemodynamic adaptations in normal pregnant women, as can be seen in the Fig. 2, or alterations in women with PregMS, as presented in the Fig. 3.

v. Exercise prevents or even reverts the alterations of the MS: Overweighed patients engaged in physical activities (i.e., activating their muscles) have a better cardiometabolic profile than those leaner (i.e., without the bad effect of fat) but sedentary. Different modes and durations of exercise have a beneficial effect on cardiometabolic

variables (VO₂max, blood pressure, IR, fasting glucose and insulin, total and visceral fat, etc.) in patients with chronic metabolic diseases [151,152,153]. The fact that exercise tackles MS, and the main executor of the actions and tasks performed during exercise is the SKM, support the idea of SKM as a protagonist in this kind of disease. The beneficial effect of exercise in the pregnant health and perinatal outcomes is well established and will be discussed below.

Exercise modulates myokines and PregMS outcomes

The exercise could be a model to test the hypothesis raised above on the active role of SKM in the pathophysiology of PregMS. Research about the effects of exercise in maternal/fetal health are not conclusive and even contradictory, due to high heterogeneity on intervention and prescription parameters [154], but in general aerobic regular training during pregnancy increases cardiac output, plasmatic volume and red blood cells counts [155], and causes an acute oxygen placental reduction which would induce adaptations in placental and fetal perfusion balance, probably augmenting nutrient delivery to placenta 24 h post-exercise [156]. As aforementioned, since oxygen arteriovenous difference can be doubled, during exercise uterine oxygen delivery would be maintained at homeostatic levels even if uterine blood flow were reduced 50% [47]. The maternofetal risk due to exercise is a very important concern. With appropriate safety measures, it should not be inconvenient to implement maximal tests in pregnant women [157,158,159].

A regular and guided aerobic exercise program is an early and effective alternative to prevent endothelial dysfunction in pregnancy [80,160] and to reduce the risk of adverse outcomes in both mother and baby, as SKM activity also moderates IR both in pregnant women and in the general population [161,162]. SKM strengthening in pregnancy is recommended to improve endurance without fatigue. SKM mass and strengthening could be protective in pregnant women since it has been inversely associated with the incidence of MS, independent of age and height [101,163], and it is safe to combine muscular strengthening with aerobic exercise, three times per week, 30 min/session, during the whole pregnancy [164]. Aerobic continuous exercise consists of several minutes without intensity changes, where the intensity can be low or moderate even high intensity (50–75% age-predicted maximal heart rate HR_{max}, or reserve HR) [65,166,167].

Pregnant women tend to believe that slight muscular exercise is safe while vigorous is not [168], but studies using low intensities usually find no effect on different outcomes, such as PE incidence, way of delivery, maternal complications, fetal growth restriction, macrosomy or prematurity [169,170]. Another study found an inverse relationship between energy expenditure (expressed as the metabolic equivalent of task -MET) and the risk of developing GDM: pregnant women exercising at more than 28 MET hours per week, reduce the risk of developing GDM by 31%, although this association did not reach statistical significance [171]. It is probable that those anodyne results were due to an insufficient stimulus to the pregnant woman, although maternofetal complications were neither elevated.

Exercise can modulate antiangiogenic/proangiogenic factors, and it seems to be related to intensity: after a three times per week intervention with bicycle (up to rating of perceived exertion (RPE) of 13 on the 6–20 Borg scale, maintained for 20 min) the pregnant whom did not exercise had lower PlGF concentrations and higher sFlt-1 and sEng concentrations than active pregnant women, while regular exercise during pregnancy is associated with higher serum PlGF and lower sFlt-1 and sEng concentrations in late gestation [80]. The reader can review above the part of “Vascular adaptation during normal pregnancy” to understand the importance of these factors in the adequate placentation, when a kind of muscle-endothelium-trophoblast axis works properly.

As for myokines and exercise in pregnancy, the effect of any intervention with exercise on the levels of myostatin or myosin in human pregnant women, healthy or with PregMS, is unknown, and severely limited in

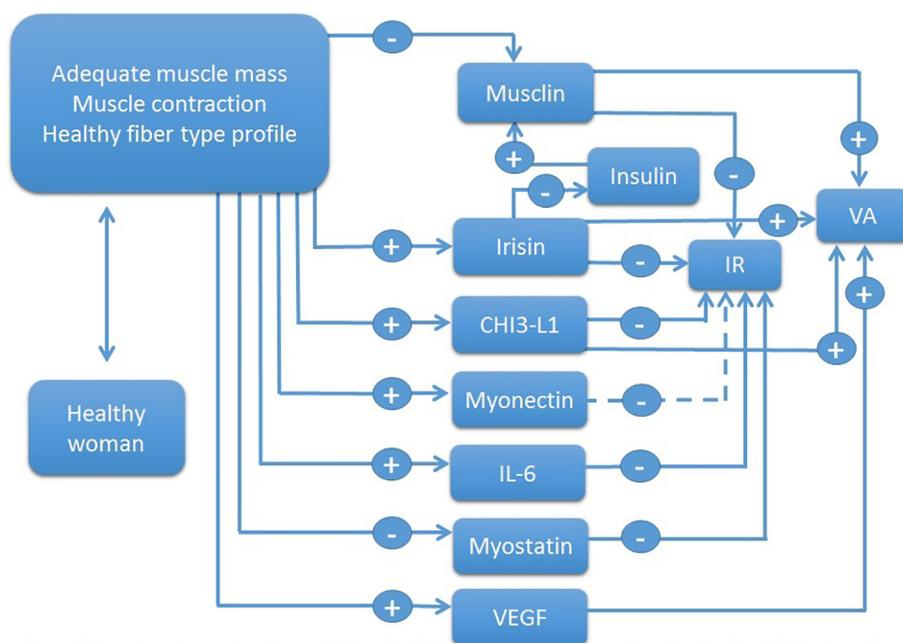


Fig. 2. Integrative scheme to show the role of skeletal muscle on insulin resistance (IR) and vascular adaptation (VA) development in healthy pregnant women. IR and VA are used as surrogates of metabolic and hemodynamic adaptations. A bidirectional relationship on the left means that muscle health impacts global health in the pregnant women and viceversa. Adequate muscle mass, a healthy fiber type composition profile and physical activity-induced muscle contraction regulate a bunch of myokines, whose increase (irisin chitinase-3 like protein 1-CHI3-L1-, myonectin, interleukin 6 -IL-6-, and vascular endothelial growth factor -VEGF-) or decrease (musclin and myostatin) avoids an excessive IR and favors the right VA. This reduces the risk of the appearance of pregMS, but allows the normal metabolic and hemodynamic adaptations expected during pregnancy. Dashed lines mean indirect effect through the reduction of circulating lipids, since a direct effect was ruled out.

the case of IL-6 and irisin, which will be reviewed in the following lines.

In overweight/obese pregnant women who performed vigorous physical activity from 15 to 32 weeks of gestation, IL-6 (and TNF α) levels were higher when compared to less active pregnant participants, and those levels were related to a lower early-phase insulin response (β -810.5 [95% CI: -1.524.5 to -96.5], $P = 0.03$). The authors concluded that there is an association between vigorous physical activity, insulin sensitivity and early-phase insulin response that could be partially mediated by IL-6 [172]. Of note, IL-6 was undetectable in a proportion of samples. Another study explored exercise and irisin at the end of pregnancy, including nine healthy pregnant with single fetus, evaluating between weeks 21 to 29 of pregnancy. The active group exercised at least 150 min/week, three times per week, of aerobic continuous exercise with intensity variations, combined with muscle strengthening. The non-active group showed a decrease of $20 \pm 13\%$ in serum irisin

levels, while the active women showed an increase of $15 \pm 28\%$. In addition, in the whole group of participants serum irisin levels correlated negatively with blood glucose and insulin levels, as well as with the percentage of glycosylated hemoglobin ($R = -0.922$; $P = 0.001$), ($R = -0.845$; $P = 0.004$) and ($R = -0.784$; $P = 0.012$), respectively [173]. Increased IL-6 and irisin levels in response to physical activity may help in pregnant women improve metabolic control and vascular function, and improve outcomes, giving support to our hypothesis.

Low intensity exercise results tend to depend on previous conditions more than high intensity exercise. For instance, recreational physical activity significantly reduced the risk of PE among Norwegian women with a pre-conceptual BMI below 25 kg/m^2 , but was not protective in overweight or obese women [174]. Conversely, overweight/obese women ($\text{BMI} > 24 \leq 28 \text{ kg/m}^2$), without health complications, whom initiate an aerobic cyclic exercise program from week 12 to 37 of

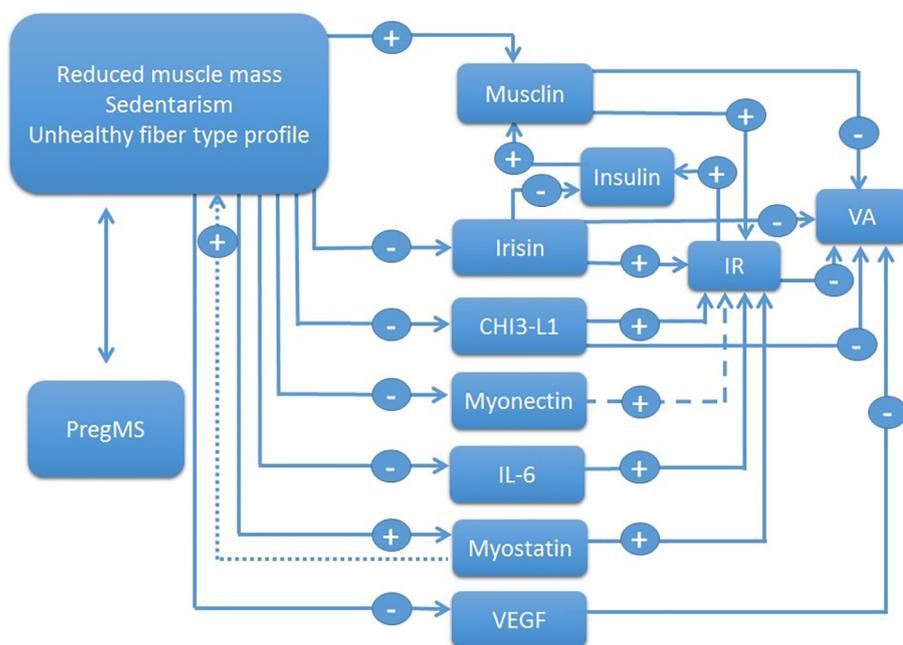


Fig. 3. Integrative scheme to show the role of skeletal muscle on insulin resistance (IR) and vascular adaptation (VA) development in pregnancy metabolic syndrome (PregMS). IR and VA are used as surrogates of metabolic and hemodynamic adaptations. A bidirectional relationship on the left means that muscle health impacts global health in the pregnant women, and viceversa. Reduced muscle mass, an unhealthy fiber type composition profile and a sedentary lifestyle regulate a bunch of myokines, whose increase (musclin and myostatin) or decrease (irisin chitinase-3 like protein 1-CHI3-L1-, myonectin, interleukin 6 -IL6-, and vascular endothelial growth factor -VEGF-) leads to an excessive IR and reduces the right VA. This in turn favors higher levels of insulin creating a loop to generate more IR, and less VA. This condition increases the risk of the appearance of PregMS. Dashed lines mean indirect effect through the reduction of circulating lipids, since a direct effect was ruled out. Dotted lines mean an autocrine effect which favors a glycolytic phenotype in the fiber types, perpetuating the unhealthy fiber types profile.

pregnancy, three times per week, 30 min/session with equivalent intensity to one Borg between 12 and 14 [167] or an aerobic combined exercise program with strengthening of general musculature, three weekly, supervised sessions of 35 min of moderate intensity walking/running until RPE of 12–15 on the Borg 6–20 scale, followed by 25 min of resistance training, showed a trend to reduction in gestational weight gain but significant in insulin levels at the middle and the end of pregnancy, compared with pregnant women whom did no exercise [175]. The latter study showed no effects on postpartum weight retention three months after delivery, but reduced circulating insulin levels, and authors attributed it to a higher proportion of women being active postpartum in the exercise group.

Another study, with slightly higher intensity (aerobic continuous exercise to 50% and 65% maximal cardiac frequency for 30 min per session, three times a week during 16 weeks) improved VR and the maximal VO_2 , without affecting weight gain in the pregnant [160]. Another study used an exercise program of 60 min per session, five days per week at intensities of 55%–60% of maximal VO_2 during the first trimester of pregnancy with subsequent reduction to 20 min until the end of pregnancy, and got better results in fetal growing than initiating with 20 min at pregnancy beginning, increasing up to 60 min at the end of it, probably due to a more functional placental capacity which improves fetoplacental growing [176]. Those positive effects in metabolic control and VR observed mainly when exercising at least at moderate intensity might be attributable to the regulation of the myokines. Low-intensity exercise might not be a good stimulus to affect muscle endocrine function. Further research to test this hypothesis is warranted.

On the other hand, high intensity interval training (HIIT) is a cardiovascular exercise strategy alternating short periods of intense anaerobic exercise with less intense recovery periods, typically lasting under 30 min. In obese nonpregnant adolescents HIIT has caused a better improvement in glycaemia and insulin regulation than low intensity exercise [161]. Vigorous aerobic exercise (95% of ventilatory threshold or 70% of maximal cardiac frequency during 40 min, three to four times per week), from week 16 to 38 of pregnancy, is equally well tolerated by active healthy pregnant than non-pregnant women with similar fitness, without increasing maternofetal risk [177]. Of note, if the pregnant has been chronically hypertensive or had previous PE, the low intensity exercise does not increase maternofetal morbidity [169]. Vigorous aerobic exercise up to 70% of reserve cardiac frequency, 40 min per session, three or four times a week from 16 to 38 weeks of gestation, have shown to improve sensitivity to insulin without inhibiting lipolysis to the end of pregnancy, suggesting that vigorous exercise would diminish GDM, PE and gestational dyslipidemia risk [177]. Diverse studies showed that cycling with aerobic continuous exercise combined with HIIT, was associated with a significant reduction in the frequency of GDM in overweight/obese pregnant women, a decrease in the gestational weight gain before the mid-second trimester and did not increase the risk of preterm birth nor reduced the mean gestational age at birth [165,166] and increases the enjoyment of exercise during pregnancy better than moderate intensity continuous exercise [178].

The participation in any kind of vigorous exercise (activities which induce more than 6 MET) impacts favorably the weekly mean weight gain from the GDM diagnosis until the end of pregnancy, and is associated with less probabilities of EGWG, when compared with no participation in the vigorous exercise [OR 0,63 (0,40, 0,99)]. In contrast, similar tendencies were not found associated with moderate intensity exercise. Those results can be related with the difference in total physical activity volume between pregnant women which did the vigorous exercise and those which did not [179]. However, another study did not find significant differences in the mean gestational weight gain between healthy pregnant with pre-pregnancy normal BMI, which did or not aerobic continuous exercise of moderate intensity combined with muscle strengthening, but women whom did not exercise had an increment on the percentage of body fat to the end of pregnancy, while

women who did exercise had an decrement on that percentage [180].

As a conclusion, regular exercise practice of moderate to high intensity during pregnancy can positively impact the mother-child outcomes with less cesarean deliveries, better mood, fitness -particularly cardiovascular- and fetal growing, as well as reduction of preterm delivery risk [176] and GDM risk [171,177,181]. We have shown above that there is evidence linking exercise and better perinatal outcomes, although the possible role of SKM mass, composition and myokines in mediating that effect has not been fully evaluated. The changes in myokines such as IL-6 and irisin are in the expected sense if they were protective against PregMS, as proposed (Fig. 3), and the lack of effect of low-intensity exercise could be explained by not enough SKM stimulation and no healthy myokines production. Then, it might be that pregnant women not practicing exercise or doing it at low intensity are missing the potential beneficial effects of SKM during their pregnancy, something that urges to be tested.

Conclusions and perspectives

GDM, EGWG and PE may be manifestations of a syndrome, which could be called the PregMS. From the clinical point of view, PE is evaluated looking for endothelial damage and hepatic, renal and/or hematologic function tests, while in GDM glycemia is evaluated and in EGWG the BMI is monitored. A syndrome could encompass the mentioned three pathologies, currently considered as individual, and this could promote a better prevention, a more timely diagnosis, and a more complete treatment.

Besides its classical functions, SKM is now recognized as a regulator of the metabolic rate and the metabolism of many tissues, including itself, mostly through its function as an endocrine organ. We have presented a model in which the mass of SKM, its composition and endocrine function, are added to several classical molecules involved in the pathophysiology of PregMS. SKM may play an important role, yet largely ignored, in PregMS development, prevention and treatment. Metabolic and hemodynamic adaptations/maladaptations function as attractors in our model. There is paucity in basic, clinical and epidemiological studies evaluating the role of SKM mechanisms to benefit pregnant women, particularly in molecular approaches to explain the eventual role if myokines of metabolism and vascular control during the normal pregnancy or the PregMS.

Since the exercise modifies the production of antiangiogenic/proangiogenic factors and myokines, and in turn, most myokines have shown endocrine functions, it would be possible that myokines mediate the proangiogenic role of exercise acting, in closed loop, with the endothelial and trophoblast cells, in a kind of muscle-endothelium-trophoblast axis. To prove it, it is necessary to measure the effects of myokines in cultured endothelial and trophoblast cells, on the production of sFlt-1, sEng, PlGF and VEGF, initially. The axis balance could be reestablished through exercise, but today there is a practically null research exploring the characteristics of physical training (frequency, intensity, duration, muscle group and type of exercise) that induces a physiologically desirable profile of myokines in the pregnant woman, in order to get better maternofetal outcomes. Clearly, it is important to develop a duly argued and controlled exercise program seeking the best impact for the mother-child binomial, with SKM in mind.

Conflict of interest

None declared.

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