Introduction:
Since the mid 1980s, a lot of attention has been dedicated to the importance of microcirculation; a part of arterial bed including arterioles, precapillary sphincters, capillaries, venules and arteriovenous shunts. It is a structure of decisive importance for an organism; in its domain an exchange of blood gases and metabolic products takes place and it contributes to thermoregulation. Mediation of vasomotor reaction and vasoarterial reflex maintaining a stable hydrostatic pressure is also an important function. Microcirculation is for its dimensions (capillary diameter approx. $5 \times 10_{-5}$ mm2, blood flow velocity around 0.4 mm.s-1) relatively difficult to access for more detailed examination, yet its impairments are very severe and dominate in many metabolic disorders. Microcirculation impairment is crucial in diabetes mellitus, where arteriovenous shunts open at the expense of nutritive bed due to a loss of sympathetic tone in peripheral circulation in diabetic neuropathy (Netten, Houben). Blood flow is therefore seemingly sufficient, but the affected tissue undergoes ischemia (warm ischemia). To what extent hyperinsulinemia contributes to this effect is not yet clearly known, one of the possible explanations may be a stimulation of sympathetic activity. Not a few studies are dealing with insulin's vasodilatory effect, however, with inconsistent findings regarding the extent of microcirculatory response at various insulin levels upon acute and chronic insulin administration.

Experimental work in rats showed an improvement in blood perfusion of sciatic nerve perineurium after a one-month insulin treatment, concurrently, amelioration in nerve conduction was proven electromyographically (Biessels). A short-term continuous subcutaneous insulin infusion (insulin pump treatment) led to an increase in capillary perfusion (Tymms) and to a decrease in venous oxygen tension in diabetic patients already after 9 days of treatment. This result suggests an existence of redistribution of skin perfusion favoring nutritive capillary bed.

Acute hemodynamic effects of insulin were tested in an experiment, where healthy young men were treated with a short insulin, locally administered into brachial artery with a rate of 1 and 5 mU/min for a period of 90 minutes in a double blinded study design. Blood flow was measured using body plethysmography. Higher insulin dose led to a statistically significant vasodilatation (20%) compared to placebo. Administration of insulin + L-glucose (metabolically inactive stereoisomer) did not produce further increase in vasodilatation in comparison with pure insulin administration. Administration of insulin + D-glucose led to an increase in perfusion in comparison with pure insulin administration (47% compared to placebo). Glucose infusion itself did not cause any significant changes in blood flow (Ueda). In diabetes type 1 patients when using different insulin infusion rates (1.5 IU/hr, 15 IU/hr) there occurred an increase in blood flow measured by laser Doppler flowmetry (LDF) at the low dose of insulin, while a decrease occurred at the higher dose (Tooke). These measurements, however, were not performed in steady state conditions during clamp examination. Conversely, no statistically significant changes in perfusion of skin microcirculation were found in an experiment in healthy volunteers, where skin perfusion was monitored during a three-step insulin clamp with gradually increasing insulinemia levels 60 – 500 IU/ml. Arteriovenous difference of glucose attained maximum value already at the lowest insulinemia, subsequently it remained constant (Utriainen).

An increase in perfusion measured using LDF was noted at supraphysiological hyperinsulinemia in anesthetized rats under clamp conditions concurrently associated with an increase in femoral artery flow (Vincent).

Some studies dealing with physiologic hyperinsulinemia (approx. to 50 mIU/l) proved an increased blood flow through muscle and skin microvascular beds and increased density of opened capillaries under this condition (DeJongh, Serne). They used LDF and video capillary microscopy for measurements. In addition, local skin administration of insulin using

iontophoresis has a vasodilatory effect in healthy volunteers, which diminishes with age (Rossi), was not proven in diabetes type 2 patients and is decreased in obese non-diabetic women (deJongh). An improvement in glycemic control leads to an increase in microvascular reactivity in diabetes type 2 patients (Forst).

To summarize the abovementioned findings; it is relatively well proven that physiological and even supraphysiological hyperinsulinemia leads to an increase in total limb blood perfusion. Study results regarding insulin influence on microcirculation vary; some authors (Utriainen) suggest an insignificant role of physiological as well as supraphysiological insulinemia, others (Tooke, Serne) observed an increase in perfusion only in physiological hyperinsulinemia. Furthermore, it is not completely clear, what part of the vascular bed contributes to the increase in perfusion; whether it is an increase in blood flow through nutritive capillaries or arteriovenous shunts.

Aim

To examine vasodilatory effect of insulin on perfusion of skin microcirculation in healthy volunteers and assess, whether this effect follows a linear trend with insulinemia.

Methods

Microcirculation was examined at rest and after stimulation by physiologically (50 mIU/l) and supraphysiologically (150 mIU/l) increased level of insulin. The examination was performed in 12 non-obese healthy volunteers with no history of diabetes in parents and siblings, with no chronic disease, with no chronic medication except hormonal contraception in women, matched in age as well as in basic anthropometric and biochemical parameters (see Table 1). Study protocol was approved by ethical committee of Medical Faculty in Pilsen, Charles University in Prague. All volunteers were fully acquainted in advance with the experiment and methods used, which they confirmed by signing an informed consent. The day before study commencement, the volunteers maintained an ordinary daily routine with the exception of heavy physical exercise, excessive consumption of saccharides, fats and alcohol, and last meal until 9 pm. The following morning, a two-step hyperinsulinemic clamp with target insulinemia 50 and 150 mIU/l was performed according to a well-established method (DeFronzo), i.e. rate of insulin infusion 2.4 IU/m2/hr and 6.0 IU/m2/hr. The order of insulinemias was inverted in one half of the sujects (i.e. first 6.0 IU/m2/hr then 2.4 IU/m2/hr). We measured skin perfusion using LDF and transcutaneous oxymetry, and respiratory quotient and energy expenditure by indirect calorimetry (V-max Sensormedics, Yorba Linda, CA, USA) according to a standard method (Weir et al.) at basal conditions and in both steady states. M-value of each clamp was calculated to assess the change in insulin resistance. Results in form of median and interquartile range were evaluated by Wilcoxon test.

Laser-doppler flowmetry

Skin perfusion was examined at basal conditions before the clamp and in steady state at both insulin levels. System Periflux 5000 (Perimed, Sweden) with PF 5010 probe emitting laser with a wavelength of 780nm and power output 1mW was used for the measurement. The probe was placed to a dorsum of non-dominant foot and measurement was performed in all subjects at stable temperature of 33°C. Subsequently, stimulation tests (Muller et al.) were employed – heating (probe heating to 44°C inducing maximal vasodilatation) and occlusion (3-minute occlusion of a limb using a sphygmomannometer cuff inflated to a pressure of 30mmHg higher than systolic blood pressure), where time necessary for attaining maximal perfusion after cuff release was measured. These stimulation tests are a standard in examination of tissue perfusion (Leahy, Walmsley, Albrecht, Wohlrab et al.) owing to a considerable time and spatial variability of plain basal perfusion measurement. Sampling rate was 31ms, firmware Perisoft (Perimed, Sweden) was used for data evaluation.

Transcutaneous oxygen monitoring

Partial pressure of oxygen was measured using tcpO2 probe PF 5040 of Periflux 5000 system

(Perimed, Sweden), based on principle of polarography (Lawall et al.). Heated Clark electrode (45°C) was attached to skin of foot dorsum at a standard location (between the 1st and 2nd metatarsus) using an adhesive ring, the space between the electrode and skin was filled with contact solution supplied by the producer. The probe was applied at least 10 minutes prior measurement commencement. Sampling rate was 31ms, firmware Perisoft (Perimed, Sweden) was used for data evaluation.

Results:

Data are clearly summarized in Table 2 and Graph 2, 3, and 4. The group, where the clamp with lower target insulinemia was performed first did not statistically differ from the group with initial higher insulinemic clamp. Statistically significant higher perfusion in skin microcirculation was achieved at physiological hyperinsulinemia in both tests (hyperemia after heating to 44°C – 1848% [984 – 2046] vs. 1599% [801 – 1836], $p < 0.05$, half time of reaching peak perfusion after occlusion release 1.2 s [0.9 – 2.6] vs. 4.9 s [1.8 – 11.4], $p < 0.05$. There occurred a statistically significant increase in tissue oxygenation ($tcpO_2$ – 48.6 mmHg [45.5 – 49.7] vs. 38.9mmHg [35.5 – 40.8], $p < 0.05$).

The perfusion of skin microcirculation was even higher at supraphysilogical hyperinsulinemia in both tests (hyperemia after heating to 44°C – 1937% [1177 – 2488] vs. 1599% [801 – 1836], $p < 0.005$, half time to reach peak perfusion after occlusion release 1.0 s [0.7 – 1.1] vs. 4.9 s [1.8 – 11.4], $p < 0.005$. There occurred a statistically significant increase in tissue oxygenation ($tcpO_2$ – 57.4 mmHg [51.7 – 66.2] vs. 38.9 mmHg [35.5 – 40.8], $p < 0.005$). The difference in perfusion and oxygenation between physiological and supraphysiological hyperinsulinemia were not statistically significant. M-value measured during the clamp for insulin resistance evaluation did not change.

Discussion:

Studies that monitored an influence of insulin on microcirculation used either local skin administration using iontophoresis (DeJongh, Serne et al.) or systemic delivery (Utriainene, Tooke, Ueda et al.). The advantage of local administration is limited local hyperinsulinemia, which does not require a clamp examination associated with fluid infusion and change in hepatic production of glucose and pancreatic production of insulin. In our study we chose the systemic administration, advantage of which is physiological insulin distribution and elimination of influence of passage of electrical current, which can induce vasoconstriction via voltage-dependent sodium and calcium channels (Figueroa et al.).

Simultaneous use of LDF and transcutaneous oxymetry methods was performed to distinguish perfusion of nutritive bed (assessed through O_2 release) from total blood flow through microcirculation (including arteriovenous shunts) in the region of interest of LDF probe, to which corresponds microvascular reactivity. However, measuring transcutaneous partial oxygen pressure can only be considered a rough indicator of nutritive bed perfusion. The exchange of oxygen between the vascular bed and tissues takes place also on other levels (larger vessels, via interstitial fluid) and there was found a discrepancy between capillary density assessed through video capillary microscopy and transcutaneous oxymetry values (Ubbink et al.).

In some older studies, (Utrainen, Tooke et al.) the authors describe no, or statistically insignificant, increase in microcirculation perfusion as a result of insulin infusion. On the other hand, more recent studies (Serne, deJongh et al.) demonstrate an increase in perfusion at physiological hyperinsulinemia. The explanation may lie in a different methodology used. In older studies the perfusion was measured by a probe only at basal conditions and no abovementioned stimulation test was employed. According to our findings the value of basal LDF perfusion showed only insignificant incremental trend (see Table 2.), which corresponds to data measured earlier (Utriainen, Tooke et al.).

Transcutaneous oxygen pressure monitoring is important for estimation of amputation wound

healing in diabetic foot syndrome (Faglia et al.) as well as for angioplasty effect monitoring in patients with critical limb ischemia (Caselli et al.), tcpO2 values at rest below 30 mmHg are an independent predictor of ischemia (Cechurova et al.). The increase in transcutaneous oxygen pressure observed in our study in consistent with previous study data (Tymms et al.), where arteriovenous difference of oxygen increased with continuous subcutaneous insulin infusion, which suggests a flow redistribution favoring functional vascular bed. On the contrary, in patients with diabetes mellitus type 2, where insulin resistance and hyperinsulinemia are present, transcutaneous oxygen pressure is inversely proportional to insulinemia – it falls with its increase (Kizu et al.). In obese patients with metabolic syndrome, but without diabetes, decreased vasomotion and reduced response to locally administered insulin was described (DeJongh et al.). These findings suggest a different behavior of microcirculatory vascular bed in hyperinsulinemic insulin-resistant patients, where the response of microcirculation to exogenous insulin administration is altered and there is no improvement of nutritive perfusion (possibly even deterioration), while in insulinsensitive

patients, insulin administration causes more nutritive capillaries to open.

Pathogenesis mechanism of reactivity changes is put in connection with oxidative stress induced by hyperlipidemia and insulin resistance, which causes vasoconstriction through augmentation of endothelin receptor activity (for thromboxane A2) in smooth muscle tissue (Xiang et al.). In this regard it will be interesting to observe insulin mediated microcirculation redistribution in patients with chronic heart failure treated with newly developed endothelin receptor antagonists, such study has not yet been done according to available literary data.

The question is, whether the fluid load itself associated with clamp examination does not lead to sympathetic activation and microcirculation reactivity increase. In literature, we can find a mention about slight microcirculatory reactivity increase associated with fast infusion of saline (Frost et al.). In our experiment, healthy volunteers were administered always a total of 1 – 1.5 l of glucose during the 2-hour clamp, there was no increase in heart rate observed. This rate of fluid infusion cannot be considered sufficient to trigger a sympathetic response. In addition, no significant difference in measured parameters was observed between the two subgroups (initially lower and higher insulinemia) despite the fact that the administered fluid volume at the first and the second steady state varied. Nevertheless, the failure to perform control examinations without glucose and insulin administration can be considered a certain limitation of the study.

Conclusion

Hyperinsulinemia causes an increase in reactivity of microcirculation as well as an increase in transcutaneous oxygen pressure in healthy volunteers upon systemic administration of insulin. This effect rises non-linearly with increasing insulinemia.